

VOLUME 31

JULY 1964

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

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OF WASHINGTON

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***Butlerius monhystera* (Nematoda, Diplogasterinae), a New
Species of Predaceous Nematode from Illinois.**

DONALD P. TAYLOR*

An undescribed species of predaceous nematode was recovered from soil collected at a commercial ornamental nursery near Edwardsville, Illinois. This population was identified as a species of *Butlerius* Goodey, 1929, a genus believed not to have been previously reported from North America.

The type species, *B. butleri*, was described by Goodey (1929) from nine specimens in alcohol-preserved, rotted banana roots. Adam (1930) described *B. filicaudatus* from a compost pile in the Netherlands. *B. okai* was described by Rahm (1938) from Hainan, and in the same year Schuurmans-Stekhoven and Teunissen (1938) based their description of *B. brevispiculatus* on one male collected in the Congo. Meyl (1957) described *B. gerlachi* from the water reservoirs of *Quesnelia arvensis* from Brazil. Lordello and Zamith (1959) described *B. singularis* from soil in Brazil, transferred *B. okai* to their newly established genus *Butleroides*, and transferred *B. brevispiculatus* to the genus *Diplogaster* Schultze in Carus, 1857. Meyl (1960) established a new genus, *Butleriellus*, into which he placed *B. filicaudatus*. J. B. Goodey (1963) rejected these latter changes and synonymized the new combinations with the originally proposed names. Thus, accepting Goodey's concept, six species of *Butlerius* are recognized: *B. butleri* Goodey, 1929; *B. filicaudatus* Adam, 1930; *B. okai* Rahm, 1938; *B. brevispiculatus* Schuurmans Stekhoven and Teunissen, 1938; *B. gerlachi* Meyl, 1957; and *B. singularis* Lordello and Zamith, 1959.

Butlerius monhystera sp. n. (Fig. 1)

DIMENSIONS: Female: (n = 5) L = 1.11 mm (1.0-1.22); a = 30.6 (26.5-33.3); b = 4.9 (4.6-5.4); c = 2.7 (2.6-2.8); V = 48% (45-50).

Male: (n = 3) L = 0.93 mm (0.77-1.07); a = 32.0 (28.2-40); b = 5.5 (4.8-6.3); c = 2.6 (2.6-2.7).

DESCRIPTION: Body slender, bluntly rounded at anterior end, tail extremely long and filiform. Cuticle thin, fine transverse striae present. No longitudinal striations noted. Lateral field not observed. Subcuticle marked with longitudinal rows of minute punctations. Amphids narrowly elliptical, just anterior to level of large dorsal tooth. Deirids not observed. Phasmids prominent, about one body width posterior to anus.

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I express my appreciation to Dr. R. V. Anderson, University of Minnesota for the loan of *Butlerius* material collected at Detroit Lakes, Minnesota, and to Prof. H. M. Darling, University of Wisconsin, for *Butlerius* specimens from Columbia, S.A.

Head bluntly rounded, possessing six setose papillae which appear single in structure. Circum-oral membrane prominent, supported by twelve terminally recurved rugae. Stoma divided into three chambers. Anterior chamber heavily sclerotized, cylindrical in cross-section. Contractile middle chamber probably not sclerotized, staining only faintly with acid fuchsin. Posterior chamber contains a large dorsal tooth and other smaller teeth and rasping structures as shown in Fig. 1B.

Esophagus heavily muscular, expanded slightly at base of stoma to form an esophageal collar. Metacorporeal swelling prominent. Conspicuous triradiate lumen from base of stoma to metacarpus, interrupted only by distinct sphincter at anterior end of metacarpus. Large tri-partate valvular apparatus in metacarpus. Isthmus and slight basal swelling not as muscular as corpus, with lumen reduced. Nerve ring just posterior to base of metacarpus. Excretory pore ventral, near level of nerve ring.

Esophago-intestinal valve projects into lumen of intestine, having at least six posteriorly directed projections. Intestinal lumen large. Rectum separated from intestine by prominent sphincter. Anus ventral, pore-like.

Tail tapers abruptly posterior to anus, forming an extremely long, filiform tip.

FEMALES: One single, anteriorly directed, reflexed ovary. Oocytes arranged as in Fig. 1A. Only one egg present in the uterus at a time. Vagina not prominent; vulva in slight ventral depression. Post-vulval uterine branch densely packed with sperm in most specimens.

MALES: Testis single, anterior end reflexed. Bursa absent, nine pairs of papillae, arranged as in Fig. 1F. Three pairs are pre-anal. Spicules paired, ventrally arcuate. Gubernaculum complex, shaped as in Fig. 1F.

DIAGNOSIS: *B. monhystera* can be distinguished from all other described species of *Butlerius* by the presence of a single ovary, by its relatively longer tail ($e = 2.6-2.8$), and by the unique shape of the gubernaculum.

HOLOTYPE: Single female on slide labeled "*Butlerius monhystera*," Slide No. T-49t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

ALLOTYPE: Single male specimen on same slide as above.

TYPE HABITAT AND LOCATION: Field soil from property owned by the Home Nursery Company, Edwardsville, Illinois.

FEEDING HABITS

The feeding of *B. monhystera* has been observed, and this species is predaceous upon other nematodes, as has been reported by Lordello and Zamith (1959) for *B. singularis*. Most specimens observed attacked nematodes having a body diameter smaller than themselves. The prey was usually ingested tail-, or less commonly, head-first. When attacking its prey, the predator's stoma became shortened, caused by a contraction of the non-sclerotized middle chamber, and teeth in the basal chamber were thus able to puncture the prey's body wall. Body contents of the prey were ingested and moved posteriorly in the lumen of the predator's procorpus. Passage of food was accompanied by extreme dialation of the lumen. Food flow was less rapid in the posterior portion of the esophagus and was not accompanied by an expansion of the lumen, indicating the probable valvular function of the sclerotized plates in the metacarpus. Failure of this species to become established on a culture of *Aphelenchus avenae* Bastian, 1865, prevented additional observations.

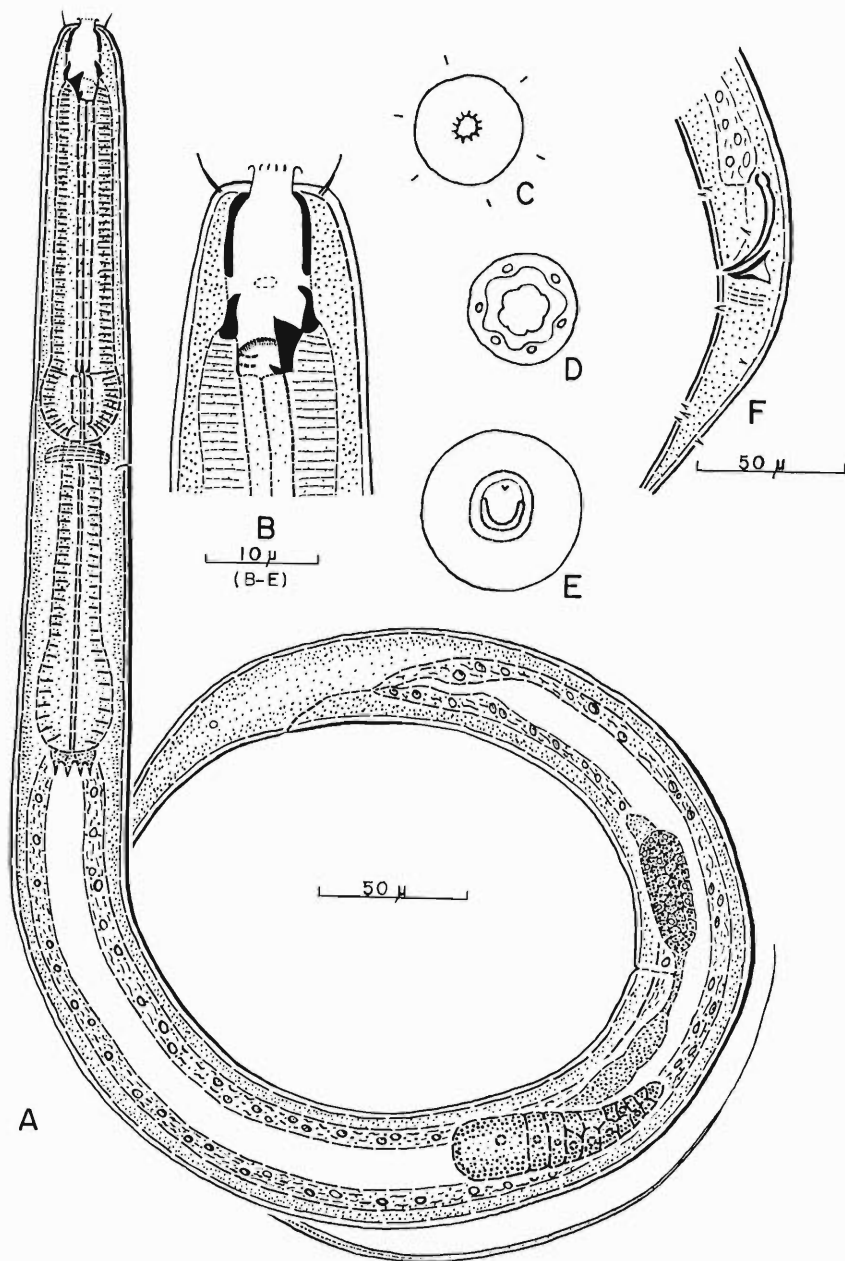


Fig. 1. *Butlerius monhystra* sp. n. A. Adult female, full length, lateral view. B. Stoma and adjacent anterior end of adult female. C-E. *En face* views: C—at level of circum-oral membrane showing rugae and papillae tips; D—at level of base of papillae; E—at level of dorsal tooth. F. Male, ventral view, cloacal region.

DISCUSSION

All previously described *Butlerius* species are didelphic, although Goodey (1963) does not mention this in the generic definition. Presence of only one ovary in *B. monhystera* n. sp. suggests that this species might represent a new genus. However, because members of this genus have so seldom been reported, it is preferred to include this species in the genus *Butlerius* until additional forms are described and their relationships investigated. Other nematode genera, e.g. *Trichodoros* Cobb, 1913, also include both monodelphic and didelphic species, and in the case of *Butlerius* such a grouping is entirely justified.

The creation of the monospecific genus *Butleroides* by Lordello and Zamith (1959) to include forms resembling *Butlerius* but in which males apparently lack a gubernaculum, seems untenable, particularly since they did not report observing the original material. Therefore, as stated by J. B. Goodey (1963), the combination *Butlerius okai* Rahm, 1938 is preferred to *Butleroides okai* (Rahm, 1938) Lordello and Zamith, 1959.

A careful review of Schuurmans Stekhoven and Teunissen's paper (1938) indicated that they were well aware of the differences between *Butlerius* and *Diplogaster* at the time they described *B. brevispiculatus*. They wrote:

"Notre matériel comprend des représentants de deux genres, *Butlerius* et *Diplogaster*.

Le genre *Butlerius* se caractérise par la texture de la cavité buccale, dont les cuticularisations pariétales sont interrompues par des portions plus faibles, caractère peu visible dans notre exemplaire à cause de l'état de contraction. . . ." (Schuurmans Stekhoven and Teunissen. Page 27).

Lordello and Zamith gave no reason for transferring *B. brevispiculatus* to *Diplogaster* and stated only: "Esta espécie, entretanto, não pode ser colocado no gênero em estudo, mas sim em *Diplogaster* Schultze, 1857."

On this basis and again in agreement with J. B. Goodey, the correct name for this nematode appears to be *Butlerius brevispiculatus* Schuurmans Stekhoven and Teunissen, 1938.

The description of *B. monhystera* brings the total number of known species in this genus to seven and alters the concept of generic characters so as to include both monodelphic and didelphic forms.

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***Xiphinema conurum* n. sp. and *Paralongidorus microlaimus*
n. sp., with a Key to the Species of *Paralongidorus*
(Nematoda : Longidoridae)**

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Plant-parasitic nematodes collected in November-December, 1961 by Mr. Keith F. Brown, Nematologist of the Shell International Chemical Co. Ltd., London, at various sites in Tunisia, contained three species of the genus *Xiphinema* Cobb, 1913. These include a new species, *Xiphinema conurum*, and two known species, *X. americanum* Cobb, 1913, (collected around roots of *Sesbania* sp. at Teboulba and apricot roots near Tunis) and *X. elongatum* Schuurmans Stekhoven et Teunissen, 1938 (around citrus and almond roots near Tunis). Mr. Brown's collection made in India yielded an undescribed species of the genus *Paralongidorus* Siddiqi, Hooper and Khan, 1963. This species was found around roots of banana plants at Vadgaon in Maharashtra State. It is named as *Paralongidorus microlaimus* n. sp. in accordance with its comparatively shorter buccal spear. This species is the third in the genus reported from India, the previously reported species being *P. citri* (Siddiqi, 1959) Siddiqi et al., 1963, and *P. sacchari* Siddiqi et al., 1963.

Xiphinema conurum n. sp. and *Paralongidorus microlaimus* n. sp. are described and a key to the species of *Paralongidorus* is provided. *Longidorus maximus* (Bütschli, 1874) Thorne and Swanger, 1936, has been considered in the genus *Paralongidorus* because it has stirrup-shaped amphids.

Xiphinema conurum n. sp. (Fig. 1, A-D)

FEMALE (Holotype) : Mounted in Glycerine. Length = 4.2 mm; a = 117; b = 10.5; c = 63; V = $9.2-49-6.4\%$.

Body much attenuated, ventrally arcuate. Body cuticle in two distinct layers; inner layer marked by transverse striae. Lateral hypodermal chords $\frac{1}{4}$ as wide as body. Amphids stirrup-shaped, half as wide as head. Head rounded knob-like, set off from body by a depression, 14 microns wide (Fig. 1, B). Lips amalgamated; labial papillae distinct, not modifying head contour. Spear 110 microns long. Spear extension distinctly flanged, 65 microns in length (Fig. 1, A). Spear guiding ring 95 microns from anterior end of body, anteriorly forming a guiding sheath measuring 35 microns long.

Oesophagus a slender tube, expanding in its posterior third to become half as wide as body width at neck base. A small triangular mucro present in the sub-ventral sector of the oesophagus 35 microns behind the base of spear extension. Nerve ring 50 microns behind spear extension, giving out nerve connection to hemizonid which is located a little anterior to its level. (Fig. 1, C). Oesophago-intestinal valve conoid-rounded. Intestine with dense food granules. Intestinal lumen distinct throughout.

Vulva a depressed transverse slit, almost equatorial in position. Vagina at right angles to body axis. Gonads paired, opposed, reflexed at the oviduct. Ovaries well-developed, with developing oöcytes in a single row. Pre-rectum 600 microns long. Rectum about one anal body width in length. Tail elongate-conoid, a little less than three times anal body width long (Fig. 1, D). Two pairs of pores on tail.

MALE: not found.

TYPE HOST AND LOCALITY: Collected from soil around roots of almond,

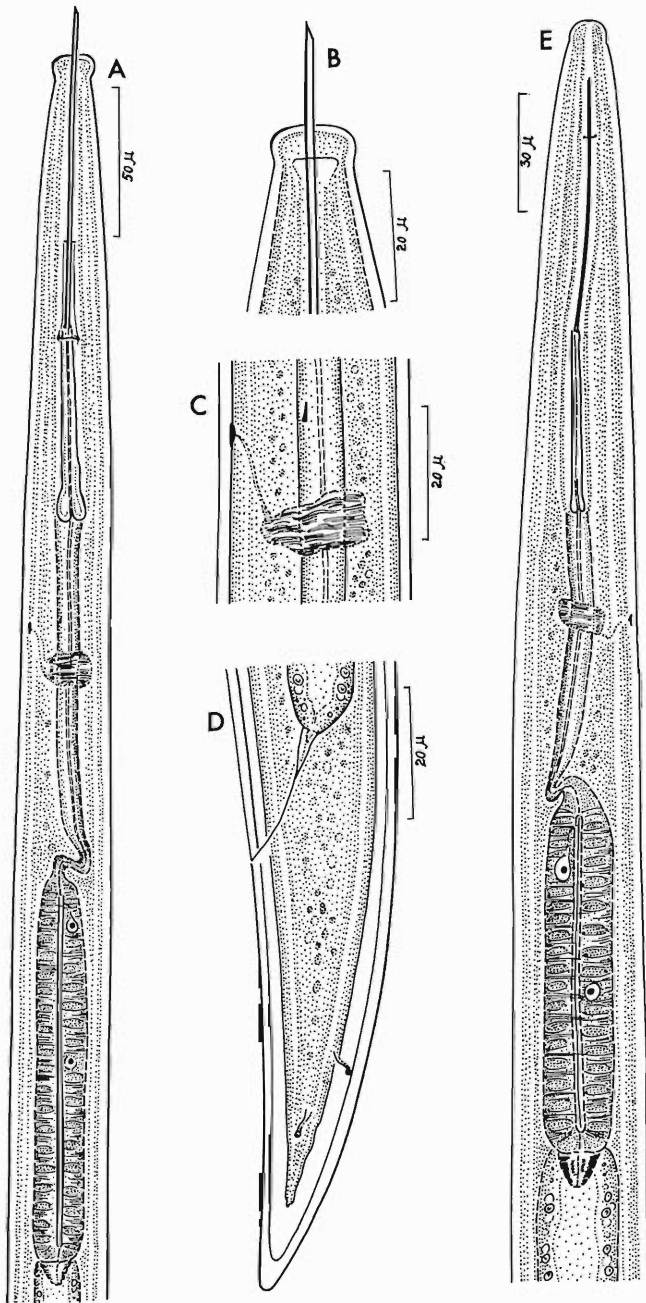


Fig. 1. A-D. *Xiphinema conurum* n. sp. A. Oesophageal region of female. B. Cephalic end of female. C. Nerve ring, hemizonid and mucro in female. D. Caudal end of female. E. *Paralongidorus microlaimus* n. sp. Oesophageal region of female.

Prunus amygdalus Batsch, near Tunis-Sausse Road, about 76 Kms. from Tunis.

DIFFERENTIAL DIAGNOSIS: *Xiphinema conurum* n. sp. resembles *X. elongatum* Schuurmans Stekhoven et Teunissen, 1938; *X. attorodorum* Luc, 1961; and *X. ifacolum* Luc, 1961. From *X. elongatum* it differs in having a longer and more attenuated body (body less than 2.5 mm long in *elongatum*), a knob-like head and a more posteriorly located vulva ($V = 40\%$ in *elongatum*).

From *X. attorodorum* it can be differentiated by its larger body size, smaller buccal spear and more posteriorly located vulva ($V = 40.1-42\%$ in *attorodorum*).

From *X. ifacolum* it differs in having a more attenuated body ($a = 50.3-62.0$ in *ifacolum*), a shorter spear and differently shaped tail (tail irregularly conoid to a subdigitate end in *ifacolum*).*

Paralongidorus microlaimus n. sp. (Fig. 1, E & Fig. 2, A-C)

FEMALE (Holotype): Mounted in glycerine. Length = 3.05 mm; $a = 76$; $b = 10.6$; $c = 98$; $V = 7.6-49-6.8\%$.

Body almost cylindrical, tapering anteriorly from neck base to lip region which become $\frac{1}{4}$ as wide as its maximum width, ventrally coiled to form a C-shape. Cuticle apparently double layered; inner layer of cuticle marked with fine transverse striae. Lateral hypodermal chords about one-third of the body width. Lateral body pores serially arranged along neck region but irregularly on body, appearing to lead into hypodermal gland-like bodies.

Head round, 10 microns wide and half as much high, slightly offset by a depression (Fig. 2, A). Amphid pouches stirrup-shaped in lateral view (Fig. 2, A). Amphidial apertures conspicuous slits, occupying $\frac{3}{4}$ of head width. Sensillar sacs at level of spear guiding ring. Spear slightly ventrally curved, 65 microns long. Spear extension simple, not flanged at base, 46 microns in length (Fig. 1, E). Spear guiding ring a little less than three cephalic widths from anterior end of body.

Oesophagus typical of the genus. Its enlarged part about $\frac{2}{7}$ of its length; dorsal and two sub-ventral glands prominent. Nerve ring at 25 microns behind base of spear extension. Hemizonid distinct, at level of nerve ring. Oesophago-intestinal valve roundly conical. Intestinal cells with dense food globules. Pre-rectum not definite. Vulva transverse. Vagina at right angles to body axis, with highly muscular walls. Gonads paired, symmetrically opposed, reflexed at oviduct (Fig. 2, B). Developing oöcytes in a single file. Rectum about one anal body width long. Tail convex-conoid to rounded terminus, slightly over one anal body width long. Two pairs of caudal pores present (Fig. 2, C).

MALE: not found.

TYPE HOST AND LOCALITY: Collected from soil around roots of banana plants, *Musa paradisiaca* L., at Vadgaon, Maharashtra State, India.

DIFFERENTIAL DIAGNOSIS: In having a comparatively smaller body-size and unconstricted head, *Paralongidorus microlaimus* n. sp. comes close to *P. sali* Siddiqi, Hooper and Khan, 1963 but can be differentiated from it in having differently shaped amphids (amphids in *P. sali* funnel-shaped with slits

**Xiphinema arenarium* Luc and Dalmaso, 1964 (Nematologica 9: 531-541, 1963) is very similar to *X. conurum* but is a little smaller in length (2.9-3.5 mm), has different body measurements and a slightly smaller buccal spear (96-105 microns long). More specimens of both species are needed to evaluate their relationship.

tending to curl inwards at their ends), a shorter buccal spear (spear 98-107 microns long in *P. sali*) and tail being longer than anal body width.

TYPE SPECIMENS: in author's personal collection.

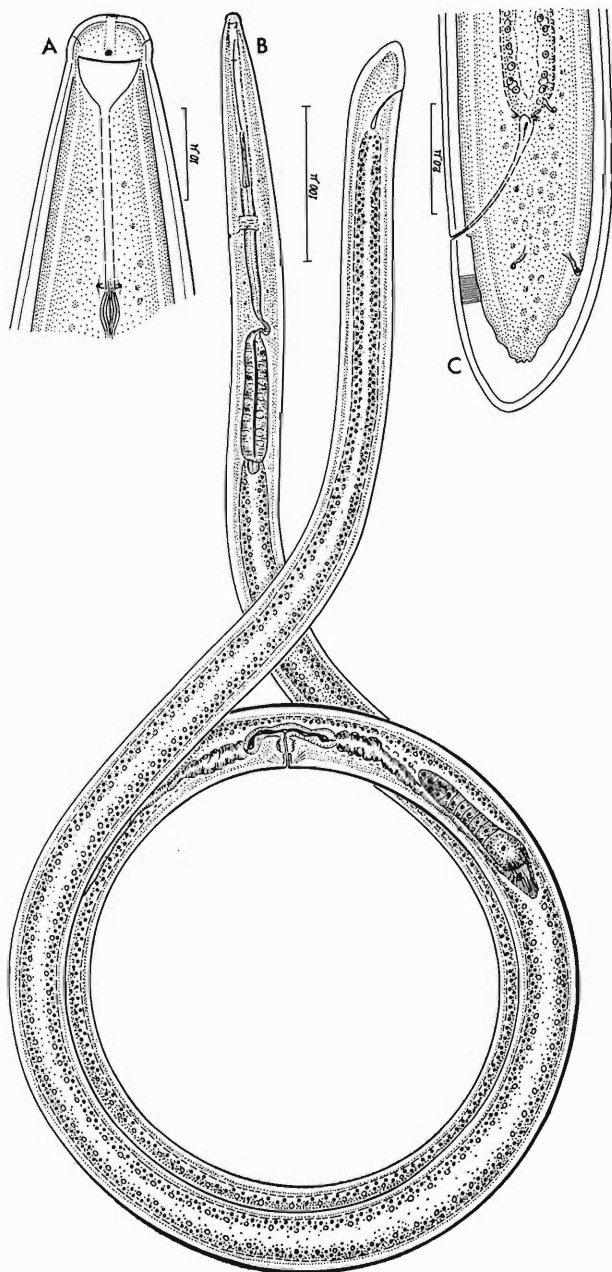


Fig. 2. A-C. *Paralongidorus microlaimus* n. sp. A. Cephalic end of female showing amphidial system. B. Female (Holotype). C. Caudal end of female.

KEY TO SPECIES OF PARALONGIDORUS

1. Head set off from body by a constriction..... 2
 Head not set off from body by a constriction..... 3
2. Tail length $\frac{1}{2}$ to $\frac{3}{4}$ anal body-width.....
 *P. maximus* (Bütschli, 1874) n. comb.
 Tail length 1 anal body-width.....
 *P. citri* (Siddiqi, 1959) Siddiqi *et al.*, 1963
3. Spear 65 microns long..... *P. microlaimus* n. sp.
 Spear about 100 microns long or more 4
4. Body-size 2.25-2.85 mm; tail less than anal body width long.....
 *P. sali* Siddiqi, *et al.*, 1963
 Body-size 4.1-5.2 mm; tail one anal body width long.....
 *P. sacchari* Siddiqi, *et al.*, 1963

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A Technique for Studying En Face View of Moderate-Sized Nematodes Without Decapitation*

SHERIDAN H. LEE

Methods of studying the *en face* view of nematodes have been described by various authors (Cobb, 1920; Chitwood and Wehr, 1934; Buhner, 1949; Tromba and Douvres, 1953; Anderson, 1958; Thorne, 1961). However, each of these methods necessitates the decapitation of the worm. Students beginning in helminthology often find themselves in a dilemma as to whether or not to decapitate the "unique" specimen when they try to identify it with the taxonomic keys found in nematological literature. The technique described here will enable one to study the *en face* view of nematodes of moderate size, e. g. *Camallanus*, *Amplicaeum ardei*, *Philometra*, *Protospirura ascaroidea*, *Litomosoides*, etc., without permanently damaging the specimen. Thus, in case a new form is discovered, the holotype can be preserved in its entirety.

MATERIALS AND METHODS

1. Fine glass rods or capillary tubing, 60 mm. long, 1 mm. or less in diameter.
 2. Standard size (25 mm × 75 mm) slide.
 3. Camel hair paint brush, size 00 or 0.
 4. Thin balsam.
 5. Glycerine or lactophenol.
- Make glass rod A about 20 mm. long and glass rod B about 40 mm. long.

*Oklahoma Baptist University, Shawnee, Oklahoma, U.S.A.
 This work was supported by NSF grant No. GE382 to the University of Oklahoma Biological Station.

With balsam draw a thin line on the clean slide perpendicular to the length of the latter. This line should be closer to one end of the slide so that there will be room to hold the worm. Place glass rod A on top of the balsam line and let it dry so that it will be securely glued to the slide.

When the worm is thoroughly cleared, transfer it directly from the clearing fluid (either glycerine or lactophenol) to the slide by means of a camel hair paint brush. Place the cephalic end across the center of glass rod A; thus glass rod A serves as a "pillow" for the worm. Place glass rod B on top of the worm. Glass rod B is now parallel with glass rod A and the two rods are as close to each other as possible. Press rod B down gently; thus the cephalic end of the worm is automatically forced upward.

A good preparation requires; (1) that the cephalic portion of the worm be held perpendicularly between the two glass rods and (2) that its very anterior tip be flush with the upper surfaces of the two rods. These requirements can be met by manipulating the posterior end of the worm, either by pulling it away from the rods or pushing it toward one side of the slide, the direction and amplitude depending on the way the head of the worm is turned. While one hand performs the manipulation, the other hand must steady glass rod B so that a continuous clamping force exists. However, care shall be taken that one does not apply too much force, especially when pulling the worm away from the rods, in order to avoid pinching off the cephalic end of the worm. One's ingenuity is encouraged to devise other means to achieve the two goals mentioned above. It is also important that there be enough clearing fluid to surround the anterior tip; therefore, one may add a very small drop of clearing fluid to the preparation after the desired position of the worm is obtained. Use a dissecting microscope with a total magnification 40X, to do the arranging. Slight adjustments can be made even under compound microscope. Detailed cephalic structures can be studied under compound microscopes with 10× oculars and 43× objectives without cover glasses.

SUMMARY

A simple method of studying the *en face* view of nematodes without decapitation of the worm is described.

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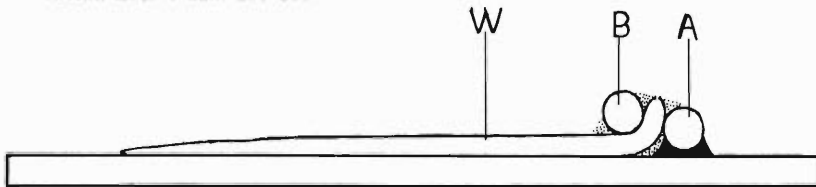


Fig. 1. A diagram of the lateral view of the final position of the worm, W, in relation to glass rods A and B.

**Studies on *Dactylogyrus corporalis* n. sp. (Trematoda :
Monogenea) from the Fallfish *Semotilus corporalis***

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ABSTRACT

Dactylogyrus corporalis n. sp. from the fallfish, *Semotilus corporalis* (Mitchill) is described along with its experimental life cycle and other biological observations. At 22°C the life cycle from egg to egg producing adult takes 13 to 14 days. After deposition the embryo develops eye spots within 2 to 3 days and hatches within 4 to 5 days. The oncomiracidium attaches to the fish within 24 hours after hatching. Eight days are required for development to maturity after attachment to the host.

In vitro oviposition rate is one egg per parasite every 17.5 min. at 23°C; site of infection is at the distal ¼ of the host gill filament and the average parasite load in nature is 4.5 worms per fish. In nature the parasite appears to be host specific for *S. corporalis*, however, under laboratory conditions it was possible to infect goldfish. Pathology consists of whitish epithelial hyperplasia with subsequent degeneration of the distal tips of infected gill filaments.

The genus *Dactylogyrus* was erected by Diesing (1850) with the generic diagnosis as follows (cf. Yamaguti 1963) : Dactylogyridae, Dactylogyrinae: Anchors supported by single rod-shaped bar. Vas deferens usually (always?) looped around intestinal limb. Vesicula seminalis formed by mere dilatation of vas deferens. Two prostatic reservoirs present. Cirrus mostly tubular, with accessory piece. Vagina single, exceptionally double, may or may not have sclerotized supporting structures, with submarginal aperture. Parasites of freshwater teleosts.

Price (1938) erected the genus *Neodactylogyrus* which has the same generic diagnosis except that the anchors are supported by two, (dorsal and ventral) bars which may be similar or dissimilar. Mizelle and Donahue (1944) rejected the genus *Neodactylogyrus*, basing their rejection on the following "the presence of the vestigial ventral bar required for assignment of species, there to, is differentially developed, not always visible in fixed specimens, and results in unnecessary synonymy." Sproston (1946) recognized the genus *Neodactylogyrus*, but Monaco and Mizelle (1955) reject it, pointing out, "that the dorsal bar supports the bases of the two anchors (dorsal) in species assigned to this category, but that the ventral bar is not associated with these large hooks." Bychowsky (1957) made no mention of the genus *Neodactylogyrus* while Yamaguti (1963) listed the original descriptions of both *Dactylogyrus* and *Neodactylogyrus*.

D. corporalis n. sp. possesses the delicate ventral bar, but it is not always visible; following the recommendation of Mizelle and Donahue (1944) we assign it to the genus *Dactylogyrus* since we regard *Neodactylogyrus* as a synonym.

This trematode appears to be specific and very common on all age groups of fallfish examined from the Opequon Creek. It was found easily on three different occasions; in June, August, and October and also on fish collected in October, but kept in the laboratory in spring water until February.

D. corporalis n. sp. causes pathology similar to *D. vastator* Nybelin (1924), which has received much attention in Russian fish culture due to its marked epizootic significance in eyprinid pond culture.

MATERIAL AND METHODS

Fish were collected from the Opequon Creek, Jefferson County, West Virginia, during the summers of 1958 and 1963. To remove the parasites for preservation in 1958, gills were dissected and placed in 0.5% urethane, after which the parasites were preserved in 10% formalin. The 1963 worms were removed mechanically and fixed in 10% formalin. For live study, individual parasitized gill filaments were removed and placed in fresh water. The following descriptions and observations are based on more than 50 specimens. The specimens were studied in the living, fixed, unstained and stained states. Specimens were stained with Harris' hematoxylin and Semichon's carmine. Measurements are in millimeters and from fixed material. Average is given with the range in parentheses.

DESCRIPTION

Dactylogyrus corporalis n. sp. (Figs. 1-11)

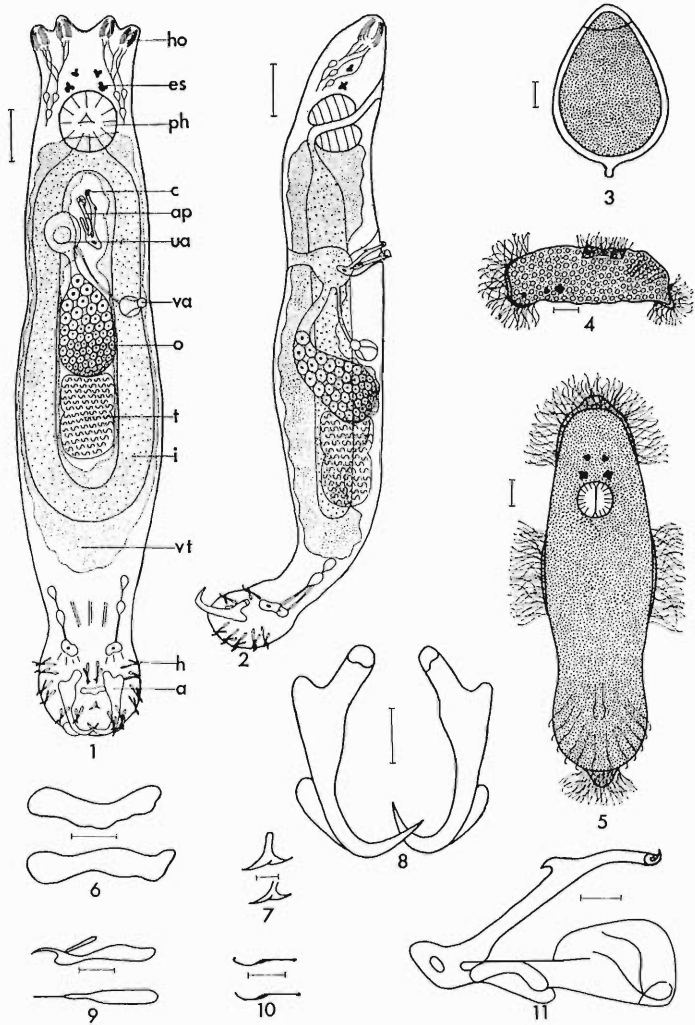
ADULT DESCRIPTION: Large daetylogyrid with smooth cuticle; length 0.800 (0.575-1.062), area of greatest width 0.110 (0.075-0.125). Eye spots four, with the posterior pair larger and farther apart. Cephalic lobes four, two on each side of mid-line and containing pyriform shaped head organs with tubes which seem to originate from secretory-type cells located lateral to pharynx. Mouth a transverse slit on ventral side behind eye spots, with tube leading to sub-circular pharynx 0.060 in transverse diameter. Intestine bifurcates into two branches directly after leaving pharynx and extends posteriorly reuniting posterior to testis. Anchors consist of base, 0.010 at greatest width, well-developed dorsal and ventral roots (dorsal the smaller), solid shaft 0.042 (0.038-0.043) long and solid point 0.016 (0.015-0.018) long. Anchor wings well developed. Dorsal bar yoke-shaped and irregular, 0.029 (0.023-0.033) by 0.005 at widest part, and articulates with anchors at area of greatest width. Delicate vestigial ventral bar not always visible, when visible tripartite with all parts approximately equal, 0.012 long.

Hooks 14 in number, approximately equal in size, 0.028 long, and normal in arrangement. Additional pair of smaller haptoral hooks lacking posterior projecting process (Mizelle and Price 1963) not seen. Each hook composed of solid elongate base, slender solid shaft and sickle-shaped termination with small opposable piece. A process projects posteriorly from the sickle-shaped termination. Ovary anterior to testis, flask-shaped in side view, with base near testis and neck extending into uterus which appears to open via uterine aperture dorsally and lateral to copulatory complex. The ovary, 0.090 (0.084-0.112) by 0.053 (0.035-0.063), contains many ova in various stages of development with larger ones toward ovary neck. Vagina opens via vaginal aperture laterally and ventrally to anterior base of ovary. Testis is ovoid, sometimes appearing bilobed, 0.080 (0.076-0.084) by 0.054 (0.042-0.070), lies ventrally against base of ovary. Vitellaria extend dorsally and laterally from the pharynx, posterior to intestine (fig. 2). Copulatory complex consists of a cirrus and accessory piece. Cirrus, 0.068 (0.053-0.079), a stout hollow tube with spiral and pointed termination. A dorsal, backward-pointing, small hollow projection, two-thirds of the way up cirrus. Accessory piece, 0.060 (0.050-0.070), and base complicated, with accessory piece forming a membranous, cradle-like structure with ringed aperture at distal end.

HOST, NATURAL: *Semotilus corporalis* (Mitchill).

HOST, EXPERIMENTAL: *Carassius auratus* (Linnaeus).

LOCATION: Distal $\frac{1}{4}$ of gill filaments.



Figures 1 to 11. Developmental stages and anatomy of *Dactylogyrus corporalis* n. sp. Figures 1 to 5 and 10 are freehand drawings made to scale. Figures 6 to 9 and 11 were drawn with the aid of microprojection. All scales represent 0.010 mm except figures 1 and 2 which represent 0.050 mm. Total body length of 1 and 2 is not the average, but falls within the range.

Abbreviations used: a, anchor; ap, accessory piece; c, cirrus, es, eye spot; h, hook; ho, head organ; i, intestine, o, ovary, ph, pharynx; t, testis, ua, uterine aperture; va, vaginal aperture; vt, vitellaria.

Figure 1. Dorsal view of composite drawing of mature diet. Figure 2. Side view of composite drawing of mature adult. Figure 3. Egg. Figure 4. Side view of newly hatched oncomiracidium. Figure 5. Dorsal view of initial parasitic stage of oncomiracidium. Figure 6. Two dorsal bars showing variability. Figure 7. Two vestigial ventral bars showing variability. Figure 8. Anchors. Figure 9. Side and dorsal views of hooks. Figure 10. Two hooks of oncomiracidium. Figure 11. Copulatory complex.

TYPE: U. S. National Museum Helm. Coll. No. 60175.

PARATYPE: Eastern Fish Disease Laboratory, Kearneysville, W. Va.

COMPARISON: *Dactylogyrus corporalis* n. sp. most resembles *D. extensus* Mueller and Van Cleave, 1932 from *Cyprinus carpio* in total body and cirrus size, but the structure of the copulatory complex is very different. *D. tenax* Mueller, 1938 from *Semotilus atromaculatus* is similar in total body size, but has a smaller copulatory complex with the cirrus being a straight tube and the accessory piece divided distally into 3 "cups."

Egg (Fig. 3): Pyriform in shape, 0.058 (0.056-0.062) by 0.047 (0.043-0.050), with a small 0.003 projection from wide posterior end. When deposited, dark brown and contains dense, finely granulated particles. Operculum and adheres quickly to substrate after leaving adult worm.

ONCOMIRACIDIUM: (Figs. 4, 5) Upon hatching, free swimming larva ca. 0.062 (0.057-0.066) long by 0.019 (0.017-0.022) wide, possesses four eye spots, and cilia on both ends and on both sides at mid-body. Bychowsky (1957) notes that, "the larva which has just emerged from the egg has a length generally 1.5 times larger than the length of the egg." Internal structures not developed except for presence of developing haptor hooks at posterior end unlike those illustrated in Bychowsky (1957). Larva enlarges after hatching, maximum size attained before requiring a fish host 0.140 (0.134-0.147) long by 0.042 (0.040-0.045) wide. At this stage of development haptor area measures 0.042 by 0.028, with peripheral hooks 0.017 long and central pair 0.014 long. A definite pharynx visible and measures 0.012 in diameter. Cilia approximately 0.011 long.

EXPERIMENTAL LIFE CYCLE (Figs. 1-5)

EGG DEVELOPMENT: Upon deposition, the eggs were pipetted to small dishes containing fresh 23°C water and held for observation. At an average temperature of 22°C (21-24) eye spots appeared in 2 to 3 days, and hatching occurred in 4 to 5 days.

ONCOMIRACIDIUM: After hatching at the above temperature, the larva reaches its maximum size in about 15 hours, and, if not afforded a fish host, dies within 24 hours. Bychowsky (1957) states, "Among representatives of the genus, the period of the free-swimming larva extends from 4 to 20 hours and the period during which the larva is capable of infecting a host is considerably shorter—does not exceed 5 hours." At hatching, parasite-free, laboratory-reared goldfish *Carassius auratus*, four months old, were exposed to the larvae to further the life cycle.

INITIAL PARASITIC STAGE: After finding a fish host, the oncomiracidium attaches itself to a filament, loses its cilia, and begins its growth and maturation period. Three days later, individual parasites were found, but they did not appear mature either in total size or internal morphology. Eight days after exposure, mature-appearing worms were found on the gills, with sex organs developed and oviposition taking place.

OTHER BIOLOGICAL OBSERVATIONS

OVIPOSITION RATE: Five small dishes were filled with 23°C water, and into each dish were placed five adult worms still attached to a freshly dissected gill filament. An individual parasite from each dish was viewed microscopically for one half hour to determine its oviposition rate. The oviposition rate of the five individuals ranged from one egg every 10 min. to one egg every 20 min. All five dishes were checked at the end of one hour for total number

of eggs deposited by the combined 25 parasites. The total number of eggs (87) was divided by the combined number of parasites (25) giving an oviposition rate of 3.4 eggs per parasite per hr, or an egg per parasite every 17.5 min. Bychowsky (1957) notes that, "a decrease in oxygen and a rise in water temperature results in an increase in the number of eggs deposited, and that is why it is so easy to acquire an intensive laying of *D. vastator* in artificial and obviously unfavorable conditions."

SITE AND INTENSITY OF INFECTION: The parasites were found on the distal $\frac{1}{4}$ of the gill filament, usually between the two rows of filaments of a gill arch. They were seldom found singly, but in groups of two or more with as many as 10 being found together at a common anchoring area on one filament. When a parasite was found by itself, it was noticed that it was not a mature worm and probably was very recently acquired. Ten fish, fresh from the stream, revealed an average parasite load of 4.5 (2-9) worms per fish.

HOST SPECIFICITY: *D. corporalis* n. sp. was not found on *Ambloplites repestis*, *Rhinichthys atratulus*, *Exoglossum maxillingua* or *Semotilus atromaculatus* taken from the same collecting area. We were able to infect laboratory-reared, four month old goldfish, *Carassius auratus*, for experimental life cycle work.

PATHOLOGY: From the point of parasite attachment and extending to the apex of the gill filament, epithelial hyperplasia takes place, causing dense whitish outgrowths. It was noticed that once the outgrowth had attained a large size, the worms were absent from that filament. In heavy infections, filaments were present with degeneration of the distal half. Observations that the worms were not present on filaments with large epithelial outgrowths, and that degenerate filaments were present are similar to those of Wunder (1929), as noted by (Bauer, 1959). Wunder (1929) drew attention to the formation of epithelial outgrowths on the top of the first order gill lamellae in cases of *D. vastator* infection, and observed that, "the parasites are unable to survive in these outgrowths and they die, the outgrowths later disintegrating." It is probable that this parasite would cause fish mortality under conditions, such as rearing ponds, which favor the reproduction of *Dactylogyrus*.

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Nematodes of the Superfamily Dorylaimoidea from East Pakistan

R. W. TIMM

In the course of a survey of nematodes associated with roots of jute (*Corchorus capsularis* L. and *C. olitorius* L.) in East Pakistan, one new genus and six new species of the Superfamily Dorylaimoidea, plus a recently reported species, were discovered in soil around the roots of jute. Five species are of the Subfamily Leptonchinae, and one each of the Subfamily Tylencholaiminae and Family Belondiridae.

Measurements of type specimens are of glycerine mounts. Measurements of all other specimen are of 5% formalin mounts, except *Calolaimus papillatus* n.g., n.sp., which were measured after killing by gentle heating in water.

Calolaimus n.gen.

Leptonchinae. Axial stylet with flanged extension; large simple guiding ring; amphids large, stirrup-shaped; esophagus with terminal bulb-like portion, containing sclerotized valve-like plates in anterior part; two ovaries, reflexed; testes, spicules and supplements dorylaimoid; male tail bearing prominent paired submedian postanal papillae.

TYPE SPECIES: *Calolaimus papillatus* n.sp.

OTHER SPECIES: *Calolaimus ditlervseni* (Micoletzky, 1922) n. comb. [= *Dorylaimoides ditlervseni* (Micoletzky, 1922) Thorne & Swanger, 1936]

The generic name is derived from the Greek words *kalos* meaning "beautiful" and *laimos* meaning "gullet."

DIAGNOSIS: This genus is closest to *Dorylaimoides* but differs in the heavier spear guiding ring, the sclerotized plates at the anterior of the esophageal bulb, the constricted accessory piece of the spicules, and the large papillae on the male tail.

Calolaimus papillatus n.sp. (Fig. 1, A-E)

FEMALES (10): L = 5.04 (4.42-6.32) mm; a = 102 (87-128); b = 15.6 (12.4-18.9); c = 32.8 (24.8-36.5); V = 39.8 (36.9-41.8) %; Ov 1 = 5.4-9%; Ov 2 = 5.5-9%.

MALE: (2): L = 4.27-4.32 mm; a = 83-89; b = 13.7-14.3; c = 28-30.

DESCRIPTION: Cuticle moderately thick, finely striated; subcuticle not striated. Head rounded, not set off by constriction; head diameter one-fourth the body diameter at esophageal base. Lips fused for most part. Six papillae in inner circle at extreme anterior and ten bluntly protruding papillae in outer circle. Amphids large, stirrup-shaped, with slit-like aperture, about 60% of head diameter wide; amphidial pouch with sensilla posterior to spear extension. Spear 9 microns long, extension 10 microns long, slightly flanged; aperture of spear about 1.5 microns. Spear guiding ring prominent, 3-4 microns wide, attached to conspicuous sheath extending to spear base. Esophageal tissue slightly swollen at base of extension. Esophagus narrow most of its length, expanding into distinct muscular terminal portion, 35-39% of esophageal length. Esophageal bulb slightly constricted near anterior, with thickened lining set off as small valve-like plates in anterior portion. Small hemispherical esophago-intestinal valve. Several cells, glandular in appearance, clustered between nerve ring and esophageal bulb. Lateral fields clear, consisting of large rectangular cells filled with

*Notre Dame College, Dacca, East Pakistan. This work is part of a survey of jute nematodes supported by a grant from the Central Jute Committee, Pakistan.

globules and granules; lateral pores not observed. Nerve ring at 50% of esophageal length. Excretory cell and pore absent. Intestine consisting of two cells in cross-section. Indistinct prerectum, about 3-5 anal body diameters long. Female reproductive system amphidelphic; ovaries reflexed; vulva transverse, surrounded by small hexagonal sclerotized plates; vagina sclerotized; ova not observed. Testes two, each about 10% of body length; spicules slightly cephalated, 50 microns long, with internal division; gubernaculum absent; broad accessory piece at side of each spicule, 12 microns long, with constriction in middle. One pair adanal papillae and two small mammillate ventro-median preanal supplements immediately anterior to anus; group of eight supplements anterior to spicules, equally spaced, anteriormost about 200 microns or 6 anal body diameters in front of anus. Four pairs of prominent ventro-submedian papillae on anterior half of male tail; one dorso-submedian pair near digitate part of tail. One pair only of dorso-submedian papillae seen on female tail, at beginning of digitate part of tail. Tail in both sexes of similar shape, conical-digitate, 4.4 anal body diameters long in male, 6.4-7.5 in female.

DIAGNOSIS: The spear guide, prominent postanal papillae in the male, and constricted accessory piece of the spicules make necessary the inclusion of *Dorylaimoides ditlerseni* in the genus *Calolaimus*. It differs from the present species in the greater number of male supplements (19).

HOLOTYPE FEMALE: Collected May 25, 1960 by G. Mazumder; personal collection, No. S15.

ALLOTYPE MALE: Collected May 19, 1960; Slide No. S16.

PARATYPES (male and female): Slides S17 and S18.

TYPE HABITAT AND LOCALITY: Soil from jute field, Kazlarpur, Dacca.

OTHER LOCALITIES: Mathertek, Dacca District, and Teligram, Comilla District.

Proleptonchus clarus n.sp. (Fig. 1, F-H)

FEMALES (5): L = 1.62 (1.43-1.73) mm; a = 32.8 (30-36); b = 7.4 (7.8-8.1); c = 66 (60-75); V = 57.2 (53.7-62.9)%; Ov 1 = 19.8-23%; Ov 2 = 4.1-5.6%.

MALES (5): L = 1.42 (1.21-1.67) mm; a = 32.7 (31-34); b = 6.6 (5.7-7.5); c = 61 (52-66).

DESCRIPTION: Cuticle smooth; subcuticle coarsely striated and rough in appearance. Head rounded at anterior, set off by a slight constriction; bases of lips distinct and somewhat protruding, with prominent papillae. Amphids almost as wide as head; pouch with sensilla opposite posterior of extension. Axial stylet 8 microns long or a little less than one head diameter long, with strongly arcuate extension 9 microns long. Stylet guide campanulate, 4 microns long, with posterior ring. Esophagus uniformly narrow to muscular swollen base, set off by a slight constriction from narrow portion; bulb approximately one-fifth of esophageal length; 3 gland nuclei seen in bulb. Four or more elongated cells, glandular in appearance, surrounding anterior of esophageal bulb. Esophago-intestinal valve triangular. Nerve ring at about 45% of esophageal length. Two faint rows of lateral pores present. Obscure prerectum, about 6 anal body diameters long in female, 10-11 in male. Anterior ovary of female reflexed; posterior ovary reduced to short sac-like structure containing sperm. One ovum with shell at a time in uterus, 85 × 40 microns. Testes two; spicules 40-50 microns long or 1.5-2 anal body diameters, not cephalated, internally divided for almost full length, with a lateral accessory piece near anterior of tip. One pair adanal papillae

and 8 pairs of ventro-medial supplements anterior to spicules; anteriormost supplement 7.5 anal body diameters anterior to anus. Tail in both sexes less than one anal body diameter long, with one pair subventral and one pair subdorsal papillae, both small.

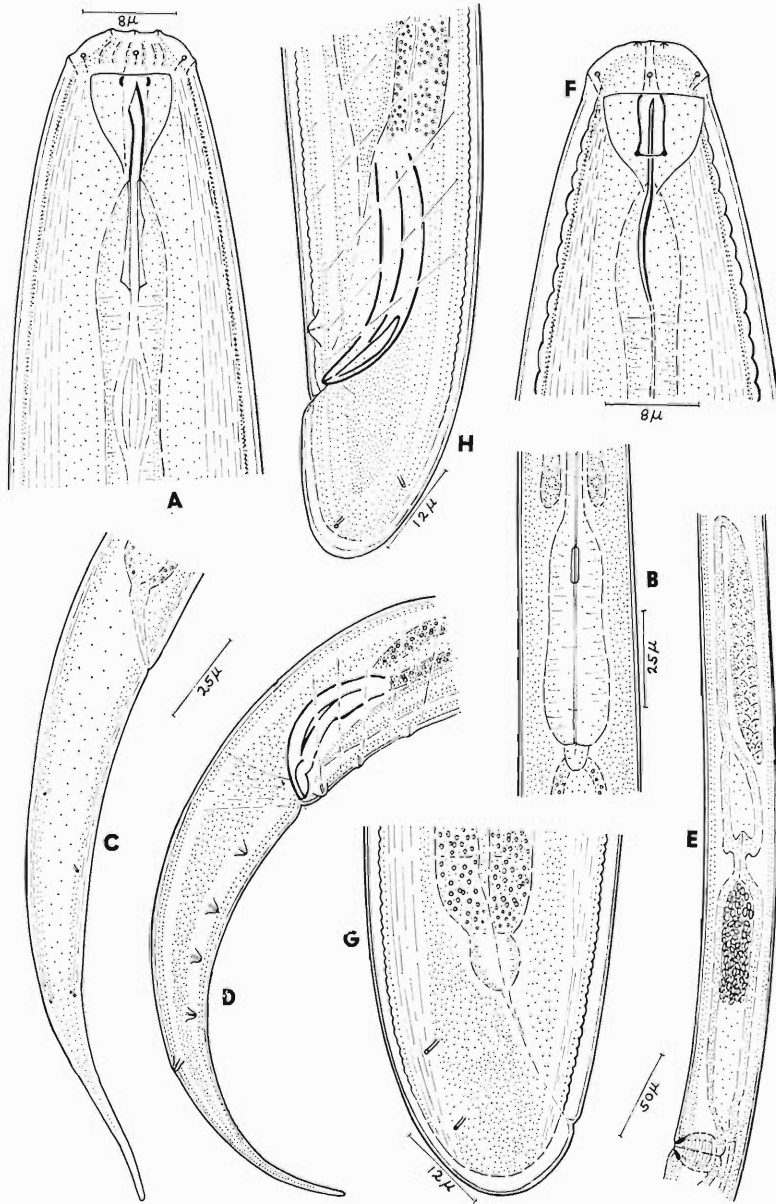


Fig. 1. *Calolaimus papillatus* n.g., n.sp. A. Female head. B. Esophageal base. C. Female tail. D. Male tail. E. Anterior part of female reproductive system. *Proleptonchus clarus* n.sp. F. Female head. G. Female tail. H. Male tail.

DIAGNOSIS: The most obvious distinguishing feature of this genus is the strongly sclerotized bell-shaped spear guide. *Proleptonchus aestivus* Lordello, 1955 apparently lacks a spear extension, but this could have been due to fixation distortion. The female of *P. clarus* n.sp. also differs from the type species in its greater length, shorter esophagus, more distinctly offset head and lips, and larger amphids. It differs from *P. saccatus* (Clark, 1962) n. comb. (= *Amphorostoma saccatum*) in the much shorter postvulvar uterine sac and in the greater number of male supplements.

HOLOTYPE MALE: Collected by M. Ameen in May, 1960; personal collection No. S23.

ALLOTYPE FEMALE: Same data as holotype; Slide No. S24.

PARATYPES (male and female): Slides S25 and S26.

TYPE HABITAT AND LOCALITY: Soil from around jute roots, Government Experimental Farm, Tejgaon.

OTHER LOCALITIES: Tumilia, Dacca District and Sahebganj, Rangpur District.

Dorylaimoides rusticus n.sp. (Fig. 2, D-E)

FEMALES (3): L = 0.95 (0.91-0.98) mm; a = 43.2 (41.5-44.5); b = 5.5 (5.4-6); c = 12.7 (12.2-13); V = 33.2 (31.4-36.2)%; Ov 1 = 1.5-6.6; Ov 2 = 14.6-23%.

MALES (5): L = 0.79 (0.69-0.89); a = 35.6 (32.3-39.1); b = 5.7 (5.3-7.3); c = 12.9 (12.1-14).

DESCRIPTION: Cuticle distinctly striated; oblique fibrils beneath surface and subcuticular striation; hypodermis containing coarse greyish granules, giving it a rough appearance. Head cap-like, set off by constriction from body; head diameter two-sevenths of body diameter at esophageal base. Lips fused; distinct labial papillae and cephalic papillae. Amphids stirrup-shaped, about 90% of head diameter wide, with narrow slit-like aperture. Axial stylet consisting of 7 micron tip and 7 micron extension, each as long as head diameter; extension flanged and inclined dorsally at its anterior. Esophagus faint, consisting of a narrow anterior portion surrounding extension without swelling, and swollen basal portion about one-fourth of esophagus long, sometimes with crenate margins. Intestinal inclusions small, pale yellow in color. Prerectum in male extending 6 anal body diameters anterior to anus, 3.5 anal body diameters in female. Female reproductive system indistinct in life; anterior ovary represented by a short sac containing sperm; posterior ovary reflexed $\frac{3}{4}$ of distance to vulva; ova not observed. Male reproductive system indistinct; two testes, 15-16% of body length. Spicules 25 microns long, dorylaimoid, with small lateral accessory piece. Paired adanal papillae and 2-3 small mammillate ventro-median supplements just anterior to spicule heads. Tails conical-cylindrical; division into two parts more apparent in female; tail ventrally curved in both sexes. Male tail 3.6-4 anal body diameters long; female tail 4.7-5 anal body diameters long.

DIAGNOSIS: This species is closest to *Dorylaimoides venustus* Andrassy, 1959, which also possesses a single posterior ovary and an anterior prevulvar sac. The tail of the latter is shorter (c = 17.8-19.8), the head is not set off by a constriction, and the swollen portion of the esophagus is much longer.

HOLOTYPE FEMALE: Collected by M. Ameen in September, 1960; Slide No. S52.

ALLOTYPE MALE: Same data as holotype; Slide No. S53.

PARATYPES (male and female): Slides S54 and S55.

TYPE HABITAT AND LOCALITY: Soil from around jute roots, Chandpur, Comilla District.

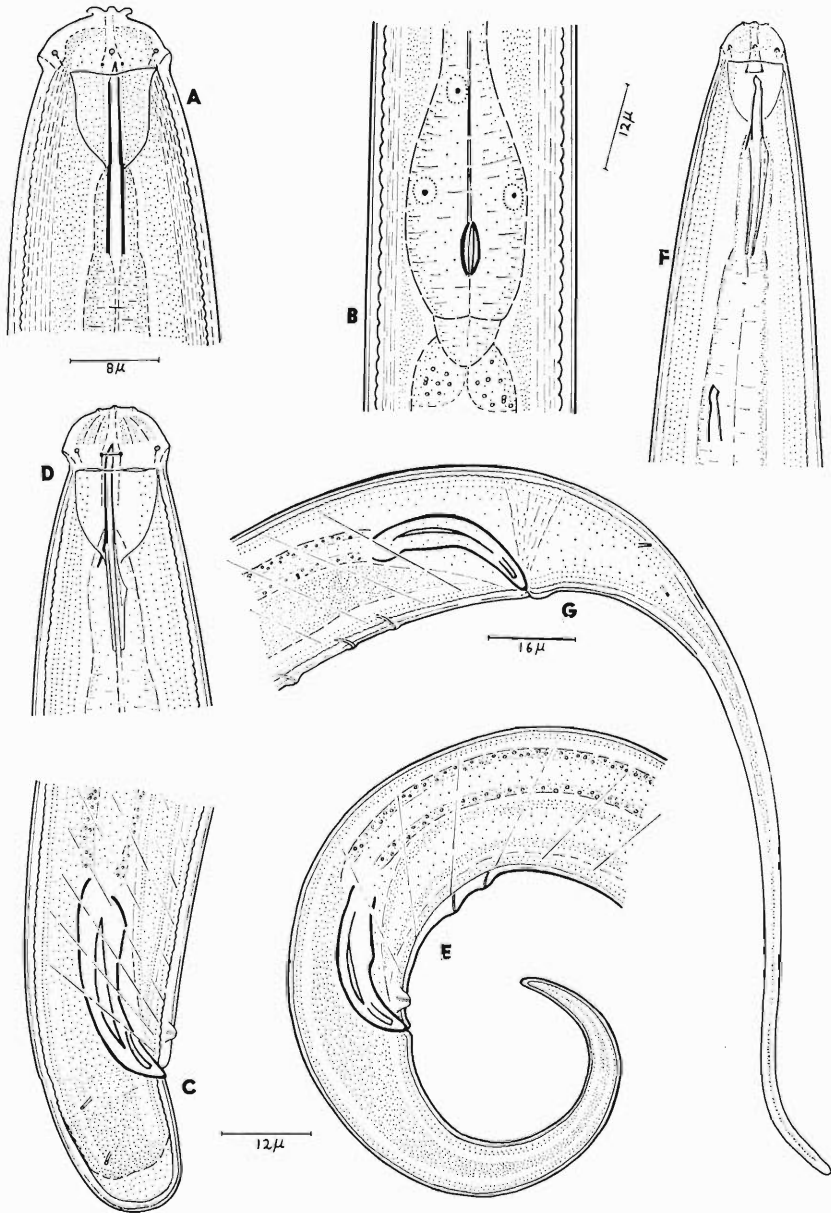


Fig. 2. *Tyleptus striatus* Heyns. A. Male head. B. Esophageal base. C. Male tail. *Dorylaimoides rusticus* n.sp. D. Female head. E. Male tail. *Dorylaimoides lepidus* n.sp. F. Anterior of second stage female. G. Male tail.

Dorylaimoides lepidus n.sp. (Fig. 2, F-G)

FEMALES (2): L = 1.26-1.79 mm; a = 40-41; b = 5.6-7.8; c = 9.4-11.9; V = 38.8-40.7%; Ov 1 = 14%; Ov 2 = 13%.

MALES (2): L = 1.51-1.59 mm; a = 34-42; b = 6-6.2; c = 10.7-11.

DESCRIPTION: Cuticle distinctly striated; subcuticular striation not observed; single row of prominent lateral pores. Head continuous with body contour. Head diameter 11 microns or $\frac{1}{3}$ the body diameter at esophageal base. Labial and cephalic papillae very distinct and protruding. Amphids about 90% of head diameter wide, as deep as wide. Stylet tip 8 microns long; extension prominently flanged, 14 microns long. Small simple guiding ring. Swollen base of esophagus about $\frac{1}{3}$ of total length. Prerectum length six times the anal body diameter in male; obscure in female. Female reproductive system double; ovaries reflexed about 60%; no distinct spermatheca. Two testes in male, each about 8% of body length. Spicules 35-42 microns long, or about 1.5 anal body diameters; accessory piece small, with parallel sides. Paired adanal papillae and 9-11 ventro-median supplements, irregularly spaced, beginning anterior to spicule head. Tails in both sexes conical-cylindrical, outstretched in death; male tail 5.5-6.4 anal body diameters long and female tail 6.1-6.8 anal body diameters long.

DIAGNOSIS: This species is closest to *Dorylaimoides stenodorus* Altherr, 1953 (= *D. paulbuchneri* Meyl, 1956), but differs in the much greater number of male supplements (5 reported by Altherr, 6 by Meyl).

HOLOTYPE FEMALE: Collected by M. Ameen in Sept., 1960; Slide No. S56.

ALLOTYPE MALE: Same data as holotype: Slide No. S57.

PARATYPES (male and female): Slide No. S58.

TYPE HABITAT AND LOCALITY: Soil around jute roots, Shahatoli, Comilla District.

Tyleptus striatus Heyns, 1963 (Fig. 2, A-C)

FEMALES (5): L = 1.02 (0.87-1.22) mm; a = 30.8 (27.1-34); b = 4.5 (4.2-4.7); c = 92 (76-100); V = 33.1 (30.3-39.5)%.

MALES (6): L = 1.07 (0.94-1.13) mm; a = 36.9 (31-41.5); b = 4.3 (3.7-4.6); c = 55.5 (49.4-59.4).

DESCRIPTION: Body curved ventrally in death. Cuticle smooth; subcuticular striation often prominent. Muscle layer of anterior sharply defined and hyaline; lateral hypodermal chords with two rows of pores. Head offset by a constriction, rounded anteriorly. Head diameter $\frac{1}{3}$ the body diameter at esophageal base. Lips fused; 6 large protruding "liplets" at extreme anterior clustered around mouth opening. Outer circle of 10 distinct cephalic papillae. Amphids campanulate, $\frac{2}{3}$ as wide as head, situated at level of head constriction, aperture consisting of two cusp-like openings. Spear tip 8 microns long or 90% of head diameter at level of head constriction; aperture a little less than $\frac{1}{4}$ its length. Spear extension 7 microns long, more thickened than spear, slightly flanged. Spear guiding ring simple. Anterior portion of esophagus narrow, slightly expanded around extension base; no constriction between narrow and expanded portions of esophagus. Basal bulb of esophagus 11% of esophageal length; sclerotized triradiate esophageal lining expanded in posterior third of bulb and abruptly ending before esophago-intestinal valve. Esophageal gland nuclei distinct; one at anterior of bulb and two at outer margins of middle of bulb. Conical esophago-intestinal valve. Globular inclusions of intestine bright greenish-yellow, in clusters. Prerectum distinct in female, 2.5-6 anal body diameters long; indistinct in

male, about 3 anal body diameters long. Reproductive systems very clear in living specimens. Posterior reflexed ovary and a short anterior sac; spermatheca expanded and set off by slight constriction from uterus. Testes two. Spicules 32 microns long or 1.6 anal body diameters, with double internal

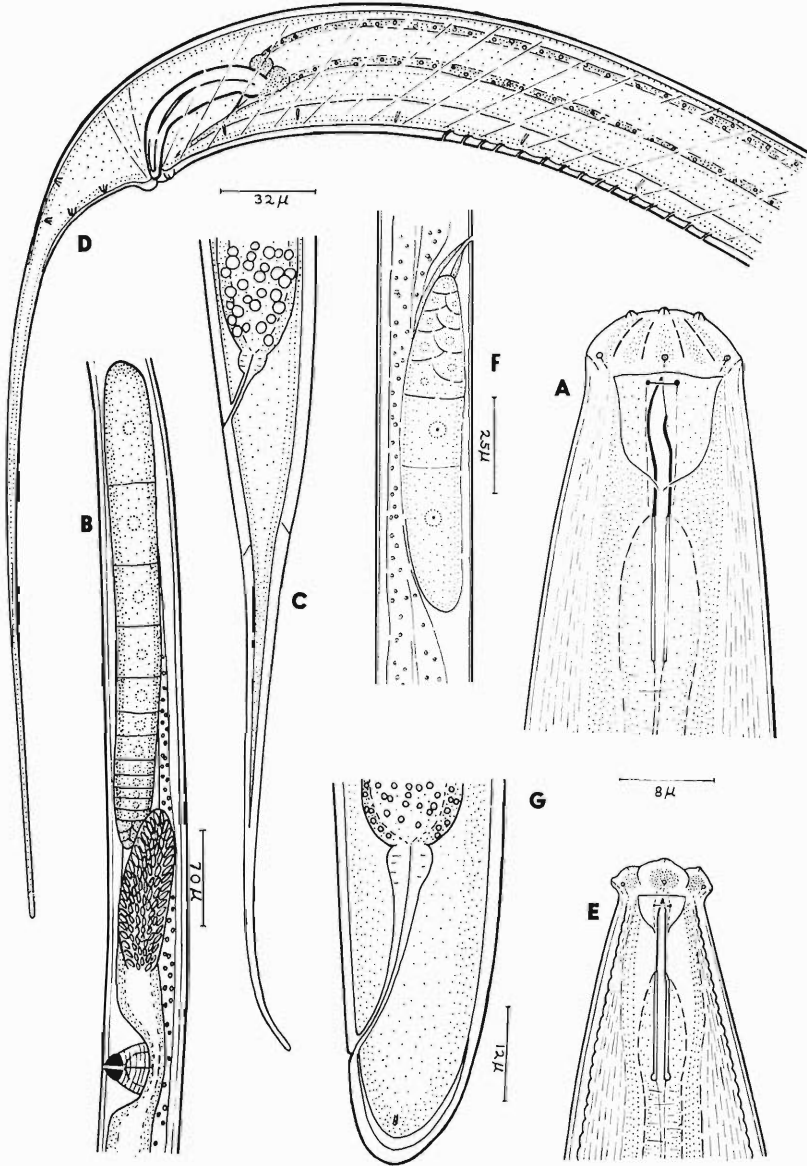


Fig. 3. *Oxydirus magnus* n.sp. A. Male head. B. Anterior part of female reproductive system. C. Female tail. D. Male tail. *Tylencholaimus pakistanensis* n.sp. E. Female head. F. Female reproductive system. G. Female tail.

division. Accessory piece 13 microns long. One pair adanal papillae and 3 small ventro-median preanal supplements, about 27 microns apart. Tails in both sexes bluntly rounded, with truncate inner cylinder; fine granulated area at tip; male tail 1 anal body diameter long, female tail 1.4-2 anal body diameters long.

DISCUSSION: Although the present specimens share all the characteristics which distinguish *Tyleptus striatus* from the type species, *T. projectus* Thorne, 1939, they are more similar to the latter in measurements, making the separation of the two species even more difficult. There are slight differences in length of amphids, spear and extension from the published description of *T. striatus* but Heyns (*in litt.*) has shown that there is some variation in these characters. Moreover, he has sent a sketch of a male from South Africa, which also has 3 ventro-median supplements.

Tylencholaimus pakistanensis n.sp. (Fig. 3, E-G)

FEMALES (4): L = 2.45 (0.42-0.5) mm; a = 19 (18-19.6); b = 3.5 (3.4-3.7); c = 31.5 (30.8-32.3); V = 45.8 (44.4-46.7)%; Ov 2 = 17.8%.

MALE: unknown.

DESCRIPTION: Cuticle moderately thick, especially thickened at tail end. Distinct transverse striation on surface of head cuticle, not seen on rest of body. Head expanded, offset from rest of body; head diameter 30% of body diameter at esophageal base; lips distinct. Inner circle of 6 prominent labial papillae; outer circle of 10 prominent cephalic papillae. Amphids small, indistinct, 30% of head diameter broad, situated opposite spear guiding ring. Spear tip 7 microns long or one head diameter; aperture about 1 micron long. Spear extension 8 microns long, almost twice as broad as spear, with small but distinct basal knobs. Simple spear guiding ring at level of head constriction. Esophagus distinctly divided into two portions; basal portion 27-33% of esophageal length, with distinct radial muscles; anterior portion not distinctly muscular. Nerve ring just anterior to expanded basal portion. Intestine ending in long rectum, 1.7 anal body diameters long. Female reproductive system single; ovary postvulvar, reflexed almost to vulva; vagina muscular, inclined posteriorly. Tail subconoid, 1 anal body diameter long; tail papillae not observed.

DIAGNOSIS: A postvulvar ovary, as found in the present species, is also present in *Tylencholaimus zeelandicus* de Man, 1876. However, that species is twice the length of the present species and the vulva is more anterior.

HOLOTYPE FEMALE: Collected August 1, 1960 by R. W. Timm; Slide No. S59.

PARATYPES: Same data as holotype; Slide No. S60.

TYPE HABITAT AND LOCALITY: Soil around jute roots, Mymensingh.

Oxyccirus magnus n.sp. (Fig. 3, A-D)

FEMALES (6): L = 5.32 (4.85-5.72) mm; a = 101 (85-117); b = 15.7 (13.2-17.1); c = 12.8 (12.1-14.7); V = 37.3 (33.3-40)%; Ov 1 = 9%; Ov 2 = 10%.

MALES (6): L = 4.67 (4.43-4.94) mm; a = 93.8 (87-103); b = 14.4 (14-15.4); c = 18.2 (16.6-20.5).

DESCRIPTION: Body long and thin. Cuticular striation very fine. Head slightly offset; somewhat truncate at anterior. Head diameter 1/5 the body diameter at esophageal base. Twelve faint non-sclerotized ribs in head region. Distinctly protruding labial and cephalic papillae, with prominent innerva-

tions. Amphids faint, stirrup-shaped, $\frac{2}{3}$ of head diameter wide, situated at level of neck constriction. Spear tip 9 microns long or 1 head diameter; aperture $\frac{1}{4}$ of spear length; extension 10 microns long. Simple spear guiding ring. Swollen portion of esophagus 32-25% of esophageal length in both sexes, surrounded by thick sheath of spiral muscles. Lateral pores faint, in 2 rows. Nerve ring at about 50% of esophageal length, faint. Esophago-intestinal valve obcordate. Intestine of 6 cells in circumference, in staggered rows. Four conspicuous rectal cells at juncture of rectum and intestine. Prerectum extending anterior to supplements in male. Ovaries paired, opposite, reflexed, inconspicuous in life, lying on opposite sides of intestine; large spermatheca about 130 microns long apparently offset from uterus, filled with elliptical sperm. Vulva conspicuous, sclerotized; vagina large and muscular, surrounded by glandular cells. Male with 2 indistinct testes. Spicules dorylaimoid, with internal division, 54-57 microns long across the are or almost 2 anal body diameters; lateral accessory piece present. One pair adanal papillae and a group of 12-16 ventro-median preanal supplements anterior to spicules, anteriormost about 6 anal body diameters anterior to anus; 5 pairs of subventral preanal papillae; 2 pairs subventral and 2 pairs subdorsal postanal papillae. Tail $\frac{1}{7}$ conical, $\frac{6}{7}$ cylindrical-filiform; division into two parts more conspicuous in male.

DIAGNOSIS: The other species of *Oxydirus* with two ovaries are *O. oxycephaloides* (de Man, 1921) Thorne, 1939; *O. denticaudatus* (Imamura, 1931) Andrásy, 1960; *O. leptus* (Cobb, in Thorne & Swanger, 1936) Andrásy, 1960; *O. japonicus* (Cobb, in Thorne & Swanger, 1936) Andrásy, 1960 (probably should not be put in this genus); and *O. elongatus* Altherr, 1963. The first four species are much shorter than the present species. It is nearest to *O. elongatus*, based on one female specimen, but the esophagus and tail of *O. magnus* are relatively shorter and the spear is shorter in absolute measurement.

HOLOTYPE FEMALE: Collected on August 2, 1960 by R. W. Thum; Slide No. S19.

ALLOTYPE MALE: Same data as holotype; Slide No. S20.

PARATYPES (male and female): Slides No. S21 and S22.

TYPE HABITAT AND LOCALITY: Soil around roots of jute, Mymensingh.

OTHER LOCALITIES: Choudhagram, Comilla District and Government Experimental Farm, Tejgaon.

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Immunization Against the Cattle Lungworm : Oral Vaccination with Infective *Dictyocaulus filaria* Larvae

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This paper is a report on three small-scale trials carried out to determine whether calves could be immunized against infection with the cattle lungworm, *Dictyocaulus viviparus*, by means of oral vaccination with infective larvae of the sheep lungworm, *Dictyocaulus filaria*. Only a brief abstract of the findings has been published heretofore (Vegors, Lucker and Douvres, 1963).

Prior to the start of the first trial in 1956, several investigators (Porter and Cauthen, 1942; Michel, 1954, 1955; Jarrett *et al.*, 1955; Rubin and Lucker, 1956) had shown that an initial patent *D. viviparus* infection usually confers on calves a strong resistance or immunity to reinfection with this species. But immunization through the induction of even a very light patent infection, though later reported to be reasonably effective (Weber and Lucker, 1959), was obviously not an ideal procedure because the immunizing agent itself could contribute to dissemination of the parasite.

We considered that the presence of reproductively active adults in the lungs probably was not essential for adequate stimulation of the protective mechanism. So, the aim of our use of *D. filaria* larvae was to obtain such stimulation from the presence of living *Dictyocaulus* capable of antigenic activity, but ordinarily incapable of maturation in the vaccinated calf. This goal was otherwise achieved by Jarrett *et al.* (1957). Their report on immunization with *D. viviparus* larvae whose potential for maturation was destroyed by X-rays appeared shortly after completion of our first trial. Nevertheless, we carried out two subsequent trials with *D. filaria* larvae in the conviction that the production of immunity against helminth infections of livestock by vaccination with larvae of related species not adapted for maturation in the host to be protected is a worthy area of investigation.

MATERIALS AND METHODS

The 10 dairy calves used were reared and kept individually in masonry pens under conditions designed to preclude extraneous exposure to helminths. All calves received the same diet.

Infective larvae were reared from first-stage larvae eliminated in the feces of infected sheep or cattle. If cultured in a medium other than feces, the first-stage larvae were preliminarily isolated by baermannization and repeatedly washed in water. Infective *D. filaria* larvae that had been reared in water a few millimeters deep were provided by our colleagues G. I. Wilson and M. L. Colglazier. The infective *D. viviparus* larvae used in trials 1, 2, and 3, respectively, were reared as follows: on moist, granular, animal charcoal; in distilled water; in distilled water (about half of each dose) and in moist feces spread about one-half inch deep in shallow, covered trays (remainder of each dose). Cultures were kept for 7 to 10 days at room temperature (about 65 to 85 F.) or in an incubator maintained at 65 to 70 F. The infective larvae were stored in water at about 44 F. until used. The period of storage varied from one day to about 3 weeks, except in the case of the *D. filaria* larvae used for vaccination II in trial 2, which were in storage about 5 weeks. Doses of larvae administered to two or more animals simultaneously were aliquots of a single suspension of larvae. The number of larvae in a dose was computed from the number of active and tightly coiled ones of normal appearance counted in samples of the suspension. The day on which any calf in a trial was first given larvae is hereinafter designated "day 0" of the trial.

Clinical observations, fecal examinations, determinations of total outputs of first-stage larvae in the feces, processing of the lungs for the recovery of *Dictyocaulus* and scoring of the degree of gross lung damage were generally carried out as described by Lucker and Vegors (1964). Worms picked out from the opened respiratory tree with forceps prior to dicing of the lungs for baermannization were counted individually.

EXPERIMENTAL DATA

TRIAL 1: A calf 4 months old on day 0 was vaccinated six times with *D. filaria* larvae. It received 21,000, 6,000, 5,000, 6,000, 10,000 and 23,500 larvae on days 0, 2, 4, 6, 8, and 10, respectively.

No first-stage larvae were found in samples of its feces collected on days 21, 22, 23, 24, 27, 29, 30, and 38 after vaccination I. Thus, this exposure apparently did not result in the development of a patent *D. filaria* infection within 38 days, which is the average duration of the prepatent periods of infection reported by Goldberg (1952), Kauzal (1934), Daubney (1920), and Guberlet (1919) in a total of 26 sheep.

Frequent clinical examinations were made only during the period from days 14 to 28 after vaccination I. On several of these days, signs of moderate respiratory distress suggestive of the presence of larvae or immature worms in the lungs were observed. The signs included elevation in respiratory rate, coughing and rales.

From a large challenge dose of *D. viviparus* larvae given about 11 weeks after vaccination VI (table 1), the vaccinated calf and a control each acquired a patent *D. viviparus* infection which was allowed to terminate naturally. However, the course of the infections differed greatly (table 2) and showed that the vaccinated calf was markedly resistant to the establishment of mature *D. viviparus*.

Respiratory distress postchallenge was somewhat more severe in the control than in the vaccinated calf. The control was listless and consumed little feed and water for several weeks, but the activity and appetite of the vaccinated calf appeared unaffected. About 3.5 months postchallenge, the control weighed 40 lbs. less, and the vaccinated calf 138 lbs. more, than on the day of challenge.

TRIAL 2: Each of the two calves was vaccinated four times with 5,000 *D. filaria* larvae per vaccination. Each was one month old on day 0. Calf 1 was vaccinated on days 0, 14, 46, and 77 and calf 2 on days 4, 25, 53 and 80.

No first-stage larvae were found in samples of feces collected on the following days after the vaccinations:

Calf	Vaccination			
	I	II	III	IV
1	29-35,38,46-49	24,32-35,52-60	24-34,46-60	24-38
2	25-39,42-56	24-35,48-55	24-27,39-52	24-35

Though patent *D. filaria* infections evidently were not established, exposure to the larvae caused severe respiratory distress. Respiratory rates reached 110-116/min. about 3-4 weeks after vaccination I and thereafter averaged about 70/min. until 2 to 3 weeks after vaccination IV, when they were briefly as high as 120/min. Coughing was infrequent and rather mild, but indications of considerable pulmonary damage were heard on auscultation of the lungs subsequent to vaccination IV. Average daily weight gain (0.8 lbs.) was poor.

Table 1. Status of calves and yearlings repeatedly vaccinated with *D. filaria* larvae and of controls when challenged with indicated doses of *D. viviparus* larvae in trials 1-3.

Trial	Number			Approx. age (months)		Wt. (lbs.)		Interval from final vaccination to challenge (days)	Number of <i>D. viviparus</i> larvae/head
	Vacc.	Cont.	Total	Vacc.	Cont.	Vacc.	Cont.		
1	1	1	2	7	5	295	250	81	28,500
2	2	2	4	5	4	205	212	42;45	10,000
3	2	2	4	16	16	817	812	20	16,000

Table 2. Results of administration of *D. viviparus* larvae to a calf repeatedly vaccinated with *D. filaria* larvae and to a control in trial 1.

Calf	Duration of patent period of infection	Number of first-stage larvae eliminated in feces postchallenge*	
	Days	Max./gm	Total to day 62**
Vaccinated	34	4	324,000
Control	90	5,600	47,500,000

*Larvae/gm of feces and weight of fecal output determined daily.

**Interval from exposure to last day of patent infection in the vaccinated calf.

About 6 weeks after vaccination IV, a fairly large challenge dose of *D. viviparus* larvae was given to each vaccinated calf and to each of two controls (table 1). The numbers of cattle lungworms recovered at necropsy 28 days

postchallenge (table 3) showed that one of the vaccinated calves was nearly immune to the establishment of mature *D. viviparus*, whereas the other was as susceptible as the controls.

On the average, the postchallenge signs of respiratory distress, effect on body weight, and degree of lung damage observed at necropsy were as severe in the vaccinated as in the control calves. The vaccinated calf which was practically immune to infection suffered the greatest adverse effects.

TRIAL 3: Each of two calves, 8 months old on day 0, was vaccinated with 2,100, 2,150, and 2,650 *D. filaria* larvae on days 0, 160, and 217, respectively.

No first-stage larvae were found in fecal samples collected from each animal usually daily from the 19th to the 35th day after each vaccination and weekly or more frequently between the 35th and at least the 47th day after each exposure.

The vaccinations did not cause appreciable elevation in respiratory rate, nor depress rate of weight gain. Vaccination II was followed by mild, infrequent coughing over a period of about two weeks.

Table 3. Results of administration of *D. viviparus* larvae to cattle* repeatedly vaccinated with *D. filaria* larvae and to controls in trials 2 and 3.

Trial	Number of lungworms (<i>D. viviparus</i>) recovered at necropsy 28 days postchallenge					
	Vaccinated cattle			Control Cattle		
	No. 1	No. 2	Av.	No. 1	No.2	Av.
2	21	872	446.5	1,269	733	1,001
3	0	11	5.5	1,298	2,898	2,098

*Calves 4 to 5 months old in trial 2; yearlings 16 months old in trial 3.

About 3 weeks after vaccination III, a substantial dose of *D. viviparus* larvae was given to each vaccinated yearling and to each of two controls (table 1). The numbers of cattle lungworms recovered at necropsy 28 days postchallenge (table 3) showed that the vaccinated cattle were immune, or nearly so, to the establishment of mature worms.

From days 13 to 26 postchallenge, respiratory rates were not much above normal in either group, but were lower in the vaccinated (max., 50/min; av., 36/min.) than in the control pair (max., 65/min; av., 49/min.). Incidence and severity of coughing were considerably less in the vaccinated than in the control pair. Postchallenge, the controls lost 0.25 lbs./head/day more than the vaccinated animals. Gross lung damage at necropsy was scored 2.0 and 3.0 in the control, *versus* 0.5 and 1.5 in the vaccinated, animals.

DISCUSSION AND CONCLUSIONS

In the three trials combined, each of five controls acquired a substantial patent *D. viviparus* infection from the challenge dose of larvae, whereas four of the five calves and yearlings that had been vaccinated with *D. filaria* larvae were highly resistant or completely immune to a patent infection. Inherent lack of capacity to develop resistance appears to be the most tenable explanation of the case in which vaccination was not effective. Lucker and Vegors (1964) have recently summarized the evidence that calves vary widely in

ability to develop resistance to this parasite.

The results of these trials suggest to us that further investigation might demonstrate that vaccination with *D. filaria* larvae can be as effective as vaccination with irradiated *D. viviparus* larvae. But they also suggest that *D. filaria* larvae may be too pathogenic for use for calftooth vaccination in practice. While the trials were in progress, Erhardová (1957) reported that a 3-month old calf given only 1,000 *D. filaria* larvae developed such severe symptoms of dictyocaulosis that it had to be slaughtered and Parfitt (1963) reported that three consecutive weekly doses of 10,000 *D. filaria* larvae were fatal to three of six calves about 2 to 3.5 months old. Our results indicate a considerably lesser level of pathogenicity. Those reported by Hildebrandt (1962) suggest an intermediate level.

Parfitt (1963) has reported that considerable numbers of *D. filaria* reached reproductive maturity in 3 of 6 calves given a total of 30,000 larvae in three doses as mentioned above. So far as was ascertained, the species did not mature in any of our test calves. Hildebrandt (1962) reported that it did not mature in calves infected by him and his review of the literature also suggests poor adaptation to cattle.

Since doses of 5,000 larvae were poorly tolerated by the calves of trial 2, which were 1 month old when first exposed, we intended in trial 3 to test doses of 2,000 larvae both for tolerance and efficacy using calves about 2 months old when first vaccinated. But circumstances necessitated a choice between indefinite postponement of the trial and use of older calves and the latter alternative was chosen. The further intent was to vaccinate only twice, as Jarrett *et al.* (1959) did with irradiated *D. viviparus* larvae and as recommended by the manufacturers* of "Dictol," which consists of such larvae. However, a third vaccination was incorporated in the trial because 160 days unavoidably elapsed between vaccinations I and II. This interval was suspected to be considerably longer than may be optimal for production of the maximum protection achievable by double vaccination; the recommended interval between vaccinations with Dictol is 4 to 6 weeks.

This trial consequently did not yield data suitable for direct comparison with the results obtained by Jarrett *et al.* (1959) by double vaccination with irradiated *D. viviparus* larvae. It did show that cattle 8 to 15 months old can be very effectively immunized by means of infection with *D. filaria* larvae and can tolerate doses of about 2,000 larvae very well.

Considerable support for this conclusion is found in Hildebrandt's observations. He reported that a dose of 12,500 larvae caused no symptoms in either of two 8-month old calves and apparently induced in them a firm resistance to infection with *D. viviparus*. One yielded only 7 and the other only 21 worms 35 days after challenge with 7,400 larvae. However, the experiment did not include control calves.

So far as we are aware, only Hildebrandt's report and the present one contain evidence that a species of livestock can be made resistant to one of its helminths by prior infection with a helminth that is not adapted for maturation in it and normally occurs and matures in another class of livestock.

However, a somewhat similar idea was tested by Allen and Samson (1961) who reported that an initial patent infection with a strain of *Haemonchus* from the pronghorn antelope caused lambs to become significantly resistant to challenge with a strain of *Haemonchus* from the domestic sheep.

*Allen and Hanburys, Ltd., Ware, Hertfordshire, England.

SUMMARY

Oral vaccination with infective larvae of *Dictyocaulus filaria* of sheep was tested as a means for the protection of calves against infection with the cattle lungworm, *D. viviparus*. In three small-scale exploratory trials, five calves were vaccinated and five served as controls. Dosages of *D. filaria* larvae, numbers of vaccinations, intervals between final vaccination and challenge, and levels of challenge, differed between trials. Each of the controls acquired a substantial patent *D. viviparus* infection from a challenge dose of larvae, whereas four of the five comparably exposed vaccinated individuals were highly resistant or immune to the establishment of mature *D. viviparus*. *Dictyocaulus filaria* apparently did not mature in the infected calves. The smallest dosage per vaccination given to young calves was 5,000 larvae. It was too pathogenic for use for immunization in early calfhood. Short yearlings tolerated repeated vaccination with about 2,000 larvae very well.

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Morphology of Somatic Setae: *Thoracostoma californicum* (Nemata: Enoplidae)

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Awareness of change in environmental conditions and the ability to successfully react to such stimuli requires that an animal have some type of receptive mechanism. Stimuli from the environment must be received by the animal and then translated into a suitable response. The type of stimuli which can be received is dependent upon the type of receptor the animal possesses. The complexity of response is determined by the association processes inherent in the animal's nervous system.

The sensory receptors of the peripheral nervous system of nematodes have received little histological attention. The sensory organs which have received the most attention are the amphids, labial papillae, cephalic papillae, cervical papillae, and genital papillae (Goldschmidt, 1903, 1908; Zur Strassen, 1904; Looss, 1905; Martini, 1916; Chitwood and Chitwood, 1933, 1950). A detailed histologic study of the setae and their nervous connections has not been done; however, studies on setae were conducted by Marion (1870), Butschli (1874), and Villot (1875). For the most part the study setae have received has been in relation to number, size and position for taxonomic organization.

The present study utilized *Thoracostoma californicum* (Steiner and Albin, 1937). Observations were also made on *Pontonema problematicum* Chitwood, 1960.

MATERIALS AND METHODS

The specimens of *Thoracostoma californicum* and *Pontonema problematicum* selected for this study were collected from holdfasts of *Egregria lacvigata* Setchell at Dillon Beach, California. Both totomounts and serial sections were employed in this study. The animals were fixed in 2½ percent formalin in sea water for 24 hours (minimum). The animals to be used in totomounts were run through the standard glycerine series, dehydrated and mounted. Those selected for sections were run according to the procedure given by Maggenti (1962). Sections were microtomed at 5 microns and stained with Baker and Jordans (1953) modification of Heidenhain's iron hematoxylin. Histochemical procedures also were employed for nucleic acids: DNA, Feulgen method (Gomori, 1952 in Jensen, 1962); DNA and RNA, methyl green and Pyronine method (Brachet, 1953 in Jensen, 1962). Histochemical controls: Differential extraction of DNA and RNA, Perchloric acid method (Erickson, Sax and Ogur, 1949 in Jensen, 1962).

The writer has followed the nomenclature of the nervous system employed by Chitwood and Chitwood (1950).

OBSERVATIONS

The labial setae and/or papillae appear to be sensory organs associated with the papillary nerves; the cephalic, somatic and caudal setae are sensory receptors connected with the somatic nerves. The somatic nerves involved could not be accurately traced; however, it appears that the sensory neurons which are located along the lateral chords are associated with the ventrolateral nerves. Connections between the cephalic setae in the anterior region of the esophagus could not be determined because of confusion with the

neurons which contribute to the ganglia of the circum-esophageal commissure (nerve ring).

At the level of the circum-esophageal commissure in the right subventral sector of the body there sometimes occurs a seta (Fig. 2, B), similar in all ways to the other somatic setae except it appears that the axon of its sensory neuron passes through the right subventral arm of the ventral nerve trunk. The position and direction of the scolopoid apparatus indicates an axon passage through the right subventral arm of the ventral nerve (Fig. 2, B). This could indicate a connection with the lateroventral somatic nerve which passes through the ventral ganglia to the ventral nerve cord and from the ventral cord loops back by way of the major lateroventral commissure and then proceeds posteriorly in the submedian line.

The somatic setae of *Thoracostoma californicum* are subdorsal, lateral and subventral in position. The lateral setae are not directly lateral but sublateral (Fig. 1, A). The position of the cephalic setae (not labial) is more difficult to ascertain because they occur singly or in groups. The pattern of setal placement is not consistent from specimen to specimen within the same species.

The detailed description presented here is based mainly on the lateral somatic setae. In the case of the subdorsal and subventral setae the construction manifested was the same as for the lateral setae, except that the connection to the sensory neuron could not be followed (Fig. 1, B). However, in the corresponding region of the hypodermis a sensory neuron could be found. This was always the case and could be easily accomplished in the mid-region of the body where sensory neurons are infrequent in occurrence. The sensory neurons are readily distinguished by their lack of a nucleolus; this structure being very conspicuous in other cells in the nematode body especially those of the hypodermis.

The bipolar sensory neuron with its associated glia is situated in the area of the lateral chords of the hypodermis. The axons and dendrites of these neurons run between the lateral and sublateral hypodermal chords. The lateral somatic longitudinal nerves run under the lateral chord (Fig. 1, A). The dendrite ending of the sensory neuron extends to the scolopoid body (sensory plate, apical body, receptaculum). From the scolopoid body a filament extends through the cuticle and on into the seta (Fig. 1 and 2). The scolopoid body associated with the setae of *Thoracostoma* and *Pontonema* differs from the sensory plate or receptaculum described by Goldschmidt (1903) at the base of the labial and cephalic papillae of *Ascaris* in that it is a more discrete body and the surrounding cytoplasm could not be ascribed to either a supporting or escort cell.

The scolopoid bodies are from 4 to 6.5 microns long and vary in width from 1 to 3.5 microns. Their typical shape is fusiform, however, this shape is also variable in that some are cylindrical and slender while others are bulb-like. An axial fiber (terminal filament) extends from the distal acute end to the seta (Fig. 1). The end of the scolopoid body from which the axial fiber extends is most conspicuous in stained specimens. Marian (1870) described a fusiform body at the base of the setae of *Thoracostoma setigerum*. The dimensions (15 by 9 microns with a central nucleus 1 micron) and their position in the somatic musculature differ from the bodies described here for *T. californicum*. The existence of these structures could not be confirmed by Butschli (1874) or Villot (1875).

The scolopoid body, when stained with iron hematoxylin and destained with

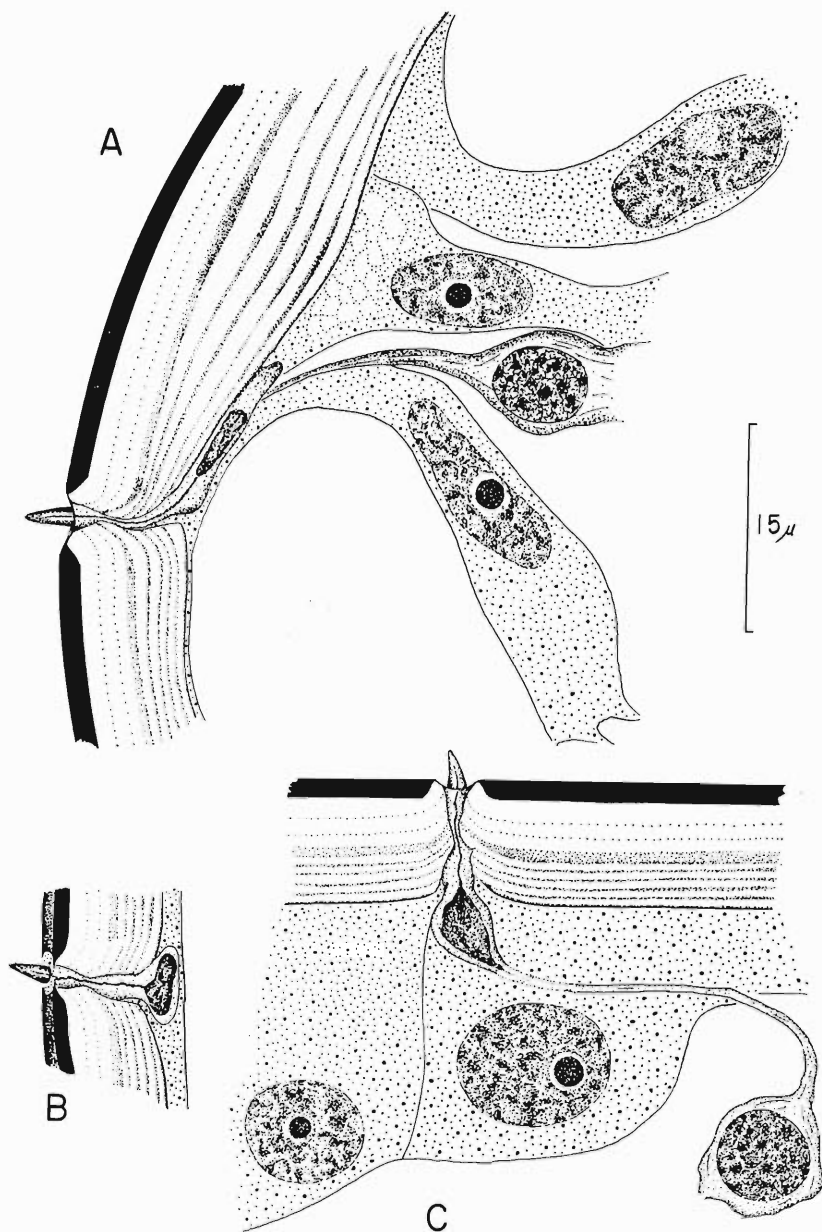


Fig. 1. *Thoracostoma californicum*. A, sublateral hypodermal seta; hypodermal chords, lateral nerves and neurocyte, cross-section. B, subdorsal seta, scolopoid body in hypodermis between cuticle and somatic muscle, cross-section. C, sublateral hypodermal seta, longitudinal section; showing neuron in relation to seta. (illustrations of equal magnification)

picric acid, has a granular appearance. It is circular in cross section and irregular in external dimensions. Each scolopoid body, rather than having a constant shape throughout the animal shows individual variability. The consistent factor is its staining properties and general appearance which is almost nuclear in character. The impression imparted is that of a small neuron which is only about one-fourth the size of the sensory neuron. Its function is unknown but it may act as an amplifier of external stimuli. In any event, it is a consistent feature of the setae of *Thoracostoma* and *Pontonema*. Histochemical procedures for DNA and RNA proved negative for the scolopoid body, thus the interpretation is that they are not nuclear in nature but rather a modification of the dendrite of the sensory neurocyte.

The scolopoid body is not always directly under the seta (Fig. 1, A). In the cephalic region the canal through the cuticle is directed posteriorly. At the seta the canal is directed into the body cavity but soon after penetrating the outer cuticular layers it turns posteriorly then upon reaching the basal layers of the cuticle it again is directed towards the body cavity. It is here that the scolopoid body is situated.

The form of the setal socket is somewhat different from that generally exhibited by the arthropods in that the thickness of the cuticle in nematodes is not proportionate to the length and breadth of the seta. However, the construction of the nematode socket and subsocket cavity compensate for this. Figures 1 and 2 show that the seta is part of or is attached to a cuticular membrane that covers the cavity filled with cytoplasm (from one or more cells). This arrangement allows the movement of the seta to transmit an external stimulus by way of the terminal filament through the scolopoid body to the nerve ending. Totomount studies do not permit the observer to comprehend this subsurface orientation of seta to cavity.

The aperture or canal through the cuticle is in the form of an "hour-glass" (Fig. 1). Into this canal extends the scolopoid body with its terminal filament and also the cytoplasm of the sublateral hypodermal cell. It sometimes appears as though the median hypodermal cell also enters this cavity. Externally, the cavity funnels out to form a socket upon which the seta is located. It, therefore, seems that the function of the hypodermal cell is to form the cuticular cavity and external membrane of the socket (Tormogen cell). If the median cell also enters then it may be the source of the seta (Trichogen cell). However, it is conceivable that either the sublateral performs both functions or that the Trichogen cell aborts after setal formation.

The hypodermal glands, though more complex in construction, show a strong similarity to the setae. The hypodermal glands in *Thoracostoma* do not open to the surface by way of a simple pore; the external opening is at the end of a short tube which resembles in all respects a cut-off seta (Fig. 2, A). This tube is set into a cuticular depression (socket) similar to that associated with setae. The canal through the cuticle is also "hourglass" shaped though less symmetrical than the canal described for setae (Fig. 1, B).

Externally, the gland-tube opening is located in one of the sublateral sectors of the lateral field. However, cross sections indicate that the gland cell itself originates from one of the lateral hypodermal cells rather than one of the subventral or subdorsal cells. Supporting evidence for this lies in the fact that where the gland tissue passes into the cuticle so also does the cytoplasm of the sublateral hypodermal cell (Fig. 2, A). It should be noted, however, that such an appearance could also be due to the displacement of cells by the gland tissue.

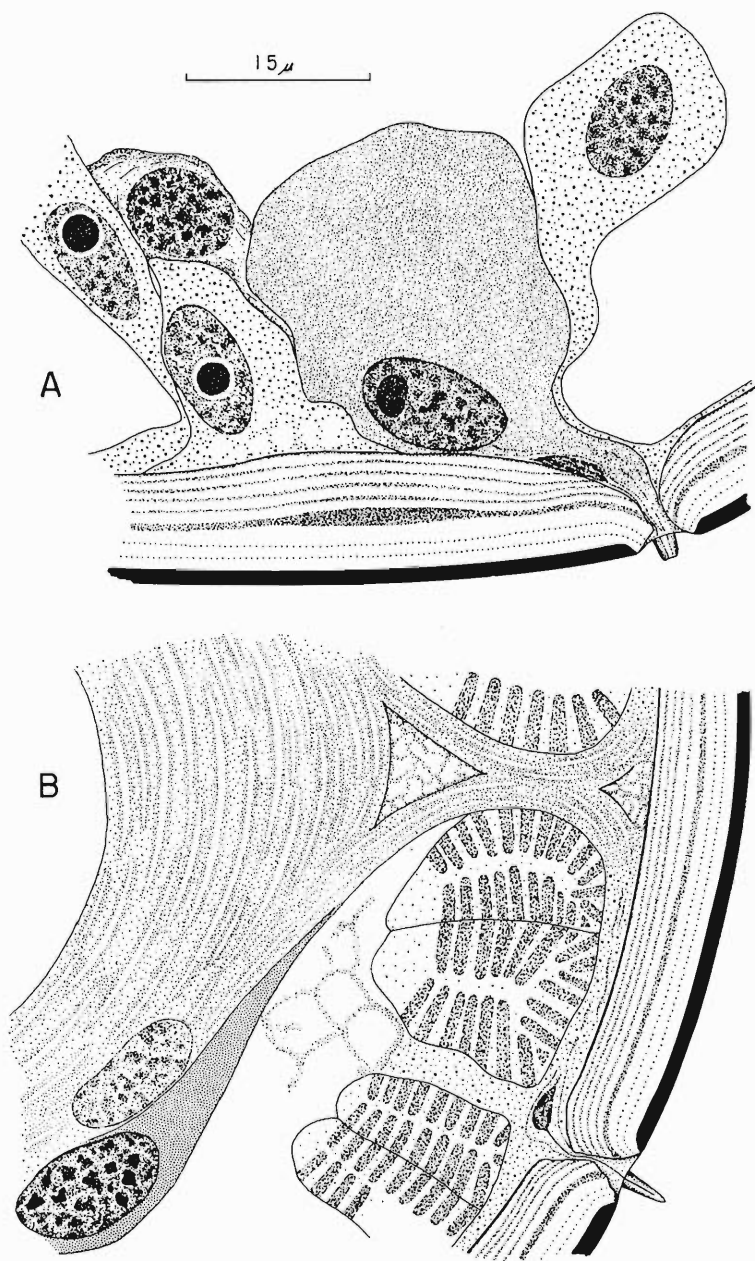


Fig. 2. *Thoracostoma californicum*. A, hypodermal gland, with neurocyte and scolopoid body, cross-section. B, subventral seta at level of nerve ring, with glia cell, cross-section. (illustrations of equal magnification)

The scolopoid body and sensory neuron also appear to be associated with the tube opening of the gland cell (Fig. 2, A). It was possible to trace a connection between the scolopoid body and the sensory neuron in serial sections.

DISCUSSION

Chitwood and Chitwood (1950) describe the ventrolateral nerves as ending posteriorly in the lumbar ganglia and connecting with the ventral nerve by way of the ano-lumbar commissures. In males the bipolar sensory neurons for the pre-anal genital papillae are situated in the ventrolateral nerve, the fibers continue posteriad to the genito-papillary commissures where they pass to the ventral nerve. The caudal papillae are innervated by the lateral caudal nerves, and by processes from the lumbar ganglia. This establishes, at least in part, that the ventrolateral nerve may serve to carry sensory impulses. It would not be inconsistent with known information to assume that a similar condition exists for the somatic setae. It is contended here that sensory impulses from somatic sensory organs are carried, at least in part, along the lateral nerves to the lateral ganglia.

From Chitwood and Chitwood's description one could conclude that impulses are not carried to the nerve ring along the ventrolateral (lateral) nerves; however, these observations are based upon the innervations of the caudal and genital papillae and not upon the somatic sensory organs. The neural paths of the labial and cephalic sensory organs are known to be different from those of the genital and caudal papillae; therefore, it is reasonable to assume that the somatic sensory organs would also have different routes of neural transmission. It would follow also that the response elicited will differ for the area of reception as well as the area of association involved.

Studies by Chitwood and Chitwood (1950) on *Ascaris*, *Cephalobellus*, *Oxyuris* and *Spironoura* have shown that 50 to 75 percent of the neurons in the lateral ganglia are unipolar. Observations on *Thoracostoma* also show that the apparent majority of neurons in the lateral ganglia are unipolar. The significance of the presence of these unipolar neurons lies in the fact that they are undoubtedly playing a major function in association. This idea was not precluded by Goldschmidt (1908) or by Chitwood and Chitwood (1950). Also they are indicative of sensory nerve endings in their respective areas. Therefore, it is assumed that the nerves of the somatic setae do not, of necessity, proceed only to the genito-papillary commissures or the ano-lumbar ganglia but may in reality send a process to the lateral ganglia and from this region the association is directed to coordinated movement. Further investigation may reveal the presence of a collateral axon on the sensory axon of tactile receptors.

It is obvious from observations of the movement of nematodes that the sensory nervous system is much more complex than described. The coordinated movement which results in the characteristic serpentine locomotion requires a highly developed association between the active muscle and the antagonistic muscle. The relaxation and contraction of the somatic muscles must be coordinated not only along the length of a single sector, but also among the various sectors. The same is true of any response to a tactile stimulus if it is to be effective. Because there is no evidence of a highly developed peripheral nervous system, as in some Crustacea, it is assumed that any coordinating transmission takes place centrally at the nerve ring and its associated ganglia, and/or the ventral nerve and its ganglia by way of the dorso-ventral commissures.

Observations of living decapitated nemas strongly suggest the presence of an immediate reflex arc. Therefore, one can conclude that the bipolar sensory neuron may have a collateral axon which carries neural transmissions to the ventral cord or directly to the muscles, the ventral cord, more likely, since the movement exhibited in response to stimulation of the remainder of the body is still general and therefore coordinated. However, such movement is not sustained to allow the animal a locomotive reaction. It is in reaction to external or internal stimuli that the lateral ganglia must be important in coordinated sustained locomotion.

The external opening of the hypodermal glands is significant as contributory evidence to both sensory organ transmutation and cephalization. It has been assumed, based upon observations of different species and genera, that such phenomena phylogenetically have taken place (Maggenti, 1963). The basic assumption has been that all labial sense organs, viz. papillae, are derived from setae; in fact a linear trend toward this can be observed in Nemata. *Thoracostoma* complements this assumption because one is led to the conclusion that the hypodermal glands find their origin in somatic setae. However, caution must be exercised for such a development may represent a specialized feature of *Thoracostoma*.

If the setiform tube opening, its associated hypodermal cells and sensory neuron with scolopoid body, less the gland cell, is considered as homologous with setae it can be concluded that the hypodermal gland is a secondary development. If the seta were secondary then it should persist and develop in those nematodes assumed to be phylogenetically higher; by observation this is not so. If the hypodermal gland is secondary it should remain in higher forms and the setae should have been lost. This latter is the observed situation in nature.

From the above arguments and the evidence known from the observation of other nematodes one can visualize the hypodermal glands of *Thoracostoma* as a phylogenetic transition from seta to gland. In this event we have one explanation whereby papillae and glands may have developed from setae.

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Studies on the Carbohydrate Metabolism of *Neoechinorhynchus* spp. (Acanthocephala)*

T. T. DUNAGAN

ABSTRACT

Neoechinorhynchus emydis and *N. pseudemydis* have large stores of glycogen which enable them to survive long periods of starving conditions. Glycogen content falls rapidly the first four days and then at a much slower rate until the worms die. When exogenous glucose is added, the rate of glycogen utalization is reduced but not completely abated. Most of the glycogen appears as lactic acid especially during the first few days of starvation. However, as time progresses, lactic acid accounts for progressively less of the glycogen used.

Only two enzymes in glycolysis were found: aldolase and lactic acid dehydrogenase. Attempts to locate phosphate intermediates gave poor results but there are large amounts of organic phosphates present. There also appears to be a relationship between age and amount of organic phosphates.

To date carbohydrate in various forms has been investigated in seven species of Acanthocephala: *Neoechinorhynchus emydis*, *N. cylindratus*, *Echinorhynchus coregoni*, *Pomphorhynchus bulbocolli*, *Leptorhynchoides thecatus*, *Macracanthorhynchus hirudinaceus*, and *Moniliformis dubius*. Quantitative studies have been limited to *M. hirudinaceus* and *M. dubius*, species that are large and available in numbers. Most acanthocephala are much smaller and more difficult to obtain in the quantities necessary for such studies but certain ones have been investigated histochemically for the distribution of glycogen and enzymes.

Marsh and Kelly (1958) observed magnesium-activated inorganic pyrophosphate activity in *M. hirudinaceus* but to a much less degree than in certain nematodes and cestodes. Previously, Bullock (1958) had found alkaline glycerophosphatase activity in all of 28 species representing 8 families but not including the Neoechinorhynchidae. He later (1949a, 1949b) noted that glycogen was deposited mostly in the subcuticula and muscles and to a less extent in reproductive organs, the lacunar system, ganglia, and lemnisci. Fat distribution paralleled that of glycogen but tests for lipase were negative. He suggested that the subcuticula of the praesoma may function in the absorption or excretion of fats. *E. coregoni* and *P. bulbocolli* showed lipase and alkaline glycerophosphatase activity and were found to have more glycogen than *N. emydis* or *N. cylindratus* but the four were similar with respect

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to distribution and site of absorption or excretion of fat. *L. thecatus* and *M. hirudinaceus* are reported by von Brand (1939b) to have the same distribution of glycogen.

According to Schwartz (1920), extracts of body fluids of *M. hirudinaceus* are able to convert potato starch to sugars. In the same species, von Brand and Saurwein (1942) observed a galactogen-like polysaccharide and found Na, K, Ca, Mg, Mn, Al, Fe, and Cu by spectrographic analysis of whole worms. Von Brand (1939a, 1940) observed more glycogen in males than females but he regularly found fermentable sugar and nonfermentable reducing substances in the body fluids of both sexes. Graff and Allen (1963) extended these observations on the unequal distribution of glycogen between sexes of *M. dubius*. They also indicated that female worms alter the metabolism of glycogen in male worms of the same host. Moreover, glycogen distribution in *M. hirudinaceus* was the same as in *N. emydis* except that the lemnisei were poor in this substance. According to von Brand (1940), Weinland and Rudolphi (1910) had found in a single experiment that glycogen consumption was less than 1.0 gm/100 gm of worm/day under conditions that were not defined but from that rate were considered by von Brand to have been almost certainly anaerobic. He further suggested that the comparative rate of glycogen consumption under aerobic and anaerobic conditions that *M. hirudinaceus* is predominantly anaerobic. Ward (1952) indicated that glycogen stores fell rapidly in starving worms from an initial 1.86% of the wet weight in females to 0.76% after 43 hours under anaerobic conditions. Miller (1943) showed that all glycogen disappeared from the acanthor of *M. hirudinaceus* after hatching; traces reappeared by the 22nd day of development in the arthropod host and thereafter increased progressively.

According to Laurie (1957, 1959), *Moniliformis dubius* possesses glycogen and trehalose as major endogenous carbohydrate sources but he found no significant differences between the sexes as to the amount of glycogen present whereas Graff and Allen (1963) always observed more glycogen in the female. *M. dubius* synthesized glycogen from glucose, mannose, fructose, and maltose but 10^{-3} M p-chloromecuri-benzoic acid inhibited fermentation of hexoses. From his date on fermentation, Laurie postulated that the worms build up an oxygen debt and suggested that the host must at least split polysaccharides to maltose before they can be absorbed or otherwise used. Read and Rothman (1958) found that *M. dubius* also required carbohydrate for growth and that starvation of the rat host produced a marked decrease in the polysaccharide content of the parasite. The rate of decrease was not given but a diurnal fluctuation of the polysaccharide occurred.

MATERIALS AND METHODS

The phosphorus content was determined as inorganic, organic, and total phosphorus, using the colorimetric method of Fiske and Subbarow (1925). Fixed specimens were used for total phosphorus, fresh ones for other determinations.

Glycogen was prepared from each sample by the method of Walaas and Walaas (1950). After weighing, the worms were digested in boiling 30% KOH for 30 minutes. Ethyl alcohol to make a final concentration of 50% was added to precipitate the glycogen which was separated by centrifuging, washing with 65% ethyl alcohol, and hydrolyzing to glucose with normal sulfuric acid. The resulting hexose was determined by the method of Roe (1955).

Aldolase was determined by the method of Sibley and Lehninger as modified by Taylor (1955). As no attempt was made to obtain a pure primary or secondary reference standard, the aldolase present could not be determined quantitatively. In controls, the enzyme was inactivated by adding the TCA to the mixture before the substrate.

Lactic acid dehydrogenase was determined by the method of Wroblewski and LaDue (1955). The optical density at 340 millimicrons was followed manually at 30 second intervals for 3 minutes with a Beckman model DU spectrophotometer at room temperature in pH 9.3 phosphate buffer. Neilands (1955) suggests pH 10.0 as the proper buffer for the reaction. He points out that the "amount of enzyme needed for the test is the order of 0.1 gamma and hence the method may be applied directly to the crude extracts without danger of non specific reduction of DPN by contaminating substrates in the enzyme solution." Commercial DPN (Sigma) was used without further purification. No activators of LDH were used; they were either not required or were present in the tissue homogenate containing the LDH. Samples prepared in buffer were immediately used for LDH determinations.

The technique of Barker and Summerson (1941) was used for determination of lactic acid. Samples were stored at -20° C. until used. Results were read spectrophotometrically at 565 millimicrons following the development of color by alkaline p-hydroxydiphenyl. In controls water replaced aliquots of the supernatant from homogenized worms.

RESULTS AND DISCUSSION

Figure 1 shows the effect of starvation on total glycogen content of *N. pseudemydis* maintained *in vitro*. The amount decreases rapidly during the first four days and more slowly thereafter. At the same time, the range in the glycogen content of the worms decreased from an initial 16% of their dry weight to about 4% at 16 days. By extrapolation of the curve, glycogen theoretically would disappear by the 25th day and 26 days was the maximum length of time that worms remained motile in Tyrode's balanced salt solution (T-BSS) alone. A wide range of variation in glycogen content occurred in the worms studied which is evident from Figures 1 and 2. These variations are probably exaggerated here since the parasites used were taken from turtles in various states of nutrition and at all seasons of the year.

Figure 2 shows that glycogen depletion is significantly decreased but is not prevented by the presence of exogenous glucose. Also, the range decreases but not as much as in T-BSS alone. With added glucose, the glycogen content at 16 days was approximately seven times that of worms in saline alone and twice as great as at 34 days when the experiment was discontinued. Also, glycogen depletion was at a uniform rate and extrapolation of the curve would intersect the abscissa at the average time for which worms remain motile in T-BSS plus 0.1% or 1.0% glucose. It thus is evident that glucose either contributes to their synthesis of glycogen or permits its conservation. This is not surprising. The question seems to be: If glucose isn't used to synthesize all the glycogen what substrate is used? The fact that glucose doesn't prevent a fall in glycogen may be due, in part, to the *in vitro* environment which undoubtedly could be improved. Although the medium was not changed during each experiment, reduction of exogenous glucose does not seem to be responsible for the gradual decrease in glycogen content, because higher molar concentrations of glucose did not prolong motility of the worms in survival experiments.

Although the term "glycogen" has been used for the alkali-stable alcohol-insoluble carbohydrate, that substance is not necessarily the same as the liver glycogens of mammals. In parasites the molecular structure of glycogens are largely unknown. It has been reported that those of *Monezia expansia* and *Ascaris lumbricoides* differ in their branching which would imply that a difference also exists in the relative activity of phosphorylase and branching enzymes during glycogen synthesis. Acanthocephala will probably follow this pattern and differ in their molecular form of glycogen; especially the "free" glycogen.

Glycogen formation and degradation in parasitic helminths has received more attention than any other aspect of their biochemistry. The rate and extent of glycogen utilization varies considerably as might be expected in such diverse forms occupying a variety of hosts and sites within these hosts. Thus, *M. dubius*, a rodent parasite, has a diurnal fluctuation in glycogen content (Read and Rothman, 1958) and any period of host starvation beyond the normal feeding pattern places severe stress on the parasite. However, no such diurnal variation was observed in *N. pseudemydis* and glycogen utilization was at a slower rate and more prolonged than reported for any other helminth thus far. The slower rate of glycogen loss in the presence of glucose is correlated with the increased longevity of the worms when glucose is present (Dunagan, 1962). That this is not an isolated event in parasites is indicated by Bueding (1950), who observed a total loss of glycogen in *Schistosoma mansoni* incubated in medium containing glucose, and Vernberg

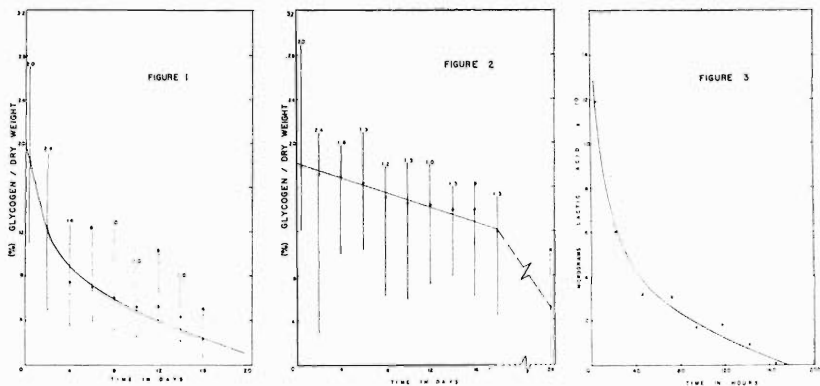


Figure 1. Glycogen depletion in females of *N. pseudemydis*, maintained in Tyrodes balanced salt solution at pH 8.2 and kept at room temperature. Each vertical line shows range of glycogen content. The number above each vertical line indicates the number of groups of four worms each used in the data. The average is indicated by a point in each range line.

Figure 2. Rate of glycogen utilization in *N. pseudemydis* maintained in Tyrodes balanced salt solution at room temperature. The media was buffered at pH 8.2 and contained 0.1% glucose. Each vertical line indicates the range of glycogen content and the number above each vertical line shows the number of groups of four worms each used to plot the data. The average is indicated by a point in each range line.

Figure 3. Rate of lactic acid decrease in female *N. pseudemydis* maintained under starving conditions in Tyrodes balanced salt solution at pH 8.2 and kept at room temperature. Each point represents the average of three determinations. Average total dry weight of worms from these three determinations was 3.1 mg.

and Hunter (1956) who found that the rate of glycogen loss in another trematode, *Gynaecotyla adunca*, was the same whether glucose was present or absent. However, Hopkins (1952) found that the plerocercoids of *Schistocephalus solidus* not only synthesized glycogen from glucose but above a threshold value, did so at a rate independent of external sugar concentration. Analysis of several species of male worms (males cannot be separated into species with any degree of assurance) whose numbers had been pooled at different times of the year indicated that a seasonal variation in endogenous glycogen occurs. However, this may simply reflect the physiological state of the turtles and the developmental state of the parasites at the time of examination.

In the present study, motility was reduced in the absence of free oxygen and yet the worms survived as long under "anaerobic" as aerobic conditions and used no free oxygen that could be detected by the Warburg technique. It thus seems that the cytochrome system as now understood, does not play a dominant role in the carbohydrate metabolism of these worms. Because of the unexpected nature of these results experiments are currently in progress to determine the oxygen tension in the intestine of the turtle host as well as in the "anaerobic" tubes used in survival studies. Considerable variability has been reported among helminths in this area.

For example, Ward (1952) observed that the metabolism of *M. hirudinaceus* maintained anaerobically and aerobically in saline for 43 hours was predominately anaerobic, even in the presence of oxygen. She also found that motility of worms under aerobic conditions was spontaneous "while those kept under anaerobic conditions moved only when stimulated by pinching with forceps." Von Brand, *et al.* (1952) found that *Trichinella spiralis* larvae under conditions of starvation lost their glycogen at the same regular rate regardless of presence or absence of oxygen and that the anaerobic metabolism of glycogen was sufficient to keep the larvae alive but not to sustain motility.

Efforts to isolate intermediates in carbohydrate metabolism by the technique of Bandurski and Axelrod (1951) were unsuccessful. Nevertheless, Table 1 indicates that organic phosphorus compounds are present in higher percentages that were reported by Read (1951) for *Hymenolepis diminuta*. The data in this table also suggest that there is a difference in concentration of organic phosphorus compounds according to age. This is difficult to evaluate, however, because of the variations in the nutritional state of the host from which the worms came. As phosphorylate intermediates were not found, determinations for certain enzymes of the Embden-Meyerhof scheme for glycolysis indicated that at least parts of this pathway exist in *Neoechinorhynchus spp.* Table 2 presents determinations of lactic acid dehydrogenase and Table 3, aldolase. The presence of lactic acid dehydrogenase is indicative of pyruvate and lactate while aldolase implies the presence of fructose-1, 6-diphosphate and triose phosphate. However, as the reader well knows, finding only part of the enzymes of Embden-Meyerhof pathway does not demonstrate the presence of the complete system as some investigators have assumed.

Although oxygen was not used in metabolism of glucose, the amount of lactic acid released to the medium by worms under starving conditions does not account for the total glycogen lost by them. As shown in Figure 3, the amount of lactic acid decreased sharply during the first 48 hours and more gradually thereafter until at 165 hours it was no longer detectable. From

Table 1. Phosphorus determinations in pooled *N. emydis*, *N. pseudemydis*, and *N. emyditoides*

Wet Weight in grams	Worm 'Age'	Total mg of P	% P (wet wt.)	mg. of inorganic P
0.998	"old"	0.64	0.06	0.04
1.024	"old"	0.68	0.07	0.06
0.416	Mature	4.62	1.10	0.11
0.322	Immature	3.48	1.08	0.15

Table 2. Lactic acid dehydrogenase determinations on buffered (pH 7.3) supernatant of homogenized female *N. emydis* (1) and *N. pseudemydis* (2). Optical density determined at 340 m μ

Species	Number of worms	Grams wet weight	Aliquot in ml.	Optical density Intervals in seconds				
				30	60	90	120	180
1	430	1.041	0.3	.206	.412	.80	1.15	1.15
1	430	1.041	0.3	.208	.410	.82	1.10	1.15
1	430	1.041	0.3	.207	.410	.81	1.15	1.15
Control	—	—	0.3	.160	.160	—	.160	.160
2	700	1.331	0.2	.215	.295	.375	.470	.530
2	700	1.331	0.2	.210	.300	.380	.468	.536
2	700	1.331	0.2	.211	.302	.391	.475	.538
Control	—	—	0.2	.200	.200	.200	.200	.201

Table 3. Aldolase determined as alkali-labile phosphorus in buffered (pH 9.0) supernatant of homogenized *N. emydis*

Reaction Mixture	Inorganic P before hydrolysis ug		Inorganic P after hydrolysis ug		Alkali labile P formed ug	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Complete (0 Time)	0	0	5	7	—	—
Complete (5 minutes)	24	30	65	80	36	43
Without cyanide	33	37	40	41	2	0

Figures 1 and 3, it can be calculated that the percentage of glycogen lost during the first 48 hours is about 6% of dry weight; however, glycogen continues to fall after lactic acid production is no longer detectable. It is probable that instead of lactic acid other substances such as formic, acetic, and/or other fatty acids were formed. Efforts to isolate the latter by paper chromatography have not been satisfactory.

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**Studies on Nygellidae n. fam. and Belondiridae Thorne, 1939
(Nematoda : Dorylaimoidea) with description of ten new species
from India**

M. SHAMIM JAIRAJPURI*

Thorne (1939) included *Belondira* Thorne, 1939; *Axonchium* Cobb, 1920; *Oxydirus* Thorne, 1939; *Swangeria* Thorne, 1939; *Nygellus* Thorne, 1939 and *Dorylaimellus* Cobb, 1913 in the family Belondiridae to which Loos (1949) added *Nygolaimellus*. The inclusion of *Nygellus* and *Nygolaimellus* in Belondiridae poses some interesting problems. They belong in Belondiridae because of having a spiral muscle sheath around the basal enlargement of the esophagus, but they also show close affinities with Nygolaimidae (Thorne, 1935) Meyl, 1960 in having a mural tooth and three glandular organs at base of esophagus. In fact, the two genera are distinctive in having a combination of belondirid and nygolaimoid characters. Clark (1961) considered the nygolaimoid characters of more importance and shifted these genera to Nygolaimidae. He kept *Nygellus* with *Nygolaimus* Cobb, 1913; *Heterodorus* Altherr, 1952 and *Sectonema* Thorne, 1930 in the subfamily Nygolaiminae Thorne, 1935; *Nygolaimellus* in having a bibulbar esophagus was put under a separate subfamily Nygolaimellinae. Goodey (1963) conforms with the classification proposed by Clark. If *Nygellus* and *Nygolaimellus* cannot be placed in Belondiridae because of having a mural tooth and three glandular bodies at esophago-intestinal junction, for similar reasons they also cannot be accommodated under Nygolaimidae since they possess a spiral muscle sheath around the expanded part of esophagus, a character found only in Belondiridae. It is thereby proposed that an entirely new family should be erected to contain these two genera which share features of both Belondiridae and Nygolaimidae.

FAMILY NYGELLIDAE n. fam.

DIAGNOSIS: Polymyarian. Spear a mural tooth. Posterior expanded portion of esophagus surrounded by a spiral muscle sheath. Three glandular organs present at base of esophagus. Ovaries paired or single.

TYPE SUBFAMILY: Nygellinae n. subfam.

OTHER SUBFAMILY: Nygolaimellinae Clark, 1961.

SUBFAMILY NYGELLINAE n. subfam.

DIAGNOSIS: Nygellidae. Esophagus unibulbar.

TYPE AND ONLY GENUS: *Nygellus* Thorne, 1939.

SUBFAMILY NYGOLAIMELLINAE Clark, 1961

DIAGNOSIS: Esophagus bibulbar.

TYPE AND GENUS: *Nygolaimellus* Loos, 1949.

DISCUSSION: Belondiridae will now include only *Belondira*, *Axonchium*, *Oxydirus*, *Swangeria* and *Dorylaimellus*. The following three new subfamilies are proposed to classify these genera.

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SUBFAMILY BELONDIRINAE n. subfam.

DIAGNOSIS: Belondiridae. Spear small, usually a little irregular in outline; extension simple. Lateral guiding pieces present.

TYPE GENUS: *Belondira* Thorne, 1939.

OTHER GENERA: *Axonchium* Cobb, 1920 and *Orydirus* Thorne, 1939.

SUBFAMILY SWANGERINAE n. subfam.

DIAGNOSIS: Belondiridae. Vestibule leading into a flat basket-like chamber with obscure cuticularized ribs. Spear dorylaimoid, proportionately longer than in other Belondiridae. Esophagus a slender, colourless tube extending back to elongate, spindle-shaped, basal portion. Cardia greatly elongated, isthmus-like, intestine attached only to its posterior end.

TYPE AND ONLY GENUS: *Swangeria* Thorne, 1939.

SUBFAMILY DORYLAIMELLINAE n. subfam.

DIAGNOSIS: Belondiridae. Spear slender; extension in two obscurely separated sections which bear broad flanges. Four cuticularized pieces occur around vestibule. Spicules very broad proximally, with an abrupt angle ventrally. Ventromedian supplements usually arranged in pairs.

TYPE AND ONLY GENUS: *Dorylaimellus* Cobb, 1913.

KEY TO SUBFAMILIES OF BELONDIRIDAE

1. Vestibule leading into a basket-like chamber; cardia elongated, isthmus-like Swangerinae
Basket-like chamber absent; cardia as in other dorylaims 2
2. Extension flanged; four cuticularized pieces around vestibule
..... Dorylaimellinae
Extension simple; cuticularized pieces absent Belondirinae

GENUS *Nygellus* Thorne, 1939

Nygellus clavatus Thorne, 1939

FEMALES (5): L = 1.0-1.2 mm; a = 44-51; b = 3.2-3.8; c = 10-13; V = 34-37.

HABITAT: About 30 females from soil around roots of sugar cane, *Saccharum officinarum* L., from Jorhat (Assam) India.

Nygellus caudatus n. sp. (Fig. 1, A-C)

HOLOTYPE (FEMALE): L = 1.3 mm; a = 41; b = 4.6; c = 60; V = 39.

DESCRIPTION: Body cylindrical, bluntly rounded at both extremities and ventrally arcuate in posterior third of body. Cuticle provided with longitudinal lines formed by dot-like structures. Lip region bluntly rounded, slightly wider than adjoining body width; lips obscure; cephalic papillae visible. Amphidial apertures occupying about half of head width. Spear a mural tooth, needle-like, about one head width long and with minute aperture. Pharyngeal cavity fairly sclerotized, narrowing posteriorly to join with the esophageal lumen. Anterior esophagus with distinct swelling and radial musculature, slightly narrowing until the nerve ring from where it gradually expands to form basal enlarged portion of esophagus which occupies about half of neck length and is surrounded by a sheath of obscurely spiral muscles. Esophageal gland nuclei obscure. Three glandular organs at base of esophagus. Nerve ring 104 microns from anterior end. Rectum less than one

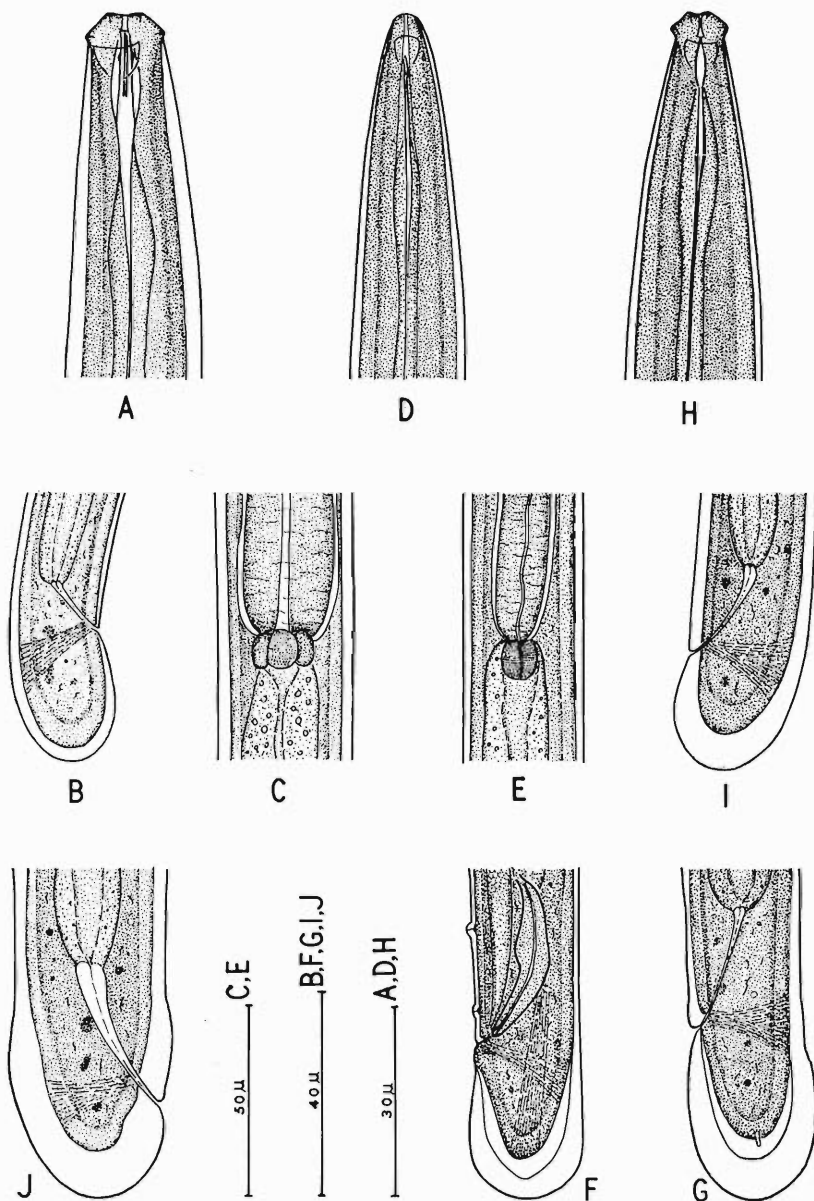


Fig. 1, A-J. A-C—*Nygellus caudatus* n.sp. A. Head end, B. Tail, C. Cardiac region; D-G *Belondira paraclava* n.sp. D. Head end, E. Cardiac region, F. Male tail, G. Female tail; H-I *Axonchium nitidum* n.sp. H. Head end, I. Tail; J. Tail of *Axonchium bulbosum* Williams, 1958.

anal body width long; prerectum length three and a half times anal body width. Vulva a transverse slit; vagina a quarter body width long. Ovaries two, short, amphidelphic and reflexed. Eggs measuring 116 x 26 microns. Tail clavate, slightly more than one anal body width long.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on August 25, 1962; deposited in the Zoological Museum of Aligarh Muslim University, Aligarh, India.

TYPE HABITAT: Soil around roots of grass.

TYPE LOCALITY: Simla (H.P.) India; height, 9,000 feet above sea level.

DIFFERENTIAL DIAGNOSIS: *Nyggellus caudatus* n.sp., comes closest to *N. symmetricus* Williams, 1958 in being didelphic, but differs in having more stout body; short esophagus; anterior location of vulva and a short tail.

KEY TO THE SPECIES OF *Nyggellus*

1. Ovaries paired 2
 Ovary single 3
2. Tail about 6 anal body widths long ($e = 15$)
 *symmetricus* Williams, 1958
 Tail slightly more than one anal body width long ($e = 60$)..... *caudatus* n. sp.
3. Tail about 8 anal body widths long ($e = 10-13$)..... *clavatus* Thorne, 1939
 Tail 2 anal body widths long ($e = 29-38$).....
 *subclavatus* Timm and Ameen, 1960

GENUS *Belondira* Thorne, 1939

Belondira ortha Thorne, 1939 (Fig. 2, I-J)

Thorne provided the description of female of *B. ortha* from young specimens and now that mature ones are available, a brief account is here given.

FEMALES (2): L = 1.5-1.6 mm; a = 36-40; b = 4.2-4.5; c = 72-74; V = 36-37.

MALE: L = 1.4 mm; a = 43; b = 4.4; c = 71.

DESCRIPTION: Body tapering slightly anteriorly to a narrow, conoid lip region continuous with body contour. Width of head a quarter of neck base. Amphidial apertures as wide as head. Basal enlarged portion of esophagus occupying three fifths of neck length. Dorsal esophageal gland nucleus large, oval. Vulva depressed transverse slit, about a quarter body width. Vagina about half body width long. Ovary opisthodelphic, reflexed. Anterior uterine branch about twice body width long, filled with sperms. Rectum equal to one anal body width long; prerectum about thrice as long as rectum. Tail hemispheroid, with thick layers of cuticle. Shape of male and female tail somewhat different from the ones shown by Thorne.

HABITAT: 4 mature and 3 immature females and a single male from soil around roots of sugar cane, *Saccharum officinarum* L., from Jorhat (Assam) India.

Belondira paraclava n.sp. (Fig. 1, D-G)

FEMALES (10): L = 1.0-1.3 mm; a = 37-42; b = 4.8-5.4; c = 37-43; V = 37-39.

HOLOTYPE (Female): L = 1.2 mm; a = 38; b = 5.2; c = 41; V = 38.

ALLOTYPE (Male): L = 1.1 mm; a = 42; b = 5.2; c = 36.

DESCRIPTION: FEMALE (holotype):—Body straight, tapering anteriorly from neck base to a narrow lip region which is not set off from body contour

in any manner. Cuticle with fine transverse striations. Lateral chords about a quarter body width. Lip region completely amalgamated, papillae obscure and not modifying its rounded contour which at level of amphidial apertures is about one fifth as wide as neck base. Amphidial apertures about half as wide as head. Spear short, dorylaimoid; extension long, simple and cuticularized. Guiding ring faint. Esophagus a very narrow, non-muscular tube

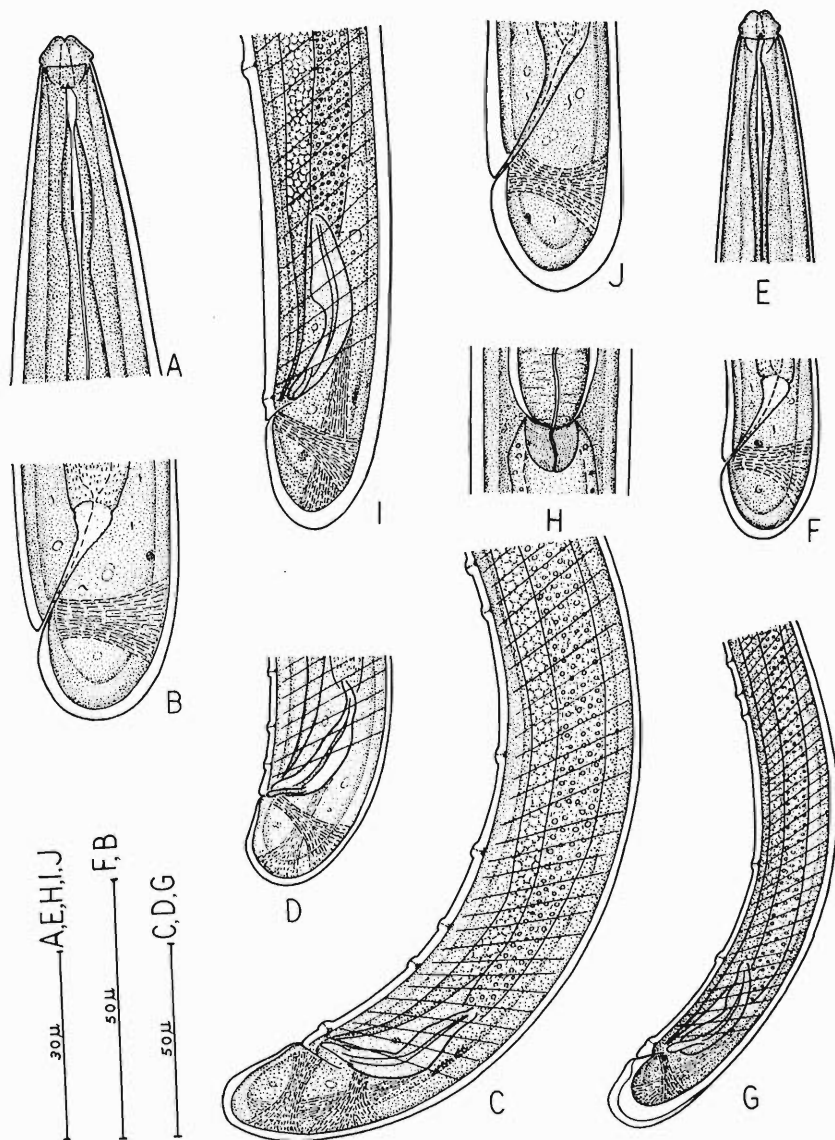


Fig. 2, A-J. A-D *Aronchium saccatum* n.sp. A. Head end, B. Female Tail, C, D. Male tail; E-H *Aronchium elegans* n.sp. E. Head end, F. Female tail, G. Male tail, H. Cardiac region; I-J. *Belondira ortha* Thorne, 1939 I. Male tail, J. Female tail.

expanding gradually behind middle to form basal expanded portion which is made up of glandular cells and surrounded by sheath of spiral muscles. Cardia spherical. Rectum as long as anal body width; prerectum length slightly more than twice anal body width. Vulva deep transverse slit; vagina straight, about half body width long. Ovary opisthodelphic, reflexed. Anterior uterine branch a short pouch, its length slightly more than one body width, filled with sperms. Tail clavate, more than one anal body width long. Cuticle of tail with unusually thick layers. Single caudal pore observed.

MALE (allotype): Similar to female in general morphology. Testes dorylaimoid. Supplements an adanal pair and a single ventromedian located at one anal body width above anus. There is trace of a rudimentary supplement at about four body widths above anus. Spicules dorylaimoid, 30 microns long. Lateral guiding pieces present. Tail one and one half anal body widths long; similar to that of female.

HOLOTYPE AND ALLOTYPE: Collected on October 25, 1962; slide deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 8 females; other data as for holotype.

TYPE HABITAT: Soil around roots of apricot, *Prunus armenica* L.

TYPE LOCALITY: Saharanpur (U.P.) India.

DIFFERENTIAL DIAGNOSIS: *Belondira paraclava* n.sp., comes closest to *Belondira clara* Thorne, 1939 but differs in having a slender body, spherical cardia, anteriorly located vulva, length of tail ($c = 66$ in *B. clara*) and in the presence of males (males unknown and sperms not present in the uteri in *B. clara*).

KEY TO THE SPECIES OF *Belondira*

1. Tail clavate 2
Tail hemispheroid or conoid 3
2. Tail one and one half anal body widths long ($c = 37-43$); males known.....
.....*paraclava* n. sp.
Tail less than one body width long ($c = 66$); males unknown.....
.....*clara* Thorne, 1939
3. Tail conoid*apitica* Thorne, 1939
Tail hemispheroid 4
4. Tail less than one anal body width long ($c = 72-75$).....*ortha* Thorne, 1939
Tail more than one anal body width long ($c = 41$).....*caudata* Thorne, 1939

DISCUSSION: The author has examined the paratypes of *Belondira singularis* Williams, 1958 and *B. perplexa* Williams, 1958 and came to the conclusion that they are not *Belondira* since they have long filiform tail and thus appear to be closely related to *Oxydirus*, but detailed study may reveal a new genus.

GENUS *Axonchium* Cobb, 1920

Axonchium amplicolle Cobb, 1920

FEMALES (5): L = 1.5-2.0 mm; a = 31-38; b = 1.9-2.7; c = 46-66; V = 51-55.

HABITAT: About 30 females collected from soil around roots of litchi, *Nephelium litchi* Cambess, from Saharanpur (U.P.), and marigold, *Tegetes erecta* L., from Barog (H.P.) India.

Axonchium nitidum n. sp. (Fig. 1, H-I)

FEMALES (10): L = 1.2-1.6 mm; a = 34-53; b = 2.5-3.0; c = 54-75; V = 52-56.

HOLOTYPE (FEMALE): L = 1.6 mm; a = 53; b = 3.0; c = 75; V = 52.

DESCRIPTION: Body ventrally arcuate, tapering to a narrow lip region. Cuticle and subcuticle with fine transverse striations. Head width one third of neck base. Lip region conoid, set off. Amphidial apertures occupying entire head width. Spear spindle-shaped, one head width long, aperture occupying one third of its length. Basal expanded portion of esophagus occupying slightly more than half the neck length. Cardia hemispheroid. Rectum length less than one anal body width; prerectum about nine anal body widths long. Vulva transverse slit; vagina half body width long. Ovary opisthodelphic, reflexed. Anterior uterine branch about half body width long. Tail hemispheroid, less than one anal body width long.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on June 25, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 9 females; other data as for holotype.

TYPE HABITAT: Soil around roots of tea plants, *Thea sinensis* L.

TYPE LOCALITY: Jorhat (Assam) India.

DIFFERENTIAL DIAGNOSIS: *Axonchium nitidum* n. sp., comes closest to *A. bulbosum* Williams, 1958 but differs from it in having a ventrally arcuate posture when relaxed, longer prerectum and shape and length of tail.

Axonchium saccatum n. sp. (Fig. 2, A-D)

FEMALES (5): L = 1.8-2.0 mm; a = 32-41; b = 2.5-3.3; c = 64-88; V = 52-55.

MALES (3): L = 1.6-1.9 mm; a = 38-44; b = 2.8-3.0; c = 52-65.

HOLOTYPE (FEMALE): L = 2.0 mm; a = 39; b = 3.1; c = 79; V = 52.

ALLOTYPE (MALE): L = 1.9 mm; a = 44; b = 2.8; c = 52.

DESCRIPTION: FEMALE (holotype): Body ventrally arcuate and tapering to a narrow lip region. Cuticle and subcuticle with fine transverse striations. Head width a quarter of neck base. Lips conoid, set off. Amphidial apertures as wide as head. Spear length nearly equal to width of lip region, aperture occupying one third of its length. Basal expanded portion of esophagus occupying three fifths of neck length. Cardia conoid. Rectum less than one anal body width long; prerectum length about ten anal body widths. Vulva transverse; vagina one third body width long. Ovary opisthodelphic and reflexed. Anterior uterine branch more than two body widths long. Tail hemispheroid, less than one anal body width long.

MALE (Allotype): Similar to female in general morphology. Testes dorylaimoid. Supplements an adanal pair and six ventromedians (7-9 in paratypes) beginning at level of anterior end of spicules (at level of middle of spicules in some paratypes) and spaced at irregular intervals. Spicules dorylaimoid, 45 microns long. Prerectum length ten anal body widths long. Tail bluntly-conoid, less than one anal body width long.

HOLOTYPE AND ALLOTYPE: Collected on June 25, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 4 females and 2 males; other data as for holotype.

TYPE HABITAT: Soil around roots of jack tree, *Artocarpus integrifolia* L.

TYPE LOCALITY: Trivandrum (Kerala) South India.

DIFFERENTIAL DIAGNOSIS: *Axonchium saccatum* n. sp., is very similar to *A. gossypii* de Coninek, 1962, but differs in having very long prerectum (only 4-5 anal body widths long in *A. gossypii*). In addition to this character the

present worms are slightly longer in body and esophagus size and male tail more than one anal body width long (less than one anal body width long in *A. gossypii*).

Axonchium elegans n.sp. (Fig. 2, E-H)

FEMALES (4): L = 1.2-1.3 mm; a = 43-49; b = 2.5-2.7; c = 58-72; V = 54-72.

MALES (2): L = 1.2-1.3 mm; a = 48-49; b = 2.7-2.8; c = 68-72.

HOLOTYPE (FEMALE): L = 1.3 mm; a = 43; b = 2.7; c = 70; V = 57.

ALLOTYPE (MALE): L = 1.3 mm; a = 48; b = 2.8; c = 72.

DESCRIPTION: FEMALE (holotype): Body cylindroid, ventrally arcuate and tapering from neck base to a narrow lip region. Cuticle and subcuticle with fine transverse striations. Head width one third of neck base. Lips conoid, set off. Amphidial apertures occupying entire head width. Spear length equal to width of lip region, aperture one fifth of its length. Basal expanded portion of esophagus occupying a little more than three fifth of neck length. Cardia spherical (conoid in some paratypes). Rectum length equal to one anal body width; prerectum about ten times as long as rectum. Vulva transverse; vagina bent posteriorad, about half of body width long. Ovary opisthodelphic, reflexed. Anterior uterine branch about three body widths long. Tail hemispheroid, less than one anal body width long.

MALE (allotype): Similar to female in general morphology. Testes dorylaimoid. Supplements an adanal pair and 5 ventro-medians beginning at level with anterior end of spicules and spaced at irregular intervals. Spicules dorylaimoid, 30 μ long. Lateral guiding pieces present. Prerectum about ten anal body widths long.

HOLOTYPE AND ALLOTYPE: Collected on June 20, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 3 females and a single male; other data as for holotype.

TYPE HABITAT: Soil around root of coconut tree, *Cocos nucifera* L.

TYPE LOCALITY: Trivandrum (Kerela) India.

DIFFERENTIAL DIAGNOSIS: *Axonchium elegans* n.sp., comes closest to *A. bulbosum* Williams, 1958 and *A. nitidum* n.sp. From *A. bulbosum* it differs in being bisexual and in having a ventrally arcuate posture when relaxed, slender body, long anterior uterine branch (almost equal to body width in *A. bulbosum*), very long prerectum and different shape of tail. From *A. nitidum* it differs in being bisexual and in having longer anterior uterine sac.

GENUS *Oxydirus* Thorne, 1939

Oxydirus gigus n.sp. (Fig. 3, A-D)

MALES (2): L = 3.7-4.3 mm.; a = 61-86; b = 12-13; c = 11-12.

FEMALES (2): L = 3.8-4.5 mm.; a = 65-66; b = 12-13; c = 10; V = 40-42.

MALE (HOLOTYPE): L = 3.7 mm; a = 61; b = 13; c = 11.

FEMALE (ALLOTYPE): L = 3.8 mm.; a = 66; b = 12; c = 10; V = 42.

DESCRIPTION: MALE (holotype): Body straight when relaxed, tapering towards both extremities. Cuticle and subcuticle apparently smooth. Amphidial apertures occupying about half head width. Lip region rounded, almost continuous with the body contour. Spear short, dorylaimoid, about one half of head width long, aperture about one third of its length. Extension simple. Esophagus slender anteriorly, enlarging in the posterior third to form basal expanded portion which is surrounded by a conspicuous sheath of spiral muscles. Cardia hemispheroid. Testes dorylaimoid. Supplements

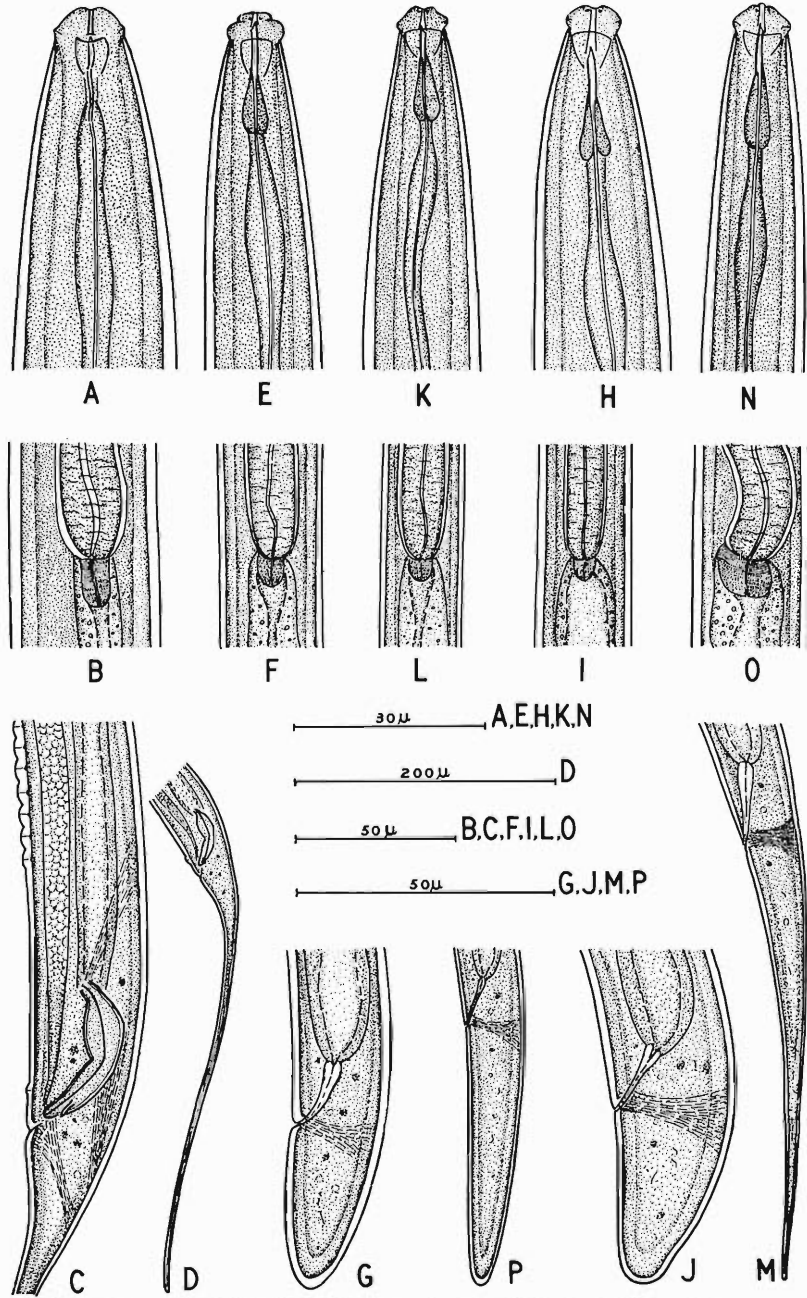


Fig. 3, A-P. A-D *Oxydirus gigus* n.sp. A. Head end, B. Cardiac region, C. Posterior portion of body of male, D. Male tail; E-G *Dorylaimellus cephalus* n.sp. E. Head end, F. Cardiac region, G. Tail; H-J *Dorylaimellus curvatus* n.sp. H. Head end, I. Cardiac region, J. Tail; K-M *Dorylaimellus filiformis* n.sp. K. Head end, L. Cardiac region, M. Tail; N-P *Dorylaimellus longicaudatus* n.sp. N. Head end, O. Cardiac region, P. Tail.

an adanal pair and about 25 ventromedians which are contiguous, the series three anal body widths above anus. Spicules dorylaimoid, 50 microns long. Lateral guiding pieces present. Tail very long, filiform with rounded terminus.

FEMALE (allotype): Similar to male in general morphology. Vulva transverse. Vagina thickwalled, about half of body width. Ovaries amphidelphic and reflexed. Rectum about one anal body width long. Prerectum length about ten anal body widths. Tail similar to that of male, about thirteen anal body widths long.

HOLOTYPE AND ALLOTYPE: Collected on October 29, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: One male, one female, and a large number of young females in various stages of development; other data as for holotype.

TYPE HABITAT: Soil around roots of guava, *Psidium guajava* L.

TYPE LOCALITY: Nainital (U.P.) India.

DIFFERENTIAL DIAGNOSIS: *Oxydirus gigus* n.sp., comes closest to *O. oxycephaloides* (de Man, 1921) Thorne, 1939 but differs from it in having a long slender body, very short esophagus and value of $c = 10-13$ ($c = 4.5$ in *O. oxycephaloides*).

GENUS *Dorylaimellus* Cobb, 1913

Dorylaimellus parvulus Thorne, 1939

FEMALES (5): $L = 0.45-0.54$ mm; $a = 28-32$; $b = 2.7-3.0$; $c = 30-32$; $V = 57-61$.

HABITAT: A widespread species in India. The present author has collected it from Aligarh, Saharanpur, Chandigarh, Nainital and Trivandrum from around roots of various plants.

Dorylaimellus cephalus n.sp. (Fig. 3, E-G)

FEMALES (5): $L = 1.0-1.3$ mm; $a = 42-50$; $b = 4.7-5.4$; $c = 34-39$; $V = 47-51$.

HOLOTYPE (FEMALE): $L = 1.3$ mm; $a = 50$; $b = 5.4$; $c = 39$; $V = 50$.

DESCRIPTION: Body ventrally arcuate when relaxed, tapering towards both extremities. Cuticle and subcuticle with fine transverse striations. Amphidial apertures about three quarters of head width. Lip region rounded, set off. Inner portion of lips forming a set off labial disc around vestibule which is about half as wide as lip region. Four small cuticularized pieces situated at base of labial disc. Lateral and ventral series of glandular organs present. Spear length equal to width of lip region, aperture minute. Extension flanged, as long as spear. Esophagus a narrow tube with a prominent swelling just below the spear extension, enlarging gradually to form basal expanded portion of esophagus which occupies slightly less than half neck length and is surrounded by a sheath of spiral muscles as thick as adjacent cuticle. Cardia hemispheroid. Vulva transverse; vagina half body width long. Ovaries amphidelphic and reflexed. Prerectum about six anal body widths long. Rectum about one anal body width long. Tail convex-conoid to a rounded terminus, slightly less than twice anal body width long.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on November 18, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 9 females; other data as for holotype.

TYPE HABITAT: Soil around roots of *Saccharum ravennae* (L.) Murray.

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

DIFFERENTIAL DIAGNOSIS: *Dorylaimellus cephalus* n.sp., comes closest to *D. projectus* Heyns, 1962 but differs from it in having long slender body, short esophagus and short convex-conoid tail, striated cuticle, spear and its extension equal in length.

Dorylaimellus curvatus n.sp. (Fig. 3, H-J)

FEMALE (5): L = 1.2-1.4 mm; a = 39-56; b = 7.3-8.0; c = 35-40; V = 50-53.

HOLOTYPE (FEMALE): L = 1.3 mm; a = 39; b = 7.3; c = 40; V = 51.

DESCRIPTION: Body ventrally arcuate when relaxed, tapering anteriorly to a narrow lip region, one third as wide as neck base. Cuticle and subcuticle finely striated. Amphidial apertures encircling entire head width. Lip region rounded, set off. Lateral series of glandular organs prominent. Four distinct, shining cuticularized pieces around vestibule present. Spear equal to head width, aperture about a quarter of its length. Extension as long as spear, broadly flanged. Basal expanded portion of esophagus occupying slightly more than one third of neck length. Cardia hemispheroid. Vulva a deep transverse slit; vagina one third body width long. Ovaries amphidelphic and reflexed. Prerectum length about three and a half times anal body width. Rectum, less than one anal body width long. Tail bluntly conoid to often slightly subdigitate in paratype, its length more than one anal body width.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on June 25, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

PARATYPES: 4 females; other data as for holotype.

TYPE HABITAT: Soil around roots of tea plants, *Thea sinensis* L.

TYPE LOCALITY: Jorhat (Assam) India.

DIFFERENTIAL DIAGNOSIS: *Dorylaimellus curvatus* n.sp., comes closest to *D. bambesae* de Coninck, 1962 and *D. imitator* Heyns, 1963. It differs from *D. bambesae* in having a smaller body, long esophagus, short prerectum and short bluntly rounded tail and from *D. imitator* in having a longitudinal vulva and in the absence of small refractive, sclerotized bodies.

Dorylaimellus filiformis n.sp. (Fig. 3, K-M)

HOLOTYPE (FEMALE): L = 0.82 mm; a = 40; b = 5.8; c = 9; V = 43.

DESCRIPTION: Body tapering anteriorly to a narrow lip region which is one third as wide as neck base. Cuticle and subcuticle apparently smooth. Amphidial apertures as wide as head. Lip region conoid, set off. Lateral series of glandular organs present. Spear slender, its length less than head width, aperture occupying a quarter of spear length. Flanged extension about twice as long as spear. Esophagus typical of the genus; basal expanded portion occupying about one third the entire neck length. Cardia hemispheroid. Vulva transverse; vagina half body width long. Ovaries amphidelphic and reflexed. Prerectum about five anal body widths long. Rectum length more than one anal body width. Tail long, filiform, about eight anal body widths long.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on September 15, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

TYPE HABITAT: Soil around roots of *Saccharum ravennae* (L.) Murray.

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

DIFFERENTIAL DIAGNOSIS: *Dorylaimellus filiformis* n.sp., is distinctive among all the previously described species of the genus in having a long filiform tail. However, it comes closest to *D. occidentalis* Thorne, 1939 but differs from it in the character of tail and in having a slender body, short esophagus and anterior location of vulva.

Dorylaimellus longicaudatus n.sp. (Fig. 3, N-P)

HOLOTYPE (FEMALE): L = 0.79 mm; a = 40; b = 4.5; c = 17; V = 47.

DESCRIPTION: Body slightly tapering towards both extremities. Cuticle and subcuticle with coarse striations. Amphidial apertures occupying entire head width. Lip region conoid, set off. Lateral glandular organs present, but indistinct. Four cuticularized pieces around vestibule present. Spear small, slender, about the width of lip region, aperture occupying about a quarter of its length. Extension about one and one half times as long as spear. Basal expanded portion of esophagus occupying about half the entire neck length. Cardia hemispheroid. Vulva transverse slit; vagina one third of body width long. Ovaries amphidelphic and reflexed. Prerectum about ten anal body widths long. Rectum length equal to one anal body width. Tail elongate-conoid, terminus rounded, its length equal to four anal body widths.

MALE: Not found; sperms not present in uteri.

HOLOTYPE: Collected on July 6, 1963; deposited in the Zoological Museum of Aligarh Muslim University.

TYPE HABITAT: Soil around roots of plum tree, *Prunus communis* Huds.

TYPE LOCALITY: Saharanpur (U.P.) India.

DIFFERENTIAL DIAGNOSIS: *Dorylaimellus longicaudatus* n.sp., comes closest to *D. striatus* N. A. Cobb in Thorne, 1939 but differs in having a short body and esophagus, long tail and anterior location of vulva.

KEY TO SPECIES OF *Dorylaimellus**

(modified after Thorne (1939) and Hooper and Cairns (1959))

- | | |
|--|---------------------------------|
| 1. Cuticle with longitudinal and transverse striations..... | 2 |
| Cuticle with transverse striations..... | 3 |
| 2. Length over 6 mm. | |
| <i>heteruris</i> Schuurmans Stekhoven and Teunissen, 1938 | |
| Length less than 2 mm..... | <i>hedickei</i> Paesler, 1941 |
| 3. Ovaries two..... | 4 |
| Ovary single..... | 17 |
| 4. Anterior portion of esophagus not constricted..... | <i>nodochordus</i> Thorne, 1939 |
| Anterior portion of esophagus constricted..... | 5 |
| 5. Cuticle coarsely striated..... | 6 |
| Cuticle finely striated..... | 7 |
| 6. Tail four anal body widths long..... | <i>longicaudatus</i> n.sp. |
| Tail two anal body widths long..... | <i>striatus</i> Thorne, 1939 |
| 7. Inner portion of lips forming labial disc..... | 8 |
| Inner portion of lips not forming labial disc..... | 9 |
| 8. Extension twice as long as spear; prerectum 1½ anal body widths long— | |
| | <i>projectus</i> Heyns, 1962 |
| Extension as long as spear; prerectum 6 anal body widths long. | |
| | <i>cephalus</i> n.sp. |

*Recently Andrassy (1963) transferred *Dorylaimellus clavicaudatus* to *Belondira* and Heyns (1963) described four new species: *D. andrassyi*, *D. directus*, *D. imitator* and *D. vector*. These papers became available only when the present one was in galley proof-stage and hence could not be included in the keys. However, *D. curvatus* n.sp., has been compared with all of them.

9. Esophagus very short (b = 7 or more).....	10
Esophagus long (b = 4.5 or less).....	11
10. Tail terminus conoid; L = 1.7-1.8 mm; b = 8.2-9.5.....	
..... <i>bambesae</i> de Coninck, 1962	
Tail terminus rounded; L = 1.2-1.4 mm; b = 7-8.....	<i>curvatus</i> n.sp.
11. Tail very long (c = 9).....	<i>filiformis</i> n.sp.
Tail short (c = 20 or more).....	12
12. Length about 1.0 mm or over.....	13
Length about 0.6 mm.....	15
13. Tail bluntly rounded.....	<i>occidentalis</i> Thorne, 1939
Tail somewhat elongate-conoid.....	14
14. Preanal supplements 4.....	<i>virginianus</i> Cobb, 1913
Preanal supplements 14.....	
..... <i>multipapillatus</i> Schuurmans Stekhoven and Teunissen, 1938	
15. Tail 2½ anal body widths long.....	<i>montenegricus</i> Andrassy, 1959
Tail less than 1½ anal body widths long.....	16
16. Tail hemispheroid; praeectum 5 anal body widths long.....	
..... <i>parvulus</i> Thorne, 1939	
Tail conoid to a rounded terminus; praeectum less than two anal body widths long.....	<i>tenuidens</i> Thorne, 1939
17. Tail clavate.....	<i>clavicaudatus</i> Williams, 1958
Tail not clavate.....	18
18. Ovary posterior to vulva.....	19
Ovary anterior to vulva.....	20
19. Anterior uterine branch present.....	<i>porosus</i> Thorne, 1939
Anterior uterine branch absent.....	
..... <i>engadinensis</i> (Altherr, 1950) Altherr, 1950	
20. Tail subdigitate; b = 4.....	<i>mirabilis</i> (de Man, 1884) Thorne, 1939
Tail conoid; b = 6.....	21
21. Tail bluntly conoid.....	<i>aequalis</i> (Cobb, 1918) Thorne, 1939
Tail acutely conoid, arcuate.....	<i>fuorni</i> (Altherr, 1950) Altherr, 1950

The genera *Miranema* and *Utahnema* are of uncertain affinities and may belong to either Dorylaimidae or Leptonchidae.

SUBFAMILY XIPHINEMELLINAE n. subfam.

DIAGNOSIS: Leptonchidae. Spear long and attenuated; extension flanged. Head provided with labial disc and basal expanded portion of esophagus set off in known forms. Ovaries paired, reflexed. Testes, spicules and supplements dorylaimoid. Lateral guiding pieces present. Tail of sexes similar.

TYPE GENUS: *Xiphinemella* Loos, 1950.

SUMMARY

Nygellidae n. fam., with Nygellinae n. subfam., having *Nygellus* and Nygolaimellinae Clark, 1961 having *Nygolaimellus* is proposed to contain those nematodes which share features of both the Belondiridae and Nygolaimidae. Belondirinae n. subfam., having *Belondira*, *Axonchium* and *Oxydirus*; Swangerinae n. subfam., having *Swangeria* and Dorylamellinae n. subfam., having *Dorylaimellus* are proposed under Belondiridae. The following ten new and four known species are reported from various parts of India: *Nygellus clavatus* Thorne, 1939; *N. caudatus* n.sp., *Belondira ortha* Thorne, 1939; *B. paraclava* n.sp.; *Axonchium amplicolle* Cobb, 1920; *A. nitidum* n.sp.; *A. saccatum* n.sp.; *A. elegans* n.sp.; *Oxydirus gigus* n.sp.;

Dorylaimellus parvulus Thorne, 1939; *D. cephalus* n.sp.; *D. curvatus* n.sp.; *D. filiformis* n.sp. and *D. longicaudatus* n.sp. Keys to the species of *Nygellus*, *Belondira* and *Dorylaimellus* are also provided.

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OUTLINE OF THE SUPERFAMILY DORYLAIMOIDEA

FAMILIES	SUBFAMILIES	GENERA
Dorylaimidae	{ Dorylaiminae Tylencholaiminae Nordianae	{ <i>Dorylaimus</i> <i>Prodorylaimus</i> <i>Amphidorylaimus</i> <i>Chrysonema</i> <i>Thornia</i> <i>Labronema</i> <i>Thorneella</i> <i>Pungentus</i> <i>Eudorylaimus</i> <i>Mesodorylaimus</i> <i>Thornenema</i> <i>Lordellonema</i> <i>Aporcelaimus</i> <i>Discolaimus</i> <i>Discolaimium</i> <i>Meylonema</i> <i>Drepanodorus</i> <i>Witoldinema?</i>
		{ <i>Tylencholaimus</i> <i>Enchodelus</i> <i>Discomyctus</i>
		{ <i>Nordia</i> <i>Longidorella</i>
		{ <i>Longidorus</i> <i>Paralongidorus</i> <i>Xiphinema</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>
		{ <i>Actinolaimus</i> <i>Aetholaimus</i> <i>Paraactinolaimus</i> <i>Metaactinolaimus</i> <i>Actinolaimoides</i> <i>Trachactinolaimus</i> <i>Trachypleurosom</i> <i>Carcharolaimus</i> <i>Mylodiscus</i> <i>Antholaimus</i>

Leptonchidae	}	Leptotonchinae	{ <i>Leptonchus</i> <i>Proleptonchus</i> <i>Dorylaimoides</i> <i>Funaria?</i>
		Tyleptinae	{ <i>Tyleptus</i> <i>Basirotyleptus</i> <i>Tylolaimophorus?</i>
		Tylencholaimellinae	{ <i>Tylencholaimellus</i> <i>Doryllium</i>
		Xiphinemellinae	{ <i>Xiphinemella</i> <i>Botalium</i>
Nygalaimidae			{ <i>Nygalaimus</i> <i>Nygalaimoides?</i> <i>Sectonema</i> <i>Stephanium</i>
Nygellidae	}	Nygelinae	<i>Nyggellus</i>
		Nygalaimellinae	<i>Nygalaimellus</i>
Belondiridae	}	Belondirinae	{ <i>Belondira</i> <i>Axonchium</i> <i>Oxydirus</i>
		Swangerinae	<i>Swangeria</i>
		Dorylaimellinae	<i>Dorylaimellus</i>
Campydoridae			<i>Campydora</i>
Aulolaimoididae			<i>Aulolaimoides</i>

***Boleodorus impar* n. sp. (Nematoda: Tylenchida) from India**

EKRAMULLAH KHAN AND M. A. BASIR²

From a collection of nematodes from soil around grass roots in Simla, India, fifteen females and seven males of a species belonging to the genus *Boleodorus* Thorne, 1941 were recovered. They represent an hitherto undescribed species for which the name *Boleodorus impar* n. sp. is proposed. They are described below.

Boleodorus impar n. sp. (Fig. 1. A-G)

MEASUREMENTS. Females (n = 14). Length = 0.504-0.6 mm (0.56 mm); a = 25-32 (28); b = 4.8-6.2 (5.3); c = 5-7 (6.5); V = 63-66% (65%); Spear = 13-14 microns (13 microns).

Males (n = 6). Length = 0.52-0.56 mm; a = 37-47; b = 4-6; c = 5-6; Spear = 12-14 microns; Spicula = 18-20 microns; Gubernaculum = 7-8 microns.

DESCRIPTION. FEMALE (Holotype): Length = 0.507 mm; a = 25; b = 5; c = 5.3; V = 65%; Spear = 14 microns.

²Contribution from the Section of Plant Nematology, Department of Zoology, Aligarh Muslim University, Aligarh, U.P. India.

The worms assume a ventrally arcuate open 'C' shape when killed in hot water. In some paratype specimens however, the body gets more or less straightened. Body marked by fine transverse striations. Head continuous with body. Lip region unstriated, conoid and elevated from body contour. An *en face* view shows six lips of which the two sub-dorsals and the two sub-ventrals are comparatively much larger than the two sub-laterals. Amphidial openings oblique slits, situated outside the sublateral lips. Cephalic frame-work hexa-radiate. Lateral field $\frac{1}{4}$ of body width, marked by four incisures of which the two outer are crenate. Lateral field arising in region of meta-carpal swelling and extending up to about 2-3 anal-body-widths posterior to anus. Buccal spear strong, bearing well-developed knob like swellings. Orifice of dorsal Oesophageal gland 3 microns posterior to the base of spear. Oesophagus consisting of a procorpus, followed by a fusiform valveless metacarpal swelling, lodging the opening of subventral glands, a short isthmus enveloped by nerve ring and an irregularly lobed basal oesophageal bulb. The base of the posterior oesophageal bulb overlaps the anterior end of intestine (Fig. 1, B, D). Excretory pore anterior to the base of posterior oesophageal bulb, 95 microns from the anterior end of body. This leads into a moderately cuticularized excretory duct which opens into the renette cell, the latter located at about $1\frac{1}{2}$ body-widths posterior to the oesophagus (Fig. 1, D). Hemizonid distinct, located at about eight body annules anterior to excretory pore.

Vulva a transverse slit, located at about two thirds of body length from anterior end. Vagina a simple tube about $\frac{1}{4}$ the corresponding body-width long. Gonad monodelphic; ovary outstretched anteriorly, oöcytes arranged in more than two rows; spermatheca oval in shape, packed with rounded sperms. Posterior uterine branch short, less than one-body-width in length.

Rectum well marked, about $\frac{1}{2}$ the anal-body-diameter in length; pre-rectum $1\frac{1}{2}$ times the anal-body-diameter long. Tail elongate tapering, slightly ventrally arcuate.

DESCRIPTION: MALE (Allotype): Length = 0.53 mm; a = 35; b = 4.2; c = 5; Spear = 13 microns; Spicula = 18 microns.

Body slender; on being killed in hot water only slightly ventrally arcuate (Fig. 1, F). Lip region more rounded than in the female; continuous with body contour. Spear well-developed as in female. Excretory pore and hemizonid located at about the same place as in female. Spicula paired, cephalated, ventrally arcuate in their distal region, with a distinct head and shaft; 18 microns in length; gubernaculum trough shaped, 8 microns long. Bursa ad-anal, arising a little anterior to the spicula and extending up to about 2 anal-body-diameters below the anus. Lateral field expanding in the region of bursa. Tail elongate, attenuated-filiform, about 9 times the anal-body-diameter in length.

HOLOTYPE: Female: deposited with the Zoology Museum, Aligarh Muslim University, Aligarh, U.P. India.

ALLOTYPE: Male: other data same as for holotype.

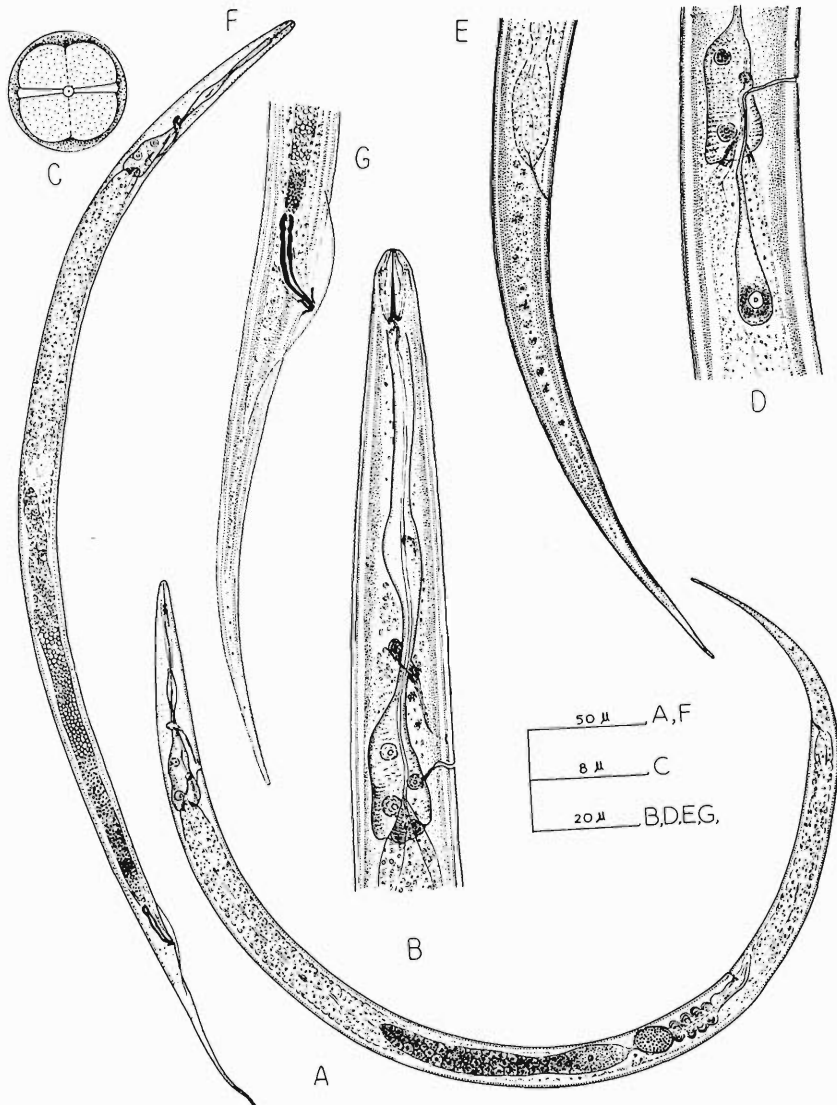
TYPE LOCALITY AND HABITAT: Collected from soil around grass, *Cynodon dactylon* Pers. roots from Kufri town near Simla, Janjab State, India (Height about 9,000 feet above sea level).

Paratypes: Fourteen females and six males, with the senior author's personal collection.

DIFFERENTIAL DIAGNOSIS: The female of *Boleodorus impar* n. sp. is distinctive in having an irregularly shaped basal oesophageal bulb, overlapping

the anterior end of intestine; orifice of dorsal oesophageal gland located close to the base of spear; lateral field $\frac{1}{4}$ of body width, with four incisures of which the outer ones are crenate, tail elongate tapering, ventrally arcuate. Males of this species showing only a slight ventral curve not forming a 'C' as in female, moreover in both the sexes the tail is proportionately longer as compared to other species of this genus.

B. impar n. sp. differs from all other known species of this genus in having



Boleodorus impar n. sp. Figure 1, A-G. Fig. 1, A. Entire female, lateral view. B. Neck region of female. C. *En face* view of female. D. Renette cell. E. Tail end of female. F. Entire male, lateral view. G. Tail end of male.

an irregular shape of the basal oesophageal bulb which envelopes the anterior end of intestine and a more or less straight and comparatively longer tail. However, it comes closest to *B. thylactus* Thorne, 1941 and *B. similis* Khan & Basir, 1963. From the former it can further be differentiated in having a more posteriorly located excretory pore and vulva, while from the latter it differs in the width and nature of lateral field ($\frac{1}{2}$ of body width with four simple lines in *B. similis*), and a comparatively longer spear with more developed knobs.

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Free-living Marine Nematodes, I. *Southerniella youngi*, *Dagda phinneyi*, and *Gammanema smithi*, new species*

D. G. MURPHY**

Southerniella youngi n. sp., *Dagda phinneyi* n. sp., and *Gammanema smithi* n. sp., representatives of rarely reported genera with few representative species are described from Pacific Ocean waters. The species are named, respectively, in honor of Drs. R. A. Young, H. K. Phinney, and F. H. Smith, all of Oregon State University.

Southerniella youngi n. sp. (Figure 1, A-C)

MALE (1): L = 0.895 mm, a = 33.1, b = 7.8, c = 10.0.

Only the one male of this species was found. The cuticle is smooth. There is a circle of six small labial papillae followed at a level about midway in the stoma by a circle of four cephalic setae, these being 5.4 microns long. Cervical and caudal setae are present as illustrated. Somatic setae are somewhat shorter than those found in the cervical and caudal regions, and distributed sparsely along four rows (dorso-laterals and ventro-laterals). The amphids are circular, 4.5 microns in diameter (45% of the corresponding head diameter); the anterior rim of the amphid is located 10 microns posteriorad. Body diameter at amphid level is 10 microns.

The stoma is tuboid, 0.9 microns in diameter and 8 microns from base to lips. The esophagus is cylindrical and can be differentiated readily into two sections which are separated by a distinct change in musculature (34 microns posteriorad). An esophageal gland (or glands) is located external of the esophagus directly behind the nerve-ring; unfortunately the specimen at hand provided only a minimum of detail. The nerve-ring is located at 65% of the esophagus. The esophagus terminates in a small, but distinct, cardia.

The excretory pore opens 22 microns posteriorad. The ampulla preceding the excretory duct is distinct; however, the greater part of the tube from the ventral gland could not be resolved. The ventral gland terminates 86 microns posteriorad from the base of the esophagus. There are four cells which appear to be associated with the ventral gland (see fig. 1, B), two located ventrally

*Part of studies supported by United States Public Health Service Grant GPD-18,939.

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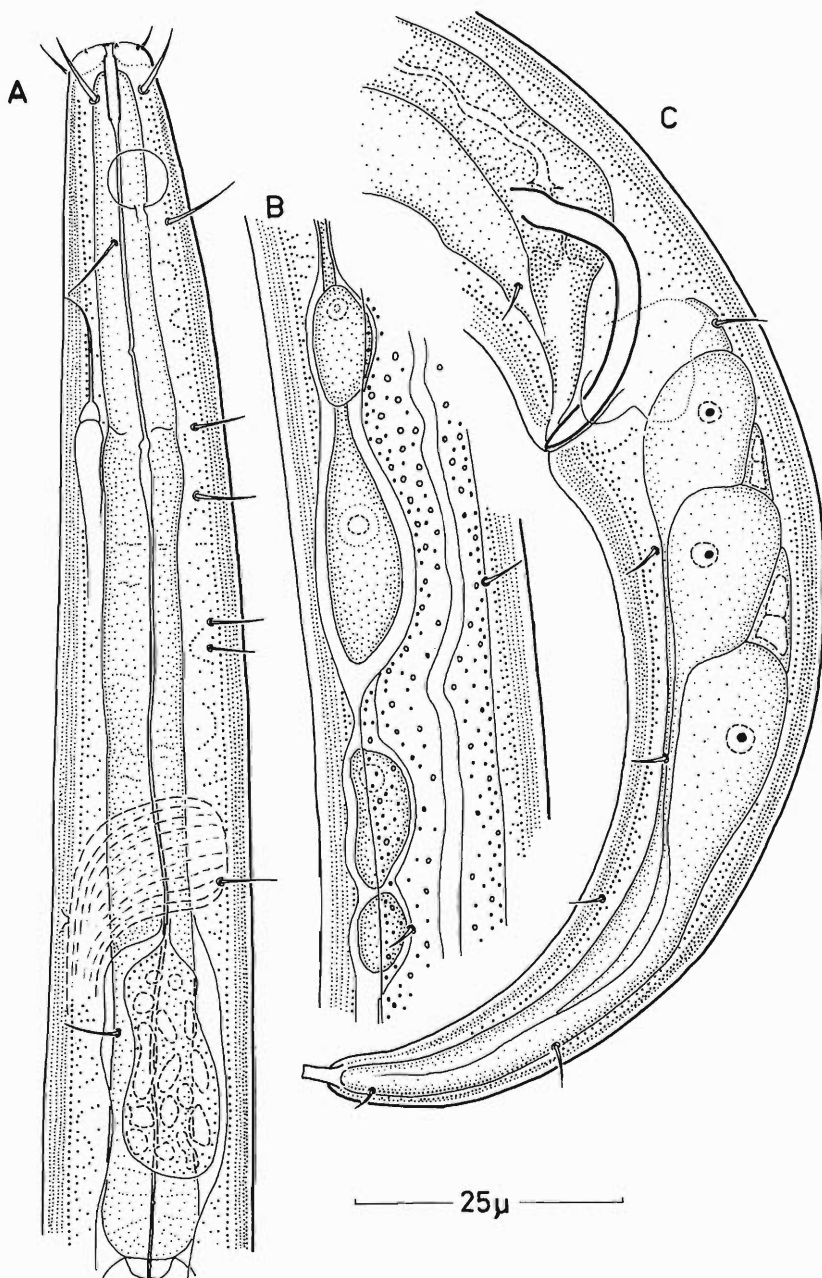


Figure 1. *Southernmiella youngi* n. sp. A, anterior region of male, lateral view. B, male, region of ventral gland, lateral view. C, male tail.

(ANT T = anterior testis, C = cells within pseudocoelom, CG = caudal gland, EG = esophageal gland, EJD = ejaculatory duct, INT = intestine, JT = junction of testes, TGZ = growth zone of testis, VD = vas deferens, VG = ventral gland.)

and positioned directly behind the ventral gland in tandem, the remaining two a pair, located ventro-laterally just anterior to the ventral gland on either side of the tubular portion of the gland.

The intestine contains many highly refractive globules; nothing definite could be determined of the contents of the lumen.

The genital system is simple; spicula arcuate, 33 microns long (arc, not chord): a gubernaculum is apparently lacking.

The tail is conoid, 4.4 anal diameters long. Body diameter at level of cloacal opening is 21 microns.

HOLOTYPE: male on slide OSU HM 98, Oregon State University nematode collection: collected 29 August 1961.

TYPE-LOCALITY: inter-tidal sand from Kawaihae, Hawaii.

REMARKS: *S. youngi* is most closely related to *S. simplex* Allgen, 1933. From this species it can be most readily distinguished by the position of the amphids, which in Allgen's description lie opposite the anterior region of the esophagus, the anterior portion of the amphid being adjacent to the cephalic setae, whereas in my specimen the amphid is entirely below the sclerotized portion of the stoma. It is distinguished from what Wieser (1956) considered to be the male of *S. simplex* by lacking a gubernaculum.

Dagda phinney n. sp. (Figure 2, A-C; 5, C-D)

MALE (1): L = 1.75 mm, a = 51.8, b = 6.6, c = 17.5.

A prominently striated nematode with a very heavy cuticle. There is pronounced tapering toward either end. Six rows of somatic setae are present, corresponding in position to the symmetry of the labial papillae. The two lateral rows do not appear to be so well developed as the latero-ventral and latero-dorsal rows. The six labial papillae are quite small. The circle of four cephalic setae lies opposite the base of the stoma: these are about 20 microns long, twice the corresponding head diameter. The stoma is small, about two times as deep as wide, and possesses a powerful, forward projecting, dorsal tooth. The latero-ventral walls of the stoma are also well sclerotized, but there is no evidence of ventral teeth. The amphid is oval, open posteriorly, and is located just behind the lips.

The esophagus terminates in a prominent, conical cardia, possesses a large, muscular basal portion (comprising 27% of the total length), and exhibits a distinct differentiation in musculature in a region about 20 microns posterior from the stoma, the latter reminiscent of the type of esophageal musculature found, for instance, in the genus *Areolaimus*, and present in *Southerniella youngi*. The nerve-ring is located at 50% of the esophagus. The excretory pore, which can be seen only with considerable difficulty, lies just posterior to the nerve-ring. The central portion of the esophagus is surrounded by numerous cells, most of which would appear to be glandular, emptying into the esophagus. Diameter at base of esophagus is 34 microns. It is difficult to determine if the intestine is soenocytic or distinctly cellular in composition. Its walls contain numerous, highly refractive granules. The prerectum is very distinct from the remainder of the intestine, the walls being relatively thin and free of refractive inclusions. The rectum and prerectum are distinctly separated, and joined by a valve-like apparatus, (more complex appearing than a normal sphincter).

The male possesses 24 tuboid supplements. The spicula are simple, arcuate, 42 microns long (arc). The lateral-pieces of the gubernaculum are broadly forked proximally, and toothed distally. The latero-ventral setae are longer

in the region of the ten supplements adjacent to the cloaca than on the remainder of the body. A large ventral papilla is located on the tail; this being preceded anteriorly by a pair of stout genital setae and followed by a similar pair of genital setae midway between the ventral papilla and the tail terminus. The caudal glands are located anterior to the forwardmost supplement. The spinnerett is particularly well-sclerotized. The tail is conical, 3 anal

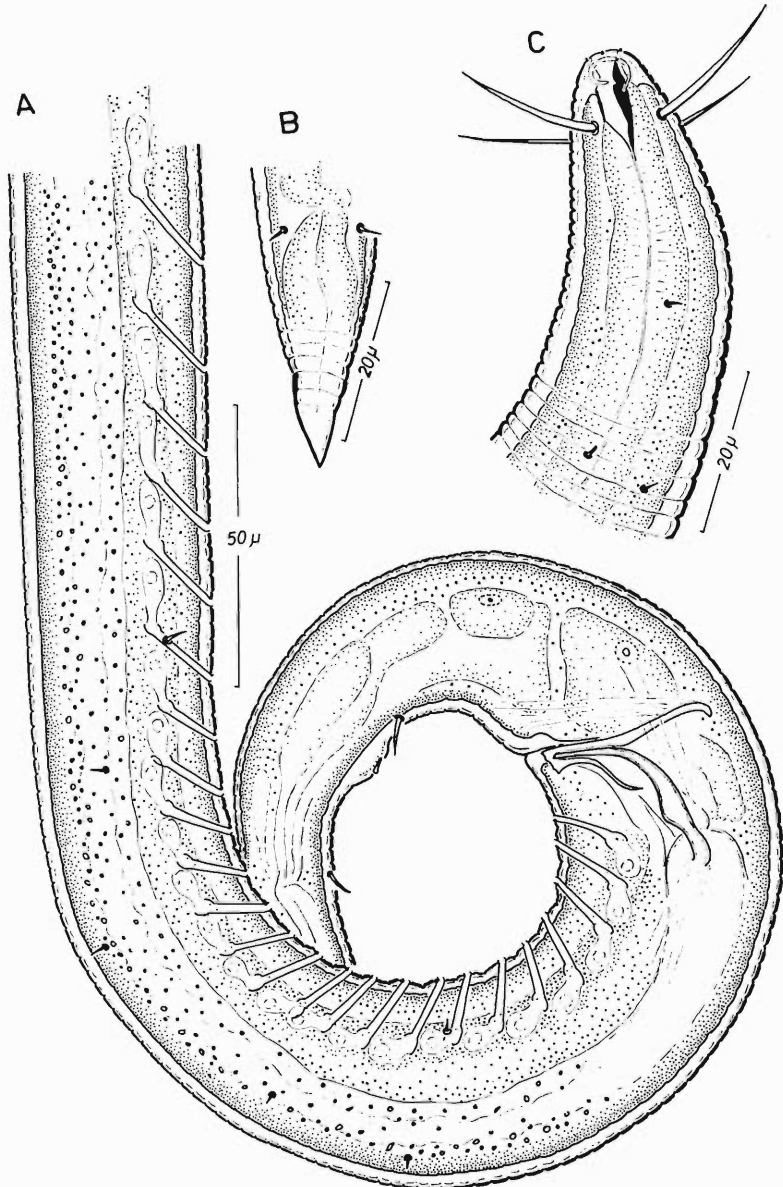


Figure 2. *Dagda phinneyi* n. sp. A, posterior region of male. B, tip of tail, male. C, anterior region of male, lateral view.

diameters long.

HOLOTYPE: male on slide OSU OM 114a, Oregon State University nematode collection. Collected 11 September 1961.

TYPE-LOCALITY: sandy-beach, inter-tidal; Umqua Light House State Park, Oregon.

REMARKS: *D. phinneyi* is the third species to be described within this genus. It is clearly a close relative to the type-species, *D. bipapillata* Southern, 1914. The remaining species, *D. asymmetrica* Gerlach, 1952, is immediately distinguished by possessing six cephalic setae rather than the normal four, and in lacking a distinct basal enlargement of the esophagus. As we become more familiar with nematodes of this group it is unlikely that Gerlach's species can remain within the genus.

D. phinneyi is one-half the length of *D. bipapillata*, bears 24 supplementary organs vs. 11 for the latter species, and possesses but one distinct dorsal tooth, lacking the prominent subventral teeth of *D. bipapillatus*.

It would appear that the presence of the prominent postanal ventral papilla would be a valid character for this genus, and the lack of the papilla may well prove to be the only valid character separating *Diodontolaimus* Southern, 1914 from *Dagda* Southern, 1914.

Gammanema smithi n. sp. (Figures 3, A-E; 4; 5, A-B)

MALE (1): L = 2.21 mm, a = 41.0, b = 7.3, c = 19.1.

Body of uniform diameter, head blunt with no appreciable tapering; tail conical, taper commencing at anus. Cuticle prominently ringed with regularly arranged punctations, these being in corresponding positions with every other row. Most of the lateral striations appear to be rings which completely encircle the nematode; however, occasionally a ring forks, thus becoming, at least partially, double.

A circle of ten cephalic setae, (6 + 4), are located on the outer rim of the forward surface of the head, these are 5 and 20 microns, respectively, in length. These setae are readily over-looked for one of two reasons: the innate obscurity, particularly of the small lateral setae, and secondly due to the ease with which they break off at the base. There is a circle of six stout labial papillae each apparently encircled by a thin, membranous sheath. A complex circumoral system of rods and membranes was to be seen, but not without difficulty (see fig. 3, E). Somatic setae are sparse, apparently present only in two latero-ventral rows, probably not exceeding 14% of the body diameter in length. Cuticular pores are present running in one lateral row down either side. These are of simple structure, lie in the annules (that is between the punctate striae, without interrupting the latter) and appear at regular intervals with about 34 striations between pores. There are as well four rows of papillae, these corresponding in position to the long cephalic setae. The papillae are not located in the annules, as are the lateral pores, but rather interrupt the striae (fig. 3, D). The interval between papillae is probably the same as that for the lateral pores, however they do not lie laterally in juxtaposition, rather appearing about midway between the pores in relative lateral position. In the cervical region there are additional papillae, however, it is difficult to interpret these as having any particular arrangement.

The amphids are prominent spirals of three turns, interrupting approximately 10 striations (there is about a 10% frequency of anastomosing and forking of striae in this region): they are 14 microns in diameter, about

27% of the corresponding body diameter. The amphids are located approximately opposite the lower portion of the stoma.

The stoma is typically tri-radiate, and divisible into a cyathiform anterior half and a more or less cylindrical posterior half. Midway between the three grooves in the forward half of the stoma (which correspond to the esophageal radii) are located paired sclerotized stomal thickenings which terminate at

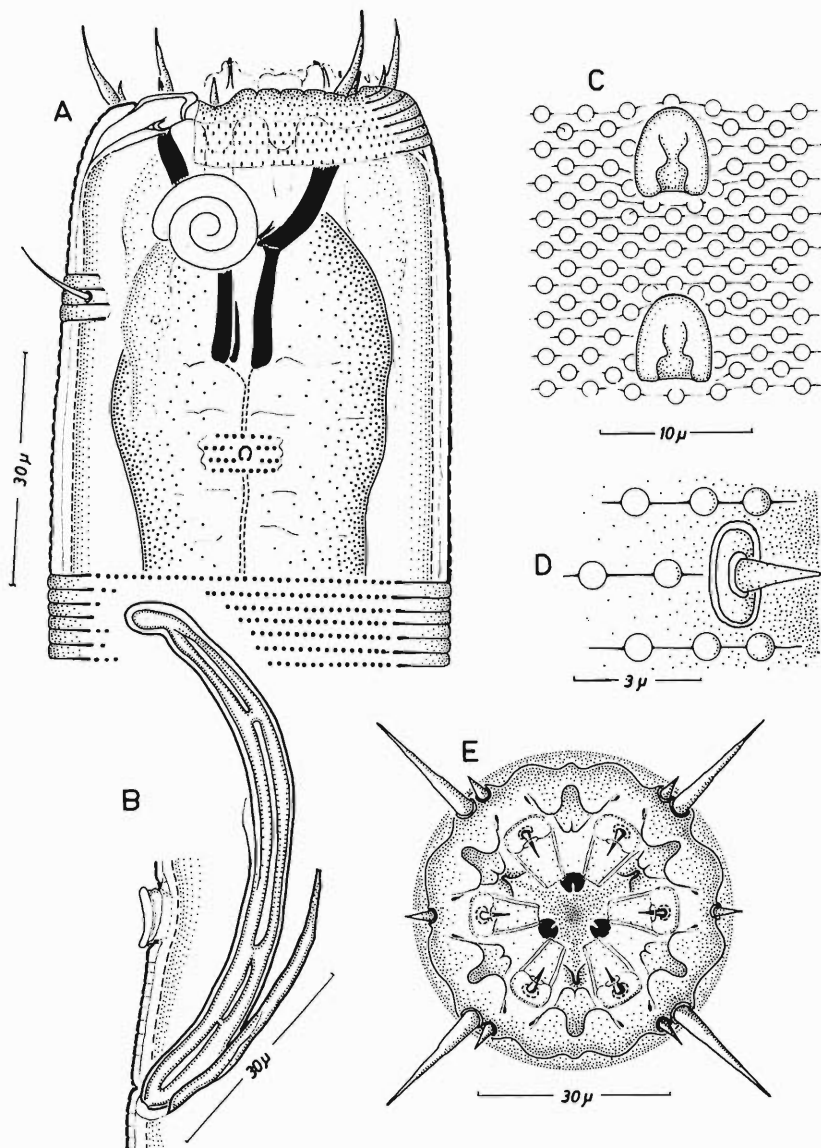


Figure 3. *Gammanema smithi* n. sp. A, male head, lateral view. B, spicular apparatus. C, ventral view of supplements. D, somatic papilla. E, face view of male.

the juncture of the two stomal halves and then project centrally and posteriorly so as to form three pairs of powerful teeth.

For the greater part of its length, the esophagus is cylindrical, increasing slightly in diameter at the posterior end to form a none-the-less distinct basal bulb. The position of the nerve-ring could be determined only with difficulty because of the abundance of refractive cells (pseudocoelomocytes?) surrounding the esophagus. Cardia ill-defined, apparently embedded within

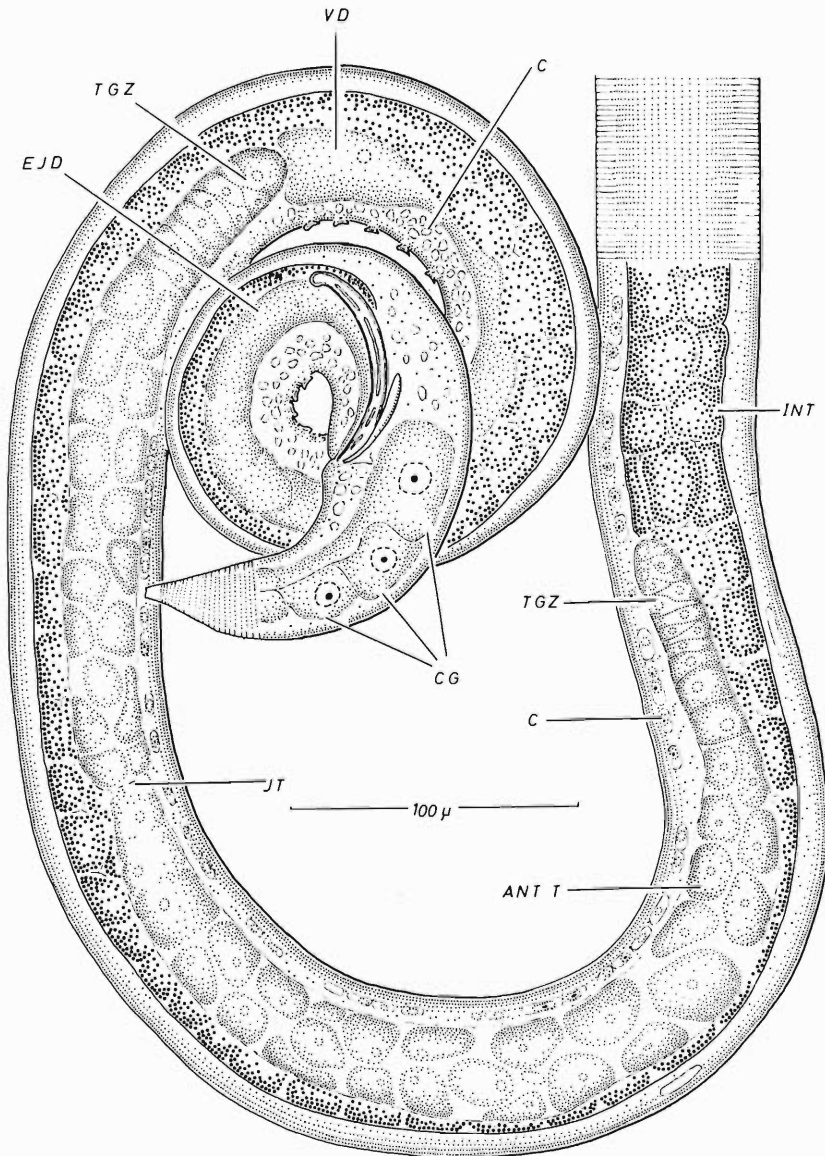


Figure 4. *Gammanema smithi* n. sp., posterior region of male.

intestinal tissue. Neither the limits of the ventral gland, nor the location of the excretory pore could be determined.

The intestine is composed of large, brightly pigmented (rust-orange) cells. Nothing could be determined of the contents of the lumen.

Testes paired, outstretched (see fig. 4), vas deferens leading to a powerful ejaculatory duct. The spicula are 78 microns long, well sclerotized, and form a moderate arc. Lying within the spicular are a series of interrupted, sclero-

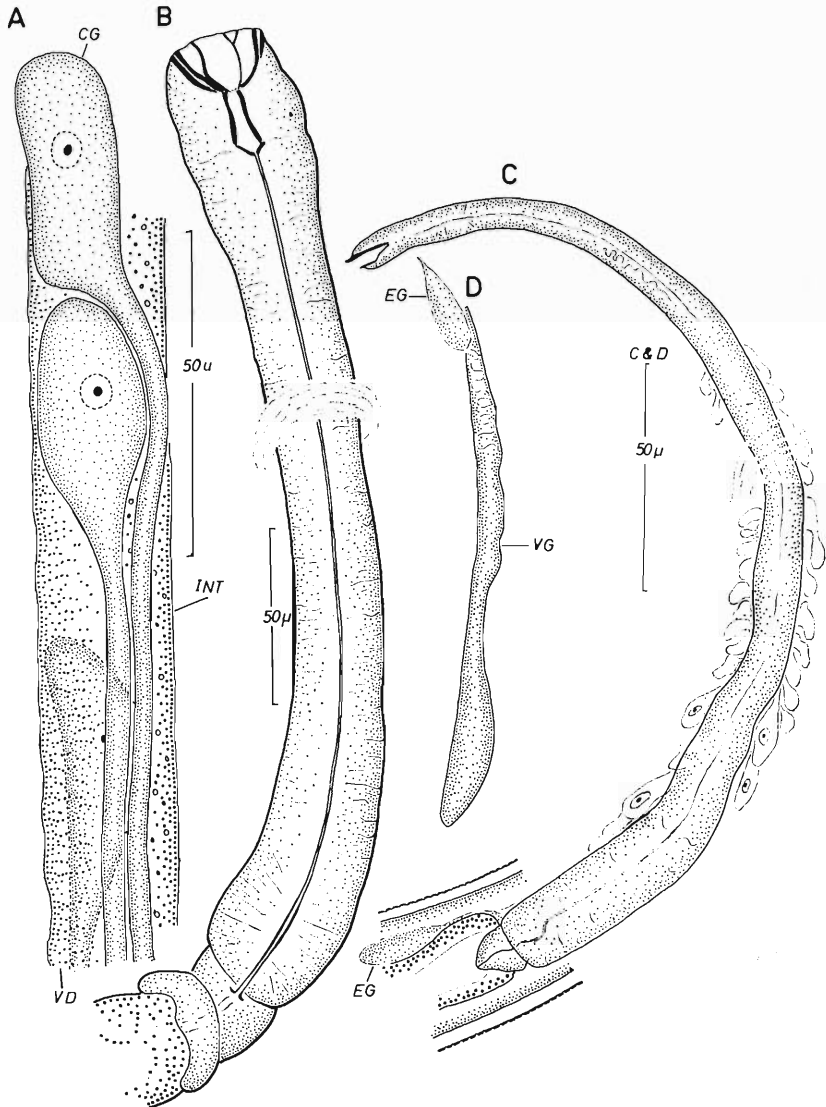


Figure 5. A-B: *Gammanema smithi* n. sp., C-D: *Dagda phinneyi* n. sp. A, caudal glands. B, esophagus. C, esophagus. D, ventral gland. (EG in both cases the same).

tized pieces. The gubernaculum is paired. There are 20 prominent, cup-shaped supplements, the anteriormost being 412 microns from the anus. No genital setae are present.

The tail is conical, about three anal diameters long. The three caudal glands are all contained within the tail.

HOLOTYPE: male contained on slide OSC WM 68c, Oregon State University nematode collection; collected on 14 September 1960.

TYPE-LOCALITY: inter-tidal sand, Bainbridge Island, Puget Sound, Washington.

REMARKS: *G. smithi* is most closely related to the type species of the genus, *G. ferox* Cobb, 1920. It is unfortunate that Cobb failed to illustrate the male genital systems inasmuch as these structures are of utmost importance in classification. The spicula of my species possess very distinct median sclerotizations, a feature as prominent as the external sclerotization of the spicula and not likely to have been overlooked by Cobb, and thus assumed not present in *G. ferox*. The latter species possesses 16 inconspicuous supplements, *G. smithi* possesses 20 (same count on both of two males studied) very conspicuous supplements. The intestine of *G. ferox* is composed of two distinctly different cell types, these containing refractive, but unpigmented granules . . . the mixing of cell types is most pronounced anteriorly: *G. smithi* appears to have but one cell type in the intestinal composition, definitely no recognizable mixture anteriorly, and the cells are brightly pigmented. The 12 labial organs illustrated by Cobb as "free" structures, i. e., setose, are present on *G. smithi*; however, from face view I believe these to be connected at least in pairs, and perhaps entirely, by a fine membrane. *G. smithi* possesses three pairs of onchia, distinct from both lateral and face views; *G. ferox* has 3 unpaired onchia. A distinct esophageal bulb is present in the former, lacking in the latter. The testes of *G. ferox* are described as being short, and outstretched (perhaps because a young animal was observed; however, Cobb's description is emphatic enough to suggest that he considered this condition normal of a mature male), in *G. smithi* they are prominent, occupy a considerable portion of the body area, and are reflexed.

Gerlach (in press) synonymizes *G. ferox* with *Halichoanolaimus rapax* Ssaweljev, 1912. The latter description was without illustration, rendering any subsequent comparison doubtful at best . . . thus I have not accepted the synonymy. In the same paper, Gerlach describes *G. rapax* (Ssaweljev, 1912) from Helgoland (Abb. 10, a-b). I believe this particular species could be readily distinguished from *G. ferox* Cobb, 1920 on the basis of a broad, short spiculum and prominent supplements. Further collections from this locality will probably enable description of a new species.

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**Two New Species of the Genus *Helicotylenchus* Steiner, 1945,
(Nematoda: Hoplolaimidae) from India**

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During a survey of the plant parasitic nematodes of North India many specimens of the genus *Helicotylenchus* were met. They were found to represent at least two hitherto undescribed species which are named as *Helicotylenchus insignis* n. sp. and *H. plumariae* n. sp. These are described below:

Helicotylenchus insignis n. sp. (Fig. 1, A-F)

MEASUREMENTS: Hermaphrodites (10): L = 0.6-0.87 mm.; a = 30.5-31.7; b = 5.7-6.9; c = 52-66.7; v = 60-63%; spear = 23-25 microns; o = 52-60%.

Hermaphrodite (Holotype): L = 0.87 mm.; a = 31.1; b = 6.9; c = 58; v = 60%; spear = 25 microns; o = 60%.

DESCRIPTION: When relaxed and killed in hot water, the worms assume a ventrally arcuate spiral shape. Body almost cylindrical, gradually tapering towards both extremities. Cuticle transversely striated, striae averaging about 1.2 microns apart near mid-body. Transverse striae interrupted laterally by four longitudinal incisures, less than one fourth as wide as body; outermost incisures crenate. Deirids not seen. Phasmids pore-like, located 3-5 annules anterior to level of anus.

Head conoid, not offset, with four annules. In *en face* view oral opening appears to be located on a rounded oral-disk, surrounded by six equal lips. Labial frame-work hexa-radiate, its outer margins conspicuous, extending about three annules into body, the inner margins forming an inverted funnel-shaped spear-guide extending posteriorly from basal plate for about eight annules (Fig. 1, A).

Spear elongate, well developed, 23-25 microns long; basal knobs with outer margins conspicuously directed forward, measuring 6 microns across (Fig. 1, A). Dorsal oesophageal gland opening into oesophageal lumen, 13-16 microns behind the spear base.

Oesophagus with a cylindrical procorpus, a muscular median oesophageal bulb and a narrow isthmus. Oesophageal glands free, extending mostly on ventral side of the intestine. Oesophago-intestinal junction forming a distinct oval chamber. Nerve ring slightly behind median oesophageal bulb. Excretory pore near level of oesophago-intestinal junction. Hemizonid conspicuous, extending along two body annules, slightly anterior to excretory pore.

Reproductive system digonic, hermaphrodite. Vulva a depressed transverse slit with a pair of lateral membranes (Fig. 1, B). Each uterus with a spermatogonium towards its distal end (Fig. 1, F). Ovaries paired, outstretched in opposite directions, with oocytes mostly arranged in single file.

Rectum short, less than one anal-body-diameter in length. Tail dorsally convex, ventrally bearing 7-11 annules. Tail ending at its tip in a short unstriated process.

DIAGNOSIS: *Helicotylenchus* with the above measurements and general description. It comes closest to *H. digonicus* Perry, 1959, and *H. dihystra* (Cobb, 1893) Sher, 1961. From the former it can be differentiated in having anteriorly concave spear knobs, a relatively smaller spear, and a more pos-

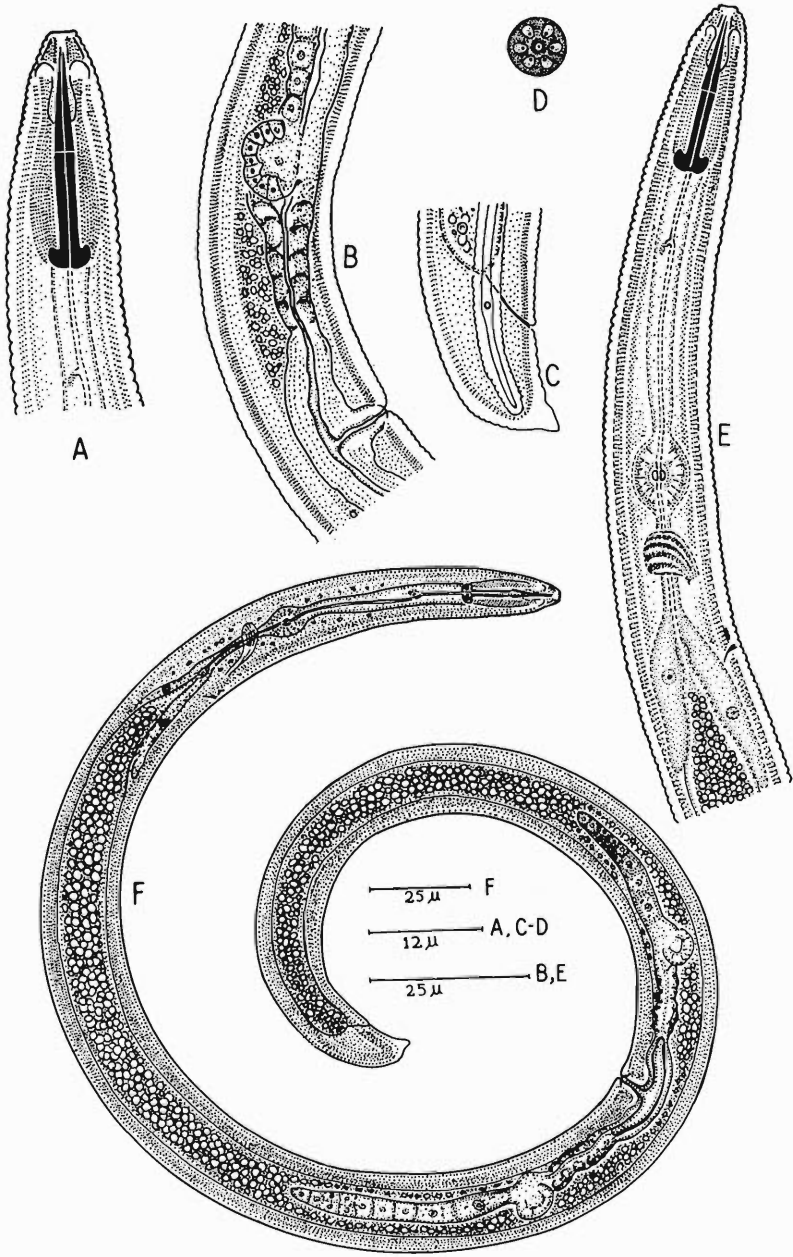


Fig. 1, A-F. *Helicotylenchus insignis* n. sp. A. Head end, lateral, B. A portion of reproductive organs of hermaphrodite, lateral, C. Tail, lateral, D. En face view, E. Oesophageal region, F. Entire body of hermaphrodite.

teriorly located dorsal oesophageal gland opening ($O = 32-36$ in *H. digonicus*). From *H. dihystra* it differs in having narrower lateral fields (lateral fields slightly over one third as wide as body in *H. dihystra*), smaller stylet (stylet 26-28 microns long in *H. dihystra*), shape of spear knobs, longer extent of outer margins of head frame-work, and the shape and size of the tail ($c = 39$ in *H. dihystra*).

TYPE HOST AND LOCALITY: Collected from soil around roots of the grass, *Cynodon dactylon*, in the lawns of Taj Mahal, Agra (U.P.), India.

TYPE SPECIMENS: Holotype and paratypes with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

Helicotylenchus plumariae n. sp. (Fig. 2, A-E)

MEASUREMENTS: Hermaphrodites (10): $L = 0.49-0.6$ mm.; $a = 23-24$; $b = 4.5-6.1$; $c = 34-42$; $v = 61-63\%$; spear = 22-26 microns, $o = 37.5-40\%$.

Hermaphrodite (Holotype): $L = 0.639$ mm.; $a = 27.7$; $b = 5.8$; $c = 42.6$; $v = 63.6\%$; spear = 23 microns; $o = 39.1\%$.

DESCRIPTION: Body tapering anteriorly and posteriorly, forming more or less a closed spiral. Cuticle transversely striated; striae completely interrupted laterally by four longitudinal incisures. Lateral fields measuring slightly less than one fourth as wide as body. Outer incisures crenate. Deirids not seen. Phasmids preanal, pore-like, about 3-4 annules anterior to level of anus, at slightly less than two anal-body-widths from caudal terminus.

Head marked with four annules, the last annule forming a slight depression at the place of junction of head with neck. Labial frame-work conspicuously sclerotized, with outer margins extending posteriorly for about two body annules from basal plate. Wall of vestibulum sclerotized, forming a spear-guide apparatus extending for about 7 annules.

Buccal spear strong, 22-26 microns long. Basal knobs of spear slightly concave anteriorly. Dorsal oesophageal gland opening into the lumen of pro-corpus 8-10 microns behind spear base. Pro-corpus a cylindrical tube; median oesophageal bulb ovate with a conspicuous refractive structure. Nerve ring in region of isthmus, slightly behind the median bulb. Subventral glands enveloping the intestine ventrally. Excretory pore at about the level of oesophago-intestinal junction, just behind the hemizonid, the latter extending for about two body annules.

Reproductive system digonic hermaphrodite. Vulva a depressed transverse slit with membranous lateral flaps. Uteri with distinct spermatogonia at their distal ends. Ovaries opposed, outstretched, with oöcytes arranged in single file.

Rectum less than one anal-body-diameter long; anus distinct. Tail dorsally convex with 10 annules on its ventral side; tail terminus having the form of an unstriated peg.

DIAGNOSIS: *Helicotylenchus* with the above general description and measurements, *H. plumariae* n. sp. can be distinguished from *H. digonicus* Perry, 1959, by its longer tail ($c = 59$ in *H. digonicus*), and anteriorly concave spear knobs. It also differs from it in its smaller body size (body length 0.62-0.71 mm. in *H. digonicus*), and a smaller buccal spear (spear 25-29 microns long in *H. digonicus*). From *H. canadensis* Waseem, 1961, it can be differentiated in having a smaller body size ($L = 0.78$ mm. (average) in *H. canadensis*), a smaller buccal spear (spear 28-30 microns long in *H. canadensis*) and a comparatively longer tail ($c = 48.7-65.0$ in *H. canadensis*).

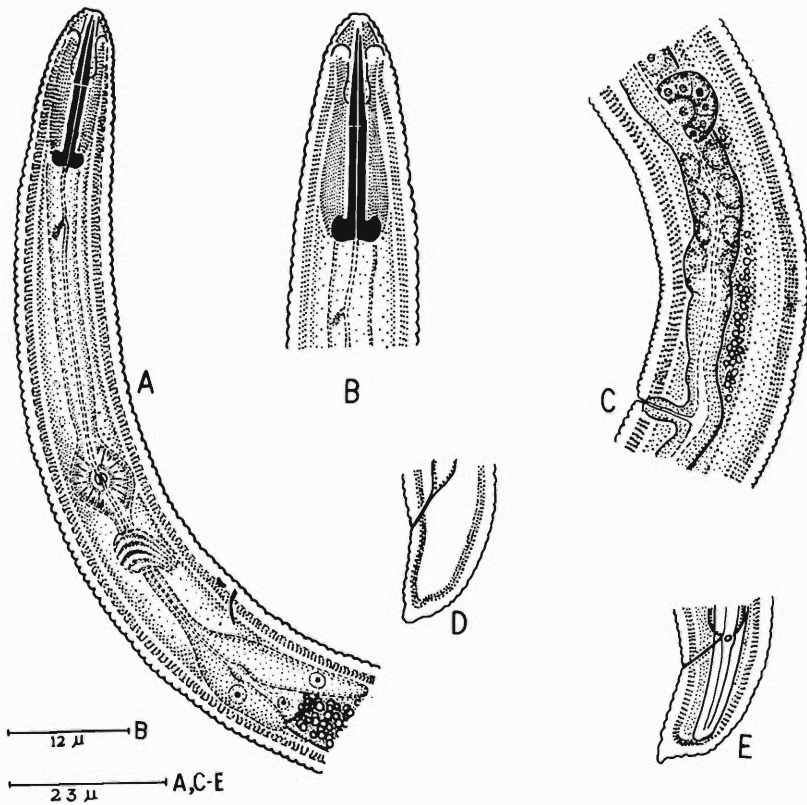


Fig. 2, A-E. *Helicotylenchus plumariae* n. sp. A. Oesophageal region, B. Head end, lateral, C. A portion of reproductive organs of hermaphrodite, lateral, D-E. Tails, lateral views.

It differs from *H. crenatus* Das 1960, in having smaller and more posteriorly located phasmids and more anteriorly located gland opening ($\sigma = 50$ in *H. crenatus*).

TYPE HOST AND LOCALITY: Collected from soil around roots of *Plumaria acutifolia* in Shahjahanpur District (U.P.), India.

TYPE SPECIMENS: Holotype and paratypes deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

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***Theristus pratti* n. sp., a Marine Nematode from Kenya**

D. G. MURPHY and A. G. CANARIS*

A here-to-fore undescribed species of marine nematode was recovered in relatively high abundance from a small collection of nematodes taken from a sandy shore in Mombasa, Kenya. *Theristus pratti* n. sp. is a member of a small, closely related, cosmopolitan group of marine and brackish-water nematodes (see: Remarks) which can be readily distinguished from the remainder of the genus through the structure of the spicular apparatus. Proper separation of this new species from closely related, previously described species would not have been possible had we not had access to the specimens from which two of the earlier descriptions were made. The successful pursuit of the systematics of such nematode groups will depend upon more thorough descriptions than those that are generally to be found in the existing literature of free-living marine nematodes: proper establishment of type-material, on permanent mounts, is essential.

The species is named in honor of Professor Dr. Ivan Pratt of Oregon State University, Corvallis.

DESCRIPTION OF MALE (figures 1 and 3 A-F, I&J).

L = 2.23 mm., a = 38.4, b = 6.1, c = 8.0 (holotype); L = 2.00 mm, a = 38.1, b = 6.0, c = 8.6; L = 1.92, a = 37.4, b = 5.8, c = 8.0.

In general appearance both sexes conform closely (figures 1 & 2B). There is no evidence of the sexual dimorphism that is occasionally manifested within the genus (in particular as a difference in the number of cephalic setae).

The cuticle bears fine striations: the annules in the mid-body region averaging 2.25 microns in width. There appears to be a consistency in the absolute number of striations to be found on the body. We attempted to count the striations lying between the head (counting from the anteriormost striation) and the anterior rim of the amphid, the diameter of the amphid measured in striations (this count including the striation on either end of the amphid to which the amphid is tangent or almost tangent), and the number of striations on the tail counted in optical section from the ventral side from the first striation posterior to the anus to the last striation anterior to the spinneret. The striations in the head region were too faint to permit an accurate count. Those encompassing the amphid consistently numbered nine. An exact count of striations on the tail was very difficult to achieve; however, for purposes of magnitude the count ranges between 135 and 140 striations. The annules are very slightly, but none-the-less distinctly, directional in the cervical and tail regions, i.e. in the cervical region the anterior portion of each annule is slightly raised in relation to the posterior portion and each annule appears to be slanted forward (optical section).

There are six, stout labial papillae of about 4 microns in length; fourteen cephalic setae in one circle, the long and the short being about 20 microns and 12 microns long respectively. Somatic setae are present in six rows corresponding in position to the cephalic setae, plus four rows which lie sublaterally: a total of ten rows. The setae in the sublateral rows are often paired and occasionally paired in the subventral and subdorsal rows. Details of genital and caudal setae are given in figures 1 and 3J.

*Respectively: guest researcher at the Zoologisches Staatsinstitut, Hamburg; and Egerton College, Njoro, Kenya on AID contract from West Virginia University. This investigation was supported in part by a Public Health Service fellowship GPD-18,939 from the Nat'l Institute of General Medical Sciences.

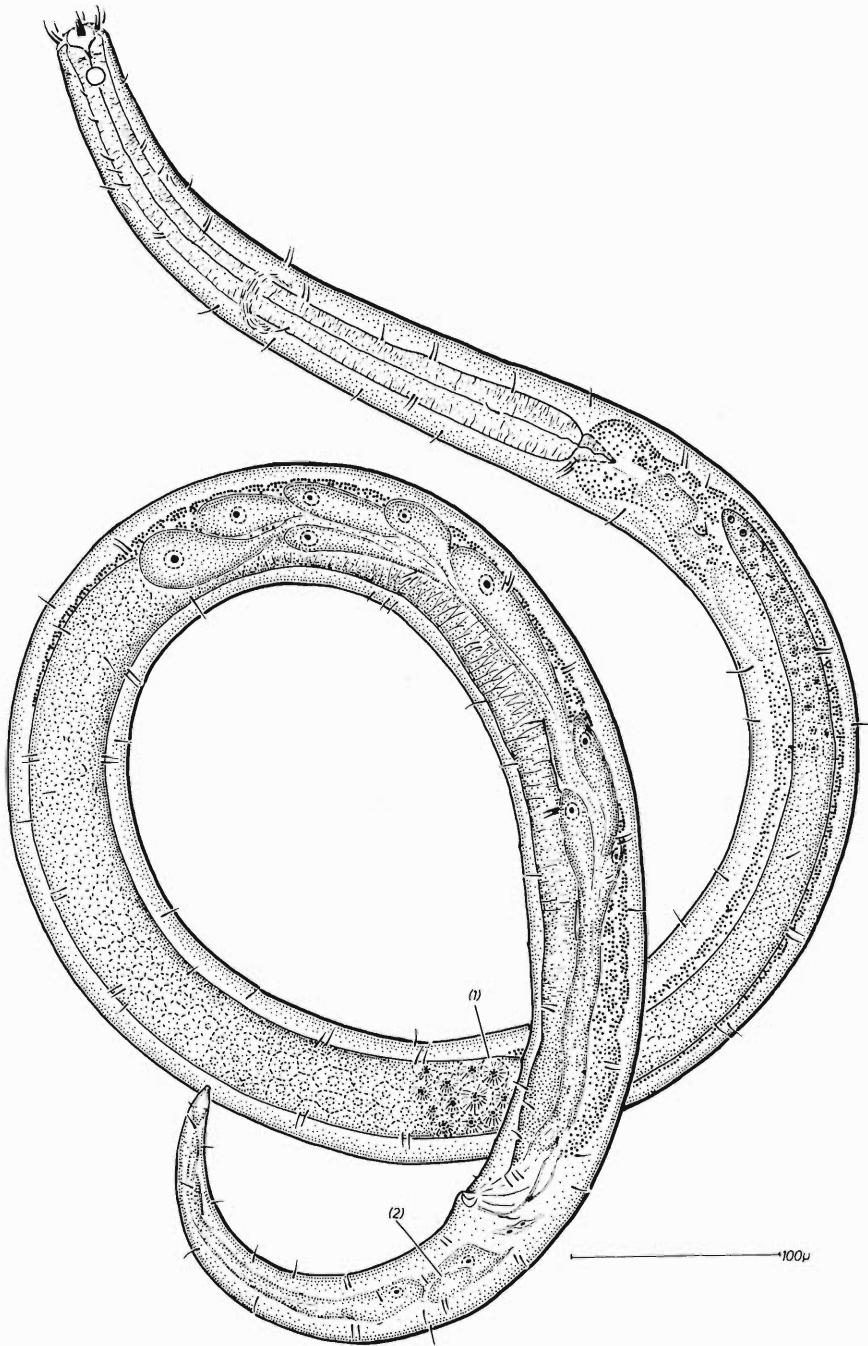


Fig. 1. *T. pratti*, holotype: male, (numerals referred to in text).

Six lips are present, corresponding in position to the labial papillae. They are very thin and transparent. Each lip is strengthened by three ridges or folds of cuticle, accounting for the four striations attributed to each lip in species of this genus when viewed laterally.

The amphid is circular, 10 microns in diameter (29% of the corresponding head diameter) and located (from anterior rim of amphid) about 32 microns posteriad.

The esophagus is cylindrical, slightly broader in the stomal region, and broadening gradually posteriorly. The basal 27% of the esophagus is often recognizable as a distinct region having a greater diameter and a slight difference in appearance in musculature. The body diameter at the base of the esophagus is 48 microns.

The excretory pore appears to lie at a level just forward of the amphid; however, it is weakly sclerotized and could have been confused with numerous hypodermal gland openings.

Several glands occupying ventral and subventral positions are found external to the anterior end of the intestine. The complex would presumably contain both the excretory and esophageal glands.

The testes are paired, outstretched. One stage of cell development in the testis is remarkable because of numerous "lines" radiating outward from the nucleus of the germinal cells, [figure 1 (1)]. The spicules are complex (figure 3F), bear a "double" lateral projection which distally becomes the dorsal portion of the spicule. What is proximally the dorsal portion of the spicule is united distally with the ventral portion. A gubernaculum is present: paired (or forked; a projection over either spicule). There is a pair of prominent lateral pieces usually considered as part of the gubernaculum, but considered by the authors as sclerotized copulatory gland openings, (a manuscript on the comparative morphology of these structures is in preparation by the senior author). A series of about eight glands (Figs. 1 & 3 D-E) extends relatively far anteriorly, and exit in the cloacal region. These may be the same glands referred to in some instances as ejaculatory glands, but it is our opinion that they exit via the lateral pieces and not into the cloaca or vas deferens. Similar glands are not to be found in females. And glands were not observed in the cloacal region of the male, but could have been obscured by spicular musculature, etc., whereas these glands are readily observed in the female.

At least five, perhaps six, preanal, tuboid supplements are present. In some cases these are as obvious as those found in the genus *Paracanthochus*. Such supplements appear to be commonly present in the genus, although usually very weakly sclerotized and thus seen only with careful study. As a rule they have been over-looked in most descriptions.

The tail is cylindro-conical, 5.4 anal diameters long. There appear to be only two fully developed caudal glands present. Cells have been observed which could be interpreted as a reduced or vestigial third caudal gland [figure 1 (2) & 2A (1)].

DESCRIPTION OF FEMALE (figures 2A-B).

L = 2.18 mm., a = 33.8, b = 6.4, c = 8.7, V = 65.6%, Ov = 45% (allotype); L = 2.14 mm., a = 30.9, b = 5.8, c = 9.0, V = 66.0%, Ov = 49%; L = 1.90 mm., a = 31.3, b = 6.2, c = 8.3, V = 66.0%, Ov = 46%.

For general morphology the reader is referred to the description of the male. Measurements for the allotype are as follows: amphid 10 microns in

diameter (interrupting a total of nine striations), located about 30 microns posteriorly. Corresponding head diameter at the level of the amphid is 30 microns. Body diameter at: base of esophagus = 48 microns, vulva = 56 microns, and anus = 39 microns.

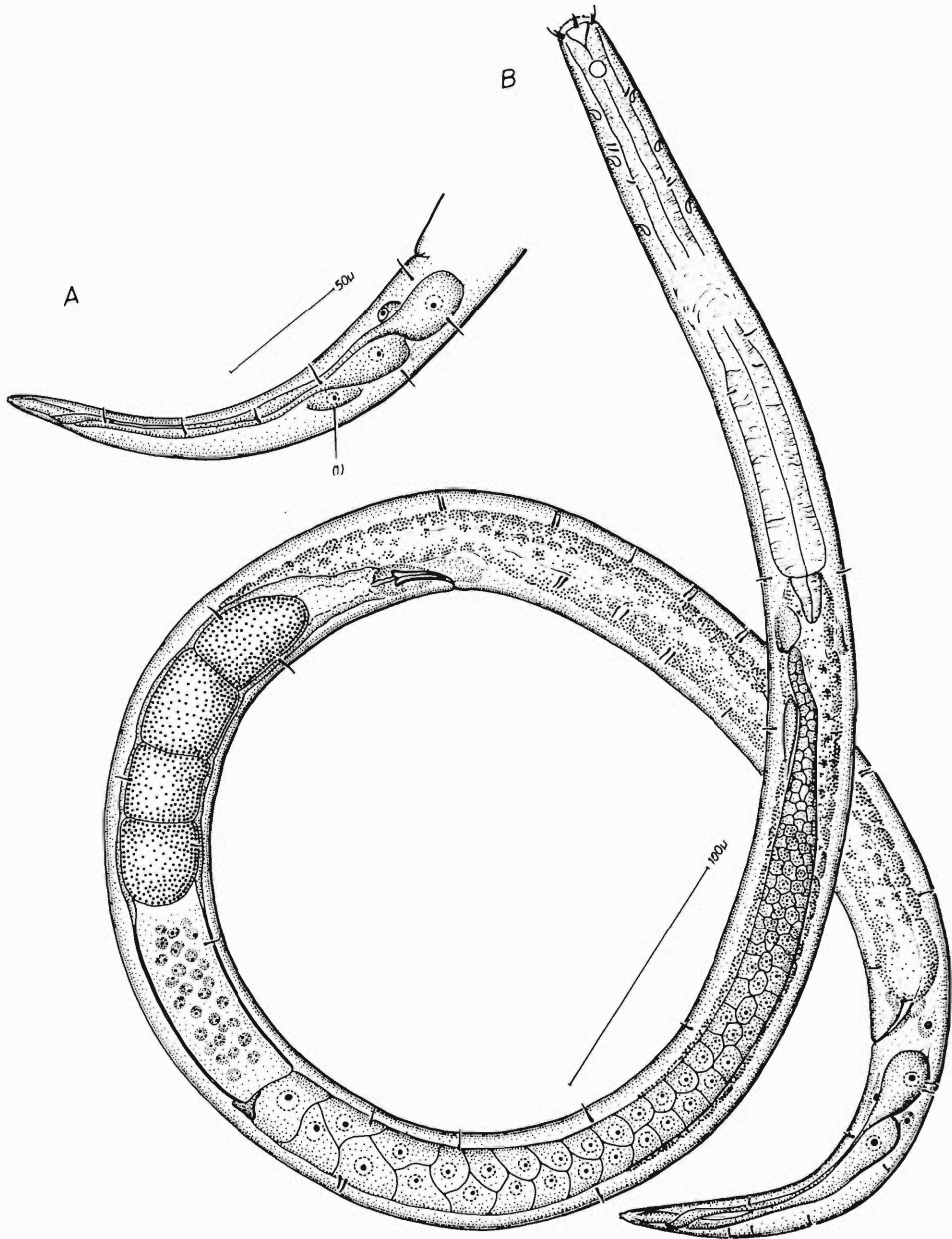


Fig. 2. *T. pratti*. A, female tail. B, allotype: female.

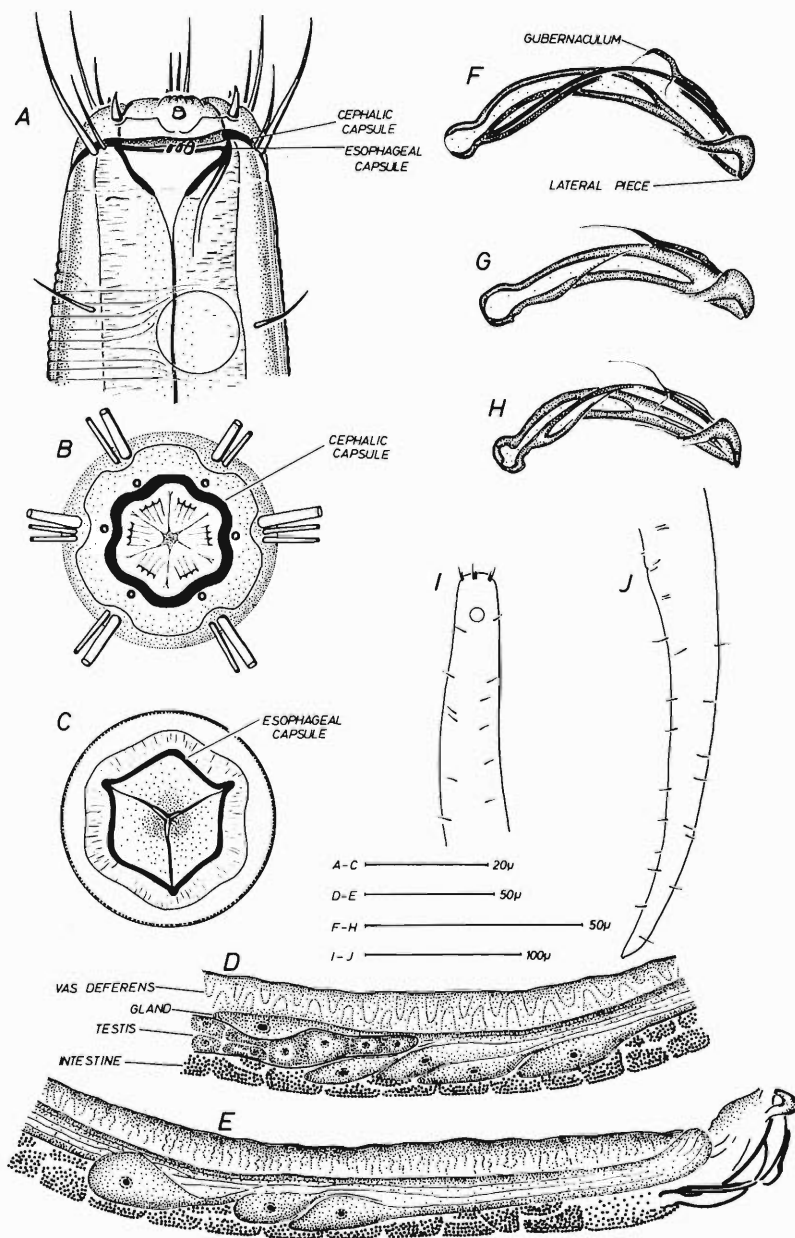


Fig. 3. A-F, I&J = *T. pratti*. A, lateral view of head; male. B, face view of male. C, optical section of head immediately below level of cephalic setae; male. D & E, continuous illustration of region of copulatory glands of male. F, spicular apparatus. G, *T. metaflevoensis*, spicular apparatus. H, *T. macroflevoensis*, spicular apparatus. I & J, respectively anterior and caudal regions showing setae arrangement of a paratype.

Setae length and arrangement as well as shape, number, and arrangement of ventral, esophageal and caudal glands approximate the description given of the male.

One outstretched ovary is present. The spermatheca is usually large and distinct. The number of eggs to be found in the uterus varies according to the development of the individual. The vagina is heavily sclerotized.

The tail is cylindro-conical, 5.6 anal diameters long.

REMARKS

HOLOTYPE, allotype, and paratypes are being maintained temporarily by the authors in collection DM-120. Institutional deposition will be published at a later date. The material was collected on 4 December 1963 by A. G. Canaris.

TYPE-LOCALITY: inter-tidal shore of the North Channel, 200 yards northeast of Fort Jesus, Mombasa, Kenya. The collection site is exposed at low-tide, but kept moist by fresh-water seepage.

RELATED SPECIES: *T. pratti* is most closely related to *T. macroflevensis* Gerlach, 1954, and *T. metaflevensis* Gerlach, 1955. Through the kindness of Prof. Dr. Gerlach, the type-material of both species was made available for comparative studies. The specimens are not particularly well preserved, and thus lack some desirable definition of detail, e.g. of glandular structure. The three species can be separated with relative ease on the basis of the structure of the male genital apparatus (Fig. 3F-H). One should note the shape of the lateral-pieces and gubernaculum, in particular in the case of the latter of *T. pratti*, the sharply bent proximal end. The strong, double lateral projection of the spicules of this species is also distinctive. *T. pratti* is further distinguished from the aforementioned species by the genital apparatus being larger, and by stouter (broader) cephalic setae, in particular the six long setae. Of these three species, *T. macroflevensis* is unique in that the vulva is more posteriorly located, about 75%.

T. flevensis Stekhoven, 1935 and *T. ambronensis* Schulz, 1936 also belong to this group of nematodes. Although they are not so closely related to *T. pratti* as the two previously mentioned species, they, too, can be distinguished at least from *T. pratti*, by the structure of the male genitalia: in particular that they both possess much weaker developed spicules than the African species. The latter is, as well, a larger nematode than the species of Stekhoven and Schulz. *T. bipunctatus* (G. Schneider, 1906) Filipjev, 1930, probably also related to these forms, if not identical to one of them, is inadequately described and is considered by the authors as a dubious species.

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***Helicotylenchus retusus* n. sp. (Nematoda: Hoplolaiminae) found
around sugar cane roots in Negros Oriental, Philippines**

M. RAFIQ SIDDIQI and K. F. BROWN*

Helicotylenchus retusus n. sp. was found in clay/loam soil from around the roots of sugar cane (variety Alunar) at Hacienda 'Biarin,' Kabankalan Sugar Co., Negros Oriental, Philippines. The new species is described hereunder.

Helicotylenchus retusus n. sp. (Fig. 1, A-E)

MEASUREMENTS. 5 females (in glycerine): Length = 0.73-0.77 mm.; a = 33-36; b = 5.7-6.0; c = 48-53; V = 61-64%; spear = 26-27 microns; 0** = 44-48.

Female (Holotype): Length = 0.75 mm.; a = 33; b = 6; c = 53; V = 64%.

DESCRIPTION. Body almost cylindrical, forming a single spiral when relaxed in hot water (Fig. 1, A). Transverse striae on body cuticle distinct, 1.4 microns apart near mid-body region. Lateral fields completely interrupt transverse striae except at the front end of body. They occupy about 2/9th the body width and are marked by four distinct incisures the outer ones of which are faintly crenate. Deirids not seen. Phasmids dot-like, about 13 body annules or slightly over one tail length anterior to anal latitude (Fig. 1, D, E). In paratypes the position of the phasmids varies from 9-15 annules anterior to anal latitude.

Lip region conoid, elevated, continuous with body contour, marked by four transverse striae. Labial skeleton strongly sclerotized, in six sectors; its outer margins extending posteriorly about two annules into body. Vestibulum sclerotized, forming a spear guiding tube which extends 6-7 annules into body.

Spear well developed, divisible into two equal parts, with three compact, anteriorly-cupped basal knobs (Fig. 1, B). Orifice of dorsal oesophageal gland 13 microns behind spear base. Procorpus of oesophagus cylindrical, narrowed at its junction with median oesophageal bulb. Latter rounded, strongly muscular, with distinct refractive inner thickening. Isthmus slender, enveloped by nerve ring anterior to its middle. Oesophago-intestinal junction at about one body width behind the level of nerve ring. Excretory pore a little anterior to level of oesophago-intestinal junction. Hemizonid three body annules long, just anterior to excretory pore (up to three annules anterior to excretory pore in paratypes).

Vulva a transverse slit, with two lateral cuticular membranes (Fig. 1, C). Vagina extending half-way into body. Gonads paired, outstretched in opposite directions. Each uterus with a muscular and a columellate part, the latter with a prominent swelling at its distal end which is possibly an spermatogonium (Fig. 1, A). Ovaries symmetrical, mostly with a single row of oöcytes. Rectum about one anal body width long. Tail obtusely rounded, sub-conoid, slightly longer than anal body width, with about 11 annules on its ventral side. Male not found.

TYPE HOST AND LOCALITY: Collected from soil around roots of sugar cane, *Saccharum officinarum* L. in Hacienda Biarin, Negros Oriental, Philippines.

TYPE MATERIAL: Collected by K. F. Brown on 2nd May, 1962; holotype female with the Rothamsted Experimental Station, Harpenden, England;

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**0 is the distance of the dorsal oesophageal gland orifice from the spear base expressed as percentage of the spear length.

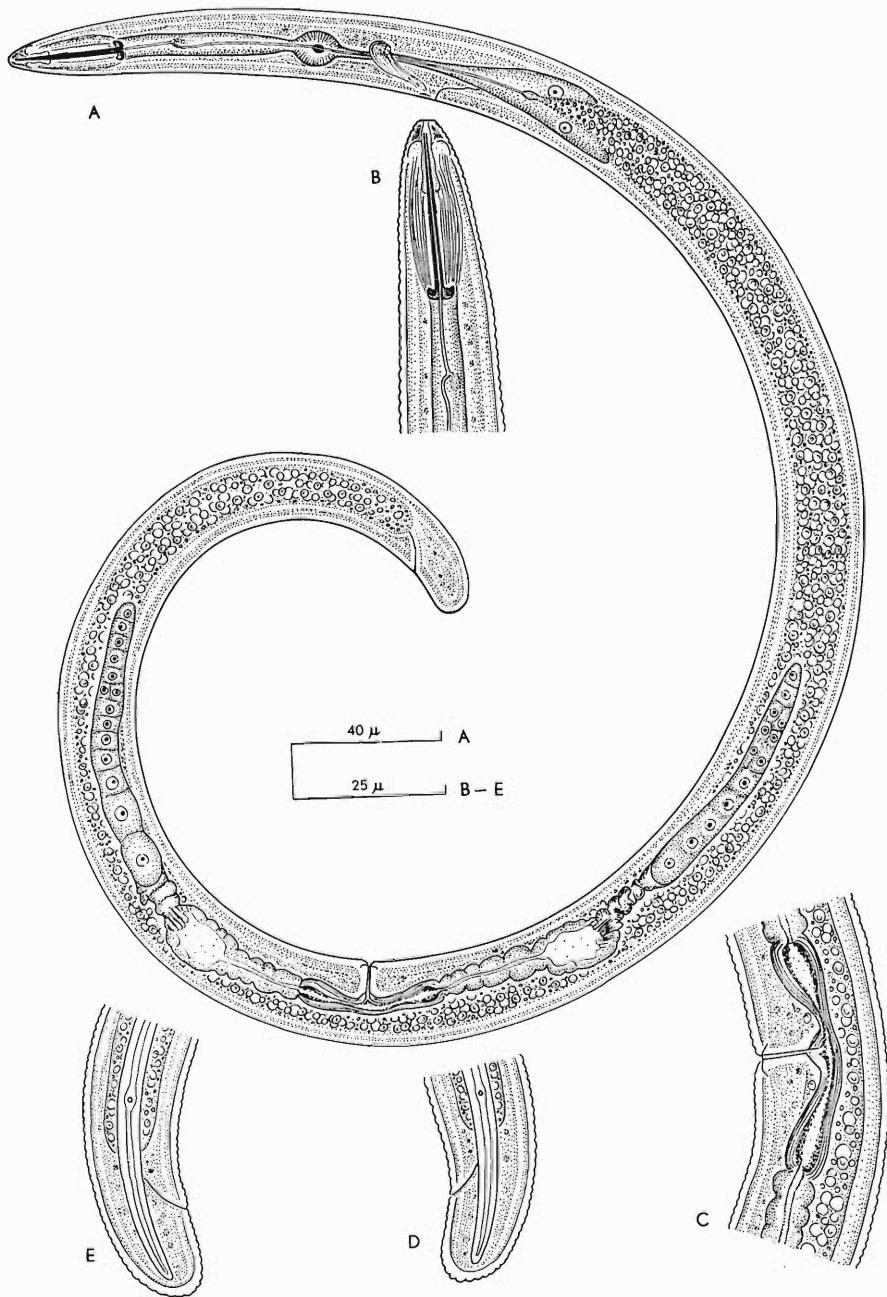


Fig. 1, A-E. *Helicotylenchus retusus* n. sp. A. Female. B. Anterior end of female. C. Vulvar region, lateral. D. and E. Female tails.

rest with the authors.

DIFFERENTIAL DIAGNOSIS: *Helicotylenchus retusus* n. sp. is distinguished by having a rounded and sub-conoid tail, 26-27 microns long spear bearing anteriorly cupped basal knobs, phasmids located 9-15 annules anterior to anal latitude, about 11 annules on ventral surface of tail and by the absence of males. It comes close to *H. platyurus* Perry, 1959; *H. canadensis* Waseem, 1961; *H. cairnsi* Waseem, 1961; *H. tunisiensis* Siddiqi, 1963; and *H. serenus* Siddiqi, 1963.

From *H. platyurus* it differs in having a smaller spear (spear = 30-40 microns long in *H. platyurus*), differently shaped tail and the phasmids located much anterior to the anal latitude.

It differs from *H. canadensis* in having a shorter spear (spear = 28-30 microns long in *H. canadensis*), a higher value for 'o' ratio and more anteriorly located phasmids (near anal region in *H. canadensis*).

From *H. cairnsi* this species can be separated by having a smaller spear, a greater value for 'o' ratio and a differently shaped tail (short, truncate in *H. cairnsi*).

It can be distinguished from *H. tunisiensis* by having a shorter buccal spears 32-36 microns long in *H. tunisiensis*), a more posteriorly located vulva (V = 56-58% in *H. tunisiensis*) and a differently shaped tail.

From *H. serenus* it differs in having a greater value for 'o' ratio (o = 31 in *H. serenus*), a shorter and differently shaped tail and phasmids located much anterior to the anal latitude (1-5 annules anterior to anal latitude in *H. serenus*).

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Three New Species of Nematodes in the Family Hoplolaimidae Found Attacking Citrus Trees in India*

M. RAFIQ SIDDIQI and ZAHID HUSAIN

During our investigations on the nematode parasites of citrus trees in India three undescribed species of the family Hoplolaimidae Wieser, 1953, were recovered. These are named and described here as *Helicotylenchus digitatus* n. sp., *H. neoformis* n. sp. and *Rotylenchus orientalis* n. sp. Other hoplolaimid species found associated with citrus in this country are *Hoplolaimus indicus* Sher, 1963; *Helicotylenchus indicus* Siddiqi, 1963; and *Scutellonema brachyurum* (Steiner, 1938) Andr assy, 1958. *Helicotylenchus digitatus* is interesting in having a small buccal spear (20-21 microns long) and

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This investigation was supported by the Council of Scientific and Industrial Research, India.

a dorsally bent digitate tail terminus and *H. neoformis* as well as *R. orientalis* have posterior branch of reproductive organs considerably reduced. No males of either species were found and it is thought that these species are digonic hermaphrodites.

All measurements were done from specimens killed by gentle heat, fixed in F.A. 4: 10 and mounted in dehydrated glycerine. The types have been deposited with the nematode collections of M. Rafiq Siddiqi at the Zoology Museum, Aligarh, India.

Helicotylenchus digitatus n. sp. (Fig. 1, A-H)

FEMALES (15): Length = 0.52-0.61 mm. (0.56 mm.); a = 21-30 (26.5); b = 4.4-4.9 (4.6); c = 21-26 (23.5); V = 59-66% (62%); spear = 20-21 microns.

FEMALE (Holotype): Length = 0.59 mm.; a = 28; b = 4.5; c = 25; V = 60%; spear = 20 microns.

DESCRIPTION: Body almost cylindrical, gradually tapers at either extremities, spirally coiled when relaxed in hot water (Fig. 1, A). Body striae 1.5 microns apart. Lateral fields $\frac{1}{4}$ - $\frac{1}{5}$ as wide as body, marked by four incisures outer ones of which are slightly crenate. Phasmids pore-like, slightly post-anal (variations in its position shown in Fig. 1, D-H). Head conoid-rounded, with four transverse striae. Outer margins of labial framework extending from basal plate two body annules. Spear short and stout, with rounded basal knobs. Orifice of dorsal oesophageal gland 8.5 microns behind spear base. Median oesophageal bulb large, spheroidal, with prominent cuticular thickening in center. Isthmus long and narrow enveloped by nerve ring at 85 microns from anterior end of body. Excretory pore a little behind level of nerve ring, just posterior to hemizonid.

Vulva a transverse slit, with lateral membranes (Fig. 1, C). Gonads paired, opposed; each uterus with a spermagonium-like swelling at its distal end. Tail dorsally convex, slightly over twice anal body width long, with 15 annules on its ventral side (13-16 annules in paratypes), ending in a long, digitate, dorsally bent terminus. A few bristle-like processes seen on tail tip in some paratypes.

TYPE HOST AND LOCALITY: Collected from soil around roots of *Citrus sinensis* (L.) Osbeck at Jog Falls, Mysore State, South India.

RELATIONSHIP: *Helicotylenchus digitatus* n. sp. can be differentiated from all the known species of the genus by having a small spear (20-21 microns long), a postanal phasmid, and tail measuring over two anal body widths in length and bearing a characteristic digitate terminus. It has some affinities with *H. africanus* (Micoletzky, 1916) Andrassy, 1958, but can at once be distinguished from it in having a smaller spear and a longer, differently shaped tail bearing a larger number of annules.

Helicotylenchus neoformis n. sp. (Fig. 2, A-C)

FEMALES (6): Length = 0.54-0.60 mm. (0.55 mm.); a = 24-33 (25.5); b = 5.0-5.4 (5.2); c = 34-57 (41); V = 74-77% (75.5%) spear = 22-23 microns.

FEMALE (Holotype): Length = 0.54 mm.; a = 26; b = 5.2; c = 45; V = 75%; spear = 22 microns.

DESCRIPTION: Cuticular striae 1.4 microns apart near mid-body. Lateral

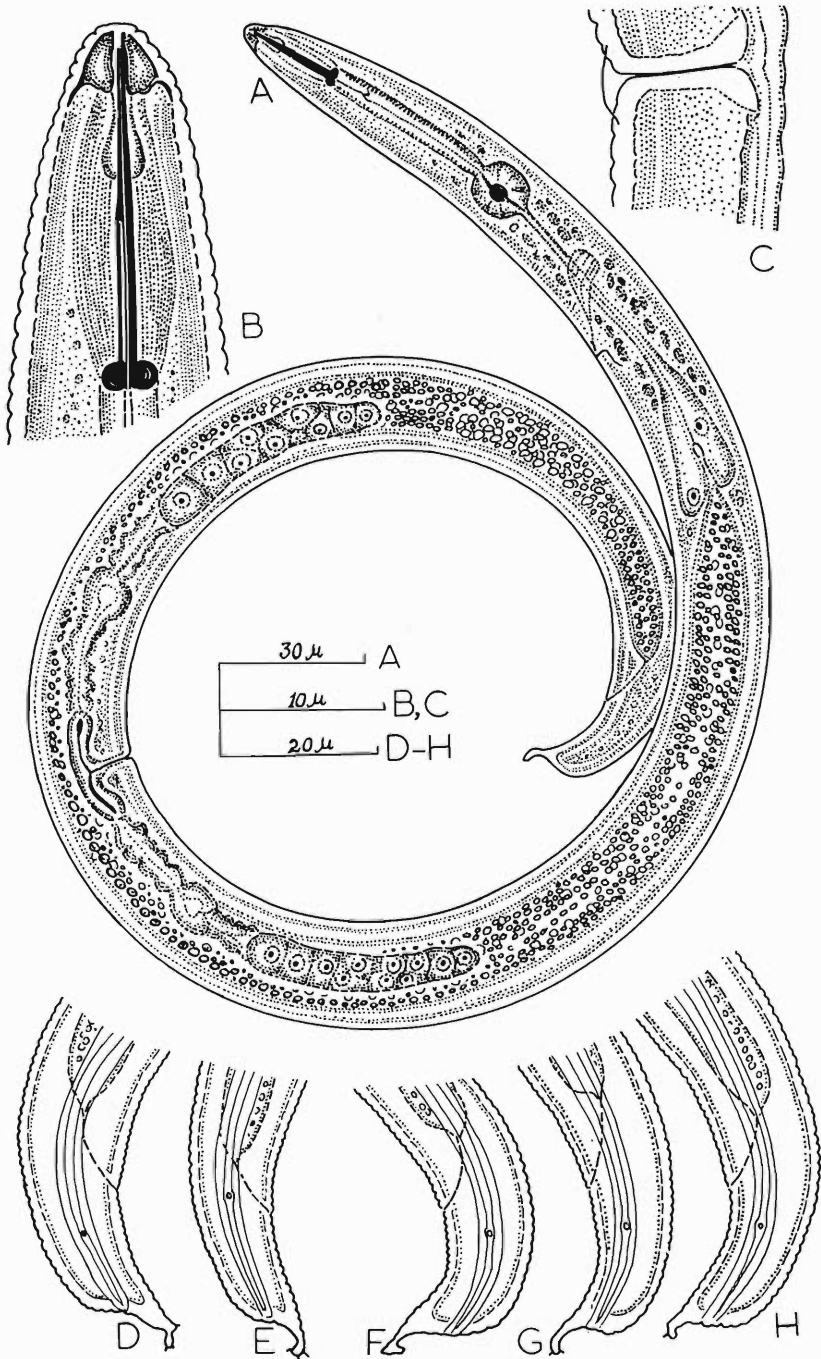


Fig. 1, A-H. *Helicotylenchus digitatus* n. sp. A. Female. B. Head end of female. C. Vulvar region. D-H. Tails of females.

fields about $\frac{1}{4}$ as wide as body. Phasmids five annules above anus (5-8 annules in paratypes). Head elevated, rounded, marked by three striae forming four annules. Outer margins of labial frame-work extending 2 annules into body. Spear slender, with anteriorly cupped basal knobs (Fig. 2, B). Outlet of dorsal oesophageal gland 9.5 microns behind spear base. Excretory pore 100 microns from anterior end of body, about one body width behind median oesophageal bulb. Oesophago-intestinal junction at level of excretory pore.

Vulva a transverse slit, with reduced lateral membranes. Anterior branch of reproductive system normal, posterior reduced (Fig. 2, A). Tail rounded, about one anal body width long, with 9 annules on its ventral side (9-11 annules on ventral side of tail in paratypes).

TYPE HOSTS AND LOCALITY: Same as for *H. digitatus* n. sp.

RELATIONSHIP: *Helicotylenchus neoformis* n. sp. comes closest to *H. intermedius* (Luc. 1960) n. comb.* from which it can be differentiated in having a larger body-size (0.394-0.523 mm. long in *H. intermedius*), a smaller spear (26-27 microns long in *H. intermedius*), a larger number of annules on tail, more highly placed phasmids and by the absence of males.

Rotylenchus orientalis n. sp. (Fig. 2, D-F)

FEMALES (5): Length = 0.68-0.76 mm. (0.72 mm); a = 27-32 (29); b = 6.2-6.7 (6.4); c = 36-56 (42); V = 66-72% (69.5%); spear = 25-28 microns.

FEMALE (Holotype): Length = 0.76 mm; a = 31; b = 6.6; c = 54; V = 70%; spear = 26 microns.

DESCRIPTION: Body spirally coiled. Cuticle with coarse striae, 1.7 microns apart near mid-body. Lateral fields $\frac{1}{4}$ as wide as body. Phasmids pore-like, pre-anal, four annules above anus. Head conoid-rounded, with five annules, continuous with body. Spear of medium built, with prominent, anteriorly cupped basal knobs. Orifice of dorsal oesophageal gland more than half spear length (15.5 microns) behind spear base (Fig. 2, D). Corpus cylindrical, narrowed at its junction with median oesophageal bulb which is oval in shape. Isthmus enveloped by nerve ring at its middle. Excretory pore a little behind level of nerve ring. Oesophageal glands forming an elongate lobe, extending over dorsal and dorso-lateral sides of the anterior end of the intestine (Fig. 2, D).

Vulva a transverse slit. Reproductive system paired, opposed; posterior set of organs reduced (Fig. 2, E). Uterus at its distal end with a prominent swelling (spermagonium?). Tail dorsally convex, rounded, with 10 annules ventrally (9-11 annules on tail in paratypes), about one anal body width long.

TYPE HOST AND LOCALITY: Collected from soil around roots of *Citrus limon* (L.) Burm. at Shillong, Assam State, India.

RELATIONSHIP: *Rotylenchus orientalis* n. sp. is unique among nominal species of the genus by having the orifice of dorsal oesophageal gland located more than half the spear length behind spear and the posterior reproductive branch being considerably reduced.

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*This species was originally described under the genus *Rotylenchoides* Whitehead, 1958. According to Luc (1960) the oesophageal glands in this species lie mostly on ventral side of the intestine like that in *Helicotylenchus*. The reduction in the size of the posterior reproductive branch is not considered of a generic value and hence the species is placed under *Helicotylenchus*.

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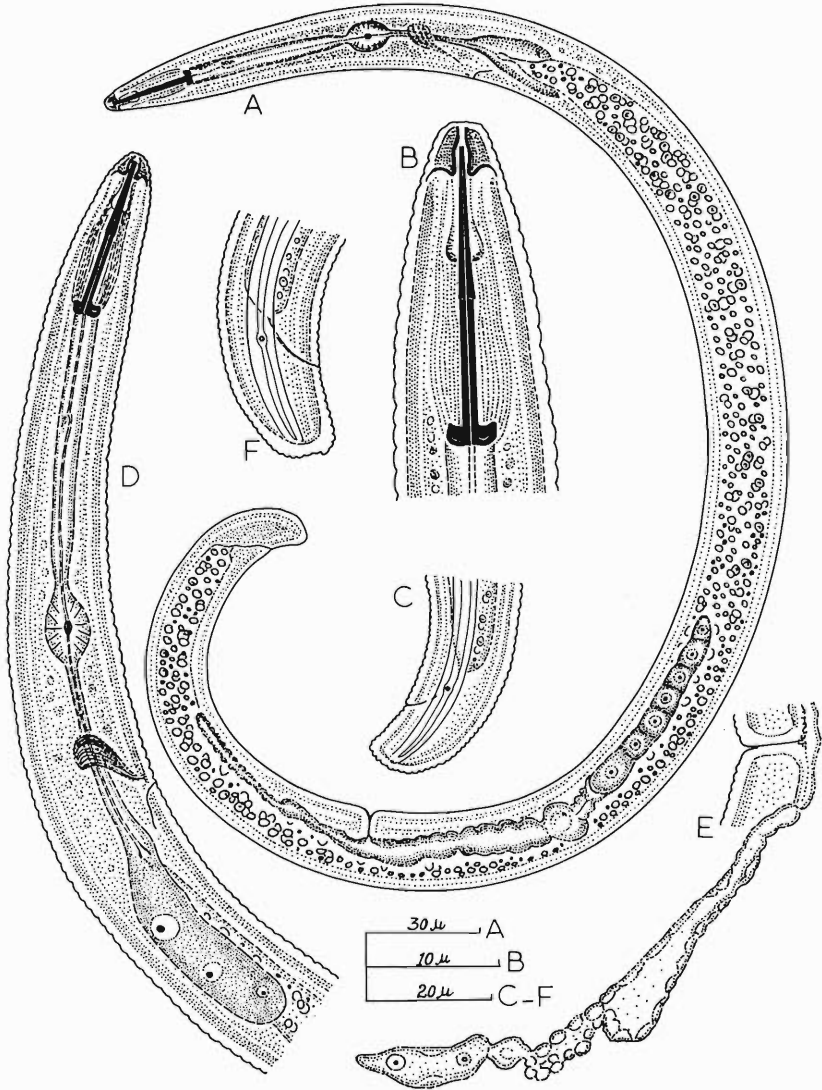


Fig. 2, A-C. *Helicotylenchus neoformis* n. sp. A. Female. B. Head end of female. C. Tail of female. D-F. *Rotylenchus orientalis* n. sp. D. Oesophageal region of female. E. Posterior reproductive branch of female. F. Tail of female.

On a Trematode, *Helicometra indica* n. sp. from the Intestine of the Gurnard Fish, *Trigla gurnardus*

VINOD AGRAWAL*

Abstract

Helicometra indica n. sp. has been described from a Gurnard fish, *Trigla gurnardus*.

Only one stained specimen of the genus *Helicometra* Odhner, 1902 was made available to me through the courtesy of Dr. S. P. Gupta, from the Helminthological collection of Professor G. S. Thapar, Lucknow University, Lucknow.

FAMILY—ALLOCREADIIDAE STOSSICH, 1903

SUBFAMILY—ALLOCREADIIINAE LOOSS, 1902

Helicometra indica n. sp. (Figs. 1-4)

HOST: *Trigla gurnardus* Linn., 1758 (Gurnard fish)

LOCATION: Intestine.

DESCRIPTION: Body elongated, aspinose, 3.23 mm. long and 1.025 mm. wide at level of posterior testis; anterior end narrower; posterior end rounded with a median notch. Oral sucker terminal, subspherical, 0.30 x 0.31 mm. in size. Ventral sucker larger than oral sucker in anterior half of body, somewhat depressed on one side, 0.42 x 0.35 mm. in size, at 0.84 mm. from anterior extremity. Prepharynx very short, 0.02 x 0.07 mm. in size; pharynx 0.1 x 0.09 mm. in size; oesophagus muscular, tubular, 0.20 mm. long bifurcating into intestinal ceca, slender, extending a little anterior to caudal end of body.

Excretory pore terminal; bladder tubular, extending to level of caudal end of posterior testis.

Genital pore median near mid-oesophagus level, between pharynx and intestinal bifurcation, at 0.54 mm. from anterior extremity.

Testes postequatorial, subequal, deeply lobed, with irregular margins, closely tandem and intercaecal. Anterior testis 0.35 x 0.53 mm. in size at 1.925 mm. from anterior extremity. Posterior testis 0.36 x 0.535 mm. in size at 0.63 mm. from caudal end. Cirrus pouch elongated, broader posteriorly and narrower anteriorly extending from genital pore up to middle region of ventral sucker, 0.6 x 0.12 mm. in size. Vesicula seminalis elongated, cylindrical, occupying $\frac{2}{3}$ of cirrus pouch, 0.38 x 0.10 mm. in size, opens into a globular pars prostatica, 0.05 x 0.04 mm. in size which continues forward as an ejaculatory duct, 0.11 mm long. Cirrus 0.065 mm. long, muscular and non spiny. A large number of prostate gland cells fill entire space in cirrus pouch around ejaculatory duct and pars prostatica.

Ovary cephalad and contiguous to anterior testis, submedian, with 4 unequal lobes, post equatorial, 0.20 x 0.31 mm. in size, at 1.75 mm. from anterior extremity. Mehl's gland, preovarian and diffuse laterally. A large pear shaped receptaculum seminis at left side of ovary, 0.4 x 0.2 mm. in size; with its anterior end prolonged into Laurer's canal. Vitellaria follicular extending a little anterior to intestinal bifurcation up to caudal end of body, mainly

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The work has been carried out under the guidance of Dr. S. P. Gupta, Department of Zoology, Lucknow University, Lucknow. The author is deeply indebted to him for his invaluable help and encouragement.

The type specimen of the form described in this paper will be deposited in Dr. G. S. Thapar's Helminthological Collection, U. P. India.

along outer margins of ceca but extending into intercaecal space caudad of posterior testis. Vitelline ducts unite in front of ovary to form a yolk reservoir that opens into ootype. Uterus arises from anterior end of ootype and

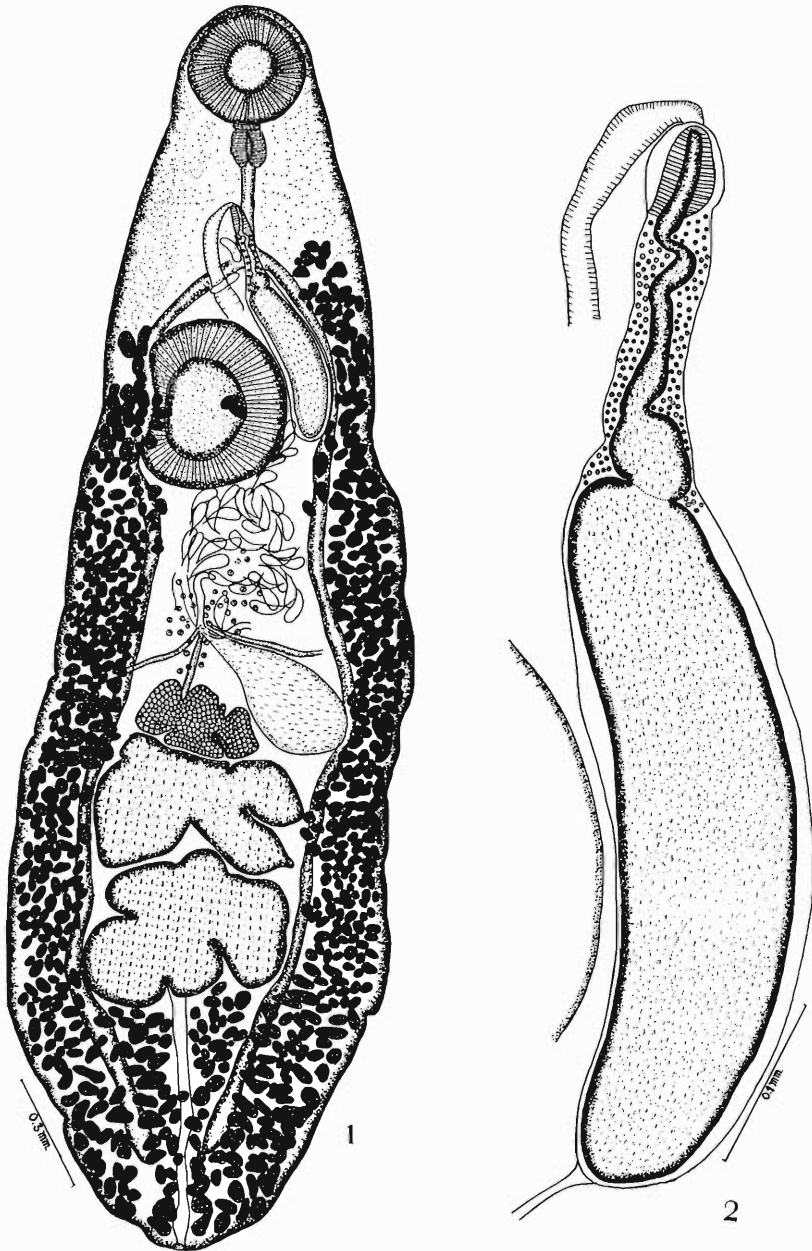


Fig. 1. *Helicometra indica* sp. nov. Ventral view. Fig. 2. Cirrus pouch enlarged.

opens at genital pore. Metraterm about $\frac{1}{3}$ length of cirrus sac passing to right of cirrus sac. Eggs oval in shape with a polar filament at one end, measuring 0.08-0.12 x 0.025-0.03 mm. in size and filament 0.09-0.15 mm. in length.

DISCUSSION: The present form has been referred to the genus *Helicometra* Odhner, 1920. Yamaguti (1958) listed 17 species from fishes under the genus *Helicometra*. *H. gurnardus* Thapar et Dayal, 1934 described from a Gurnard, *Trigla gurnardus* from India appear to be a *nomen nudem* since no description of it was given.

Of the known species of the genus *Helicometra*, the new form shows close affinities to *H. pulchella* (Rud., 1819) Odhner, 1902, *H. fasciata* (Rud., 1819) Odhner, 1902, *H. epinepheli* Yamaguti, 1934, *H. hypodytis* Yamaguti, 1934 and *H. markevitschi* Pogorelzeva, 1954 in having deeply lobed testes one behind the other and in the extension of vitellaria from beyond bifurcation of intestinal caeca to end of body to posterior end of posterior testis. The new form differs from all the above mentioned species in the extension of cirrus pouch from mid acetabular region to a short distance behind the pharynx and in the structure of vesicula seminalis. The new form is very similar to *H. hypodytis* Yamaguti, 1934 and *H. bassensis* Woolcock, 1935 in having genital pore near the middle of the oesophagus but however differs from both of them in the extent of viteline glands. In *H. hypodytis* the vitellaria extend up to pharynx and in *H. bassensis* up to ventral sucker. Further the new form can also be distinguished from *H. bassensis* in having testes and ovary deeply lobed and in the extent of cirrus pouch from genital pore to mid region of ventral sucker. These differences are sufficient to create a new species *H. indica* sp. nov.

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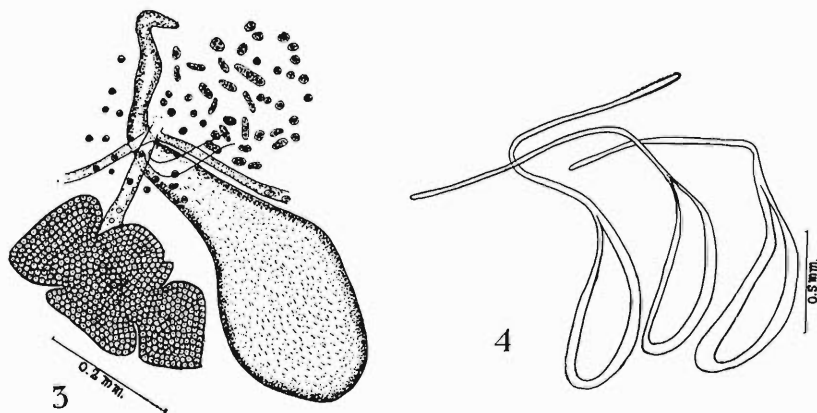


Fig. 3. Ootype enlarged. Fig. 4. Eggs enlarged.

A trematode, *Prosotocus mastacembeli* n. sp. (Subfamily Prosotocinae Yamaguti, 1958) from the intestine of a fresh water fish, *Mastacembelus armatus* (Lacep.) from Lucknow

VINOD AGRAWAL*

ABSTRACT

Prosotocus mastacembeli sp. nov. is described [for the first time] from a fresh water fish *Mastacembelus armatus* from the river Gomti at Lucknow.

Prosotocus mastacembeli n. sp.

Only one specimen of this form was collected from the intestine of a fresh water fish, *Mastacembelus armatus* (Lacep.), from the river Gomti at Lucknow.

DESCRIPTION: Body elongated, aspinose, rounded at extremities, 1.03 x 0.462 mm. in size. Oral sucker terminal and circular, 0.12 x 0.13 mm. in size. Prepharynx small and thin walled; pharynx globular, muscular, 0.05 mm. in diameter; oesophagus tubular, 0.078 mm. long bifurcating into two intestinal caeca extending up to posterior end of ovary. Ventral sucker circular, equatorial, equal to oral sucker, 0.12 x 0.13 mm. in size at 0.465 mm. from anterior extremity.

Genital pore lies on right margin of body at level of pharynx at 0.255 mm. from anterior extremity.

Excretory pore terminal. Excretory bladder V-shaped with a median stem with cornua extending up to $\frac{1}{4}$ of body length.

Testes entire, subequal, most extra-caecal, preacetabular and situated obliquely on either side of body. Left testis 0.145 x 0.11 mm. in size at 0.355 mm. from anterior extremity. Right testis smaller than left testis, a little anterior to it close to median line, 0.12 x 0.11 mm. in size at 0.25 mm. from anterior extremity. Cirrus sac claviform, 0.364 x 0.116 mm. in size extending from genital pore up to ventral sucker. Vesicula seminalis bipartite, fills basal saecular part of cirrus sac. Proximal portion 0.08 x .095 mm. in size and distal portion 0.1 x .08 mm. in size. It opens by narrow small duct into an elongated pars prostatica, 0.17 x 0.04 mm. in size. Ejaculatory duct, 0.15 mm. long, narrow, straight running in distal narrow portion of cirrus sac and passes terminally into a small cirrus. Space between vesicula seminalis and pars prostatica in cirrus sac is filled with prostate gland cells.

Ovary globular, in acetabular zone, smaller than left testis and overlapping left intestinal caecum measuring 0.096 x 0.12 mm. and gives of a short oviduct from its inner margin. Receptaculum seminis pear shaped lying behind ovary dorsal to acetabulum, 0.065 x 0.042 mm. in size. Vitellaria follicular extending from hind end of pharynx to anterior margin of ventral sucker. They occupy obliquely lateral areas between pharynx and intestinal bifurcation, with a few follicles occupying space between intestinal bifurcation and ventral sucker. Uterus postacetabular with much coiled descending and ascending limbs. The metraterm is thick walled and lies between left testis and distal portion of cirrus sac. Eggs oval, 0.028-0.032 x 0.12-0.18 mm. in size.

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The work has been carried out under the direction of Dr. S. P. Gupta, Department of Zoology, University of Lucknow, Lucknow. The author is greatly indebted to him for his valuable help and encouragement.

The type specimen of the form described in this paper would be deposited in Dr. G. S. Thapar's Helminthological Collection, Lucknow, U.P. India.

DISCUSSION: Ten species of the genus *Prosotocus* Looss, 1899 have been described from a mammal and amphibian hosts namely *P. confusus* (Looss, 1894) Looss, 1899, (*Distoma clavigerum* Dujardin, 1845 renamed); *P. indicus* Mehra et Negi, 1928; *P. fuelleborni* Travassos, 1930; *P. vespertilionis* Modlinger, 1930; *P. infrequentus* Srivastava, 1933; *P. himalayai* Pande 1937; *P. kashabia* Kaw, 1943; *P. partapus* Kaw, 1950; *P. sigalasi* Beilenger et Chanseau, 1954; and *P. dorsoporus* Murhar, 1960. *P. vespertilionis* Modlinger, 1930 is the only one described from a mammalian host, the rest occurring in the amphibian hosts. The present form is interesting as it records the presence of the genus from a fresh water fish for the first time. The new form differs from *P. sigalasi*, *P. confusus*, *P. fuelleborni* and *P. vespertilionis* in having cirrus sac fully preacetabular instead pre to post-acetabular. Further the new form differs from *P. dorsoporus*, *P. himalayai*, *P. kashabia* and *P. paratapus* in the possession of bilateral vitellaria instead unilateral. The new form stands closer to *P. indicus* and *P. infrequentus* in having vitellaria bilateral and cirrus sac fully acetabular but however differs from *P. infrequentus* in having ventral sucker equal to oral sucker. The new form resembles closely *P. indicus* in having suckers of equal size but differs from it in the position of genital pore at the hind end of pharynx instead at the level of intestinal bifurcation, in the extension of vitellaria from pharynx up to anterior border of ventral sucker instead of from pharynx up to posterior margin of intestinal bifurcation, in having cirrus pouch closer to ventral sucker instead of away from it and in having testes overlapped by intestinal caeca instead extra-caecal. Accordingly it is regarded as new with the specific name *P. mastacembeli* sp. nov.

HOST: *Mastacembelus armatus* (Lacep.).

LOCATION: Intestine.

LOCALITY: Lucknow.

KEY TO THE SPECIES OF THE GENUS *Prosotocus* LOOSS, 1899

Cirrus sac pre to post acetabular.....	2
Cirrus sac fully preacetabular.....	5
2. Body covered with papillae.....	<i>P. sigalasi</i>
Body without papillae.....	3
3. Genital pore testicular.....	<i>P. confusus</i>
Genital pore post testicular.....	4
4. Ovary entire.....	<i>P. fuelleborni</i>
Ovary lobed.....	<i>P. vespertilionis</i>
5. Vitellaria bilateral.....	6
Vitellaria unilateral.....	8
6. Ventral sucker larger than oral sucker.....	<i>P. infrequentus</i>
Ventral sucker equal to oral sucker.....	7
7. Vitellaria extending from pharynx up to intestinal bifurcation.....	<i>P. indicus</i>
Vitellaria extending from pharynx up to ventral sucker.....	<i>P. mastacembeli</i> n. sp.
8. Intestinal caeca extend beyond ventral sucker.....	<i>P. himalayai</i>
Intestinal caeca do not extend beyond ventral sucker.....	9
9. Cirrus sac overlapping anterior part of ventral sucker.....	<i>P. partapus</i>
Cirrus sac preacetabular.....	10
10. Uterus with a few loops.....	<i>P. kashabia</i>
Uterus well developed.....	<i>P. dorsoporus</i>

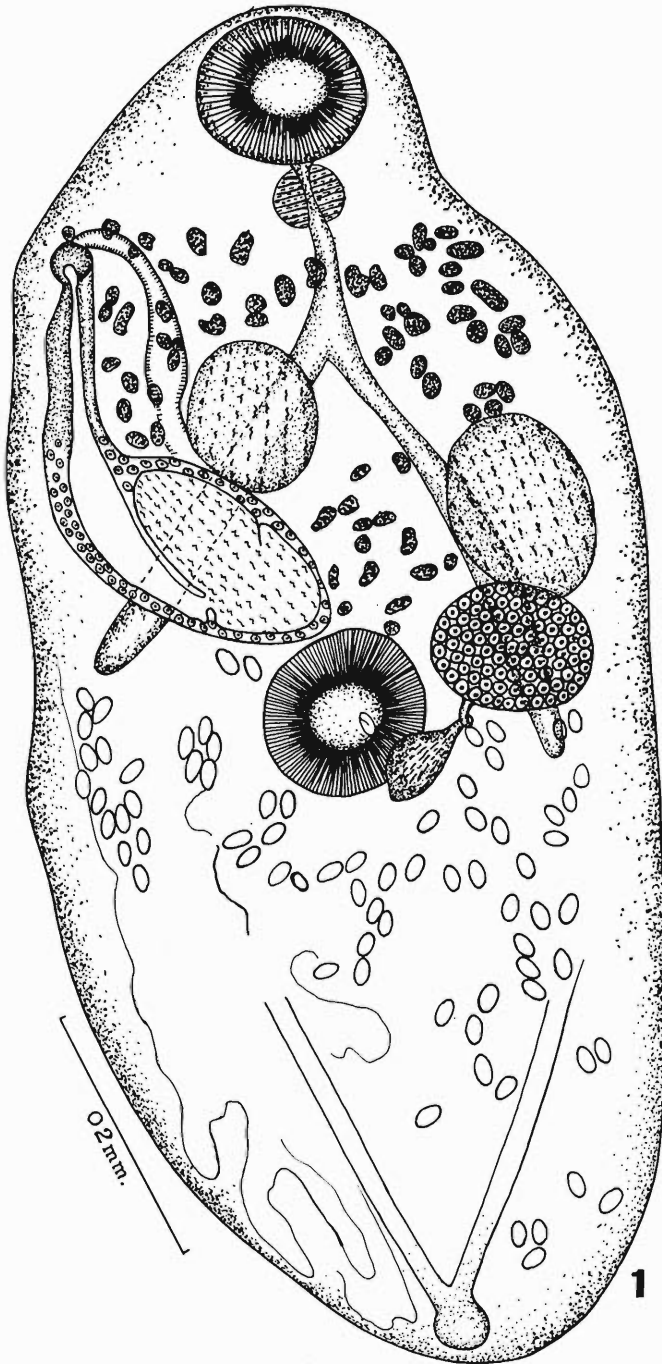


Fig. 1. *Prototocus mastacembeli* n. sp. Ventral view.

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***Dorella mira* n. gen., n. sp., Nematoda: Dorylaimoidea) from India**

M. SHAMIM JAIRAJ PURI*

A new genus and species of Leptonchidae was found in soil around roots of citrus plants from Nainital, U.P., (elevation about 6,500 feet). The name *Dorella mira* n. gen., n. sp., is proposed for its reception.

GENUS *Dorella* n. gen.

DIAGNOSIS: *Tylencholaimellinae*. Spear dorylaimoid, with a short ventral stiffening piece arising from spear base. Spear extensions poorly knobbed. Esophagus a slender tube with a short basal bulb set off by a distinct constriction. Vulva transverse, postequatorial; anterior sexual branch normal, posterior rudimentary; ovary reflexed. Testes and spicules dorylaimoid. Supplements an adanal pair and a ventromedian one located well above spicules. Tail of sexes similar.

TYPE AND ONLY SPECIES: *Dorella mira* n. sp.

RELATIONSHIP: *Dorella* n. gen., comes closest to *Tylencholaimellus* M. V. Cobb, 1915 and *Doryllium* Cobb, 1920 but differs from both in having a short ventral stiffening piece on spear and type of reproductive organs.

Jairajpuri (1964) included *Tylencholaimellus*, *Doryllium* and *Botalium* Heyns, 1963 under *Tylencholaimellinae* Jairajpuri, 1964 but later on (1964-in press) transferred *Botalium* to a new subfamily Xiphinemellinae of Leptonchidae. *Dorella* n. gen., comes under *Tylencholaimellinae*.

KEY TO GENERA OF TYLENCHOLAIMELLINAE

1. Spear without a stiffening piece *Doryllium*
Spear with a stiffening piece 2
2. Stiffening piece dorsal equal to spear length; posterior sexual branch normal *Tylencholaimellus*
Stiffening piece ventral, about one third of spear length; anterior sexual branch normal *Dorella*

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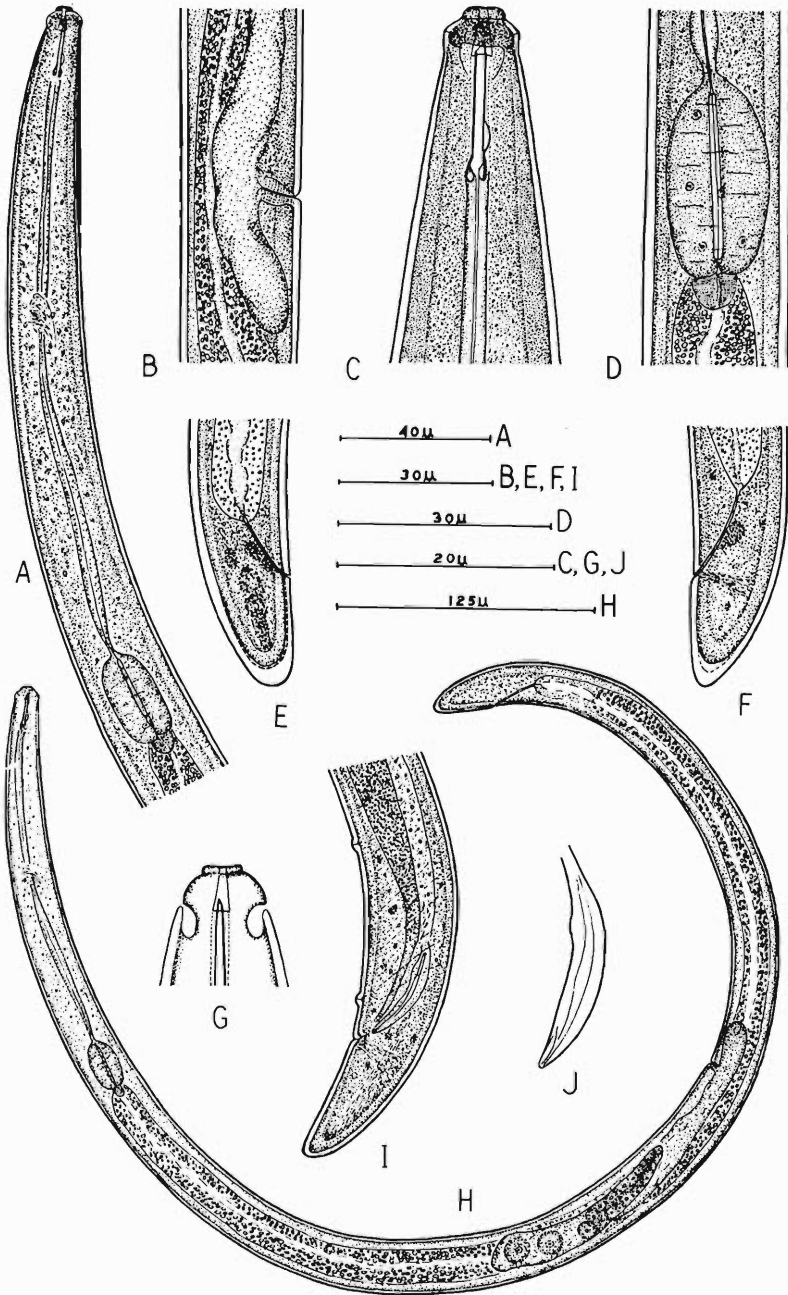


Fig. 1, A-J *Dorella mira* n. gen., n. sp. A—Esophageal region, B—Vulval region, C—Head end (lateral), D—Basal esophageal bulb, E & F—Female tail, G—Head end (dorsoventral), H—Female (entire), I—Male tail, J—Spicule.

Dorella mira n. sp. (Fig. 1, A-J)

FEMALES (7): L = 0.65-0.85 mm.; a = 32-45; b = 4.0-4.8; c = 34-42; V = 61-68.

MALES (4): L = 0.70-0.84 mm.; a = 39-46; b = 4.2-4.7; c = 34-38.

HOLOTYPE (female): L = 0.80 mm.; a = 33; b = 4.2; c = 38; V = 67.

ALLOTYPE (male): L = 0.84 mm.; a = 40; b = 4.6; c = 35.

DESCRIPTION: FEMALE (holotype): Body cylindroid, blunt at extremities and forming a 'C' shape when relaxed. Cuticle and subcuticle apparently smooth. Lateral chords half of body width. Lip region set off, one third as wide as neck base and forming a labial disc around vestibule. Amphids cup-like, their apertures about half as wide as head. Spear length one and one half times of head width. Stiffening piece about one third of spear length (very faint or indiscernible in two paratypes). Extensions less than half spear length, poorly knobbed. Stoma faint; guiding ring difficult to observe. Length of basal esophageal bulb about twice its width and one ninth of neck length. Nerve ring above middle of anterior slender part of esophagus. Cardia hemispheroid. Vulva a transverse slit; vagina less than half body width and with thick cuticular walls. Anterior sexual branch normal, ovary reflexed. Posterior uterine sac about corresponding body width long (its length slightly variable in paratypes) packed with sperms. Sperms also present in normal uterine branch. Rectum length more than one anal body width. Prerectum length about twice anal body width (up to four anal body widths long in some paratypes). Tail slightly conoid to rounded terminus (markedly conoid in one paratype). No caudal pores observed in lateral or dorsoventral views.

MALE (allotype): Similar to female in general morphology. Testes dorylaimoid. Supplements an adanal pair and a single ventromedian one located well above range of spicules. Spicules dorylaimoid, 20 microns long. Lateral guiding pieces present. Prerectum length about six anal body widths. Tail bluntly conoid, about one and one half of anal body widths long.

HOLOTYPE, ALLOTYPE AND PARATYPES: Collected on August 7, 1963; deposited in Zoology Museum, Aligarh Muslim University, Aligarh, India.

SUMMARY

Dorella n. gen. (Nematoda: Dorylaimoidea) has short ventral stiffening piece on spear, poorly knobbed spear extensions, set off basal esophageal bulb, anterior sexual branch normal, posterior rudimentary and only a single ventromedian supplement located well above spicules.

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**The Life History of *Protospirura numidica*
Seurat, 1914 (Nematoda: Spiruroidea)***

JAMES R. CROOK AND ALBERT W. GRUNDMANN

ABSTRACT

The life cycle of *Protospirura numidica* Seurat, 1914, in the Bonneville Basin, Utah, is shown to include *Eleodes tuberculata patralis* Blaisdell, 1918, as the natural intermediate host. This life cycle, under insectary conditions of 25°C. and 50% relative humidity, required approximately 40 days to develop to an infective larval stage in the intermediate host and worms in the stomachs of deer mice required approximately 36 days to commence egg production.

Life history studies on *Protospirura numidica* Seurat 1914 were conducted by the authors as part of a larger study organized to determine the influence played by intermediate hosts on the ecological distribution of a number of parasitic species throughout the major habitats of the Bonneville Basin, Utah.

Seurat (1914) established the genus *Protospirura* with *P. numidica* from the stomach of *Felis ocreata* Bate 1905, as the type species. Later, Schuurmans-Steekhoven (1937) described *Spiroxys gedoelsti* from the same host but in 1938 made this form a synonym of *P. numidica*.

In the Bonneville Basin of western Utah, *P. numidica* has been found to parasitize eight mammal species. Butler and Grundmann (1954) reported this species from the small intestine of the coyote, *Canis latrans lestes* Merriam. Later work, Grundmann, 1957, and Grundmann and Frandsen, 1960, has reported *P. numidica* to be present in the stomachs of the common deer mice, *Peromyscus maniculatus sonoriensis* Le Conte, and *P. m. rufinus* Merriam; the canyon mouse, *P. crinitus pergracilis* Goldman; the pinion mouse, *P. truei nevadensis* Hall and Hoffmeister; the grasshopper mouse, *Onychomys leucogaster utahensis* Goldman; two subspecies of the Ord kangaroo rat, *Dipodomys ordii celeripes* Durrant and Hall, and *D. o. marshalli* Goldman; the harvest mouse, *Rheithrodontomys m. megalotis* (Baird), and the least chipmunk, *Eutamias minimus pictus* Allen.

The life histories of several species of *Protospirura* have been studied. Leuckart (1867) pioneered in helminth life cycles by demonstrating for the first time the role of insects as intermediate hosts and vectors of parasites with his elucidation of the life cycle of *P. muris* (Gmelin, 1790). In his research he discovered that the nematode cysts would develop in meal worms (*Tenebrio*) after they had eaten *P. muris* ova. The infective cycle was completed and adults produced when the infected meal worms were eaten by *Mus decumanus*. Miyata (1939) demonstrated that the life cycle of *P. muris* could be completed using cockroaches, *Xenopsylla* (rat fleas), and *Ceratophyllus* (skin moth) as intermediate hosts. Foster (1939) using opossums as definitive hosts, found that cockroaches would serve as suitable intermediate hosts for *P. muricola* Gedoelst, 1916. Brumpt (1931) worked out the life cycle of *P. bonnei* Ortlepp, 1924, from rats. He discovered that *Rhypparobia maderae*, *Blatta germanica*, and *Periplaneta orientalis* (cockroaches) served as suitable intermediate hosts for this parasite. Cram (1926) found the larvae of

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This study was supported by National Science Foundation Grant No. G23398.

P. columbiana Cram, 1926, encysted in *Blatella germanica*. Hall (1929) showed the intermediate host of *P. gracilis* Cram, 1924, to be *Aphodius fmetarius*. With the exception of the studies reported by Hall, all life history investigations were carried out using common laboratory-reared insects which probably are not the hosts of these species under natural conditions.

MATERIALS AND METHODS

Protospirura numidica eggs used in these experiments were obtained from naturally infected deer mice, *Peromyscus maniculatus sonoriensis* (LeConte, 1853) and *P. m. rufinus* (Merriam, 1890) both collected from the Bonneville Basin, Utah (Fig. 1). Mouse feces containing parasite eggs were fed to 15 species of native arthropods which occurred in association with the definitive hosts in their natural habitats of the Bonneville Basin. These species were selected from the arthropod fauna of the area because they either lived in or around rodent burrows where access was had to host feces, or upon trial, indicated a preference for this food in the diet. Species tested were *Eleodes hispilabris sculptilis* Blaisdell, 1909, *E. nigrina* LeConte, 1858, *E. omissa pygmaea* Blaisdell, 1909, *E. tuberculata patralis* Blaisdell, 1918, *Iphthimus lewisi* Horn, 1870, *Carabus taedatus oregonensis* LeConte, 1854, *Chlaenius leucoscelis* Chevrolat, 1834, *Cymindis unicolor* Kirby, 1837, *Discoderus amoenus* LeConte, 1863, *Harpalus* (*Lasioharpalus*) *fraternus* LeConte, 1884, *Pterostichus* (*Hypherpes*) *protractus* LeConte, 1860, *Silpha* (*Thanatophilus*) *lapponica* Herbst, 1793, *Ceuthophilus utahensis* Thomas, 1876, (*Utahensis* group), *Melanoplus femur-rubrum* DeGeer, 1773, and *Hippiscus corallipes* Haldeman, 1852. Four common laboratory arthropods, *Tribolium confusum* Duval, *Tenebrio molitor* (Linné), *Blatta orientalis* Linné, and *Gryllus* sp., were also fed infected feces.

Ten specimens of each species were used as controls to determine if natural infections were present; 10 each were fed feces containing *P. numidica* eggs. Because rodent hosts are coprophagic, other groups of each arthropod species were fed "recycled" feces containing eggs which were being passed through deer mice a second time. Each insect group was placed in a covered container with the infective fecal meal for a period of 10 days after which the arthropods were fed poultry laying mash for the remainder of the test period. Tests were conducted in a constant temperature-humidity insectary at 25° C. and 50% relative humidity. To follow the course of possible infection, one specimen of each insect species and one control were dissected at ten day intervals. Infected beetles were later fed to uninfected deer mice. Also, infective larvae isolated from the beetles were fed via pipette to young, parasite-free *Peromyscus maniculatus*. These mice were checked daily for eggs commencing 10 days after feeding. Developing parasite larvae were preserved in AFA (Van Cleave, 1953), dehydrated, cleared in oil of cloves, transferred to toluene, and mounted in Permount (Fisher).

RESULTS

Tests to determine the intermediate host or hosts of *Protospirura numidica* were begun on 6 August 1963. Ten days after exposure, test dissections of one member of each species revealed larval worms and small cysts in the hemocoel of *Eleodes tuberculata patralis*, both those fed "normal" and "recycled" feces. The cysts observed were irregularly spherical and were located among the malpighian tubules. These cysts measured 222.4 to 291.9 microns in diameter and contained one to two larval worms. Average measurements

of 11 of the free larval worms were 112 microns long and 8.4 microns wide. Gastrointestinal contents of *E. tuberculata patralis* revealed numerous *P. numidica* eggs with and without larval worms inside, and free larvae. Six of these larvae measured approximately 101 microns long and 8.2 microns wide (Fig. 2). Dissection of other test insect species and controls revealed no infection. Twenty days after exposure, the cysts in *E. tuberculata patralis* had grown to 382 to 394 microns in diameter while maintaining the irregular spherical form previously noted. No free worms were observed in the hemocoel at this time. A number of these cysts were fed to laboratory reared uninfected mice without achieving infection, indicating that the larval worms were not mature. Other insect species tested, again proved negative. Thirty days after exposure, the cysts in *E. tuberculata patralis* had grown to 552 to 559 microns in diameter, and again these cysts were fed to mice without resulting in an infection. The remaining insect species being tested and the controls revealed no infection. Forty days after exposure, the cysts from *E. tuberculata patralis* had reached 630 to 638 microns in diameter (Fig. 3) and were mostly free in the thoracic and abdominal hemocoel with only a few adhering to muscles and malpighian tubules. The hemocoels of dissected specimens were completely congested with cysts. Approximately 25 of these cysts were fed to two mice via pipette and 34 days later, the first eggs were passed in the feces. The remaining insect species being tested and the unexposed controls remained negative. Subsequent insect dissections 50 and 60 days after exposure revealed that no further cyst growth had taken place in *E. tuberculata patralis* which indicates that little growth occurs after the cyst matures and that the larvae pass into a quiescent state. One control, *E. tuberculata patralis*, collected from the Tushar Mountains in southern Utah, and dissected on 1 October, produced one fully developed *P. numidica* cyst in the abdominal hemocoel indicating a natural infection. Two laboratory infected *E. tuberculata patralis* were fed to each of two mice and were readily ingested except for the forewings and legs. Following this feeding, the mice feces were checked daily for ova. After 36 days, one mouse passed the first *P. numidica* ova in its feces while the second did not begin to pass ova until 39 days later. One mouse was force-fed cysts via pipette and died in two hours due to some of the material being aspirated into its lungs. The stomach of this animal was examined immediately after the mouse died, and revealed numerous excysted immature worms. These worms measured 3.0-3.8 mm. long and 97 microns wide (Fig. 4).

On 11 November 1963, a new group of 25 *E. tuberculata patralis* were fed feces containing numerous *P. numidica* eggs. Ten days later, one beetle was dissected with the same results obtained in the first experiment. Dissections 20, 30, and 40 days after exposure resulted in findings similar to those from the previous experiment. Mature cysts from the remaining beetles used in this test were fed to mice in order to establish a laboratory colony.

Tests on common laboratory arthropods showed that in *Tribolium confusum* there was only partial development of *P. numidica* larvae. Larval worms of this parasite were observed to be released from eggs in the gut of this beetle and to penetrate into the abdominal hemocoel in the same manner as is accomplished in the proved intermediate host. Cyst development continued normally for 10 to 15 days, but failed to develop beyond this point, resulting in a degenerate cyst. Other species listed in Materials and Methods were negative.

DISCUSSION

Protospirura numidica is widespread and may be found present in all major habitats from the vegetated dunes of the Great Salt Lake Desert at 4200 feet to the alpine tundra at 11,500 feet. Since the deer mouse is ubiquitous throughout the range, has an eight percent infection rate in the region and has been colonized for laboratory work, this species was selected as the donor for infective eggs and as the host animal for the mature larval stages obtained from laboratory infected intermediate hosts.

Time intervals of the *Protospirura* spp. life cycles are typified in the sequence given by Cram (1926) for *P. columbiana*. The intermediate host (*Blatella germanica*) ingested the eggs and in 41 days infective cysts were obtained, as compared with approximately the same result in *P. numidica* from *Eleodes tuberculata patralis*. Infective *P. columbiana* cysts develop into sexually mature worms in the stomachs of white rats after about 115 days, as compared with about 36 days for *P. numidica* to become sexually mature in the stomachs of deer mice.

It is significant that but one of the insect species commonly associated with rodent burrows was found to be the intermediate host for *P. numidica*, and it is of further importance that this beetle species was found distributed throughout the range of habitat, from desert to alpine tundra, of the region. This wide distribution and apparent ecological tolerance to extremes of heat and cold can be readily explained when one considers the somewhat uniform conditions of humidity and temperature existing in burrows regardless of elevation or external conditions. The pattern of intermediate and definitive host infection adds further evidence to the statements of Baylis (1924) that hosts having broad host range and tolerance as adults will have a narrow range and tolerance among potential intermediate hosts.

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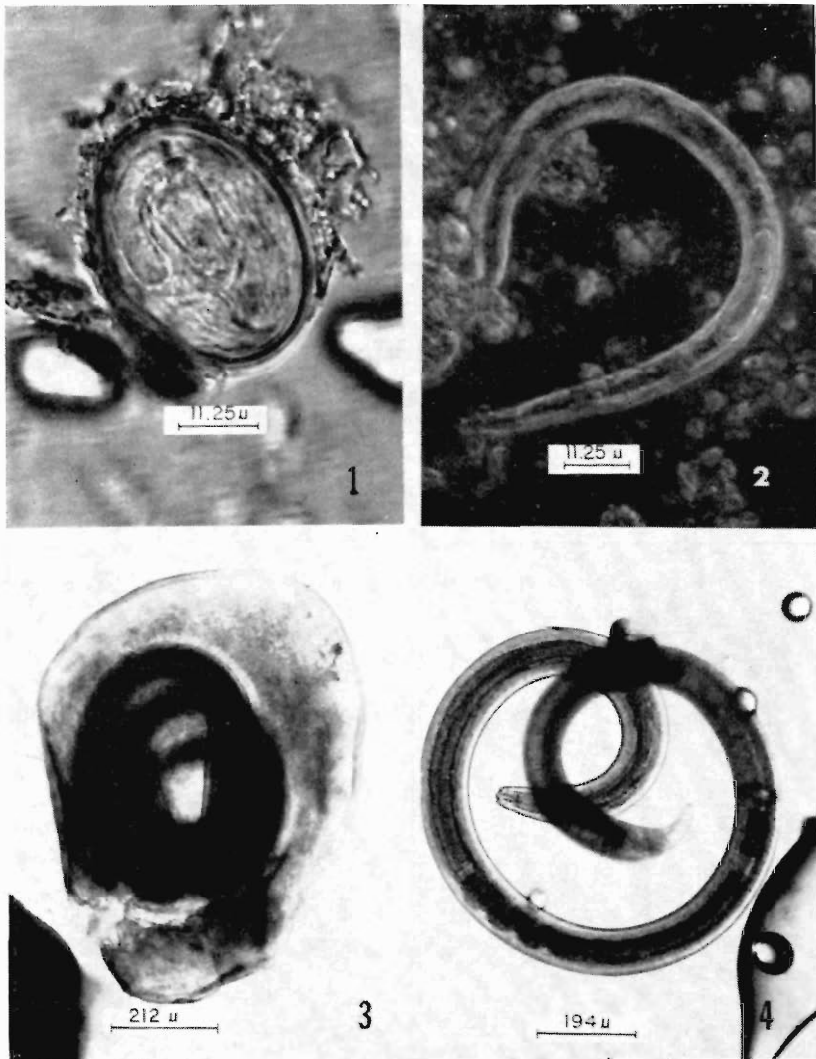


Fig. 1. *Protospirura numidica* egg.

Fig. 2. *P. numidica* larval worm after release from egg, recovered from the gut of *Eleodes tuberculata patralis*.

Fig. 3. Infective cyst of *P. numidica* from the abdominal hemocoel of *Eleodes tuberculata patralis*.

Fig. 4. Excysted larval worm of *P. numidica*, three hours after ingestion, from the stomach of *Peromyscus maniculatus sonoriensis*.

A Monogenetic and Seven Digenetic Trematodes of Amphibians and Reptiles from Palawan Island, Philippines*

JACOB H. FISCHTHAL and ROBERT E. KUNTZ**

The flukes of this report were part of a collection of parasites made by the junior author while a member of the U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan, and serving as a guest investigator on the Silliman University-Bishop Museum Expedition to Palawan Island, Republic of the Philippines. Parasites were washed in saline, killed in hot water and transferred immediately to FAA fixative. After 4-8 hours they were stored in 70 percent alcohol plus 2 percent glycerine. Staining was in Harris' hematoxylin, carmalum and fast green, or Gower's carmine. All were mounted in balsam. Measurements are in microns.

FAMILY POLYSTOMATIDAE

Polystomoides cyclemydis n. sp. (Figs. 1, 2)

HOST: *Cyclemys dentata* (Testudinidae).

HABITAT: Large intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 25 May 1962.

TYPES: USNM Helm. Coll. No. 60192 (1 slide of holotype and 1 of paratype).

DIAGNOSIS (based on 2 specimens): Body proper (without haptor) 1,733 to 2,178 by 859 to 882 (ovarian level). Haptor 706 to 851 by 815 to 994; bearing 6 cuplike suckers 215 to 295 by 225 to 295, suckers more or less equidistant from one another; armed with 2 pairs of large hooks between posterior pair of suckers and varying number of larval hooklets; outer pair of large hooks 72 to 91 by 5 to 6, shaft wider at beginning two fifths, next two fifths narrower and uniformly wide almost to hook, then narrowing abruptly and gradually tapering to curved, sharp pointed tip; inner pair of large hooks 32 to 47 by 4 to 5; larval hooklets 17 to 23 (longitudinal extent), 1 in each sucker of both specimens, remainder lost in paratype while 4 present posteromedial to anterior pair of suckers in holotype. Eyespots absent. Oral sucker 242 to 295 by 324 to 390. Pharynx 222 to 265 by 242 to 310, wider than long; esophagus very short; ceca extending to posterior end of

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This work was supported in part by funding under Public Law 480, Section 104(c) and in part by ARPA Appropriation 97x0400.1304.

From Bureau of Medicine and Surgery, Navy Department, Research Task MR 005.09.-1606.

The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Navy Department or the Naval Service at large.

The authors are indebted to Prof. D. S. Rabor, Silliman University, Dumaguete, Negros Oriental, leader of the Silliman University-Bishop Museum Expedition, for general support in field activities; also, to Mr. Max Thompson, Bishop Museum, for assistance in collecting hosts, to Dr. William E. Duellman, Associate Curator, Museum of Natural History, University of Kansas, and Dr. Alan E. Leviton, California Academy of Sciences, for host identifications, to Mr. Chin-tsung Lo, Museum of Zoology, University of Michigan, for assistance in host examinations, to Mrs. R. Grace Hsu, The George William Hooper Foundation, University of California, San Francisco, for technical assistance in preparation of parasites for study, to Miss Janet E. Brown, Associate Librarian, Harpur College, State University of New York, for obtaining interlibrary loans, and to Dr. George R. LaRue and Dr. Allen McIntosh, Beltsville Parasitological Laboratory, U.S. Department of Agriculture, for comments on the specimens of *Meristocotyle*. Special recognition is due Captain Robert A. Phillips, MC, USN, Commanding Officer, U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan, for continuous scientific, administrative and financial support which enabled the staff of its Parasitology Department to take part in field studies in the Philippines.

body proper. Testis 340 to 350 by 210 to 270, median, at beginning of posterior half of body proper; pharynx to testis 460 to 468; posttesticular space of body proper 490 to 850. Seminal vesicle 136 to 150 by 65 to 85. Cirrus sac 99 to 136 by 92 to 106; pharynx to cirrus sac 84 to 111. Genital pore median, immediately postbifurcal. Genital coronet of 32 hooks in both specimens; hooks of two alternating size ranges, larger ones 30 to 37 long, smaller ones 23 to 24 long, width of both nearly uniform. Ovary 126 to 182 (longitudinal extent) by 92 to 97, comma shaped to elongate oval, sinistral; pharynx to ovary 276 to 322. Vaginal openings ventral, near body margins, at level of posterior part of ovary. Genito-intestinal canal opening into cecum on ovarian side. Vitellaria extending from pharynx to posterior end of body proper, filling space not occupied by ceca and reproductive structures. Uterus in each specimen with 1 egg, 232 by 181 and 242 by 162.

DISCUSSION: This monogenetic trematode appears closest to *P. ocellatum* (Rud., 1819) Ozaki, 1935. Yamaguti (1963) noted the latter's occurrence in several genera of turtles in Europe and in *Clemmys japonica* in Japan. *P. cyclemydis* differs from *P. ocellatum* in the shape of the outer pair of large haptoral hooks; Strankowski (1937) and Palombi (1949) illustrated them for the latter species. Also, although the genital hooks of *P. ocellatum*, as noted by Strankowski, are of 2 size ranges (50 to 55 and 34 to 36 microns, respectively), they are much larger than for our species.

FAMILY MESOCOELIDAE

Mesocoelium sociale (Lühe, 1901) Odhner, 1911

SYNONYMS: *Distomum sociale* Lühe, 1901; *Mesocoelium meggitti* Bhalerao, 1927.

HOST: *Bufo biporcatus philippinicus* (Bufonidae).

HABITAT: Small intestine, and rarely in liver.

LOCALITY: Puerto Princessa, Palawan Island, Philippines.

DATES: 21 and 23 May 1962.

SPECIMENS: USNM Helm. Coll. No. 60193 (4 slides with 1 specimen each).

MEASUREMENTS and some pertinent data (based on 26 specimens, 8 measured): Body 2,383 to 2,840 by 913 to 1,135; forebody 598 to 759, hindbody 1,610 to 1,894; preoral body 37 to 85 long; ventral pit opening anterior to oral sucker, extending dorsal to latter; spines usually on anterior body half but may extend to posterior end, very sparse posteriorly, spines on dorsal surface of preoral body but absent laterally and ventrally; oral sucker 224 to 298 by 199 to 265, acetabulum 152 to 224 by 147 to 222, sucker length ratio 1:0.68 to 0.83; prepharynx 18 to 26 long; pharynx 81 to 132 by 88 to 136; esophagus 22 to 81 long; ceca extending posterior to vitellaria; right testis 166 to 250 by 123 to 232, left testis 155 to 232 by 129 to 272; testes usually at acetabular level but anterior in 1 and posterior in 2, usually overlapping only 1 cecum but sometimes both or entirely intercecal; vas deferens usually relatively long, sometimes short; parts of vasa efferentia, their junction, and/or vas deferens may be inflated into small vesicles; cirrus sac 186 to 314 by 74 to 110, posterior end overlapping acetabulum as much as 25 or up to 44 anterior to it; posterior chamber of bipartite internal seminal vesicle 93 to 158 by 63 to 94, anterior chamber 41 to 70 by 38 to 52; prostatic vesicle present, 28 to 43 by 21 to 30; cirrus 32 to 87 by 9 to 15, straight, slightly thick walled and muscular; genital pore median in 3, left of midline in 9, right of midline in 12, usually at pharyngeal level but sometimes just anterior to posterior margin of oral sucker or up to 118 posterior to latter at

esophageal level; ovary 155 to 245 by 173 to 265, dextral in 11, sinistral in 13; seminal receptacle 72 to 166 by 77 to 115, anteromedial to ovary, overlapping it dorsally; vitellaria usually interrupted on ovarian side opposite latter and/or testes; metraterm usually straight, 224 to 422 long, usually slightly longer than cirrus sac, commencing at acetabular level, ascending on side opposite ovary; 33 eggs measuring 32 to 37 by 21 to 24.

DISCUSSION: Our partial description provides some previously unrecorded data. Much morphological variation is evident. As *M. sociale* this form has been reported from toads, *Bufo melanostictus* from India (Lühe, 1901; Sewell, 1920), Indonesia (Odhner, 1911), and Burma (Meggett, 1927; Bhalerao, 1936; Chatterji, 1940), *B. crucifer* from Brazil (Travassos, 1924), and *Bufo* sp. from Paraguay (Odhner, 1911); from frogs, *Rana trigrina* from Burma (Meggett, 1927; Bhalerao, 1936); and from snakes, *Ptyas mucosus* from Burma (Chatterji, 1940). As *M. meggitti* it has been reported from lizards, *Mabuia dissimilis* from Burma (Bhalerao, 1927; Chatterji, 1940), and *M. multifasciata* from Luzon Island, Philippines (Tubangui, 1931). Chatterji (1940) declared *M. meggitti* a synonym of *M. sociale*; we concur. Dollfus (1954), on the basis of geographical distribution, questioned the presence of *M. sociale* in South America inasmuch as its hosts are neither migratory nor transported by man or birds. Babero and Okpala (1962) in noting considerable morphological variation in *M. monodi* Dollfus, 1929, indicated its probable synonymy with *M. sociale*. It is possible that Dollfus' (1954) arguments also may be applicable in the latter instance. Yamaguti (1958) listed *M. sociale*, *M. meggitti*, and *M. monodi* as distinct species as did Skrjabin and Morozov (1959) and Cheng (1960) in their reviews of *Mesocoelium*. The key given by the latter is unworkable as our specimens, depending on the combination of varying characteristics, could be keyed to *M. sociale*, *M. microon* Nicoll, 1914, *M. meggitti*, *M. monodi*, *M. americanum* Harwood, 1932, and *M. megaloon* Johnston, 1912. Extensive life history studies are necessary to determine the extent of synonymy in *Mesocoelium*.

FAMILY PLAGIORCHIIDAE

Haematoleechus sibiricus (Issaitschikoff, 1927) Ingles, 1932

SYNONYM: *Pneumonoeces sibiricus* Issaitschikoff, 1927.

HOST: *Oecidozyga laevis laevis* (Ranidae).

HABITAT: Small intestine (probably should be lungs).

LOCALITY: Tarabanan Concepción, Palawan Island, Philippines.

DATE: 12 May 1962.

SPECIMEN: USNM Helm. Coll. No. 60194.

MEASUREMENTS (1 specimen): Body 3,819 by 287 at anterior margin of vitellaria and 636 at seminal receptacle; forebody 1,395, hindbody 2,262, posttesticular space 944, postvitellarian space 491; oral sucker 139 in diameter, acetabulum 162 by 126, sucker length ratio 1:1.16; prepharynx 34 long; pharynx 81 by 88; anterior testis 429 by 221, posterior testis 394 by 243; acetabulum to anterior testis 493, to posterior testis 876; ovary 228 by 245, overlapping acetabulum; seminal receptacle 291 by 239, 184 postacetabular; vitellaria extending 865 preacetabular and 1,771 postacetabular; right uterine fold extending 383 and left 356 preacetabular; 10 eggs measuring 19 to 21 by 12 to 15.

DISCUSSION: Odening (1958) reviewed the subfamily Haematoleechinae Freitas and Lent, 1939 (syn. Pneumonoecinae Mehra, 1937), listing 4 sub-

species of *H. sibiricus* from Siberia, China, Korea, and Japan. Skrjabin and Antipin (1962) also reviewed the subfamily, accepting these subspecies.

FAMILY DICROCOELIIDAE

Paradistomum gregarium Tubangui, 1929

SYNONYMS: *Paradistomum magnum* Tubangui, 1928, nec Travassos, 1919; *Paradistomoides gregarium* (Tubangui, 1929) Travassos, 1944.

HOST: *Hemidactylus frenatus* (Gekkonidae).

HABITAT: Gall bladder.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 22 May 1962.

SPECIMENS: USNM Helm. Coll. No. 60195 (4 slides with 1 specimen each).

MEASUREMENTS and some pertinent data (based on 8 young and old adults from 1 host, 7 measured): Body 891 to 2,293 by 440 to 1,219; forebody 294 to 498, hindbody 464 to 1,322; preoral body present or not; oral sucker 128 to 250 by 123 to 228, acetabulum 133 to 158 by 144 to 247, sucker length ratio 1:0.92 to 1.06; pharynx 52 to 107 by 57 to 104; esophagus 29 to 129 long; right testis 79 to 158 by 88 to 247; left testis 79 to 199 by 76 to 206; cirrus sac 112 to 236 by 54 to 95; muscular cirrus protrusible; genital pore to oral sucker 32 to 155, to acetabulum 101 to 235; ovary 66 to 206 by 98 to 261; seminal receptacle 49 to 103 by 49 to 107, dorsal to ovary; metraterm present; 26 eggs measuring 29 to 40 by 17 to 26.

DISCUSSION: This species was originally described by Tubangui (1928)

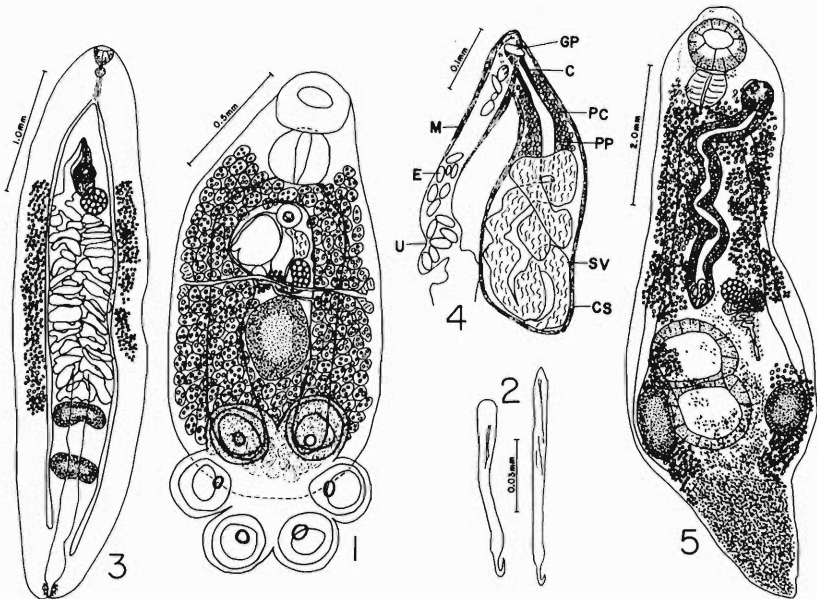


Fig. 1. *Polystomoides cyclemydis*, holotype, ventral view.

Fig. 2. Same. Outer large haptor hooks; from holotype on left, paratype on right.

Fig. 3. *Telorchis philippinensis*, holotype, dorsal view.

Fig. 4. Same. Terminal genitalia of holotype, dorsal view. C, cirrus; CS, cirrus sac; E, egg; GP, genital pore; M, metraterm; PC, prostate cells; PP, pars prostatica; SV, seminal vesicle; U, uterus.

Fig. 5. *Meristocotyle varani*, holotype, ventral view.

from the same host from Luzon Island, Philippines, and redescribed by Bhalerao (1929) from *Hemidactylus gleadori* from Burma. Much variation is evident in our specimens: oral sucker terminal or subterminal, smaller or larger than acetabulum; testes symmetrical to slightly oblique, smooth to lobed, same size or smaller or larger than ovary, right testis same size or smaller or larger than left testis; posterior end of cirrus sac preacetabular or at anterior margin of acetabulum or overlapping latter; ovary smooth to lobed; vitellaria commencing at testicular level or pretesticular. Baer (1957) listed *Paradistomoides* Travassos, 1944, as a synonym of *Paradistomum* Kosack, 1910. Skrjabin and Evranova (1953), reviewing the family, and Yamaguti (1958) considered them distinct. As a result of study of various populations of *Paradistomum orientalis* Narain and Das, 1929, Arora and Agarwal (1960) and Arora, Agarwal and Agarwal (1962) also concluded that *Paradistomoides* was invalid. The latter authors presented an expanded diagnosis of *P. orientalis* in which *P. moghei* Bhalerao, 1936, and *P. banaransensis* Bangh, 1956, were considered synonyms. As examples the following species also would fit this diagnosis: *Paradistomum mutabile* (Molin, 1859) Dollfus, 1922, *P. gregarium*, *P. geckonum* Bhalerao, 1929, *P. palaoensis* Tubangui, 1933, *P. excalotes* Tubangui and Masilungan, 1936; *Paradistomoides orotermosum* (Bhalerao, 1929) Travassos, 1944, *P. intestinalis* Simha, 1958, *P. lanceolatus* Simha, 1958, *P. spatulatus* Simha, 1958. Probably other species would also fit. Extensive life history studies are necessary to determine the extent of synonymy in *Paradistomum*.

Euparadistomum varani Tubangui, 1931

SYNONYM: *Platynotrema varani* (Tubangui, 1931) Chatterji, 1948.

HOST: *Varanus salvator* (Varanidae).

HABITAT: Gall bladder.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 22 May 1962.

SPECIMEN: USNM Helm. Coll. No. 60196.

MEASUREMENTS and some pertinent data (1 specimen): Body 2,945 by 1,917; forebody 1,479, hindbody 943; oral sucker 843 long, acetabulum 523 by 548, sucker length ratio 1:0.62, acetabulum at level of posterior two thirds of body length; pharynx 158 by 170; esophagus 85 long; right testis 275 by 188, left testis 230 in diameter; cirrus sac 237 by 102, thin walled, anterior-most margin 26 anterior to genital pore, containing thin walled, tubular, slightly winding seminal vesicle, a short, cell lined pars prostatica surrounded by prostate cells, and a thick walled, muscular, slightly winding cirrus 109 (longitudinal extent) by 27; anterior margin of genital atrium lying 18 posterior to anterior margin of cirrus sac and 8 anterior to genital pore; genital pore bifurcal (probably postbifurcal as anterior end of body somewhat contracted forcing bifurcation posteriorly), pharynx to genital pore 70, latter to acetabulum 567; gland cells surrounding genital atrium and anterior part of cirrus sac; ovary 255 by 195; seminal receptacle 125 by 90; metraterm quite muscular, ventral to cirrus sac, opening into genital atrium posterior to cirrus opening; 15 operculate eggs measuring 43 to 55 by 22 to 24; excretory bladder Y-shaped, main stem bifurcating 200 postovarian, arms extending anterolaterally dorsal to ceca to ovarian level; excretory pore surrounded by conspicuous sphincter muscle.

DISCUSSION: This form was first described from the same host from Luzon Island, Philippines. Capron, Deblock and Brygoo (1961) reported *E. varani*

var. *madagascariensis* from *Chamaeleo* spp. from Madagascar. Our partial description of this parasite provides some previously unrecorded data. In our specimen the acetabulum is situated posterior to the middle of the body length rather than being equatorial. Chatterji (1948, 1952) declared *Euparadistomum* Tubangui, 1931, a synonym of *Platynotrema* Nicoll, 1914, stating that the preacetabular extension of the uterine coils was not of generic significance. Baer (1957) also listed them as synonymous. Buckley and Yeh (1958) and Singh (1958) considered *Euparadistomum* valid, noting the significance of the preacetabular extension of the uterine coils in the systematics of digenetic trematodes. Skrjabin and Evranova (1953) and Yamaguti (1958) considered them distinct genera. From the description of *Euparadistomum* by Tubangui (1931) and *Platynotrema* by Nicoll (1914) we agree that the former is valid in possessing a Y-shaped excretory bladder, whereas the latter has a tubular one.

FAMILY TELORCHIIDAE

Telorchis philippinensis n. sp. (Figs. 3, 4)

HOST: *Cyclemys dentata* (Testudinidae).

HABITAT: Stomach.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 25 May 1962.

TYPES: USNM Helm. Coll. No. 60197 (1 slide of holotype and 2 slides with 1 paratype each).

DIAGNOSIS (based on 3 specimens): Body 3,919 to 4,418 by 828 to 1,074, elongate, widest at body middle, extremities round. Cuticle thick, spined; spines quincuncially arranged, terminating at posterior testis level; anteriorly spines wider, coarser, numbers greater; progressing posteriorly spines becoming longer, thinner, more pointed, distribution sparser. Forebody 889 to 920, hindbody 2,846 to 3,321, ratio 1:3.20 to 3.61; posttesticular space 744 to 966. Short preoral body present or not. Oral sucker 140 to 153 by 156 to 160; acetabulum 175 to 184 by 170 to 188, at about anterior body fourth; sucker length ratio 1:1.20 to 1.26. Prepharynx 29 to 33 long; pharynx 92 to 102 by 85 to 92, round to longer than wide, surrounded by gland cells; esophagus 160 to 191 long, muscular, surrounded by gland cells; cecal bifurcation 455 to 525 preacetabular; ceca narrow, internally lined with conspicuous cells, terminating variably in posttesticular space 300 to 545 from posterior extremity. Excretory bladder Y-shaped; main stem tubular to saccular, extending almost to ovary; narrow, muscular canal surrounded by gland cells leading from bladder to terminal pore.

Testes 2, tandem, smooth to slightly lobed, much wider than long, in posterior three fourths to overlapping posterior fourth of body, may very slightly overlap cecum ventrally; anterior testis 170 to 222 by 335 to 453, posterior testis 195 to 261 by 345 to 422; testes contiguous in 1, 152 and 184 apart in 2. Cirrus sac 441 to 445 (longitudinal extent) by 107 to 147, thick walled, muscular, much wider posteriorly, arching to right over acetabulum, extending from 130 to 161 preacetabular (genital pore) to 92 to 129 postacetabular (ovarian level), may overlap ovary dorsally, containing seminal vesicle, pars prostatica, prostate cells, and cirrus. Seminal vesicle tubular, much coiled, longitudinal extent 273 to 335. Pars prostatica thick walled, slightly muscular, straight, surrounded by prostate cells. Cirrus short, straight, thick walled, muscular. Genital pore slightly submedian to left, 130 to 161 preacetabular, 294 to 395 postbifurcal.

Ovary 200 to 225 by 175 to 221, round to slightly elongate, 6 to 38 post-acetabular, right of midline next to cecum, in posterior end of anterior body third, anterior extremity to ovary 1,077 to 1,135, ovary to anterior testis 1,419 to 1,480. Ootype complex large, posteromedial to ovary. Vitelline follicles in lateral fields, extracecal, few follicles overlapping ceca ventrally; vitellaria usually commencing at level of posterior margin of acetabulum to 38 anterior to this margin, level of right field may vary from left; vitellaria ending at level of anterior testis to 590 pretesticular, level of right field varying from left. Uterus intercecal, few loops may slightly overlap ceca ventrally, descending on right, ascending on left, between acetabulum and anterior testis. Metraterm 213 to 245 long, thick walled, entirely preacetabular or may extend to middle of acetabulum, about half as long as cirrus sac, ascending left of latter. Eggs numerous, operculate, 15 measuring 30 to 38 by 12 to 16.

DISCUSSION: In one of our specimens the oral sucker was absent, the anterior end of the body appearing normal in all other respects; the mouth opened into the pharynx. In another specimen the left vitelline field commenced 38 microns anterior to the posterior margin of the acetabulum, whereas the right field commenced 268 microns posterior to this margin (37 microns postovarian).

Telorchis philippinensis appears closest to *T. clemmydis* Yamaguti, 1933 (syn. *Paracercorchis megacotyle* Fukui and Ogata, 1933) from *Clemmys japonica* and *Geoclemmys reevesi* from Japan. It differs from the latter in having a metraterm considerably shorter than the cirrus sac and not extending postacetabular, the posttesticular space about twice as long, the genital pore farther preacetabular, the vitellaria commencing at the acetabular level, the ovary much closer to the acetabulum, and in lacking pharyngeal lappets. Skrjabin and Antipin (1963) reviewed the family.

FAMILY ?

Meristocotyle n. gen.

DIAGNOSIS: Body large, cuticle thick, unarmed. Oral sucker subterminal, surmounted by preoral lobe. Prepharynx, pharynx, and esophagus present. Ceca terminating short distance postacetabular. Acetabulum divided internally into separate, contiguous, muscular, anterior and posterior portions enclosed by common rim. Excretory pore terminal; bladder tubular, extending almost to cecal bifurcation. Testes 2, symmetrical, on each side of posterior acetabulum. Cirrus sac preacetabular, elongate, extending to pharyngeal level; bipartite, separated by transverse septum, seminal vesicle and pars prostatica in long posterior portion, cirrus and retracted genital atrium in short anterior portion. Genital atrium with outer longitudinal and inner circular muscles; sphincter at each end. Genital pore sinistral to pharynx. Ovary anterolateral to anterior acetabulum. Mehlis' gland well developed. Seminal receptacle and Laurer's canal present. Vitelline follicles in lateral fields, confluent just postpharyngeal, interrupted at anterior acetabular level. Uterus coiled in hindbody and dorsal to acetabulum. Eggs large, operculate, numerous. Adult parasitic in stomach (?), immature forms in lungs, of lizard.

Meristocotyle varani n. sp. (Fig. 5)

HOST: *Varanus salvator* (Varanidae).

HABITAT: Adult in stomach (may represent postmortem wandering); immature specimens in lungs.

LOCALITY: Tarabanan Concepción, Palawan Island, Philippines.

DATES: 12 (adult) and 14 (immature) May 1962.

SPECIMENS: USNM Helm. Coll. No. 60198 (1 slide of holotype adult, and 2 slides with 1 immature specimen each).

DIAGNOSIS (based on 1 adult and 4 immature specimens; adult measured): Body 9,452 by 2,709, widest at acetabulum; anterior end round, with prominent fleshy preoral lobe 184 by 626; posterior end tapering to blunt point. Cuticle thick, unarmed, forebody 5,161; hindbody 2,363. Parenchymal glands on each side of pharynx, ducts leading anteriorly to open on anteroventral surface of preoral lobe. In immature specimens pigment granules scattered in parenchyma from oral sucker to just postacetabular; none in adult. Oral sucker 782 by 828, subterminal. Acetabulum divided internally into separate, contiguous, muscular, anterior and posterior portions enclosed by common rim, anterior portion 1,008 by 1,273, posterior portion 1,005 by 1,254, longitudinal extent of acetabulum 1,928; surrounded by body fold in immature specimens. Prepharynx 75 by 440, glands on each side. Pharynx 499 by 541. Esophagus slightly elongate. Ceca short, terminating just postacetabular. Excretory pore terminal; in immature specimens bladder tubular but inflated from midhindbody to just preacetabular, extending almost to cecal bifurcation.

Testes 2, smooth, elongate oval, symmetrical, 1 on each side of posterior acetabulum; right testis 856 by 481, left testis 675 by 537; in immature specimens fundaments of testes in similar position. Cirrus sac 3,236 (longitudinal extent) by 391, thick walled, muscular; bipartite, with smaller anterior part (437 by 375) separated from much longer posterior part by thin, muscular septum; sinuous, commencing 140 preacetabular, ascending in midline to cecal bifurcation, then proceeding anterointrally, terminating sinistral to pharynx; containing seminal vesicle, pars prostatica, prostate cells, cirrus, and portion of genital atrium; in immature specimens fundament of cirrus sac present from level of pharynx to just postbifurcal. Seminal vesicle 339 (longitudinal extent) by 96, much coiled, tubular, thick walled, muscular, at proximal end of cirrus sac. Pars prostatica 2,623 (longitudinal extent) by 132; composed of outer, thin, longitudinal and middle, much thicker, circular muscles, and inner cell layer. Prostate cells dense, numerous, from midlevel of seminal vesicle to distal end of cirrus sac. Cirrus in anterior part of cirrus sac, muscular, protruding into genital atrium. Latter conspicuously muscular, composed of outer longitudinal and inner circular muscles, with sphincter at each end; atrium retracted into anterior part of cirrus sac, leaving thin walled channel leading to genital pore. Genital pore 100 posterior to anterior margin of cirrus sac, at its dextral margin.

Ovary 351 by 401, smooth, preacetabular, dextral, at level of proximal end of cirrus sac; in immature specimens ovary at level of anterior acetabulum. Oviduct from posterior end of ovary, muscular. Mehlis' gland 345 by 506, posterolateral to and slightly overlapping ovary. Seminal receptacle 265 (longitudinal extent) by 147, postovarian but slightly overlapping it dorsally. Laurer's canal sinuous, muscular, surrounded by gland cells, proceeding posteriorly to dorsal surface, opening sinistral to anterior acetabulum. Vitelline follicles mainly in lateral fields from postceally to pharynx, confluent just postpharyngeally, interrupted on left from ovarian level to short distance pretesticular and on right from anterior margin of acetabulum to level of anterior third of anterior acetabulum, dorsal to ceca, testes, parts of uterus and of cirrus sac; right and left vitelline ducts uniting ventral to

Mehlis' gland, forming small, thick walled reservoir 126 by 87. Uterus much coiled in hindbody and dorsal to acetabulum, descending on left, ascending on right; could not be traced preacetabularly to genital pore. Eggs numerous, yellow, operculate, 16 measuring 77 to 94 by 47 to 56.

DISCUSSION: The generic name, *Meristocotyle* (Gr. *meristos*, divided; Gr. *kotyle*, cup), refers to the internal division of the acetabulum, forming 2 portions. We are erecting a new subfamily for this genus, but can not place it into any known family.

MERISTOCOTYLINAE n. subfam.

DIAGNOSIS: Body large, cuticle thick. Oral sucker subterminal, surmounted by fleshy lobe. Prepharynx, pharynx, and esophagus present. Cecae terminating postacetabular. Acetabulum divided internally into separate, contiguous, muscular, anterior and posterior portions enclosed by common rim. Excretory pore terminal; bladder tubular, extending preacetabular. Testes symmetrical, at acetabular level. Cirrus sac long, preacetabular, divided by septum into long posterior portion containing seminal vesicle and pars prostatica, and short anterior portion containing cirrus and retracted, muscular genital atrium. Genital pore sinistral, at pharyngeal level. Ovary preacetabular, sinistral. Seminal receptacle and Laurer's canal present. Vitelline follicles in lateral fields, confluent postpharyngeal, interrupted at anterior acetabular level. Uterus coiled in hindbody and acetabular region. Eggs large, numerous.

FAMILY PARAMPHISTOMIDAE

Diplodiscus amphichrus Tubangui, 1933

SYNONYM: *Diplodiscus sinicus* Li, 1937.

HOST: *Rana cancrivora* (Ranidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princessa, Palawan Island, Philippines.

DATE: 24 May 1962.

SPECIMEN: USNM Helm. Coll. No. 60043

MEASUREMENTS and some pertinent data (1 specimen): Body 3,781 by 951; preoral body 70 long; oral sucker 305 by 353; oral diverticula 206 by 162 to 166; esophagus 379 long, surrounded by sparsely distributed gland cells, with slight muscular bulb; acetabulum 760 by 913; testis 460 by 560, slightly constricted in middle of its width; cirrus sac 119 by 121; ovary 350 by 324, smooth; cecal bifurcation to genital pore 422, to testis 890, to ovary 1,242; vitellaria confluent anteriorly and posteriorly, commencing at cecal bifurcation and terminating short of cecal ends, follicles irregular to round in shape, somewhat interrupted at testis level with 25 follicles anterior to interruption and 19 posterior; 8 eggs measuring 103 to 121 by 63 to 77.

DISCUSSION: This parasite, first described by Tubangui (1933) from *Rana* spp. from Luzon Island, Philippines, has also been reported from the same host genus from India, Manchuria, and Korea. Our specimen readily keyed to *D. amphichrus* in the key given by Bravo (1941).

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Embryology and Reproduction of *Ditylenchus destructor* Thorne, with Emphasis on Gonad Development*

R. V. ANDERSON** AND H. M. DARLING***

Knowledge of the embryonic development of nematodes has been derived largely from studies of permanent mounts or from temporary water mounts of living specimens. Observations thus are restricted to specific developmental stages and lack the continuity needed to correlate the many intricate sequential processes of development. Subtle transitions in development are often difficult to obtain or are obscured by fixation and stains complicating interpretations, particularly relative to function. Prolonged observations of active specimens under high magnifications when supplemented by cytological studies would be preferred. The potato rot nematode feeds and reproduces on fungi and can be aseptically cultured in the laboratory. Opportunity thus is provided for continuous observations at all stages of development. The present study traces the development of *Ditylenchus destructor* from fertilized egg to adult, with emphasis on gonad development as a means of distinguishing sex and age of larvae.

MATERIALS AND METHODS

Nematodes used for this study were selected from fungus-reared populations established from single gravid females originally extracted from diseased potato tubers (Faulkner and Darling, 1961). Development of the potato rot nematode from fertilized egg to adult was observed with living specimens grown on the fungus *Chaetomium indicum* Corda in micro-observation chambers (Anderson and Darling, 1964). These chambers, containing an agar substrate and fungus mycelia, permitted continuous observations at magnifications up to 900 x to 1025 x. Up to the second molt, nematodes tended to remain at the interface between agar and coverslip providing opportunity for uninterrupted observations of individual specimens. Observations of advanced stages were interrupted more often between feeding periods and molting because of more vigorous movements of larvae. Intermediate developmental sequences required repeated and prolonged observations.

*This paper is approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

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To assure maximum opportunity for observing all developmental stages, active males and females at different stages were introduced into the chambers. In a single chamber it was possible to see all the transitions between molts, as well as copulation, fertilization, and egg development. Extended observations for periods as long as a month provided a substantial amount of data.

EMBRYOLOGY

CLEAVAGE AND DEVELOPMENT OF THE EMBRYO: Egg production of *D. destructor* was lower in micro-observation chambers than in petri plates. To assure maximum egg production, 1 or more young females recently molted and several adult males were added to each chamber. Under these conditions a single female laid as many as 12 eggs within 24 hours. Usually half this number was produced. Older females laid as few as 1 or 2 eggs in 24 hours.

Eggs usually were deposited unsegmented and each contained a large transparent nucleus which sometimes was granular (Fig. 1 D, F). The nucleolus disappeared just prior to mitosis. Nuclei at metaphase were conspicuous and served as a convenient marker for observing cleavage. When at metaphase, the chromatin material was massive and rope-like in appearance (Fig. 1 B, C). Chromosomes aligned in any direction across a nucleus and divided cells were arranged correspondingly (Fig. 1 C, D). Usually cells remained at this stage for 30 minutes or more before the chromosomes split. Within 2 minutes after separation, the chromatin disappeared from view. The cytoplasm soon began to constrict along the equatorial plate which was laid down between the separated chromosomes. Cleavage was completed in 10 minutes. Divisions early in cleavage did not occur consecutively. At the 2-cell stage, mitosis was delayed in one cell while the other blastomere continued to divide. Not before the 5-cell stage did the former cell undergo mitosis (Fig. 1 F, G). Following this stage, divisions were in sequence and 2 or more cells were never observed to divide at the same time. As blastomeres increased in number, there was a corresponding decrease in size of cells, nuclei, and cytoplasmic globules (Fig. 1 A-H). Gastrulation occurred soon after the 16-cell stage. The time interval between divisions up to the 7- or 8-cell stage usually was from 2½ to 5 hours. Sometimes 7 hours lapsed between divisions while other blastomeres cleaved within 1 hour. Beyond 8 cells, the time interval between cleavages decreased to about 1 hour.

Tensions appeared to be produced within the egg during cleavage which caused the entire egg to rotate spasmodically 90°, 180°, and 360°. Occasionally an egg was seen to turn instantly end over end. This phenomenon was observed first at the 6-cell stage and persisted up to cell differentiation.

Cell specialization became obvious soon after the 16-cell stage as evidenced by differential mitotic divisions and cytoplasmic changes between groups of cells. When about 16 celled, most of the peripheral cells divided, became ½ the size of cells in the previous stage, contained fewer and smaller globules, and were less granular (Fig. 1, I). By contrast, the large inner cells became darker and more granular than previously and the globules tended to aggregate. Some enlarged, but all appeared to have thicker, refractive walls. This stage represents a phase in gastrulation.

The embryo was discernible within 2½ hours after cell differentiation was first apparent (Fig. 1 J). The cell walls at this stage began to break down and 2 distinct areas were recognizable. The anterior half of the young embryo was transparent, finely granular, and contained few globules (Fig. 1, J-L).

The ectodermal epithelium and stomodaeum were conspicuous. This region developed into the anterior portion of the larva which contained the esophagus. Within the darker, more coarsely globular half, the midgut endoderm and germinal primordium were recognizable which gave rise to the intestine and reproductive system, respectively. The young embryo was small, revealing the partially collapsed vitelline membrane (Fig. 1 K-L). Movement of the embryo, particularly in the anterior region, was first noted at this time. The ectodermal epithelium and stomodaeum were clearly delineated by a fine arched line (Fig. 1, K-L). Often, a portion of the coeloblastula was visible at the apex of the embryo between the ectodermal epithelium and stomodaeum. The stomodaeum persisted until late in larval development but became indistinct before formation of the esophageal lumen. During this developmental phase, the epithelium developed around the darker posterior portion (midgut endoderm) which became progressively smaller and more compact (Fig. 1 K-L). Small uniformly distributed elliptic nuclei were visible in the stomodaeum mass. At this stage, the embryo soon began to elongate and again filled the vitelline membrane. The posterior region elongated more rapidly than the anterior region and within 20 hours, began to reflex anteriorly (Fig. 2, M-N). Development from the unsegmented egg to this stage took about 48 hours.

Once the tail of the embryo reflexed, the embryo began to elongate rapidly. Most of the increase occurred in the posterior $\frac{2}{3}$'s of the body and was accompanied by a decrease in body width. Movements also quickened and, within about 18 hours the first molt began. The body now was not easily accommodated within the vitelline membrane and was tightly compressed and looped 3 or 4 times (Fig. 2, Q-T). For this reason details of its development were not easily discernible. However, during the molt, the body was relaxed and development more easily followed.

The esophagus developed relatively late in first stage larvae (Fig. 2, O-T). Prior to molt, the basal bulb of the esophagus and dorsal gland nucleus were very prominent in first stage larvae as was the nerve ring (Fig. 2, Q-R-S). The dorsal gland duct outlet first became visible after the spear apex had formed. At this stage, the esophageal lumen was faintly visible.

At the beginning of the molt, a short tube within the head became sclerotized (Fig. 2, R). This structure later was shed with the cuticle. Next, the median bulb began to develop and the esophageal lumen became quite distinct. Later in the molt, the median bulb valve appeared and was most conspicuous about the time the spear apex formed. Development of the median bulb, however, was not completed until late in the molt. The excretory duct also was visible at this time. By the time the cuticle was shed, about 20 hours after initiation of the molt, these structures had become functional.

POST-EMBRYONIC GONAD DEVELOPMENT: Gonads arose from a genital primordium visible in early first stage larvae (Fig. 1, J). It contained a single germinal nucleus which enlarged and became spherical during first molt. The primordium elongated slightly tapering both anteriorly and posteriorly (Fig. 2, T). A small somatic nucleus appeared anterior and one posterior to the germinal nucleus early in second stage larvae (Fig. 3, A). These divided twice during the second molt and usually became arranged with 3 nuclei anterior to the germinal nucleus, 3 posterior, and 2 along the dorsal side (Fig. 3, C). During the third larval stage, 6 nuclei moved to one end of the primordium (Fig. 3, D). In females, these moved posteriorly while in males the direction was anterior toward the nematode head. Of the 2

remaining nuclei, 1 became the cap nucleus while derivatives of the other gave rise to the epithelium of the ovary or testis.

GONAD DEVELOPMENT AT THIRD MOLT: Female and male reproductive systems are now easily discernible and increased rapidly in size and development during the third molt and later phases of growth. The major regions of the female gonad were distinct at the beginning of the third molt. The ovary was the largest portion of the gonad, was relatively well developed, and contained up to 7 nuclei including the germinal nucleus and cap nucleus (Fig. 3 F). The immature uterus was evident as a short region, ventrally expanded, and contained up to 3 nuclei. The uterus was separated from the ovary by a short narrow region in which the quadricolumella and spermatheca eventually developed. The post-uterine branch was rudimentary and usually contained 1 nucleus (Fig. 3 F). At this stage, 4 specialized ventral chord nuclei were present along the ventral side of the distal end of the gonad (Fig. 3 F).

The gonad increased in length during this molt and its various regions became more discernible. The most marked developmental changes occurred at the uterus. Eight specialized ventral chord nuclei were present now which were $\frac{1}{2}$ the size and double the number (Fig. 3 G) of the previous chord nuclei (Fig. 3 F). These presumably arose by mitotic divisions though none were observed dividing. During this process, somatic nuclei within the uterus enlarged. The one lying most ventral moved from the uterine wall and separated the chord nuclei into 2 groups of 4 nuclei (Fig. 3 H). This nucleus was at first triangular, contained a nucleolus, and its cytoplasm was distinctly granular. It appeared to function in the initiation of the vagina and henceforth is referred to as the vaginal initial. Towards the end of this molt, 16 specialized ventral chord nuclei were present; 8 lying ventrally anterior and 8 posterior to the vaginal initial (Fig. 3 H). The vaginal initial now became spherical, more heavily granular, and began to move into the uterus.

Development of the male gonad during third molt proceeded in the opposite direction to that of the female gonad. The testis, with the proximal end toward the nematode tail (Fig. 3 K), contained a large germinal cell about midway in the testis and was surrounded by numerous small nuclei (Fig. 3 K-L-M). The distal portion of the gonad (gonoduct) consisted of 3 regions early in the molt (Fig. 3 K). The apical segment contained 3 darkly granular nuclei similar in appearance and size to those of the ejaculatory duct of the adult (Fig. 5 B). The adjoining regions of the gonoduct consisted of 2 large granular nuclei, and 2 smaller nuclei, respectively (Fig. 3 K). Serial mitotic divisions of these 4 nuclei throughout the molt resulted in a double row of small nuclei (Fig. 3 L-M). These eventually gave rise to the various regions of the gonoduct. Divisions always were perpendicular to the longitudinal axis of the gonad (Fig. 3 M-N). Development and elongation of the gonad occurred largely in the undifferentiated gonoduct. At the completion of the molt, the elongating portion of the gonad began to reflex and grew posteriorly (Fig. 3 M).

Early phases in the development of the copulatory apparatus were first apparent shortly before the third molt, evident as a concentric compact group of 8-10 nuclei which formed adjacent to the dorsal side of the cloaca and rectum (Fig. 3 P-Q). These nuclei appeared to be contained within a membrane which disappeared during the final molt and after the gubernaculum had formed (Fig. 5 C-D).

Gonad development during the fourth larval stage: During this develop-

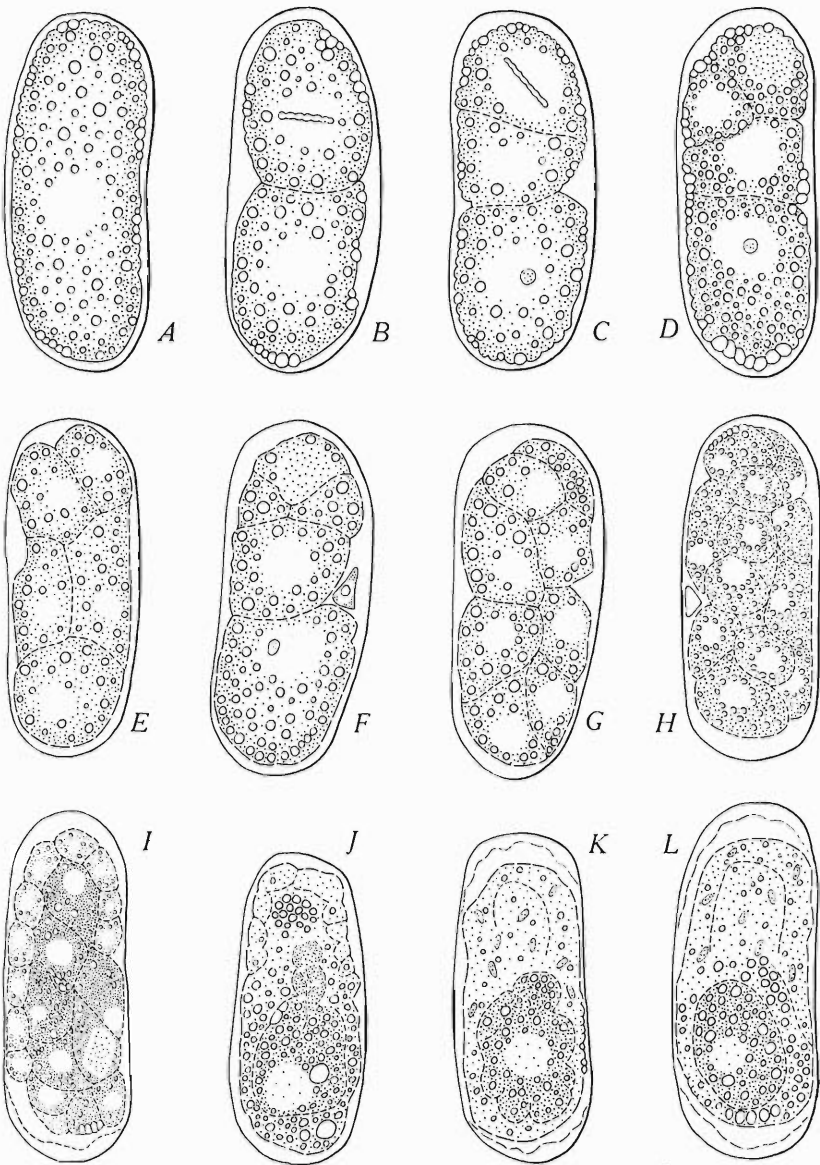
mental stage, there was a rapid increase in the length and differentiation of male and female gonads. The somatic nuclei increased in number within the gonoduct which usually showed some structural organization by the fourth molt (Fig. 3 J). Formation of the vagina was initiated just after the third molt and developed slowly until completed late in the fourth molt. Early in the fourth stage larvae, the vaginal initial nucleus moved into the uterus to the dorsal wall (Fig. 3 I). It was followed closely by pairs of specialized ventral chord nuclei, one from each side chain. These nuclei progressed inward and met at the dorsal side of the uterus forming a flask-shaped structure (Fig. 3 J). A cuticular covering now appeared which enclosed the nuclei, separating them from the body cavity and uterine contents. As the vaginal walls developed, the nuclei became arranged at different levels. Shortly before the final molt, all nuclei had moved within the uterus (Fig. 4 A). Most of the nuclei were located within the thicker portions of the immature vagina walls. The walls of the cup-shaped portion of the vagina were continuous at this stage, but the thin base lying against the dorsal wall of the uterus eventually broke down (Fig. 4 A). Two bands of closely associated nuclei encircled the vagina. The nuclei within the vagina eventually disappeared.

The uterus underwent considerable development during this developmental stage. As the gonad lengthened, nuclei in the uterus increased in number. These eventually became compressed within the uterus and post-uterine branch. By the end of the fourth stage, the nuclei began to disappear and a large central cavity formed which extended much of the uterus length and width (Fig. 4 A). The quadricolumella and spermatheca now began to differentiate about midway between the ovary and vagina (Fig. 3 J).

Development of the male gonad proceeded more slowly than the female gonad and usually was not completed until after the final molt. After reversion of the testis was completed, the posterior portion elongated rapidly and grew toward the rectum. Two large hyaline "cells" appeared at the apex of the gonoduct occupying its entire width (Fig. 3 O). Several small granular nuclei were present adjacent to the hyaline cells; the remainder of the gonoduct contained numerous scattered nuclei of different sizes. A gubernaculum was initiated during this molt along the ventral wall of the concentric cluster of nuclei posterior to the rectum (Fig. 5 B, C, D).

GONAD DEVELOPMENT AT FINAL MOLT: Gonad development was completed during the final molt. Somatic cell divisions usually had ceased by the end of the molt and these specialized cells became incorporated into their respective structures. The cells which composed the various structures did not attain their full size until after the molt, about the time the gametes matured.

The uterus proper of the young adult consisted of 2 distinct regions: a large celled anterior portion and a non-cellular homogeneous portion continuous with the post-uterine branch (Fig. 4 B). In heat relaxed specimens, the contents of this portion broke down and moved freely throughout its length when pressure was applied to the coverslip. Though the uterus was discernible in second stage larvae, it did not begin to develop until the vagina initiated during the fourth larval stage. The uterus was practically empty early in the fourth molt with only a thin layer of cytoplasm lining its walls. Several nuclei soon appeared within the layer. Cytoplasm began to accumulate around them and slowly progressed inward (Fig. 4 A). Eventually, the cytoplasm met at the center and fused to form an unsegmented structure (Fig. 4 B). The nuclei had disappeared by this time. The anterior portion



Abbreviations for Figures: ej—ejaculatory duct; gm z—germinal zone; gr z—growth zone; m ret—molting rectum; m z—maturation zone; ov det—oviduct; q c—quadricolumella; sp—spermatozoa; spi p—spicular pouch; spthe—spermatheca; ut—terus proper; v def—vas deferens; vag—vagina; v.i.—vaginal initial.

Fig. 1. Egg cleavage and early embryo development. A—unsegmented. B—2-celled egg, I at metaphase. C—3-celled, I at metaphase. D—4-celled. E, F—5-celled. G—8-celled. H—16-celled. I—early gastrulation. J, K, L—embryo. $\times 2000$.

of the uterus proper was composed of large thin-walled epithelial cells.

Early in development of the uterus, the thin base of the immature vagina disappeared (Fig. 4 A). The thick-walled vagina began to shorten in depth, became cylindrical, and its ventral surface flattened against the sub-cuticle. Prior to molting, the cuticle dissolved along the narrow compressed vaginal lumen forming the gonopore. The quadricolumella, spermatheca, and oviduct had formed by this time.

Spicules began to form just prior to the last molt within 2 pouches located between the molting rectum and gubernaculum (Fig. 5 C, D). Within each pouch there were at least 3 nuclei which at first were similar in size and appeared enclosed within a membrane. At the beginning of the molt, fine refractive strands appeared in the distal tapered end of each pouch (Fig. 5 C). Within 2 hours, a long tapering cell had formed around the most distal nucleus of the pouch (Fig. 5 D). The fine refractive strands were present also within this cell, but soon disappeared. This portion of the spicular pouches corresponded to the position and shape of the spicule apices (Fig. 5 C-D).

The ventral side of the spicules formed adjacent to the rectum wall, the distal portion developing first. The proximal portion of the spicules developed later in a ventromedian direction (Fig. 5 A). In the adult the dorsal curved part of the spicules was more lightly sclerotized than the ventral side (Fig. 5 B). The proximal part to which the muscles are attached became as heavily sclerotized as the ventral side of the spicule. A cluster of nuclei of unknown function surrounded the spicular primordium during development of the spicules.

Towards the end of the molt, a lumen became discernible through the center of the ejaculatory duct and vas deferens. Free, globular-like bodies and nuclei within this region soon became arranged throughout its length. Their development was not completed until after the molt. In the adult, the ejaculatory duct contained 12 granular, hemispherically-shaped nuclei. These nuclei were arranged in rows of 3 around the lumen and adjacent nuclei were staggered (Fig. 5 B). At fourth molt, and shortly after, these nuclei may be spherical and opposite, but always remained arranged in a similar grouping.

The vas deferens consisted of 2 regions (Fig. 5 B). The region adjoining the ejaculatory duct contained 8 large, clear globules which formed progressively from smaller ones. As the male ages, each may contain 2 to 4 vacuole-like bodies. In heat relaxed specimens, these globules were oblong and the contents granular. The adjoining portion of the vas deferens was expanded and contained at least 6 similar globules that were about half the size of those in the posterior portion of the vas deferens (Fig. 5 B). An undetermined number of smaller globules of unknown function also were present throughout this region.

SPERMATOGENESIS: Spermatogonia evolved from mitotic divisions of the germinal nucleus which remained inactive until about midway between the third and the final molt. Certain small epithelial cells were seen to migrate within the testis and became attached to the posterior and anterior ends of the spermatogonia (Fig. 3 O, Fig. 5 A). Larger epithelial nuclei were also observed in the testis. Spermatogonia matured as they moved down the testis; their nuclei and nucleoli increased in size (Fig. 5 A). After molt, when the testis was full of spermatoocytes, mitosis ceased and all appeared alike. Meiosis of the posterior spermatoocyte began shortly after the molt.

Prior to the first meiotic division, elongate globules developed; first within the posterior spermatocyte and later in the adjacent anterior cell. These refractive globules developed around the nucleus first and gradually spread out to fill the entire cytoplasm (Fig. 5 B). During meiosis, these shortened, but were retained in the cytoplasm of the spermatozoa.

The first meiotic division occurred perpendicular to the longitudinal body axis. The second divisions were parallel to the longitudinal body axis resulting in 4 spermatids of equal size. The posterior most cell from the first meiotic division usually divided first. Spermatids accumulated in a zone (maturation zone) where they became arranged in a double row (Fig. 5 B). Here they underwent maturation and asymmetrical nuclei appeared. Mature spermatozoa were stored in a seminal vesicle and were arranged in a single row. As sperm accumulated, they became compressed and discoid. If not soon ejected at copulation, they began to accumulate in the maturation zone and may eventually form a double row throughout much of the seminal vesicle. Spermatoocytes moved down the testis to replace those that divided.

OÖGENESIS: The germinal nucleus began to divide in fourth stage larvae and subsequent divisions continued through the fourth molt. Oögonia appeared to form in the same way as spermatogonia. Small free epithelial cells became attached to the oögonia apparently providing their cytoplasmic shells. Nuclei and nucleoli of oögonia increased in size as they moved down the

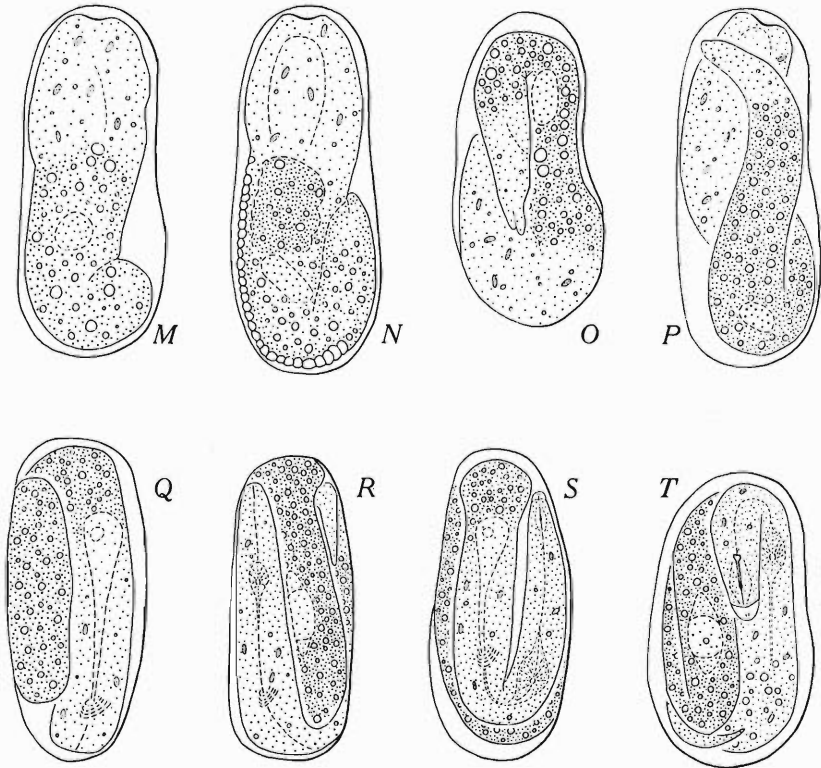


Fig. 2. Development of the first stage larva. M, N, O, P—initial stages of elongation. Q, R, S, T—development during molt. $\times 2000$.

ovary and their cytoplasmic contents became progressively more granular. By the end of the final molt, the ovary was full of oöcytes. In young fertilized females, the posterior oöcytes increased in size and globules began to form within the cytoplasm. The first meiotic division occurred prior to its passage into the spermatheca as judged by disappearance of the nucleoli.

REPRODUCTION

Copulation and egg production were most frequent shortly after final molt. Females thus were selected soon after or prior to molt for this study, being detected easily under the dissecting microscope. Only males containing sperms were selected. Despite numerous observations, actual copulation and subsequent migration of sperms within the female were seen in relatively few instances.

Females became receptive to males for about a week after final molt. Males remained sexually active for a longer time and were capable of copulation at least 3 weeks after final molt. A single female may copulate several times during her receptive period and with more than one male. Several hours usually lapsed between copulations.

Amphids of the male probably function in part as sensory organs for locating the female and orientation prior to copulation. As the male approached the female, he proceeded directly to the perineum. The male head first touched the vulva, then twisted away and began to revolve in a spiral parallel to the female with the ventral side in contact with the female perineum. The bursa partially encircled the female and functioned to hold the male in place as well as a guide for orientation prior to copulation. The female tail was bent frequently ventrad which assisted also in guiding the male over the vulva. Several passes of the male generally occurred before the vulva and spicules became aligned and copulation took place. After each pass, the head again moved to the vulva, then twisted away with its body revolving against the female. If copulation did not take place within 5 or 10 minutes, the male moved away.

At copulation, the male and female heads were never parallel, but away from each other. The duration of copulation was very short. The curved spicules, held in place by the bursa, were inserted deeply into the vagina with the apices extending a short distance into the uterus. Insertion of the spicules was followed immediately by ejection of the sperms into the posterior portion of the uterus. As few as 6 or as many as 20 sperms were transferred within 1 second. The male moved away immediately after copulation.

STRUCTURE AND FUNCTION OF THE SPERMATHECA: The spermatheca served as a storage organ for sperms and was composed of thin-walled epithelial cells capable of considerable expansion. The inner walls protruded into the lumen and appeared thinner than the outer walls. Those cells surrounding the posterior and anterior ends of the spermatheca were flattened.

Sperms became attached to the spermathecal walls as they entered singly from the quadricolumella and remained attached to one another by mucous-like membranes (Fig. 4. B). Sperms were arranged in tandem within the spermatheca and when more than 7 or 8 sperms were present, the row was reflexed and opposite with at least one side of the ellipsoidal sperms in contact with the inner walls of the spermatheca (Fig. 4. B). The inner ends of the sperms were attached to a thin membrane which separated the 2 rows. Sometimes the reflexed row of sperms was on a different level and then both sperm ends adhered to the inner spermathecal walls. Most anterior sperms

appeared to be fixed, but those in the posterior portion of the spermatheca sometimes migrated slowly from side to side though always moving as a unit. Their movement was more rapid and erratic during the entrance of an oöcyte.

The inner walls of the spermatheca rhythmically rippled between the sperms. Movement resulted from an invagination that formed just below an

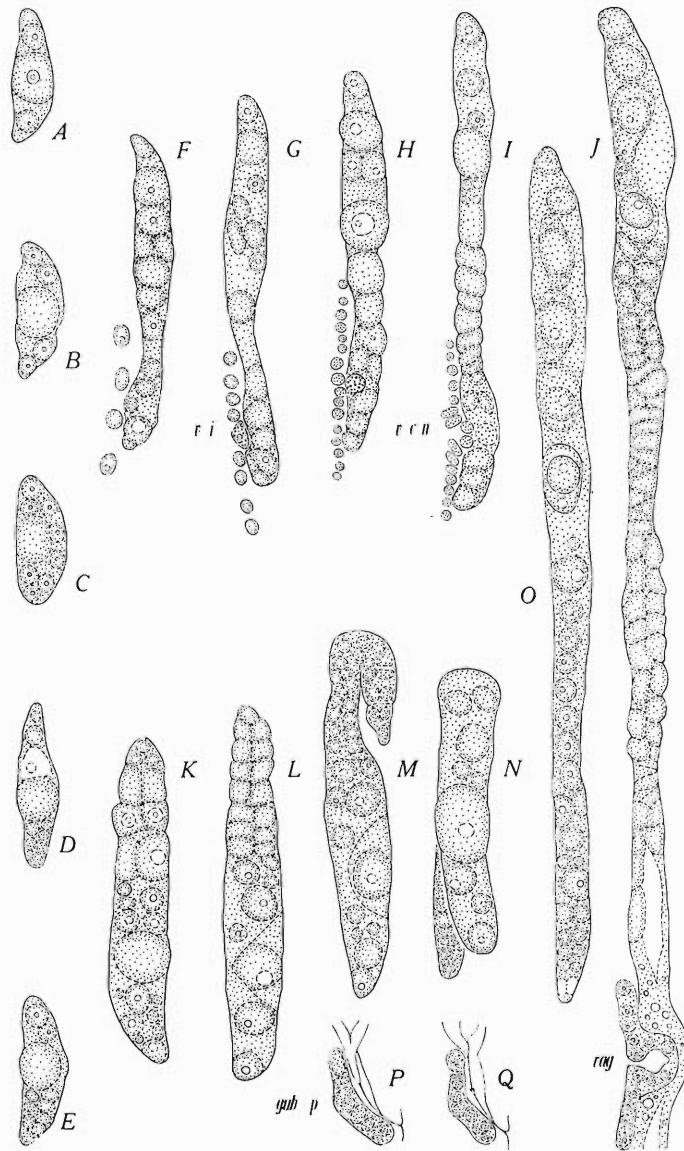


Fig. 3. Post-embryonic gonad development. A—germinal primordium of second stage larvae. B, C—during second molt. Female: D, E—early third stage. F, G, H—during third molt. I—at fourth stage. J—late in fourth stage. Male: K, L, M, P—during third molt. N, Q—early fourth stage. O—midway in fourth stage. $\times 2000$.

attached sperm and moved slowly toward the lower sperm. The invagination first extended practically the width of the spermathecal cell and was $\frac{1}{2}$ to $\frac{1}{3}$ as wide as it was long; becoming smaller as it approached a lower sperm. A small spheroid globule sometimes was seen discharged from the wall at the termination of each "peristaltic" wave. In young females about 6 waves occurred a minute. The lower sperm was pulled forward at its point of attachment as the wave approached and returned to its original position when the wave terminated. This created the impression that the sperms moved rhythmically back and forth. No movement of the inner spermathecal walls was observed when devoid of sperms.

SPERM MORPHOLOGY AND MIGRATION: Spermatozoa were spherical within the seminal vesicle at first, but became compressed and discoid as they accumulated (Fig. 5. B). Cytoplasm of the sperms was densely packed with large refractive elongate globule-like bodies. Each sperm contained an asymmetrical nucleus, which varied in shape: some consisting of 2 to 4 distinct segments, whereas others were unsegmented and U-shaped (Fig. 5 B).

At copulation, sperms passed rapidly through the lumen of the vas deferens, ejaculatory duct, cloaca, and through the vagina into the uterus. As they passed into the uterus, the sperms became tightly compressed and discoid. They remained in the uterus about a half hour where they underwent certain changes before migration anteriorly. They soon separated from one another and for the first time, were seen to be connected by a thin, transparent mucous-like membrane. Sperms were now ellipsoidal and the globules within the cytoplasm were fewer, smaller, and less refractive than in the seminal vesicle. Sperms then moved slowly with an amoeboid motion into the thin-wall portion of the uterus proper where they became attached to the walls by mucose-like extensions as in the spermatheca (Fig. 4 B). After 30 minutes, sperms began to move slowly anteriorly, through the quadricolumella and into the spermatheca. Migration proceeded without interruption for about 2 hours. If an egg was present in the gonoduct, the sperms easily moved around it. Migration of sperms terminated in the spermatheca. The first to enter became attached to the inner walls near the oviduct.

Sperms lost by impregnation of oöcytes were replenished by sperms stored in the quadricolumella, uterus proper, or sometimes post-uterine branch. Occasionally young females were seen with groups of 10 or more sperms in each of these structures; attached to their walls as in the spermatheca. They migrated forward when those in the quadricolumella moved into the spermatheca. Migrations of sperms stored in the quadricolumella usually occurred when sperms in the spermatheca were reduced to eight or less. The first to enter from the quadricolumella joined with the posterior sperm of the existing chain. When all sperms had moved into the posterior end of the spermatheca, the free end moved along the existing chain to a position opposite the anterior most sperms.

IMPREGNATION AND DEVELOPMENT OF THE EGG WITHIN THE FEMALE: Development of the oöcytes and their subsequent fertilization and expulsion from the uterus were followed in over 25 females and substantiated by numerous observations made at each of the various phases in egg maturation. An oöcyte moved into the spermatheca when it had attained the approximate size of a fully developed egg, usually within 2 hours after the nucleolus disappeared. When fully developed, its cytoplasm was largely globular. Passage of an oöcyte into the spermatheca took 1 or 2 minutes. The oviduct expanded little, allowing only a narrow stream of globules to pass. Oöcyte contents were thus disorganized as it entered the spermatheca. The contents reorgan-

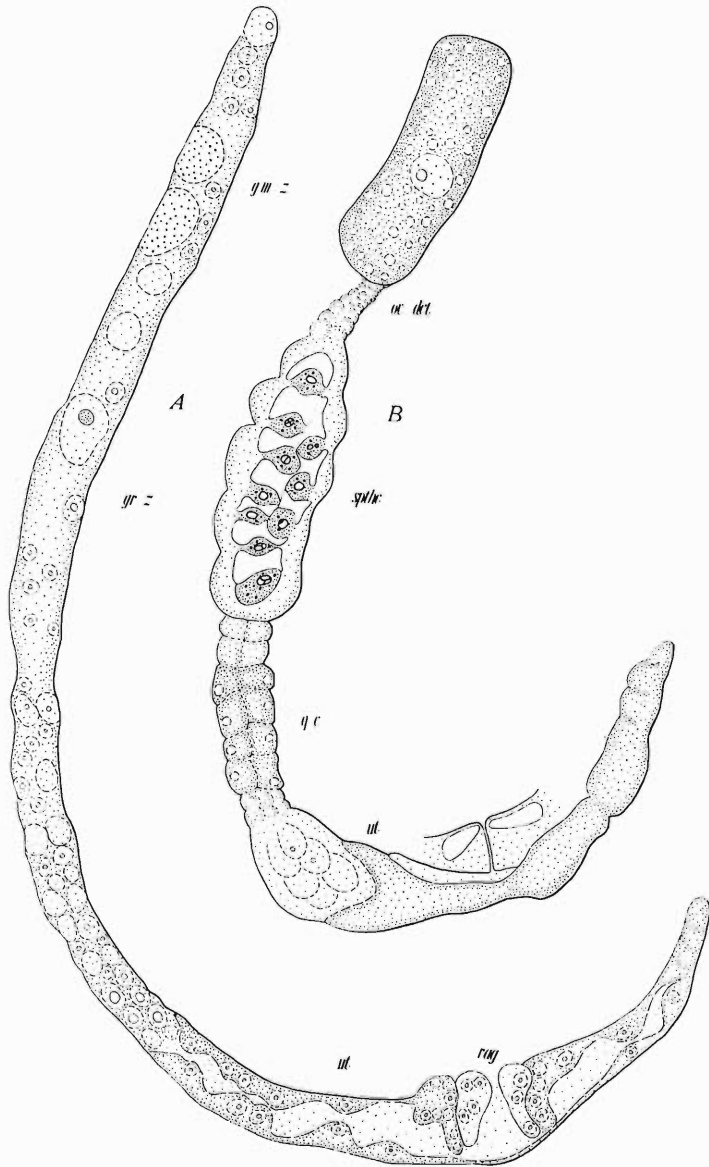


Fig. 4. Female gonads of *D. destructor*. A—fourth molt. A germinal nucleus has just completed division. B—mature gonad with spermatozoa adhering to the spermathecal walls. $\times 2000$.

ized to the shape of the spermatheca and the nucleus remained visible as a clear area about midway in the oöcyte, but to one side. The remaining oöcytes in the ovary slowly moved down to fill the vacated space and the posterior most cells began to increase in size. A second oöcyte moved into the spermatheca within 4 or 5 hours.

Impregnation of oöcytes always was observed to take place in the spermatheca from a single sperm which became detached from the end of the row of sperms. The sperm lost its identity within 1 or 2 minutes after penetrating the anterior end of the oöcyte and a clear area formed in its place. The impregnated oöcyte remained within the spermatheca about 5 minutes before moving out. One sperm was always missing from the spermatheca after passage of an oöcyte.

Subsequent migration of the egg was slow and usually it did not arrive in the quadricolumella for 5 to 10 minutes. Fertilization apparently caused a hardening of the egg membrane as it retained its shape during migration through the narrow tube from the spermatheca and into the quadricolumella despite pressures exerted by these expanded structures. Frequently 3 or 4 sperms followed the egg into the quadricolumella, but these eventually moved back into the spermatheca. The membrane connecting the sperms was most clearly seen at this point as it became stretched during the sperms passage through the narrow tube connecting the spermatheca and quadricolumella.

The larger granular cells of the quadricolumella expanded greatly as the egg advanced. After entrance, the end walls closed over the egg and completely encased it. Those cells covering the ends of the egg were stretched greatly in length and became flattened. Most of the tension was on the median cells which separated slightly to accommodate the egg. The egg usually remained in the quadricolumella for at least 1 hour. During this period, secretions were observed flowing between the egg and walls of the quadricolumella. Soon after entrance of the egg, the sperm nucleus began to migrate slowly, increasing in size as it approached the egg pronucleus. Migration of the sperm pronucleus was not always seen and fusion of the pronuclei was not observed. In one case, however, the sperm nucleus was observed opposite the egg pronucleus prior to migration of the egg into the uterus. Both nuclei were the same size, appearing as large vacuoles.

Migration of the egg from the quadricolumella into the uterus took about 15 minutes in young females where it may remain 30 minutes before deposition. The uterine walls were expanded by the egg, particularly the thinner-walled anterior portion (Fig. 4 B). This structure may be stretched to twice its size by an egg, but returns to its original size about 3 hours after the egg is expelled from the uterus.

The walls of the vagina began to twitch periodically as the egg approached and advanced slightly beyond the vagina. When in this position, muscular contractions began which opened the vagina slightly for periods of several seconds. With each contraction, a small portion of the egg wall protruded into the vagina. Usually several contractions occurred before the egg was deposited. Expulsion requires about 1 second during which time the egg flowed out of the vagina which opened to only $\frac{1}{4}$ of the egg diameter. Egg contents appeared disorganized for a time after its expulsion, apparently due to its passage through the narrow vagina.

As females age, the rate of egg migration through the gonoduct and egg oviposition decreased. Consequently, several eggs may accumulate in the uterus in different stages of cleavage. One female observed after 2 weeks, for exam-

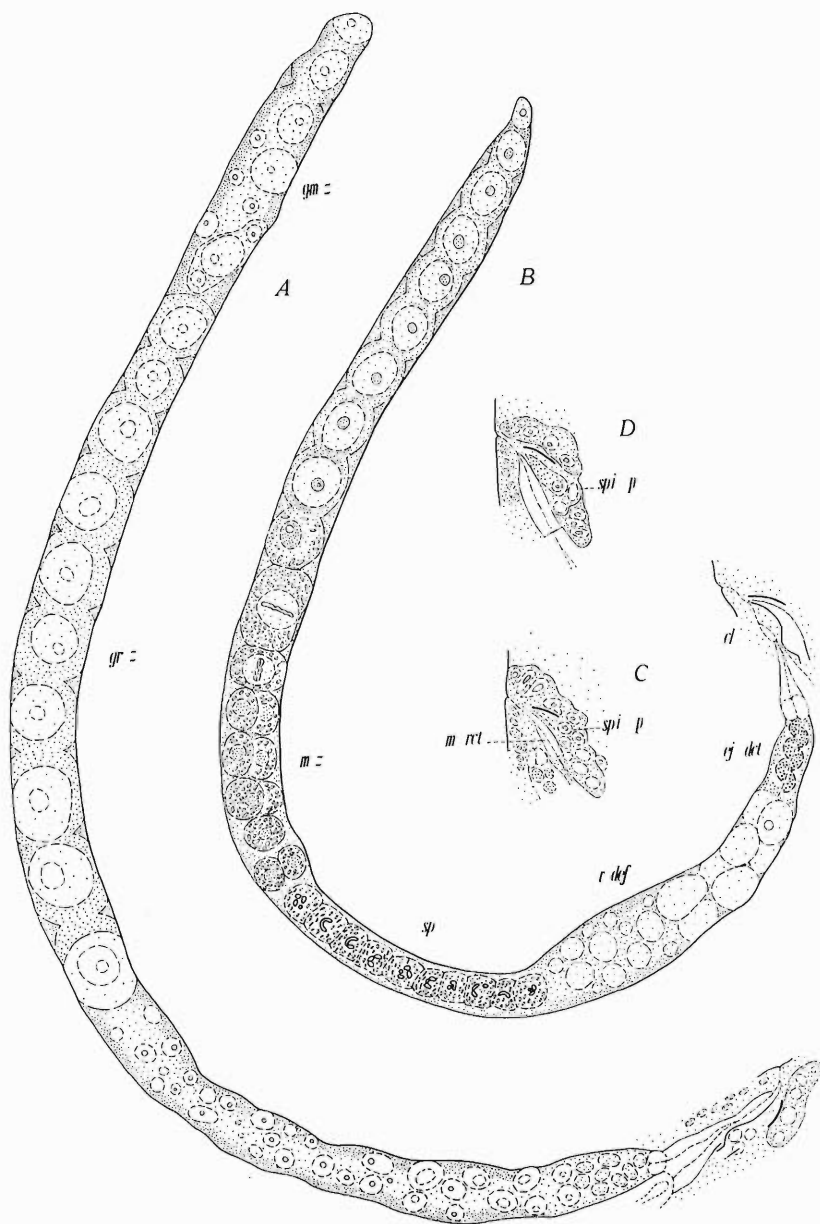


Fig. 5. Male gonads of *D. destructor*. A—fourth molt, note small epithelial cells adhering to a germinal nucleus. B—adult gonad with spermatocytes undergoing meiosis. C—spicular development at beginning of the fourth molt before the spear shaft fades. D—Spicular development 2 hours later. $\times 2000$.

ple, contained 3 eggs in the uterus with another in the quadricolumella. One egg over the vagina contained 3 blastomeres. The other 2 laid tandem in the anterior portion of the uterus proper. One of these contained 2 blastomeres while the other was unsegmented. In another female, an egg was found in the uterus which contained a fully developed larva. It was eventually deposited after 5 hours.

Size of the oöcytes appeared to be reduced in females approaching senility which may result in deformed larvae that die before the first molt within the egg. One such female, for example, deposited 2 eggs that were half the normal egg size. Both eggs were able to cleave, but died soon after gastrulation. This female was observed for 1 week and did not lay additional eggs.

Senility in females was characterized largely by dark, densely globular intestinal cells and arrested reproduction. The intestinal walls of old females became distended and turgid, exerting considerable pressure on the reproductive system. When the body bent, the intestine was pushed against the gonad forcing it out of position. Movements of nematodes at this stage were slow which in part may be due to turgidity of the distended intestine. Though senile females lost their ability to lay eggs, oöcytes continued to be produced for a time which accumulated in the distended base of the ovary. These were never observed to enlarge or move into the spermatheca.

DISCUSSION

Recent studies of gonad development have provided reliable criteria for distinguishing the sex and various stages of larval development (Raski, 1950; van Gundy, 1958; Triantaphyllou and Hirschmann, 1960; van Weerd, 1960; Yuksel, 1960; Hirschmann, 1962). Sex in some nematodes has been discernible in second stage larvae by the presence or absence of spicular primordia (van Gundy, 1958) and by the number of gonads (Triantaphyllou and Hirschmann, 1960). Hirschmann (1962) first reported the role of specialized ventral chord nuclei in vaginal development. Their presence late in second stage larvae of *Ditylenchus trifurcatus* separates females from males. She found that differences in the structure and orientation of male and female gonads were conspicuous at second molt and at subsequent stages of larval development. Sexual differentiation can be expected to vary to some extent with the species or environmental conditions. In *D. destructor*, differential development of gonads did not occur until shortly after second molt. The specialized ventral chord nuclei were seen first just prior to the third molt.

Development of the vagina begins during the third molt and appears to be initiated by a specialized nucleus within the uterus. During third molt, this nucleus separates from the ventral uterine wall midway between the specialized ventral chord nuclei. As it moves into the uterus, it is followed closely by 8 pairs of chord nuclei thus appearing to provide the (entrance and) establishment of the vagina. Subsequent development of the vagina proceeds in much the same manner as described in *D. trifurcatus* by Hirschmann (1962). Development of the gubernaculum and spicules appears similar to their development in other nematodes (Chitwood and Chitwood, 1950).

Genital primordia are recognizable in first stage larvae and may contain 1 germinal nucleus (Hirschmann, 1962) or 2 germinal nuclei (Chitwood and Chitwood, 1950; van Weerd, 1960; Chuang, 1962; Hechler, 1963). At this developmental stage, 2 somatic nuclei are present within the primordium: 1 anterior to the germinal nucleus and 1 posterior. Chuang (1962) determined that these were mesodermal cells acquired early in first stage larvae of *Pelodera (Pelodera) teres* Schneider, 1866 (Dougherty, 1955). In *D.*

destructor at first molt, the ovate genital primordium contains 1 germinal nucleus, enclosed by a membrane which becomes continuous with the epithelium of the reproductive system. Somatic nuclei appear within the primordium only in second stage larvae. These divide twice during the second molt and become specialized in function during the third larval stage.

The germinal nucleus, first apparent during gastrulation, divides in fourth stage larvae giving rise to the oögonia and spermatogonia. Usually no more than 3 mitotic divisions occur in males at this stage of development and no more than 1 in females. In no case did the cap cell nucleus undergo division and apparently does not function in gametogenesis in this species. This agrees with the observations of other workers (Chitwood and Chitwood, 1950; Hirschmann, 1962). Epithelial cells are conspicuous between the germ cells in the germinal zones of the gonads. These vary in size and function. The large epithelial cells contribute to the development and expansion of the epithelium (Hirschmann, 1962). The smaller epithelial cells, as seen in *D. destructor*, adhere posteriorly and anteriorly to the germinal nuclei and may add to the cytoplasmic mass of the oögonia and spermatogonia. Subsequent development of the germ cells is similar in males and females.

Meiosis is observed easily in males with the compound microscope. Prior to meiosis, elongate refractive globules appear in the cytoplasm which are similar to those described in *Spirina parasitifer* by Cobb (1928). These shorten during maturation of the spermatids, but are retained in the spermatozoa. Spermatids temporarily accumulate within the gonoduct anterior to the seminal vesicle where they undergo maturation before moving into the seminal vesicle. The posterior portion of the gonoduct consists of a vas deferens of 2 sections and an ejaculatory duct terminated by 2 large hyaline cell-like bodies. Copulation and subsequent amoeboid migration of sperms in the female gonoduct are described.

Sperms stored within the spermatheca are bathed in secretions exuded from its moving inner walls with which the sperms are intimately associated. These secretions probably serve as nourishment for the sperms and prolong their longevity. The quadricolumella, described by Wu (1958), and the uterus proper function in a similar manner when containing sperms. When an egg is present, fluids were seen to move around it, particularly in the quadricolumella. These fluids may serve as a "lubricant" which aids in the passage of the eggs.

SUMMARY

Unsegmented eggs are deposited by young females. Cleavage and embryonic development occasionally may be found within the body of older females. Cleavage normally begins soon after the egg is laid and is delayed in one blastomere until the 5 cell stage. Divisions occur during a 2½ to 5 hour period up to the 8 cell stage, but occur within an hour in subsequent division. Contents of eggs often rotate spasmodically during cleavage and sometimes the eggs turn end over end. Gastrulation is evident soon after the 16 cell stage and the moving embryo is discernible within 2½ hours. Developmental stages of first stage larvae is described. Larval development is complete in about 48 hours from first cleavage.

Sex is discernible in third stage larvae by the presence or absence of specialized ventral cord nuclei and by orientation, size, and structure of the gonad and germinal nucleus. In females, 4 specialized ventral chord nuclei appear late in development of third stage larvae and increase to 16 by the

end of the third molt. These move into the uterus in pairs during fourth stage. A specialized uterine nucleus separates from the ventral uterine wall, providing an entrance for the specialized ventral chord nuclei. Vaginal development is completed during final molt. Testes develop first toward the tail of the nematode, reflex during the third molt and early fourth stage, and by late in the fourth stage are orientated anteriorly. Spicules and the gubernaculum develop during the fourth stage and is completed during final molt. The gubernaculum forms along the ventral side of a concentric group of nuclei first seen during the third molt. Spicules form during final molt, each within a pouch of nuclei located between the molting rectum and gubernaculum. The spicule apex appears first along the rectum wall. The proximal portion develops in a ventromedian direction from this region. The morphology of the adult male and female reproductive system is described.

Gametes arise from a germinal nucleus which divides during the fourth larval stage and into the fourth molt. Soon after mitosis certain small epithelial cells become attached to the germinal nuclei synchronous with the appearance of the membranes enclosing the oögonia and spermatogonia. Meiosis follows distinct changes in the cytoplasm and nuclei of the germ cells. Sperms are stored primarily in the spermatheca, but may be present in the quadricolumella, uterus proper, and post-uterine branch. Following copulation, they migrate with an amoeboid movement within the female gonoduct as a group, connected to one another by mucous membranes. Sperms adhere to the inner walls of the spermatheca which rhythmically ripple, giving off secretions which bathe the sperms. Oöcytes are impregnated by 1 sperm as they move into the spermatheca.

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**A new species of trematode belonging to the genus *Maxbraunium*
Caballero et Zerecero, 1942 (Lecithodendriidae), from
Malayan bats**

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In a previous paper on trematodes from Malayan bats, the author described a trematode from *Rhinolophus steno* ANDERSEN, and assigned it tentatively to the genus *Cephalotrema* (see ROHDE, 1963). The description was based on a single specimen and although the worm could not be included in a known species, the establishment of a new species was postponed until more material was available.

In the meantime, 8 other specimens of the same species were found, of which 5 are mature. One of these was sectioned and stained with HEIDENHAIN's Azan, while the others were stained and studied as whole mounts.

Though there is an astonishing degree of similarity between this trematode and *Cephalotrema* BAER, 1943, a closer study of the cirrus pouch, especially from sections, showed that it is a new species of the genus *Maxbraunium**.

FAMILY: Lecithodendriidae ODHNER, 1910.

SUBFAMILY: Maxbrauniinae YAMAGUTI, 1958.

GENUS: *Maxbraunium* CABALLERO et ZERECERO, 1942.

M. baeri n. sp.**

DESCRIPTION (based on whole mounts of eight specimens and serial sections of one specimen): Shape of living specimens variable. Dorsally convex and ventrally concave. Ventral surface completely covered with large spines, dorsal surface spinose only in its anterior part and laterally on side of genital opening (seen only in sectioned specimen!) Oral sucker subterminal, large and round. Pharynx present, esophagus practically absent. Caeca not quite reaching to posterior end, their proximal portion thin-walled, their distal portion thick-walled. Acetabulum larger than oral sucker, approximately in center of body. Ovary round or oval, on right side of body, overlapping caecum or touching it at its medial side, at level of anterior part of acetabulum (sometimes reaching into zone just in front of it). Small yolk reservoir and MEHLIS' gland medio-posterior or posterior to ovary. LAURER's canal present, opening dorsally near ovary. Receptaculum seminis neither in whole mounts nor in sections. Vitellaria lateral (mostly extracaecal and caecal, sometimes partly intercaecal) from level of posterior margin of oral sucker or as far back as anterior margin of acetabulum to level slightly behind posterior margin of acetabulum. Transverse yolk-duct crossing acetabulum. Uterus fills space between posterior margin of acetabulum and end of body, overlapping caeca on both sides. Anterior branch of uterus crosses acetabulum, more or less strongly coiled, and joins male genital duct a very short distance in front of genital opening. Testes mainly intercaecal, sometimes overlapping caeca, on both sides of posterior part of acetabulum and behind it, weakly lobed or straight-margined.

CIRRUS POUCH LARGE, CONSISTING OF THREE PARTS: (1) DISTAL PART formed by relatively small, thin-walled vesicle, which is sometimes coiled, being sur-

*I wish to express my gratitude to Dr. George Dubois, Neuchâtel, for the examination of part of the material and his valuable advice.

**After Professor Jean G. Baer, Neuchâtel.

rounded by connective tissue fibres and circular and longitudinal muscles. Prostatic gland-cells (very compact and stained deeply red with HEIDENHAIN's Azan), large cells and third, smaller type of cell (colorless with Azan, situated close to surface of vesicle) are situated at dorsal side of terminal portion of this part.

(2) MIDDLE PART of cirrus pouch consists of duct (more or less narrow according to number of spermatozoa present) surrounded by thin, striated

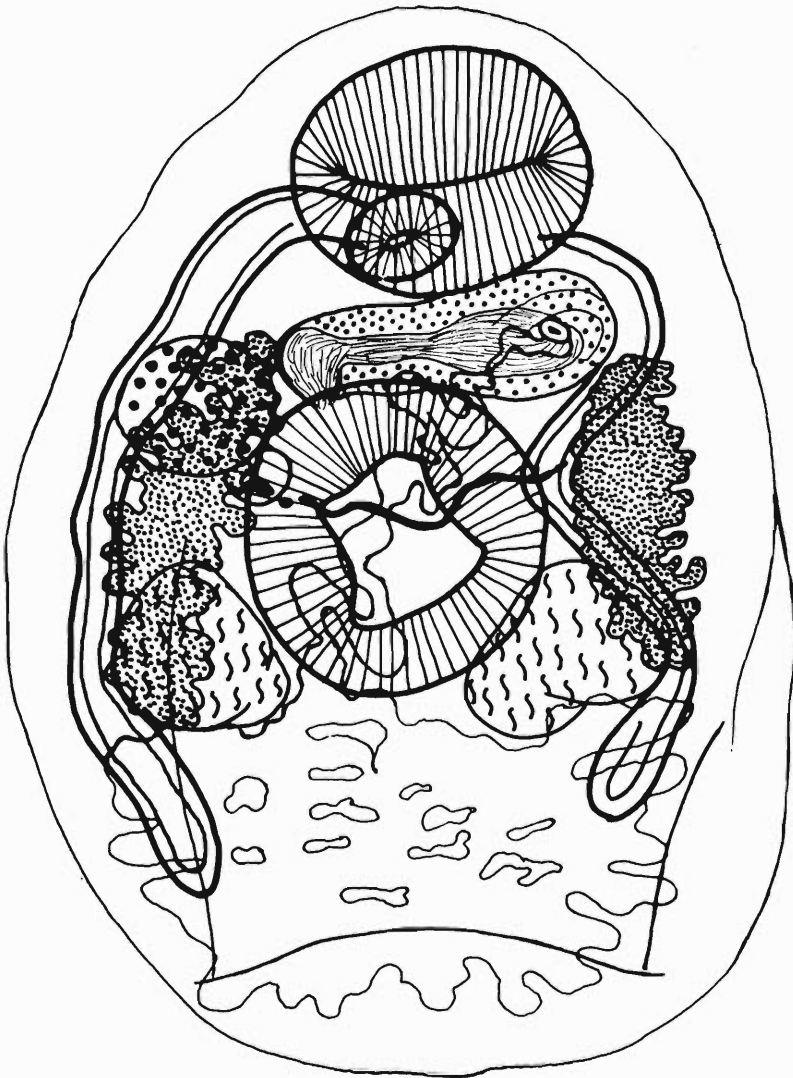


Fig. 1. *Maxibraunium baeri* n. sp. from the intestine of *Tylonycteris* sp. Whole mount, only slightly pressed. (Ventral view).

epithelium, and well developed internal circular and external longitudinal muscles. Many prostatic glands form thick and compact layer around duct. Separated from latter by thin layer of syncytial parenchyma with some large cells; externally limited by muscle-fibres and connective tissue.

(3) THIRD, PROXIMAL PART of cirrus pouch formed by large vesicle, into which all prostatic glands open, including those surrounding distal and middle

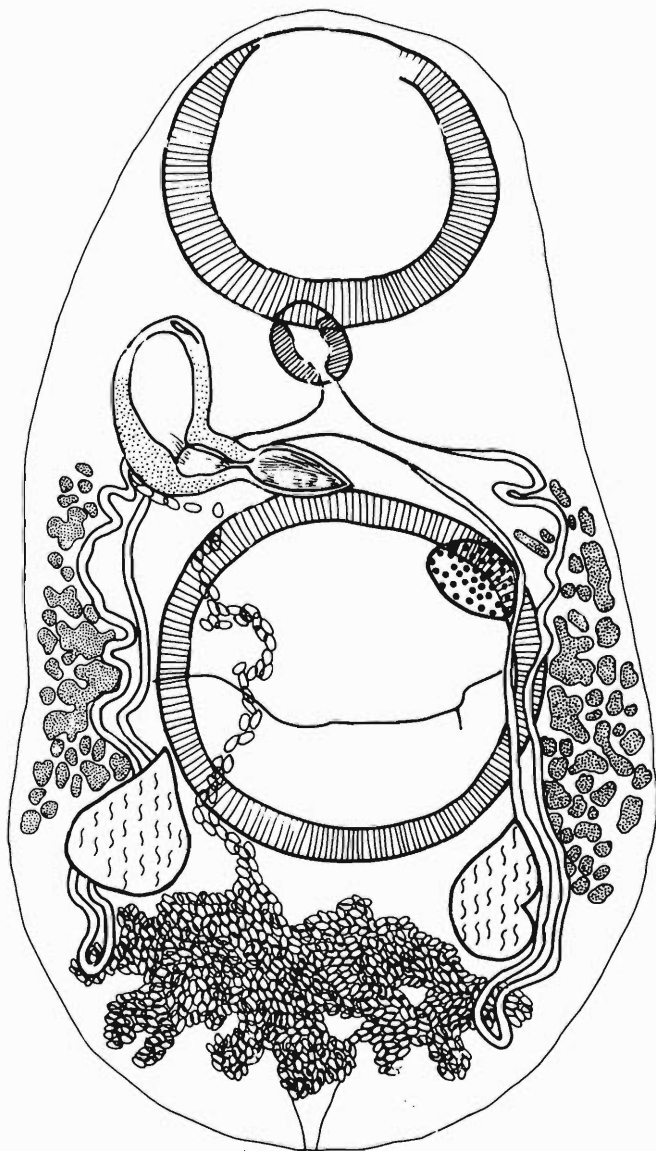


Fig. 2. *M. baeri* n. sp. (holotype) from the intestine of *Rhinolophus* Andersen. Whole mount, strongly pressed. (Dorsal view).

parts of cirrus pouch. Ventral and lateral portions of this vesicle surrounded by large mucous glands (blue with Azan). Openings of mucous glands proximal to those of prostatic glands. Some large cells dispersed among prostatic glands. Wall of first half of vesicle thin, consisting of connective tissue, its second half formed by thick cuticle with large scales. Scales broader at their base and more irregular in shape than thin spines on surface of body. Structure of second vesicle suggests that it is eversible, serving as a cirrus.

Distal and middle parts of cirrus pouch have a more or less transverse position, former part being sometimes oblique or directed backwards; proximal part bent either ventrad (in contracted specimens), or antero-ventrad (in extended and pressed specimens). Genital opening situated approximately half-way between median line and left margin of body at level of posterior end of oral sucker.

Excretory bladder V-shaped, excretory opening terminal; eggs small and numerous, oval.

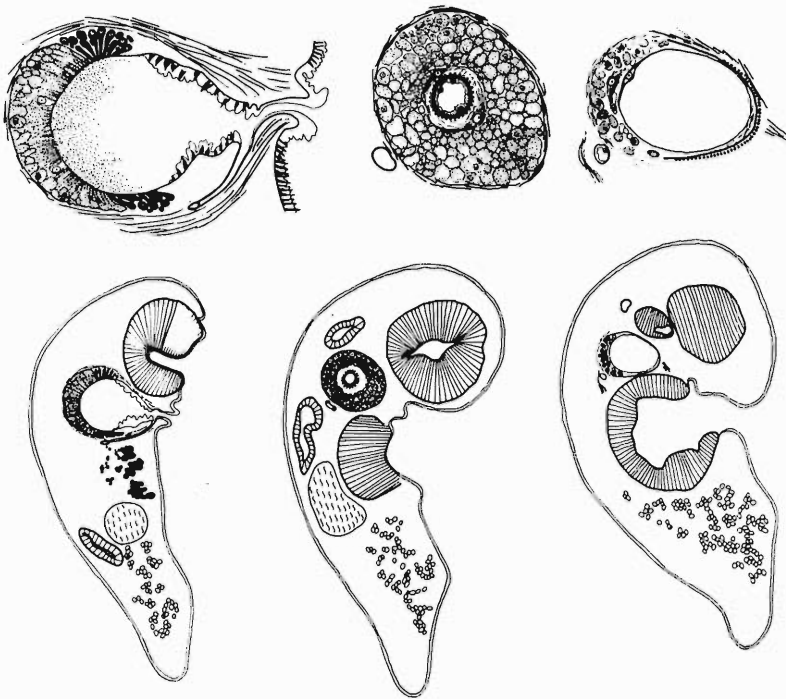


Fig. 3. Sections (approximately longitudinal) through *M. baeri* n. sp. (below) and its cirrus pouch (above). Sections from left to right at levels of (1) genital pore; (2) middle part of cirrus pouch; (3) distal part of cirrus pouch.

Note: (1) Proximal part of male genital duct widened, with cuticular lining at its terminal portion, and surrounded by mucous glands (black) and serous glands (dotted). (2) Middle part consists of narrow duct, lined by epithelium and surrounded by circular and longitudinal muscles, and serous glands. (3) Distal part of cirrus pouch consists of internal seminal vesicle, surrounded by circular and longitudinal muscles; partly surrounded by serous glands.

Measurement of mature specimens: (In millimeters: longitudinal diameter first, in oblique organs longest diameter first. Specimen 1 strongly pressed. Specimen 1 from *Rhinolophus stheno*, Specimens 2-5 from *Tylonycteris* spp.).

Length	Maximum width	Oral Sucker	Acetabulum	Pharynx	Cirrus pouch (length of transverse portion)			
					Ovary	Testis (Ovarial)	Testis	Eggs
1) 1.2	0.63	0.30x0.35	0.41x0.45	0.09x0.07	0.25	0.10x0.09	0.17x0.11	0.15x0.11
2) 1.55	1.14	0.36x0.44	0.44x0.47	0.17x0.12	0.48	0.19x0.20	0.25x0.20	0.15x0.27
3) 1.33	1.10	0.42x0.46	0.45x0.51	0.15x0.11	0.48	---	0.37x0.21	0.21-0.027x0.012-0.015
4) 2.62	---	0.51x0.48	0.58x0.57	-- x0.18	0.73	---	---	---
5) 2.16	1.27	0.42x0.48	0.51x0.47	0.17x0.15	0.48	0.21x0.24	0.32x0.22	0.25x0.19

HOSTS: *Rhinolophus stheno* ANDERSEN (Type host), *Tylonycteris malayana* CHASEN, *Tylonycteris* sp., *Myotis mystacinus* (KUHL), *Kerivoula hardwickei* (HORSFIELD), *Kerivoula pusilla* THOMAS.

LOCALIZATION: Stomach, intestine.

LOCALITIES: Gunong Pengasih, 900 m. above sea level, Kajang District, Selangor (type locality); Kuala Senyul, Ulu Kelantan District, Kelantan; Fort Brook, Ulu Kelantan District, Kelantan; Janda Baik, Bentong District, Pahang; Pantai Valley, Kuala Lumpur, Selangor.

HOLOTYPE: Helminthological Collection No. M.287; Paratypes: M.639-M.642, Zoology Department, University of Malaya, Kuala Lumpur.

Immature specimens are deposited in Helminthological Collection No. M.643-645, sections in Helminthological Collection No. M.646, Zoology Department, University of Malaya, Kuala Lumpur.

DISCUSSION

The species, described above, was originally placed tentatively in the genus *Cephalotrema* BAER, 1943, because the arrangement of all the organs corresponds closely to that of this genus (ROHDE, 1963). The only differences seemed to be the larger suckers, the more anterior position of the ovary, and the slightly more posterior position of the genital opening. It was especially striking that the cirrus pouch in both forms consists of 2 parts, one being located transversely, the other being hooklike directed anteriorly.

A closer study of the cirrus pouch of the Malayan form, however, showed that its terminal part is directed anteriorly only in strongly pressed specimens, while it has ventrad direction in contracted specimens. Furthermore, the cirrus pouch of the Malayan form is rather compact and contains a distal seminal vesicle and a proximal second widening of the male genital tract, which are connected by a narrow duct. According to BAER's description, in *Cephalotrema* there is only one distal seminal vesicle, which is divided into two parts by an invagination of its wall; besides, there is a terminal elongated prostatic part in *Cephalotrema*. The presence of an unarmed cirrus in *Cephalotrema* and of an armed distal part of the cirrus pouch, probably serving as a cirrus, in the Malayan species, is also significant. These features clearly characterize the Malayan form as belonging to the genus *Maxbraunium*. Hitherto only 1 species of this genus was known, i.e. *M. tubiporum* (BRAUN, 1900) CABALLERO et ZERECERO, 1942. The Malayan species differs from *M. tubiforme* in the following characters: (1) larger suckers, (2) acetabulum larger than oral sucker, (3) more anteriorly situated ovary. These differences justify the establishment of a new species, which I name *M. baeri* in honor of Professor JEAN G. BAER, Neuchâtel.

SUMMARY

A new species of the genus *Maxbraunium* (Lecithodendriidae, Maxbrauniinae), from Malayan bats, is described. It differs from the only other known species, *M. tubiporum*, in the presence of relatively larger suckers and a more anterior position of the ovary. Besides, its acetabulum is larger than the oral sucker, while in *M. tubiporum* both suckers are of equal size or the oral sucker is slightly larger than the acetabulum.

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Studies on the *in Vitro* Cultivation of *Histomonas meleagridis* with Three Different Mixed Bacterial Flora

ELLIOTT LESSER

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This investigation was undertaken to learn whether enteric bacterial flora from readily available laboratory animals could be used as successfully as turkey cecal bacteria (TCB) for the *in vitro* cultivation of *Histomonas meleagridis*.

MATERIALS AND METHODS

Cecal material from the golden hamster (HCB), fecal pellets from the Mongolian gerbil (GFB), and chick cecal bacteria (CCB),* were each added to separate tubes of plain nutrient broth. The tubes were maintained at 40°C and subcultured thrice weekly.

Histomonads for this study were obtained from existing modified "199" cultures containing antibiotic treated TCB (Lesser, 1960b). The "199" medium was used for all cultures in this experiment.

All bacterial cultures were treated with antibiotics (Lesser, 1960a) before being inoculated in 0.5ml amounts into histomonad cultures.

All other experimental conditions, unless otherwise noted, were the same as those previously reported by the author (1960b). Inoculations were made as follows:

GFB were inoculated into ten histomonad cultures.

HCB were inoculated into twelve histomonad cultures.

CCB were inoculated into seven histomonad cultures; two of these utilized 0.2 ml of a 5 mg/ml suspension of cholesterol stearate and 0.3 ml of a 1% solution of sodium taurocholate in lieu of cream; two others contained 0.2 ml of a 5 mg/ml suspension of cholesterol palmitate. (Previous studies by the author (1961) had shown that the palmitate and stearate esters of cholesterol could replace cream in the modified "199" medium). TCB were inoculated into 29 control cultures; controls for two CCB cultures containing cream contained: a) cholesterol palmitate and sodium taurocholate in one and b) cholesterol palmitate in the other, all in the above noted concentrations. Comparable controls were used for all other experimental cultures.

*Supplied in a special medium by Mr. C. N. Huhtanen, American Cyanamid Co., Princeton, N. J.

Transfers were made three times per week (Monday, Wednesday, Friday), as long as even one histomonad could be found on a slide by direct microscopic examination.

Cultivation was considered to be successful if the histomonads could be transferred 22 consecutive times with a particular bacterial flora.

RESULTS

With GFB, only one out of ten cultures could be transferred 22 consecutive times; histomonads in the remaining cultures survived from one to eleven consecutive transfers.

With HCB, only three out of twelve cultures could be transferred 22 consecutive times and in two cultures the histomonads failed to develop at all; histomonads in the remaining cultures survived from one to eleven consecutive transfers.

With CCB, five out of seven cultures could be maintained through 22 consecutive transfers; two were in medium containing cream, two were in medium containing cholesterol stearate and sodium taurocholate, and one was in medium containing cholesterol palmitate. In one culture containing cream, the histomonads survived seven consecutive transfers, whereas in the remaining culture containing cholesterol palmitate the histomonads survived five consecutive transfers. The average histomonad growth, however, even in the successful CCB cultures never was as good as that in their respective controls. The average number of histomonads per microscopic field (20x × 15x) in all of the successful CCB cultures was 1.07; in the control group the average was 2.44.

CONCLUSIONS

Mixed enteric bacterial flora from the hamster and gerbil could not be relied upon to support the continuous *in vitro* propagation of *H. meleagridis*. However, the CCB could be used for this purpose with some success. Nevertheless, these bacteria also seem to lack a growth stimulating factor present in the TCB. This may be a possible reason for the usually greater severity of histomonad infections in turkeys than in chickens.

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Studies on the *in Vitro* Growth of *Histomonas meleagridis* with Single Species of Bacteria

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Until it was demonstrated that certain fresh hamster tissues could be used for the *in vitro* propagation of *Histomonas meleagridis* (Lesser, 1961, 1963), mixtures of bacteria were the only means employed for this purpose. Nevertheless, as far as can be determined, no studies were conducted to identify the bacterial species in these mixtures and to ascertain the value of each to the media.

One of the above mentioned mixtures was obtained by the author by culturing fresh turkey cecal droppings in plain nutrient broth. The resulting bacterial flora, which was capable of supporting the *in vitro* growth of *H. meleagridis* in various media (Lesser, 1960a, 1960b, 1960c), was selected for bacteriological analysis. The species of bacteria found were: *Proteus mirabilis** *Escherichia freundii**, *E. Coli*,*† and a lactobacillus designated as T16†.

MATERIALS AND METHODS

The modified "199" medium (Lesser, 1960c) was used for the cultivation of histomonads with all of the above named bacteria. In addition, modified media "107" and "109" (Lesser, 1960b) were also employed with *E. freundii*. Each species of bacteria was maintained separately in plain nutrient broth. The histomonads were obtained from existing cultures containing the mixed turkey cecal bacterial flora (TCB).

Unless otherwise noted below, the bacteria were treated with antibiotics (Lesser, 1960a) and inoculated in 0.5 ml. or 1.0 ml. amounts into histomonad cultures as follows: eight with *P. mirabilis*; ten with *E. coli*; eight with *E. coli* not treated with antibiotics; ten with T16; nine with *E. freundii*; seven with *E. freundii* not treated with antibiotics; five modified "107" cultures with *E. freundii*; three modified "109" cultures with *E. freundii*. Sixty-two cultures serving as controls (one for each experimental culture) were inoculated with TCB.

All cultures were transferred three times per week (Monday, Wednesday, Friday), as long as even one histomonad could be found on a slide by direct microscopic examination.

Cultivation was considered to be successful if the histomonads could be transferred 22 consecutive times with a particular species of bacteria. All other experimental conditions were the same as those previously reported by the author (1960e).

RESULTS

The histomonads could not be successfully transferred 22 consecutive times with any of the bacterial species used (see Table 1).

In the presence of the antibiotic-treated *E. coli*, histomonads survived from one to four consecutive transfers; with the non-antibiotic-treated *E. coli*, the

* Isolated and identified at Beltsville, Md., by K. L. Heddlston, National Animal Disease Laboratory, Animal Disease and Parasite Research Division, ARS, USDA, Ames, Iowa.

† Isolated and identified by Mr. C. N. Huhtanen, American Cyanamid Company, Princeton, New Jersey.

histomonads also survived from one to four consecutive transfers, but did not develop at all in one culture.

In the presence of T16, five histomonad cultures survived only one transfer and in five other cultures the histomonads did not develop at all.

Histomonads survived from three to 21 consecutive transfers in the presence of antibiotic-treated *E. freundii* in the modified "199" medium; in the modified "107" medium, they survived from three to 18 consecutive transfers, but did not develop at all in one culture; in the modified "109" medium, they survived from three to ten consecutive transfers. With the non-antibiotic-treated *E. freundii*, the histomonads survived from one to 20 consecutive transfers.

The histomonads in control cultures could be transferred indefinitely. In addition, the histomonads were much more numerous in the control cultures than in the experimentals.

Table 1. Survival of *H. meleagridis* in the presence of single species of bacteria

Bacteria	Medium	Number of Cultures	Number of successful consecutive transfers
<i>Proteus mirabilis</i>	modified 199	8	1-9
<i>Escherichia freundii</i>	modified 199	9	3-21
<i>E. freundii</i> not treated with antibiotics	modified 199	7	1-20
<i>E. freundii</i>	modified 107	5	0-18
<i>E. freundii</i>	modified 109	3	3-10
<i>E. coli</i>	modified 199	10	1-4
<i>E. coli</i> not treated with antibiotics	modified 199	8	0-4
T16	modified 199	10	0-1
Controls*		62	22+

* Controls for each experimental culture, using comparable media, contained antibiotic-treated turkey cecal bacteria.

DISCUSSION AND CONCLUSIONS

Except for the T16, the bacterial species employed in this study could maintain *H. meleagridis* through a limited number of consecutive transfers. Growth of the protozoan, however, was poor. Therefore, these species of bacteria individually cannot be used for the continuous *in vitro* propagation of histomonads.

It is possible that not all of the bacteria in the TCB were isolated. Similarly, a delicate balance of two or more bacterial species may be required to furnish the histomonads with their growth requirements. These findings illustrate the need for much more investigation into the relationships between enteric bacteria and *H. meleagridis*.

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Studies on Freshwater Larval Trematodes. Part VII. Observations on a New Gymnocephalic Cercaria, *C. sanlorenzensis*, from Venezuela*

PIR NASIR AND AMADO ACUÑA CEDEÑO

In order to study the trematode infestations of freshwater snails, a general survey of the various species of mollusks from Cumaná and its neighborhood is being conducted. Nasir (1964a; 1964b; 1964c; 1964d; 1964e) has already described five new species of cercariae with experimental observations on various stages in the life cycle of one of them.

All observations were made on freshly emerged cercariae, only measurements (in mm.) were taken on specimens fixed by addition of an equal volume of 10% hot formalin. *Intra vital* stains like neutral red and methylene blue were occasionally applied to elucidate various organ-systems.

Cercaria sanlorenzensis n. sp. (Fig. 1-4)

DESCRIPTION: Body and tail aspinose. Body with five "hair-like" processes on each side. Tail with three "hair-like" processes on each side. Both suckers isodiametric. Prepharynx present. Pharynx muscular. Esophagus dividing immediately anterior to ventral sucker. Intestinal caeca very thin, not extending beyond equatorial level of ventral sucker. Both esophagus and intestinal caeca without cellular inclusions. Cystogenous glands with "rod-like" contents, rendering body extremely opaque. At extreme anterior end of body six openings, apparently of ducts emerging from some sort of glands but no glandular equipment could be associated with them. Excretory vesicle a transversely distended structure, lying at posterior end of body. Antero-dorsal to excretory vesicle lying an accessory excretory vesicle into which open main lateral excretory tubes after forming a V-shaped union. Main lateral excretory tubes, in pre-acetabular region only, enclosing refractile excretory granules of double nature. Each of main excretory tubes after forming a loop in pharyngeal region running posteriorly as a secondary excretory tubule as far back as post-equatorial level of ventral sucker where it divides into an anterior and a posterior excretory tubule. Caudal excretory duct divides into two lateral branches in caudal region of tail. Flame-cell formula: $2((2+2+2)+(2+2+2))=24$. Genital rudiments represented by a diffuse cellular mass in front of ventral sucker and other posterior to it. Two masses being connected by a cellular strand running around one side of ventral sucker. Musculature of tail very well pronounced consisting of a pair of dorsal longitudinal muscle bands and a pair of ventral longitudinal muscle bands. Circular muscle fibres characteristically arranged. No particular time of emergence. Negatively phototactic. Measurements of ten specimens: Body, 0.150-0.216 by 0.105-0.144. Tail, 0.171-0.234 by 0.042-0.057. Oral sucker, 0.045-0.057 in diameter. Prepharynx, 0.013-0.015 long. Pharynx, 0.015-0.027 in diameter. Esophagus as long as prepharynx. Pre-acetabular extent, 0.075-0.123. Post-acetabular extent, 0.021-0.042. Development in rediae having a pharynx, a poorly developed collar and a saccate gut. In

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The authors are indebted to Dr. J. P. E. Morrison, Associate Curator, Division of Mollusks, Smithsonian Institute, Washington, D. C., for identifying the molluscan intermediate host of *Cercaria baldai* Nasir (1964) as *Marisa cornuarietis* (L.) which was mistakenly taken as *Australorbis glabratus* (Say). Thanks are due also to Dr. Morrison for identifying the other molluscan hosts, the trematode infestations of which are being studied by the authors.

most of rediae posterior locomotor appendages absent but when present poorly developed. Measurements of living rediae: Length, 0.396-1.5; breadth, 0.120-0.156. Pharynx, 0.045-0.060 by 0.039-0.054.

HOST: *Pomacea glauca* (L).

LOCALITY: Irrigation canal, San Lorenzo, about forty miles east of Cumaná.

RELATED SPECIES: Cercaria of *Fasciola hepatica* as described by Thomas (1883), Wright (1927), Rees (1932), *C. gracilis* O'Roke (1917), *C. indicae* XLI Sewell (1922), cercaria of *Psilotrema spiculigerii* as described by Mathias (1925), *C. redicystica* Tubanguí (1928), *C. helvetica* XVIII Dubois (1929), *C. helvetica* XIX Dubois (1929), *C. chitinostoma* Faust (1930), *C. catenadena* Faust (1930), *C. tuberculata* de Filippi 1857, as described by Wesenberg-Lund (1934), *C. grandis* as described by Wesenberg-Lund (1934), *C. helvetica* XIX as described by Wesenberg-Lund (1934), *C. papillosa* Ercolani, 1882, as described by Wesenberg-Lund (1934), *C. durbanensis* Porter (1938), *C. fasciolae giganticae* Simroth and Hoffman, 1928, as described by Porter (1938), *C. broederstroomiae* Porter (1938), *C. morijae* Porter (1938), *C. klarbosiae* Porter (1938), cercaria of *Ribeiroia* (= *Psilodistomum*) *ondatrae* (Price, 1931), as described by Beaver (1939), *C. htomasi* McMullen (1938), as described by Kuntz (1951), *C. palegae* Goodman (1951), *C. dollfusi* Fain (1953), *C. ituriensis* Fain (1953), *C. amnicolensis* Etges (1956) and *C. albinea* Kahn (1960) are other gymnocephalic cercariae with which *C. sanlorenzensis* may be compared. Of all these 25 species of cercariae only *C. albinea*, found in *Bithynia tentaculata*, Bushy Park, London, England, resembles *C. sanlorenzensis* very closely in the following points: aspinose body as well as tail, lack of papillae, unarmed suckers, "rod-like" contents of cystogenous glands, pattern of excretory system in body, number of flame cells and structure of rediae. However, the two species of cercariae differ fundamentally in certain other characters: *C. albinea*, as the name suggests, is "absolutely white" in comparison with *C. sanlorenzensis* which is rendered so opaque with cystogenous glands that it is a hard job to make out various anatomical details. Moreover, the former is characterized with 12 openings of penetration ducts, at anterior border of body, in contrast with 6 orifices met with in the latter. At the same time *C. albinea* has a long esophagus, ceca extending to posterior end of body and the ventral sucker is larger than the oral sucker. All these features are in a direct conflict with their counterparts found in *C. sanlorenzensis*. Last but not least is the disagreement in extension of caudal excretory duct. The caudal excretory duct in *C. sanlorenzensis* extends almost as posteriorly as the caudal tip while the same is limited only up to middle level of the tail in *C. albinea*.

In view of the foregoing comparative study *C. sanlorenzensis* is regarded as a new species and named after the locality of its occurrence.

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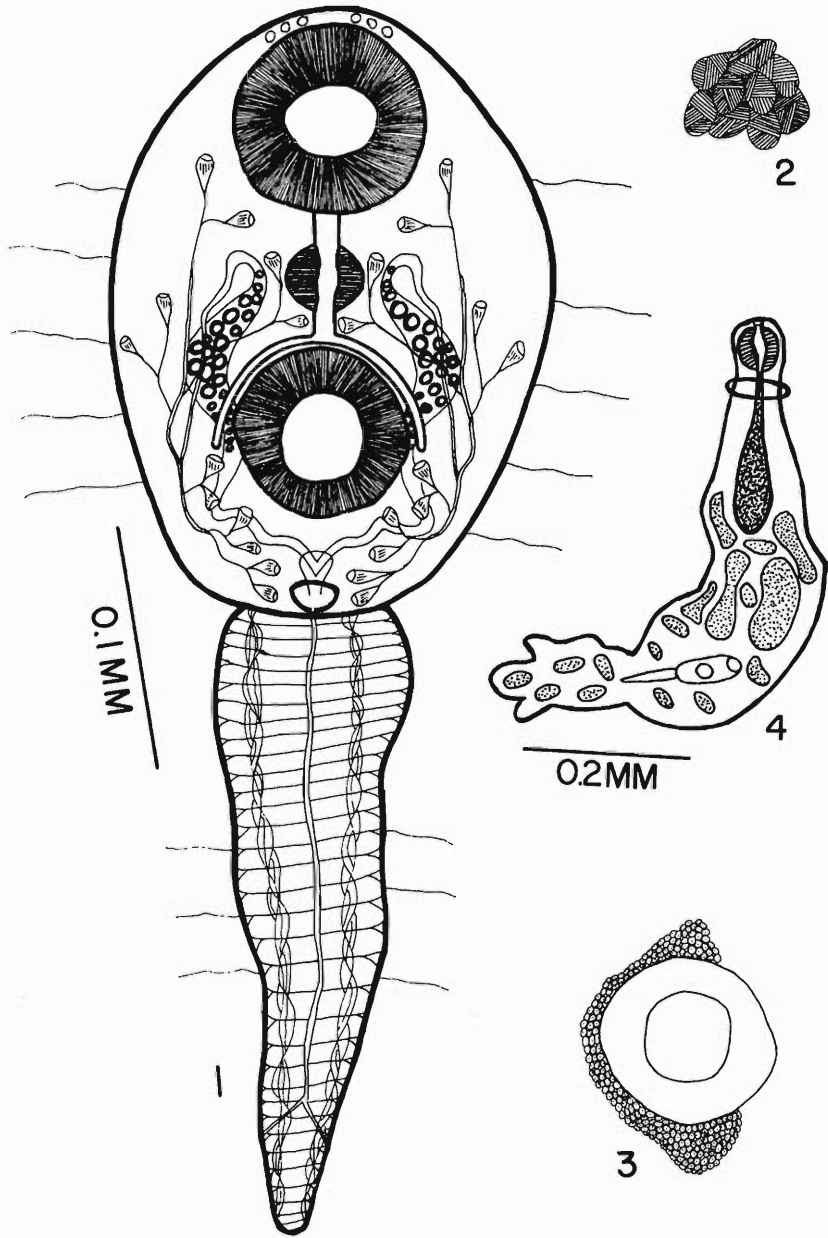


Fig. 1. *Cercaria sanlorenzensis* n. sp. showing various organ-systems.
 Fig. 2. Some of the cystogenous glands showing "rod-like" contents.
 Fig. 3. Ventral sucker with encompassing genitally rudiments.
 Fig. 4. Redia of *C. sanlorenzensis*.

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A Compendium of the Genus *Tylenchorhynchus* (Tylenchidae: Nematoda)*

A. C. TARJAN

SUMMARY: A key and pertinent morphological data on 68 valid species of *Tylenchorhynchus* are presented. *Tylenchorhynchus alatus*, *T. bucharicus*, *T. caromatus*, *T. coffeae*, *T. graminicolus*, and *T. sexamammilatus* are transferred to species inquirendae.

ZUSAMMENFASSUNG. Ein Bestimmungsschlüssel und zutreffende morphologische Angaben werden für 68 gültige *Tylenchorhynchus* Arten gegeben. *Tylenchorhynchus alatus*, *T. bucharicus*, *T. caromatus*, *T. coffeae*, *T. graminicolus* und *T. sexamammilatus* werden zu species inquirendae gestellt.

The incredible influx of 32 nominal species into *Tylenchorhynchus*, since Allen (1955) published his important paper, has been discouraging to many. Loof (1959) made a worthwhile contribution in his publication of a key encompassing 8 species that had been proposed since 1955 and including 2 earlier species not discussed by Allen. The present paper attempts to continue this trend by presenting a key including 23 additional species published to date, by tabulating pertinent details of all species in the genus, and by transferring 6 additional species into *species inquirendae*.

To the excellent list of departures from *Tylenchorhynchus* cited by Baker (1962) should be added *T. spinicaudatus* Schuurmans Stekhoven, 1944 which has recently been placed in *Hirschmanniella* by Luc and Goody (1963). *Tylenchorhynchus browni* Kreis, 1929, *T. styriacus* (Micoletzky, 1922), and *T. symmetricus* (Cobb, 1914) were placed in *species inquirendae* by Allen (1955) while *T. paucus* Kirjanova, 1951 was similarly relegated by Meyl (1961). It is proposed that the following 6 species also be placed in *species inquirendae*: *T. alatus* (Cobb, 1930) because it was based only on a single male specimen; *T. bucharicus* (Tulaganov, 1949) and *T. caromatus* (Tulaganov, 1949) each of which was based only on a single specimen for which descriptions are quite meager and lacking in pertinent data; *T. coffeae* Siddiqi and Basir, 1959 for which no figure or adequate description was given and which has not been subsequently referred to by either author; and *T. graminicolus* Kirjanova, 1951 and *T. sexamammilatus* (Kirjanova, 1938) because of poor figures and inadequate descriptions that preclude adequate comparisons to other species.

Siddiqi (1961) synonymized *T. acti* Hopper, 1959 to *T. capitatus* Allen, 1955 based on "personal correspondence" with Hopper who felt that the two species were identical. Hopper has indicated *in litt.* that the referred-to personal correspondence had not occurred and he regards each of the two species as distinct, as apparently does Marinari (1962). For these reasons, *T. acti* is reinstated as a valid species.

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Florida Agricultural Experiment Stations Journal Series No. 1889.

The assistance of D. L. Coker, who aided in the compilation of data, is appreciated. I am grateful to the following for their comments on the manuscript: M. W. Allen, R. P. Esser, B. E. Hopper, and P. A. A. Loof.

The following key** contains 68 species of *Tylenchorhynchus* and is based solely on female characteristics as indicated by a search of the literature. A stringent attempt has been made to base separations on objective rather than subjective characters. The author feels that the more important of the diagnostic criteria are: 1) number of lines in the lateral field, 2) number and relative position of longitudinal striations, 3) labial region continuous or offset, 4) tail terminus annulated or smooth, 5) stylet length, 6) ratio of the tail divided by the width of the body at the anus (T/ABW), 7) number of labial annules, and 8) number of tail annules. When necessary, subjective characteristics such as degree of labial framework sclerotization, relative tail shape, etc. have also been used but such separations are usually given with sufficient latitude between choices so as to eliminate borderline cases. An inspection of the following key will indicate some species that are now inadequately separated by rather meager characters. This alludes to the extreme urgency of a thorough generic review before any prospective author proposes a new species.

The following key represents only a tool by which species may be tentatively, and not conclusively, identified. Precise identification must be made by reference to Table 1 and/or the original publication.

KEY TO FEMALES OF *Tylenchorhynchus*

- | | |
|---|--------------------------------------|
| 1. Lateral lines 3-4 | 2 |
| Lateral lines 5-6 | 42 |
| 2. Lateral lines 3 | 3 |
| Lateral lines 4 | 8 |
| 3. Labial region continuous | 4 |
| Labial region offset | 6 |
| 4. T/ABW ratio 5.6 | <i>rhopalocercus</i> Seinhorst, 1963 |
| T/ABW ratio 2.3-2.5 | 5 |
| 5. Labial annules 2-3, labial framework
sclerotization very conspicuous, tail
annules 10-11 | <i>sculptus</i> Seinhorst, 1963 |
| Labial annules 4, labial framework
sclerotization inconspicuous, tail an-
nules 14-16 | <i>triglyphus</i> Seinhorst, 1963 |
| 6. Tail annules 42-46 | <i>bifasciatus</i> Andrassy, 1961 |
| Tail annules 10-27 | 7 |
| 7. Stylet 16-17 microns long, tail annules 20-27 | <i>divittatus</i> Siddiqi, 1961 |
| Stylet 21-22 microns long, tail annules 10-14 | <i>trilineatus</i> Timm, 1963 |

** In correspondence received after this paper had been submitted for publication, Dr. I. Andrassy informed me that the number of longitudinal striae for *T. quadriifer* Andrassy, 1954, had been erroneously given in the original publication as 60 when it should have been 30; this correction has been entered in Table 1 which follows. He further felt that his species may be identical with *T. ornatus* Allen, 1955. *Tylenchorhynchus trilineatus* Timm, 1963, according to Andrassy, is identical with *T. triglyphus* Seinhorst, 1963 (discounting the very slight labial constriction of the latter species).

A recent paper has been brought to the writer's attention (Siddiqi, M. R., 1963. *Z. Parasitenk.* 23: 397-404) in which *T. rugosus* is proposed. This species has the following criteria: L = 0.8-0.9 mm; a = 30-32; b = 4.4-5.1; c = 15.5-17.0; V = 55-56%. Labial region continuous; 6-7 labial annules; labial framework inconspicuously sclerotized; stylet 23 microns long with posteriorly inclined knobs; 6 lateral lines; 32-36 longitudinal striae at mid-body; 24-28 tail annules; subcylindrical tail shape; subhemispherical, smooth tail terminus; T/ABW ratio about 2.7-3.0; spicule 28 microns long; and gubernaculum 6 microns long. The species keys out to *T. ornatus* Allen, 1955 (possibly identical with *T. quadriifer* as proposed above) and is regarded a likely synonym by Andrassy.

Wallace and Greet (*Parasitology* 54: 129-144, 1964) have recently separated *Tylenchorhynchus macrurus* (Goodey, 1932) *sensu lato* into a small form, which retains the species name, and a large form, *T. icarus*. *Tylenchorhynchus macrurus* now has the following criteria: L = 0.8-1.2 mm, a = 19-25; b = 4.8-6.3; c = 14-20; V = 54-59%; stylet = 25-34 microns; 39-47 tail annules. *Tylenchorhynchus icarus*: L = 1.5-2.0 mm; a = 29-34; b = 5.9-6.9; c = 19-25; V = 50-57%; stylet = 34-42 microns; 50-59 tail annules. In most other details, the two species are very similar. In the key to species, *T. icarus* is separated from *T. macrurus* by a longer stylet (34-42: 25-34 microns) and by a greater number of tail annules (50-59: 39-47).

8. Cuticle with longitudinal striae 9
 Cuticle without longitudinal striae 14
9. Cuticle with 29 longitudinal striae *claytoni* Steiner, 1937
 Cuticle with 8-20 longitudinal striae 10
10. Stylet 14-17 microns long 11
 Stylet 21 microns long 12
11. Cervical region with 8 longitudinal striae,
 tail annules 40-41 *brevilineatus* Williams, 1960
 Cervical region with 12 longitudinal striae,
 tail annules 32-36 *indicus* Siddiqi, 1961
12. Labial region continuous, T/ABW ratio 1.9 *lamelliferus* (de Man, 1880)
 Labial region offset, T/ABW ratio 2.2-3.0 13
13. Tail terminus annulated, stylet 21 microns
 long *judithae* Andr ssy, 1962
 Tail terminus smooth, stylet 24-27 microns
 long *microphasmis* Loof, 1959
14. Labial region offset 15
 Labial region continuous 20
15. Tail terminus annulated, tail annules 46-48 *dubius* (B tschli, 1873)
 Tail terminus smooth, tail annules 14-33 16
16. T/ABW ratio 3.7, labial annules 3 *martini* Fielding, 1956
 T/ABW ratio 2.2-2.7, labial annules 4-6 17
17. Stylet 16-17 microns long 18
 Stylet 20-27 microns long 19
18. Labial annules 4-5, tail annules 18-33 *brassicae* Siddiqi, 1961
 Labial annules 6, tail annules 14-15 *latus* Allen, 1955
19. Labial annules 4, tail annules 18-26,
 stylet 20-23 microns long *agri* Ferris, 1963
 Labial annules 5, tail annules 15-16,
 stylet 24-27 microns long *cylindricus* Cobb, 1913
20. T/ABW ratio 1.1 *brevicaudatus* Hopper, 1959
 T/ABW ratio 2.0 or greater 21
21. Female tail hook-shaped with cuticular flaps *hursifer* Loof, 1959
 Female tail not hook-shaped 22
22. Tail terminus annulated 23
 Tail terminus smooth 28
23. Tail shape clavate, T/ABW ratio 3.7 *clavicaudatus* Seinhorst, 1963
 Tail shape not clavate, T/ABW 3.0 or less 24
24. Tail shape conoid, tail terminus bluntly
 pointed, labial annules 4 *eremicolus* Allen, 1955
 Tail shape cylindrical, tail terminus
 hemispherical, labial annules 5-7 25
25. Stylet 27-30 microns long, labial frame-
 work sclerotization very conspicuous *magnicauda* (Thorne, 1936)
 Stylet 24 microns or less, labial frame-
 work sclerotization inconspicuous 26
26. Body length 1.0-1.4 mm, stylet 21-24
 microns long *maximus* Allen, 1955
 Body length 0.7-0.9 mm, stylet 17-19
 microns long 27
27. T/ABW ratio 2.0, labial annules 5 *huesingi* Paetzold, 1958
 T/ABW ratio 3.0, labial annules 7 *parvus* Allen, 1955

28. Body length 1.7 mm, stylet 31-39 microns
 long *galeatus* Litvinova, 1946
 Body length 1.2 mm or less, stylet 15-31
 microns long 29
29. Stylet 28-31 microns long, tail annules 51-58 *kegenicus* Litvinova, 1946
 Stylet 15-26 microns long, tail annules
 38 or less 30
30. Labial annules 2 *nudus* Allen, 1955
 Labial annules 3 or more 31
31. Tail annules 35-38 *manubriatus* Litvinova, 1946
 Tail annules 30 or less 32
32. Stylet 23-26 microns long, body length
 0.8-1.0 mm *silvaticus* Ferris, 1963
 Stylet 22 microns or less, body length
 0.8 mm or less 33
33. Labial annules 5-6 34
 Labial annules 3-4 37
34. Stylet 21-22 microns long, labial frame-
 work sclerotization very conspicuous *ebriensis* Seinhorst, 1963
 Stylet 15-18 microns long, labial frame-
 work sclerotization inconspicuous 35
35. Tail annules 10-15 *clarus* Allen, 1955
 Tail annules 20-28 36
36. Body ventrally contracted posterior to
 vulva, stylet knobs not anteriorly
 inclined *contractus* Loof, 1964*
 Body not ventrally contracted posterior to
 vulva, stylet knobs anteriorly inclined *striatus* Allen, 1955
37. Tail annules 25-30, labial framework
 sclerotization moderately conspicuous 38
 Tail annules 25 or less, labial framework
 sclerotization inconspicuous 39
38. Tail annules 25-26, T/ABW ratio 3.2 *dactylurus* Das, 1960
 Tail annules 29-30, T/ABW ratio 2.7 *digitatus* Das, 1960
39. T/ABW ratio 3.6 *crassicaudatus* Williams, 1960
 T/ABW ratio 3.0 or less 40
40. T/ABW ratio 3.0, tail annules 23-25 *elegans* Siddiqi, 1961
 T/ABW ratio 2.4-2.5, tail annules 13-19 41
41. Stylet 17-18 microns long, tail terminus
 bluntly pointed *mashhoodi* Siddiqi & Basir, 1959
 Stylet 18-20 microns long, tail terminus
 hemispherical *ewingi* Hopper, 1959
42. Lateral lines 5 43
 Lateral lines 6 47
43. Labial annules 6-8, tail annules 32-56 44
 Labial annules 4-6, tail annules 15-23 46
44. Tail terminus annulated, stylet 20-24
 microns long *goodeyi* Marinari, 1962
 Tail terminus smooth, stylet 16-18
 microns long 45

45. Tail terminus bluntly pointed, tail annules
32-33.....*capitatus* Allen, 1955
Tail terminus hemispherical, tail annules
42-53.....*acti* Hopper, 1959
46. Labial annules 6, body length 0.6-0.7, an-
terior surface of stylet knobs inclined
anteriorly.....*acutus* Allen, 1955
Labial annules 4-5, body length 0.5-0.6,
anterior surface of stylet knobs inclined
posteriorly.....*curvus* Williams, 1960
47. Cuticle with longitudinal striae.....48
Cuticle without longitudinal striae.....52
48. Tail terminus annulated.....*tessellatus* Goodey, 1952
Tail terminus smooth.....49
49. Labial region continuous, about 32 longi-
tudinal striae at mid-body.....*ornatus* Allen, 1955
Labial region offset, about 24-30 longi-
tudinal striae at mid-body.....50
50. Labial annules 5, about 30 longitudinal
striae at mid-body, tail shape cylindri-
cal.....*quadrifer* Andr ssy, 1954
Labial annules 6, about 24-28 longitudinal
striae at mid-body, tail shape conical.....51
51. T/ABW ratio 3.9, stylet 18-20 microns
long.....*lenorus* Brown, 1956
T/ABW ratio 1.9, stylet 24 microns long.....*tartuensis* Krall, 1959
52. Labial region offset.....53
Labial region continuous.....61
53. Stylet 39-67 microns long.....54
Stylet 21-30 microns long.....57
54. Stylet 67 microns long, T/ABW ratio 1.3.....*superbus* Allen, 1955
Stylet 39-48 microns long, T/ABW ratio
2.6-3.7.....55
55. T/ABW ratio 2.6, labial annules 7.....*macrodens* Allen, 1955
T/ABW ratio 3.5-3.7, labial annules 9.....56
56. Tail shape conoid, tail terminus bluntly
pointed, labial framework sclerotization
inconspicuous.....*conicus* Allen, 1955
Tail shape subcylindrical, tail terminus
hemispherical, labial framework sclero-
tization very conspicuous.....*alpinus* Allen, 1955
57. Tail terminus annulated.....58
Tail terminus smooth.....59
58. T/ABW ratio 2.8, stylet 21-23 microns
long.....*bogdanovikatjkovi* (Kirjanova, 1941)
T/ABW ratio 4.1, stylet 23-27 microns
long.....*leptus* Allen, 1955
59. T/ABW ratio 4.0, tail annules 27-32.....*obscurisulcatus* Andr ssy, 1959
T/ABW ratio 2.8-3.1, tail annules 36-44.....60
60. Labial framework sclerotization very con-
spicuous, tail shape conical.....*grandis* Allen, 1955
Labial framework sclerotization incon-
spicuous, tail shape subcylindrical.....*lineatus* Allen, 1955

61. Tail terminus smooth	62
Tail terminus, annulated	64
62. Stylet 14-16 microns long, T/ABW ratio	
2.9	<i>brevidens</i> Allen, 1955
Stylet 26-30 microns long, T/ABW ratio	
2.3 or less	63
63. T/ABW ratio 1.1-1.5, tail annules 10-12	<i>brachycephalus</i> Litvinova, 1946
T/ABW ratio 2.3, tail annules 28-33	<i>affinis</i> Allen, 1955
64. Stylet 12-18 microns long	65
Stylet 24-35 microns long	66
65. Stylet 12-15 microns long, T/ABW ratio	
3.8, labial annules 7	<i>nanus</i> Allen, 1955
Stylet 16-18 microns long, T/ABW ratio 3.0,	
labial annules 6	<i>nothus</i> Allen, 1955
66. Stylet 30-35 microns long, labial annules	
8-10, labial framework sclerotization	
very conspicuous	<i>macrurus</i> (Goodey, 1932)
Stylet 24-27 microns long, labial annules	
5-7, labial framework sclerotization in-	
conspicuous	67
67. T/ABW ratio 3-2, tail annules 53-58,	
labial annules 7	<i>obscurus</i> Allen, 1955
T/ABW ratio 2.0-2.8, tail annules 35-39,	
labial annules 5-6	<i>socialis</i> Andr�ssy, 1962

Table 1 is a synopsis of morphological measurements and other information of diagnostic value on the species presented in the key as well as those now in *species inquirendae*. This table is based on data concerning females except for the last 2 columns which concern males. When specific information was not presented in the original publication, this was obtained from the accompanying drawing, if possible, e.g. spicule length, number of tail annules, etc. If certain information on a species is unknown, a question mark is inserted in the table.

An explanation of the code symbols used is presented at the end of the table.

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* Description to appear in *NEMATOLOGICA*, Vol. 10, 1964.

Table 1. Diagnostic data on *Tylenchorhynchus* spp.

Tylenchorhynchus	Length in mm.	a	b	c	Labial Region	Labial Annules	Frm. Sel.	Stylet Length in μ	Sky. Cli.	Lateral Lines	No. Long. Striae	No. Tail Annules	Tail Shape	Tail Terminus	T/ABW	Spicule Length in μ	Gubern. Length in μ
<i>acti</i>	0.65-0.71	30-36	4.6-4.9	15-16	55-57	OFF	8	INC	17	LAT	5	42-53	CON	HEM-SMO	3.0	—	—
Hopper, 1959																	
<i>acutus</i>	0.63-0.70	31-38	4.8-5.3	17-22	56-57	OFF	6	INC	16-17	ANT	5	17-22	CON	BLP-SMO	2.1	—	—
Allen, 1955																	
<i>affinis</i>	0.80-0.90	30-33	4.2-5.0	14-15	55-58	CNT	8	VCO	26-27	POS	6	28-33	SCL	SHM-SMO	2.3	27	9
Allen, 1955																	
<i>agri</i>	0.06-0.77	28-33	4.7-5.5	15-21	55-58	OFF	4	INC	20-23	LAT	4	18-26	SCL	HEM-SMO	2.6	22-25	13-14
Ferris, 1963																	
<i>alatus</i>	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
(Cobb, 1930)																	
<i>alpinus</i>	0.83-0.95	26-27	5.0-5.3	11-13	52-54	OFF	9	VCO	39-42	POS	6	54-58	SCL	HEM-SMO	3.7	?	?
Allen, 1955																	
<i>bifasciatus</i>	0.65-0.73	26-31	5.6-5.9	13-17	51-52	OFF	5-6	INC	19-20	POS	3	42-46	SCL	HEM-ANN	2.6-2.7	25	12
Andrassy, 1961																	
<i>bogdanovikadjkovi</i>	0.50-0.70	23-27	4.0-5.0	13-14	54-58	OFF	7-9(?)	MCO	21-23	POS	6	47(?)	CON	SHM-ANN	2.8	—	—
(Kirjanova, 1941)																	
<i>brachycephalus</i>	0.66-0.77	26-28	4.4-5.04	35-42	52-60	CNT	4-6	VCO	28-30	POS	6	10-12	CON	DIG-SMO	1.1-1.5	37	11
Litvinova, 1946																	
<i>brassicae</i>	0.58-0.72	26-35	5.0-6.0	14-17	52-58	OFF	4-5	INC	16-17	LAT	4	18-33	CON	BLP-SMO	2.4-2.7	18-21	9-11
Siddiqui, 1961																	
<i>brevicaudatus</i>	1.28-1.37	34-36	6.7-6.9	34-44	57-59	CNT	8	VCO	37-38	LAT	4	13-14	SCL	HEM-ANN	1.1	32	13
Hopper, 1959																	
<i>brevitatus</i>	0.54-0.60	23-27	4.2-5.2	11-13	52-58	CNT	6	MCO	14-16	POS	6	42-49	SCL	HEM-SMO	2.9	15	10
Allen, 1955																	
<i>brevilineatus</i>	0.56-0.65	34	5.6	14	53-56	OFF	5-6	INC	16	POS	4	8 CER	SCL	HEM-SMO	2.7	23	12
Williams, 1960																	
<i>browni</i>	0.67-0.92	35-54	5.1-8.2	15-20	52-58	OFF	?	MCO	16-21	POS	4(?)	?	SCL	HEM-SMO	3.2	18-23	10-13
(Kreis, 1929)																	
<i>buchanicus</i>	0.53	29	4.2	27	57	OFF	5	INC(?)	16	POS	?	14	CON	BLP-SMO	2.5	—	—
(Tulaganov, 1949)																	
<i>bursifer</i>	0.56-0.66	25-30	4.3-5.1	13-17	54-56	CNT	5(?)	INC	22-24	POS	4	22-30	SCL	DIG-SMO	2.9	23	10
Loof, 1959																	

capitatus Allen, 1955	0.63-0.85	30-38	5.0-5.8	12-17	51-58	OFF	8	INC	16-18	POS	5	—	32-33	CON	BLP-SMO	2.8	19	7
caronatus (Tulaganov, 1949)	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	15	10
clarus Allen, 1955	0.49-0.61	28-33	4.0-5.0	16-20	57-61	CNT	5	INC	15-17	ANT	4	—	10-15	CON	SHM-SMO	2.7	—	—
clavicaudatus Seinhorst, 1963	0.54-0.72	31-43	4.5-5.9	12-18	53-59	CNT	3	INC	18-19	POS	4	—	31-35	CLA	HEM-ANN	3.7	19	12
claytoni Stener, 1937	0.64-0.73	24-25	5.0-6.2	18-19	55-57	OFF	3	?	?	?	4	29	MID	CON	SHM-SMO	1.7	25	15
coffea Siddiqi & Basir, 1959	?	?	?	?	?	CNT	4	?	?	?	4	?	?	CYL	? -SMO	?	?	?
conicus Allen, 1955	0.78-1.10	31-39	4.4-5.8	11-14	52-56	OFF	9	INC	40-45	POS	6	—	49-55	CON	BLP-SMO	3.5	32	9
contractus Loof, 1964	0.42-0.63	22-30	3.8-5.0	13-15	56-60	CNT	5-6	INC	16-18	LAT	4	—	29-28	CON	SHM-SMO	2.5	17-18	8-9
crassicaudatus Williams, 1960	0.58-0.69	28-33	4.9-5.7	14-15	53-57	CNT	3	INC	20	POS	4	—	18-19	CYL	HEM-SMO	3.6	23	12
curvus Williams, 1960	0.49-0.63	29-37	4.6-5.5	15-18	52-57	OFF	4-5	INC	17	POS	5	—	15-23	CON	BLP-SMO	2.5	—	—
cylindricus Cobb, 1913	0.65-0.99	28-35	4.2-6.0	13-20	54-65	OFF	5	MCO	24-27	ANT	4	—	15-16	CON	BLP-SMO	2.5	25	16
dactylurus Das, 1960	0.64-0.71	31-33	4.6-5.0	15-16	52-54	CNT	4	MCO	20-21	LAT	4	—	25-26	SCL	HEM-SMO	3.2	22	13
digitatus Das, 1960	0.67-0.72	24-27	4.7-5.2	14-17	52-74	CNT	4	MCO	20	LAT	4	—	29-30	SCL	HEM-SMO	2.7	23	13
divittatus Siddiqi, 1961	0.55-0.72	32-38	5.4-6.4	16-19	53-55	OFF	5	INC	16-17	LAT	3	—	20-27	SCL	DIG-SMO	2.8	17	8
dubius (Butschli, 1873)	0.62-0.78	30-35	5.0-6.0	13-16	54-57	OFF	7	INC	18-19	POS	4	—	46-48	SCL	SHM-ANN	3.2	24	12
ehrensii Seinhorst, 1963	0.52-0.59	23-29	4.9-5.4	14-16	54-56	CNT	5	VCO	21-22	POS	4	—	25-30	CON	BLP-SMO	2.6	21	10
elegans Siddiqi, 1961	0.56-0.70	25-29	4.4-5.2	15-16	54-55	CNT	3-4	INC	15-18	LAT	4	—	23-25	SCL	SHM-SMO	3.0	20	13
eremicolus Allen, 1955	0.70-0.76	28-30	4.5-5.8	14-16	55	CNT	4	MCO	19-20	ANT	4	—	27-29	CON	BLP-ANN	2.8	23	9
ewingi Hopper, 1959	0.55-0.75	30-35	4.3-5.5	13-16	53-59	CNT	3	INC	18-20	POS	4	—	15-19	SCL	HEM-SMO	2.5	22	12

Table 1. Diagnostic data on *Tylenchorhynchus* spp.—Continued.

Tylenchorhynchus	Length in mm.	a	b	c	V ^C %	Labial Region	Labial Annules	Frm. Scl.	Styl. Len. in μ	Styl. Cli.	Lateral Lines	No. Long. Striae	No. Tail Annules	Tail Shape	Tail Terminus	T/ABW	Spicule Length in μ	Gubern. Length in μ
<i>galeatus</i> Litvinova, 1946	1.68	31	7.3	19	54	CNT	9-12	?	31-39	POS	4	—	45-47	CON	BLP-SMO	2.2	51-53	13-18
<i>goodeyi</i> Marinari, 1962	0.50-0.81	24-29	4.4-5.4	13-16	52-56	CNT	6-7	MCO	20-24	POS	5	—	49-56	CON	HEM-ANN	2.8-3.2	25-29	8-10
<i>grammicolus</i> Kirjanova, 1951	1.02	40-42	6.0-7.0	15-18	51-52	CNT(?)	?	?	25-28	LAT	6(?)	—	?	CON	BLP-?	3.9	28-30	8
<i>grandis</i> Allen, 1955	0.96-1.11	34-41	5.0-6.4	13-16	50-54	OFF	6	VCO	26-30	POS	6	—	36-43	CON	SHM-SMO	2.8	30	9
<i>huasingi</i> Peitzold, 1958	0.78-0.91	26-31	5.0-5.9	15-21	53-55	CNT	5	INC	18-19	POS	4	—	32-36	CYL	HEM-ANN	2.0	30	14-15
<i>indicus</i> Siddiqi, 1961	0.50-0.67	24-33	4.3-5.7	12-16	53-57	OFF	6	INC	14-17	POS	4	12 CER	32-36	SCL	SHM-SMO	2.5-2.8	26	11
<i>judithae</i> Andrassy, 1962	0.88-0.97	33	6.7-7.2	17-23	54-56	OFF	6-7	INC	21	LAT	4	14	36-40	CON	SHM-ANN	2.2-2.6	—	—
<i>kegenicus</i> Litvinova, 1946	0.79-1.20	25-35	4.3-6.8	10-14	42-55	CNT	6.9	?	28-31	POS	4	—	51-58	SCL	SHM-SMO	3.3-4.8	26-31	9
<i>lamelliferus</i> (de Man, 1880)	0.86-1.10	25-31	4.3-6.8	16-24	46-53	CNT	6	INC	24-28	LAT	4	14 CER	41-45	CON	SHM-ANN	1.9	31	16
<i>latus</i> Allen, 1955	0.58-0.70	30-40	4.9-5.4	18-22	56-61	OFF	6	INC	16-17	ANT	4	—	14-15	CON	SHM-SMO	2.2	—	—
<i>lenorus</i> Brown, 1956	0.63-0.78	22-29	5.2-6.7	12-15	52-58	OFF	6	INC	18-20	POS	6	24 Mid	28-30	CON	SHM-SMO	3.9	27	7
<i>leptus</i> Allen, 1955	0.64-0.96	26-34	4.5-5.7	11-12	51-56	OFF	9	INC	23-27	POS	6	—	50-58	CON	SHM-ANN	4.1	—	—
<i>lineatus</i> Allen, 1955	0.85-0.86	30-33	4.8-5.0	12-13	51-63	OFF	6	INC	25-28	POS	6	—	42-44	SCL	HEM-SMO	3.1	29	11
<i>macrodens</i> Allen, 1955	1.02-1.12	30-32	5.1-6.0	14-15	50-55	OFF	7	VCO	43-48	POS	6	—	34-37	SCL	SHM-SMO	2.6	37	11
<i>maurus</i> (Goodey, 1932)	0.85-1.68	22-31	5.5-10.3	14-19	55-57	CNT	8-10	VCO	30-35	POS	6	—	46-58	CYL	HEM-ANN	2.0	35-38	9
<i>magnicauda</i> (Thorne, 1935)	0.79-1.00	23-32	4.4-6.0	13-19	50-62	CNT	6	VCO	27-30	ANT	4	—	31-33	CYL	HEM-ANN	2.2	—	—
<i>manubriatus</i> Litvinova, 1946	0.75	29	5.4	15	55	CNT	6	?	18	POS	4	—	35-38	CYL	HEM-SMO	3.3	—	—

<i>martini</i>	0.75	31	5.0	14	54	OFF	3	?	19	LAT	4	—	22-27	SCL	HEM-SMO	3.7	—	—
Fielding, 1956																		
<i>masbhoodi</i>	0.62-0.76	26-30	4.9-5.5	16-19	55-58	CNT	3	INC	17-18	POS	4	—	13-16	CON	BLP-SMO	2.4	22-24	12-13
Siddiqi & Basir, 1959																		
<i>maximus</i>	0.98-1.40	37-47	5.4-8.0	16-20	47-54	CNT	7	INC	21-24	POS	4	—	38-41	CYL	HEM-ANN	2.6	—	—
Allen, 1955																		
<i>microphasms</i>	0.77-0.94	31-39	5.2-6.1	15-18	49-55	OFF	6-7	INC	24-27	LAT	4	16-20	53-55	CON	SHM-SMO	3.0	29	14
Loof, 1959																		
<i>nanus</i>	0.52-0.64	27-31	4.5-5.3	10-12	52-57	CNT	7	INC	12-15	POS	6	—	55-60	CON	SHM-ANN	3.8	23	8
Allen, 1955																		
<i>nothus</i>	0.55-0.70	24-30	4.0-5.1	10-11	53-57	CNT	6	INC	16-18	POS	6	—	39-48	CON	SHM-ANN	3.0	?	?
Allen, 1955																		
<i>nudus</i>	0.71	30	4.1-4.8	14-15	54-56	CNT	2	INC	19-23	ANT	4	—	18-20	SCL	HEM-SMO	3.0	23	13
Allen, 1955																		
<i>obscuroscutatus</i>	0.79	50	5.8	17	55	OFF	6	INC	22	POS	6	—	27-32	CON	SHM-SMO	4.0	—	—
Andrassy, 1959																		
<i>obscurus</i>	0.63-0.79	26-27	4.6-5.3	13-16	53-58	CNT	7	INC	24-27	POS	6	—	53-58	SCL	SHM-ANN	3.2	29	8
Allen, 1955																		
<i>ornatus</i>	0.63-0.67	28-33	4.0-4.8	14-21	54-56	CNT	6	INC	18-19	POS	6	24 CER	19-21	SCL	HEM-SMO	2.0	24	8
Allen, 1955												32 MID						
<i>parvus</i>	0.65-0.72	25-30	4.8-5.6	13-16	52-57	CNT	7	INC	17-18	LAT	4	—	35-43	CYL	HEM-ANN	3.0	12	5
Allen, 1955																		
<i>paucus</i>	0.67	23	7.0	9	68	CNT	?	MCO(?)	9	LAT(?)	?	—	?	CYL	DIG-?	4.3	—	—
Kirjanova, 1951																		
<i>quadifer</i>	0.64-0.73	26-28	5.0-5.9	17	57-59	OFF	5	INC	20-22	?	6	30 MID	?	CYL	HEM-SMO	?	—	—
Andrassy, 1954																		
<i>rhopalocercus</i>	0.62-0.81	38-46	6.5	11-13	49-55	CNT	7-8	INC	17-19	POS	3	—	42-45	SCL	HEM-ANN	5.6	21	8
Seinhorst, 1963																		
<i>sculptus</i>	0.54-0.59	23-25	4.9-4.7	18-19	56-57	CNT	2-3	VCO	22	ANT	3	—	10-11	SCL	SHM-SMO	2.3	22	12
Seinhorst, 1963																		
<i>sexamamillatus</i>	1.30	34	7.8	12	52	CNT	?	?	15	?	?	?	?	CON	DIG-?	?	34	12
(Kirjanova, 1938)																		
<i>silvaticus</i>	0.80-1.00	24-33	5.5-6.3	18-23	53-58	CNT	4	INC	23-26	ANT	4	—	17-23	CYL	HEM-SMO	2.5	29	18
Ferris, 1963																		
<i>socialis</i>	0.89-0.92	29	5.2-5.6	14-20	59-60	CNT	5-6	INC	24-26	LAT	6	—	35-39	SCL	HEM-ANN	2.0-2.8	28-29	8
Andrassy, 1962																		
<i>striatus</i>	0.58-0.72	29-34	5.0-5.3	13-16	55-57	CNT	5	INC	16-17	ANT	4	—	20-27	SCL	SHM-SMO	2.9	20	12
Allen, 1955																		
<i>styriacus</i>	0.45	22	7.3	7	?	?	?	?	?	?	?	?	?	CON	DIG-?	?	?	?
Micoletzky, 1922																		

Table 1. Diagnostic data on *Tylenchorynchus* spp.—Continued.

Tylenchorynchus	Length in mm.	a	b	c	V	Labial Region	Labial Annules	Frm. Scl.	Stylet		Lata Lines	No. Long. Striae	No. Tail Annules	Tail Shape	Tail Terminus	T/ABW	Spicule Length in μ	Gubern. Length in μ
									Sty. in μ	Cli. in μ								
superbus Allen, 1955	1.12	28	5.6	22	56	OFF	8	INC	67	POS	6	—	26-29	SCL	SHM-SMO	1.3	33	12
symmetricus (Cobb, 1914)	0.60	26	4.9	9	?	OFF	?	?	19	?	?	?	?	CON	DIG-?	3.4	?	?
tartuensis Krahl, 1959	0.76-1.04	31-35	4.6-5.1	17-19	55-56	OFF	6	VCO	24	POS	6	24-28	30-37	CON	HEM-SMO	1.9	—	—
tessellatus Goodey, 1962	0.90	29	6.0	15	51	OFF	6	?	20	POS	6	?	36	SCL	HEM-ANN	2.4	34	11-12
triglyphus Sainhorst, 1963	0.58-0.68	24-30	4.6-5.6	16-19	55-58	CNT	4	INC	20-23	LAT	3	—	14-16	CON	HEM-SMO	2.5	22	10
trilineatus Timm, 1963	0.67-0.75	31-36	5.1-5.6	19-25	56-58	OFF	4	INC	21-22	LAT	3	—	10-14	SCL	SHM-SMO	2.9	21-22	12-14

CODE

Labial Region:

CNT = Continuous

OFF = Offset or constricted

Frm. Scl. = Labial framework sclerotization:

VCO = Very conspicuous

MCO = Moderately conspicuous

INC = Inconspicuous

Sty. Cli. = Inclination of anterior surface of stylet knobs:

ANT = Anterior

LAT = Lateral

POS = Posterior

No. Long. Striae = Number (and position) of longitudinal striations:

CER = At cervical region

MID = At mid-body

PVU = Posterior to vulva

No. Tail Annules = Range of number of annules cited in the description and counted in the accompanying drawing.

Tail Shape:

CON = Conoid

CYL = Cylindrical

SCL = Subcylindrical

CLA = Clavate

Tail Terminus:

HEM = Hemispherical

SHM = Subhemispherical

BLP = Bluntly pointed

DIG = Digitate

SMO = Smooth

ANN = Annulated

T/ABW = Tail divided by width of body at anus

Quantitative Studies on Development of *Nippostrongylus brasiliensis* after Different Routes of Infection*

JOSIAH OLATUNJI SIMAREN**

In the past four decades, biological journals have contained many extensive investigations and observations on the behavior and development of the rat nematode, *Nippostrongylus brasiliensis*. Literature reveals that the most commonly used method of infection has been subcutaneous injection of larvae.

Chandler (1936, 1937), Gharib (1955) and Twohy (1956) are a few who have performed many experiments on tissue migration with this rat nematode, using the percutaneous and subcutaneous routes. They concluded that the skin penetration is not essential to the development of the parasite, and that the subcutaneous injection of larvae does not alter their early migration and growth. Twohy (1955) found that *Nippostrongylus* larvae injected into the femoral vein developed normally. Brackett and Bliznick (1949) successfully infected mice with larvae by intraperitoneal route. Yokogawa (1922) and Africa (1931) have demonstrated the establishment of a few adult worms in rats after oral ingestion of the filariform larvae and Spindler (1936) after administration by duodenal tube. Schwartz and Alicata (1934) confirmed this finding.

However there is comparatively little information on the development of *N. brasiliensis* after subcutaneous, intravenous, intraperitoneal and oral routes of infection within a single strain of rats. This investigation was designed to compare the efficiency of the above four methods of producing infection with *N. brasiliensis* in a single strain of laboratory rats.

MATERIALS AND METHODS

Helminth free white laboratory rats of the Sprague-Dawley strain between 6 and 7 weeks old were used as experimental hosts. Infective larvae of *N. brasiliensis* were obtained from moist chamber cultures of rat feces and granulated animal charcoal kept at room temperature (72°-75°F) for 7 days. The recovered larvae were isolated and washed three times in 0.85 per cent sodium chloride solution, centrifuged at low speed, and the supernatant removed. The centrifuge tube of larvae was agitated several times and a 0.1 ml. sample quickly withdrawn and counted on a slide with a dissecting binocular microscope. An average of five samples was made and the amount of solution adjusted to yield the number of larvae needed for infection. All rats were infected with 500 larvae in 0.3-0.4 ml. of suspension regardless of the route of infection.

Subcutaneous infection was by injection in the posterior neck region beneath the loose skin and the intravenous infection was in one of the tail veins. The intraperitoneal infection was made with the syringe held in a perpendicular position. For oral infection the larvae were introduced gradually into the stomach by means of a urinary catheter attached to the needle of the syringe and followed by another 3-4 drops of water to ensure that all given larvae were swallowed.

The number of *Nippostrongylus* eggs passed in every 24 hour fecal collec-

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tion was determined by a modification of Stoll's (1923) dilution egg-count method.

For worm counts, rats were fasted overnight except for water, and chloroformed. The small intestine, lungs, trachea, heart, and fluids from the thoracic and abdominal cavities were examined for worms using binocular dissecting microscope.

A modification of Haley's (1961) method was used to measure the worms. Since the worms have marked tendency to coil they were individually teased out straight on Shark skin filter paper with a very fine needle and thereafter measured with an ocular micrometer in a stereoptic microscope.

RESULTS AND DISCUSSION

A set of four experiments were performed to compare the development of infections produced by 500 *N. brasiliensis* larvae given subcutaneously, intravenously, intraperitoneally and orally established that some differences in the efficiency of different routes do occur.

The patent period following all four different routes of infection was observed from the sixth day, through the fourteenth day in rats orally and subcutaneously infected but the patent period in rats intravenously and intraperitoneally infected extended only to the thirteenth day after infection (Table 1).

Most of the rats infected subcutaneously showed a peak egg production on the seventh day after infection. Slightly delayed peaks of egg production were noticed in the groups infected intravenously, intraperitoneally and orally.

The subcutaneous infection also gave the highest average worm burden and egg production. A decreased number of worms and eggs resulted from the intravenous and intraperitoneal routes. The oral routes produced only a very light worm burden and egg count (Table 1 and 2).

Slightly more female than male worms were recovered from all four different infection routes 10 days after infection (Table 2). Yokogawa (1922) has previously reported that only a small proportion of larvae fed orally to rats were able to reach intestine. Africa (1931) demonstrated that rats infected orally produced fewer eggs than those given by cutaneous infections, and

Table 1. Comparison of average egg count in thousands from rats (8 per group) infected with 50 larvae of *N. brasiliensis* by various routes.

Experiments	I	II	III	IV
Routes of Infection	Subcutaneous	Intravenous	Intraperitoneal	Oral
Infection:				
Days after				
5	0	0	0	0
6	54	7	1	2
7	178	100	19	10
8	167	170	75	13
9	112	122	74	12
10	75	112	31	8
11	41	21	17	5
12	13	3	3	3
13	5	0.1	0.1	3
14	2	0	0	3

Table 2. Number of *N. brasiliensis* from each of four groups of rats (3 per group) recovered 10 days after infecting 6-7 week old rats by four different routes.

Route of Infection	Average daily Egg Production in thousands from days 6 to 14	Worms per rat		Mean No. Worms Per rat	Percent Females	Percent Development
		M	F			
Subcutaneous	647	178	218	345	56	69
		163	238			
		113	124			
Intravenous	535.1	98	180	274	61.3	55
		119	141			
		101	184			
Intraperitoneal	220.1	64	141	245	60.3	49
		121	150			
		104	156			
Oral	59	19	16	70	62.9	14
		34	76			
		26	40			

that both routes yielded fewer males than female worms. The present results confirm those findings and also furnish additional supporting evidence that the intraperitoneal and oral routes of infection are not good routes for the development of *Nippostrongylus*, even though some worms do reach the intestine and mature.

The variation in the length of *Nippostrongylus* adults had a similar pattern from all different routes of infection. The length in every case was within the limits reported by Yokogawa (1920) in his original description, namely males 3-4 mm. and female 4-6 mm. (Table 3).

Microscopic examination of autopsied rats showed no worms trapped or retained in organ tissue or peritoneal cavity. There was no evidence of pathological injuries or inflammatory reactions of migrating larvae.

SUMMARY

The ability of the rat nematode, *Nippostrongylus brasiliensis*, to develop in young rats after infection by the intravenous, intraperitoneal, and oral routes was compared with those resulting from subcutaneous infection.

The first appearance of eggs occurred uniformly in all four groups of rats on the sixth day of infection. The daily number and average number of eggs produced during the sixth through fourteenth days of infection were consist-

Table 3. Size of *N. brasiliensis* on day 10 after infection by four different routes. A total of 50 males and 50 females were measured from the worms recovered from two rats in each group.

Route of Infection	Sex of Worms	Length in mm.		% Deviation from subcutaneous
		Mean	Range	
Subcutaneous	M	3.5	2.3-4.3	-
	F	4.3	2.8-5.6	-
Intravenous	M	3.6	2.3-4.6	+3
	F	4.0	2.3-5.6	-7
Intraperitoneal	M	3.8	3.1-4.4	+8
	F	4.5	3.3-5.3	-5
Oral	M	3.5	2.1-5.0	0
	F	4.4	2.8-5.9	+2

ently highest in the subcutaneously infected rats, and progressively smaller in the intravenously, intraperitoneally, and orally infected rats. The largest number of adult worms was found in the intestine in the subcutaneously infected rats, and progressively smaller number in the intravenously, intraperitoneally, and orally infected animals. There were no significant statistical differences in the mean length of either male worms, or the female worms recovered from the four groups of rats infected by different routes. The recovered worms were all apparently normal in morphology and size.

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Esterase Enzymes in Two Free-living Nematodes

D. L. LEE*

Linford (1937) studied the feeding habits of certain hollow stylet nematodes including *Dorylaimus* sp. and *Actinolaimus* sp. Rhoades & Linford (1961) and Hechler (1962) continued these studies on phytophagous nematodes but these studies were on the mechanics of feeding and did not investigate the nature of the secretions observed. Tracy (1958) detected a cellulase and a chitinase in homogenates of three species of *Ditylenchus* but not in *Turbatrix aceti*. Krusberg (1960) carried out a thorough investigation of the hydrolytic and respiratory enzymes of two species of *Pratylenchus*. Goffart & Heiling (1962) detected amylase, invertase and pectin dissociating enzymes in the secretion from the salivary glands of *Ditylenchus* spp. and *Heterodera* spp.

This investigation has been carried out on two free-living cryptobiotic (Keilin, 1959) nematodes, *Actinolaimus hintoni* and *Dorylaimus keilini* (Lee, 1961, 1962b) which are found in shallow rock pools in Nigeria. The aim of this work was to determine the localization of esterase enzymes, as detected by histochemical methods, and to compare the distribution of the enzymes in these two nematodes which appear to have different methods of feeding and different foods. *A. hintoni* is presumed to be a predator (Goodey, 1951) as it possesses a spear and jaws (onchia) while *D. keilini*, which possesses a spear but no jaws, is presumed to feed on vegetable matter as the intestine contains green material in the lumen.

MATERIALS AND METHODS

The nematodes arrived and were stored in the original dried mud in which they were found. They were obtained from the dry mud by shaking it with distilled water and picking them out on a fine needle when they were fully extended. They were then transferred directly to the incubating media.

The substrates used to detect non-specific esterase were α -naphthyl acetate—Fast blue B salt; naphthol AS acetate—Fast red TR salt; and 5-bromo-indoxyl acetate. When 5-bromoindoxyl acetate was used the body wall gave an intense blue result making it difficult to determine the localization of esterase in internal structures, and α -naphthyl acetate gave generally poor localization whereas these problems did not arise when naphthol AS acetate was used therefore most of the work was done using this substrate. The substrate used to detect cholinesterase was acetylthiocholine iodide. All methods used are given in Pearse (1960). The nematodes were incubated in naphthol AS acetate medium for 15 to 30 min. or for 2 to 3 hr. in the acetylthiocholine iodide medium (Gomori's modification, *cf.* Pearse, 1960) at 24°C, and were eventually mounted in glycerin after slow evaporation in glycerin and water. The inhibitors used to determine the nature of the esterases were 10⁻⁴M and 10⁻⁵M eserine and 10⁻⁶M E600 ("Paraoxon"; diethyl-p-nitrophenyl phosphate). The nematodes were incubated for 1 hr at 30°C in the inhibitor before incubation in the substrate which contained the same concentration of inhibitor. Control nematodes were incubated in distilled water at 30°C before incubation in the substrate.

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These studies were supported in part by research grant AI-04275 from the National Institutes of Health, U.S. Public Health Service.

Esterases which hydrolysed acetylthiocholine iodide and naphthol AS acetate and which were inhibited by 10^{-5} M eserine and by 10^{-6} M E600 were identified as cholinesterases. Esterases not inhibited by 10^{-5} M eserine were classed as non-specific esterases. Non-specific esterases which are sensitive to 10^{-6} M E600 but not to 10^{-5} M eserine were classified as B-type esterases.

RESULTS

Cholinesterase is present in the nerve ring in both *A. hintoni* and *D. keilini* and it is also present around the base of the jaws and spear of *A. hintoni* (Fig. 1) but there is no equivalent localization around the spear of *D. keilini*. There is also a B-type esterase around the base of the jaws and spear of *A. hintoni*. In several specimens there was a weak or negative result for esterase around the jaws and spear of *A. hintoni*.

The intestinal cells of *D. keilini* gave a much stronger result for esterase than did the intestine of *A. hintoni*. In both species there was weak esterase activity in the oesophagus and in the body wall but localization was poor.

As these nematodes, in their normal environment, are subjected to moderately high temperatures when drying out (40°C) and presumably to even higher temperatures when in the dry state in the dry season, it was decided to find out what the effect of wet and dry heat was on the esterase enzymes as detected by the naphthol AS acetate method.

Samples of the dry soil containing the nematodes were placed in a dry oven at 100°C for periods up to 6 hr. and further samples were placed in distilled water for 10 min. at 90°C . Control nematodes were kept at room temperature (20°C) in the dry soil and also in distilled water at room temperature for the equivalent periods of time. The nematodes were then tested for the presence of esterase.

There was normal distribution of esterase in both species after being subjected to dry heat at 100°C for 6 hr., but after 10 min. at 90°C in distilled water there was no detectable enzyme activity. In both wet and dry control nematodes there was normal distribution of esterase.

DISCUSSION

Several workers have demonstrated cholinesterase or acetylcholinesterase in nematodes (for review see Fairbairn, 1960; and Lee, 1962a) and Rhode (1960), using histochemical methods, found acetylcholinesterase in the nervous system and associated sensory structures of several plant parasitic nematodes. This study has demonstrated the presence of cholinesterase in the nerve ring of both *Actinolaimus hintoni* and *Dorylaimus keilini*. However, cholinesterase has also been demonstrated around the base of the jaws and spear of *A. hintoni* and although it is probably associated with the muscles which operate these powerful jaws there is a possibility that it may have some other function. A non-specific esterase is also present around the base of the jaws and spear and again this may be associated with the muscles but it is interesting that in several specimens this esterase is weak or absent in the region of the jaws whereas the distribution of esterases in the rest of the body is of normal intensity. It is possible that this non-specific esterase and the cholinesterase are injected into and used to paralyze and, or, digest the prey of the nematode and that those nematodes which gave a weak or negative result around the jaws had discharged the enzymes into prey just before the habitat began to dry out. The fact that the esterase in the intestine of *A. hintoni* gives a much weaker result than the intestine of *D. keilini*

indicates that *A. hintoni* carries out extracorporeal digestion of the prey. *D. keilini*, which has much stronger esterase activity in the intestine presumably carries out more intestinal digestion than *A. hintoni*. Thus the distribution of esterase in these two nematodes may be associated with their methods of feeding as species of *Actinolaimus* are presumed to be active predators (Goodey, 1951) and need to paralyze the prey and carry out a certain amount of extracorporeal digestion before sucking up the semi-digested contents while the majority of species of *Dorylaimus* feed on vegetable matter and carry out more intestinal digestion, although some species are predators.

It is interesting to find that the esterases in these cryptobiotic nematodes, when in the dry state, are resistant to dry heat but not to wet heat. If the other enzymes present in these nematodes are similarly resistant it may help to explain how these nematodes are able to survive in their natural environment during the hot dry season.

SUMMARY

The distribution of esterase enzymes in whole mounts of *Actinolaimus hintoni* and *Dorylaimus keilini* is described. There is more esterase activity in the intestine of *D. keilini* than in *A. hintoni* but there is pronounced esterase activity around the stylet and onchia of *A. hintoni* and little around the stylet of *D. keilini*. It is suggested that this distribution of esterases may be related to the feeding habits of these two species and that *A. hintoni* carries out more extracorporeal digestion than *D. keilini*. The enzymes in these nematodes, when they are in a cryptobiotic state, are resistant to dry heat but not wet heat.

I wish to thank Dr. H. E. Hinton for the supply of material and for his continued interest in this work, and Dr. P. Tate, in whose department this

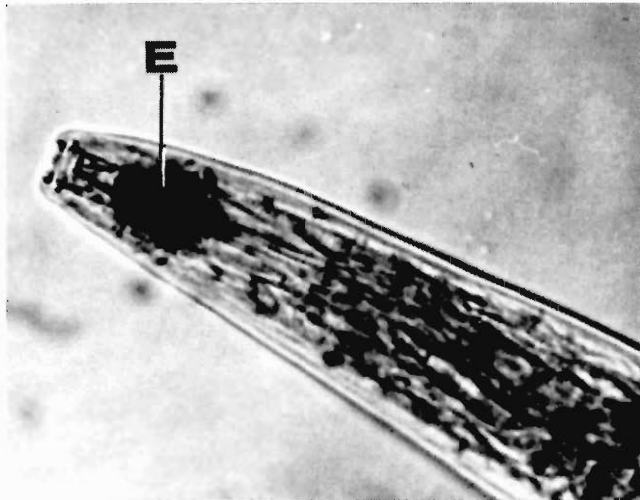


Fig. 1. Anterior end of *Actinolaimus hintoni* showing the concentration of esterase (E) around the mouth spear and onchia.

work was carried out, for much useful advice. This work was carried out during the tenure of a Research Fellowship at Christ's College, Cambridge.

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***Quadriplotriaena hypsokysta* sp. n. (Nematoda: Filariidae) from
the Eastern Meadowlark, *Sturnella magna magna***

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Filaroid nematodes, constituting a new species, were found in the body cavities of 3 eastern meadowlarks (*Sturnella magna magna* L.) collected in Licking County, Ohio in April and May of 1963 by Mr. James Deck. The material studied consisted of a series of 16 males and 33 females: meadowlark (1) 4 males, 10 females; (2) 6 males, 12 females; (3) 6 males, 11 females. The holotype and allotype were chosen from bird No. 2. Some specimens were examined alive under the phase microscope; the majority were heated gently in a physiological salt solution until all movement ceased and fixed with AFA. Preserved specimens were cleared with glycerine alcohol and mounted in glycerol under supported cover slips. The latter were studied with both phase and regular light microscopes. *En face* preparations were cleared with both glycerine and lactophenol. Measurements are given as an average followed by the range in parentheses.

Quadriplotriaena hypsokysta sp. n. (Figs. 1-8)

DESCRIPTION: Filariidae Claus, 1885; Diplotrieninae Skrjabin, 1916; *Quadriplotriaena* Wehr, 1939 (Wehr, 1939). Oral opening dorsoventral slit, surrounded by oval circumoral membrane. Four pairs of large, submedian, cephalic papillae present. Amphids large, lateral, between papillae. Tridentes very weakly cuticularized, extremely difficult to discern. Tips of 4 manubria of tridentes project finger-like above cephalic surface, two on each side of oral opening. Esophagus clearly divided into anterior, short, muscular part and posterior, long, broad, glandular part. Cuticle with delicate, regular transverse striations and widely scattered small bosses.

MALE (10 specimens): Length 61.7 (50-72) mm. apically attenuate, especially anteriorly. Maximum width 0.51 (0.49-0.51) mm., attained near middle of body. Nerve ring 0.22 (0.16-0.25) mm. from anterior end, surrounding muscular part of esophagus. Muscular esophagus 0.38 (0.33-0.43) mm. long; glandular esophagus 4.9 (4.3-5.6) mm. long, maximum width at junction with intestine 0.27 (0.26-0.29) mm. Total length of esophagus 5.3 (4.6-6.0) mm. Intestine broad, particularly in region following junction with esophagus. Testis reflected, extends to 0.43 (0.43-0.44) mm. from anterior end. Spicules morphologically dissimilar, markedly unequal in length. Long spicule 1.33 (1.05-1.48) mm., thin, delicate, slightly arcuate; slightly grooved toward anterior end, finely winged in posterior half, tip appears to have opening to internal channel. Short spicule 0.62 (0.54-0.66) mm., stout, twisted twice, winged. Both spicules cephalated, pointed distally with clear tips, surrounded along part of length by faint but discernable sheath of tissue. Cloacal aperture ovate, subterminal. Five pairs of unsymmetrical caudal papillae, very difficult to discern. Caudal end rounded slightly swollen. Caudal alae absent.

FEMALE (15 specimens): Length 185 (155-210) mm., tapering at both ends. Maximum width 0.96 (0.93-0.98) mm., attained near middle of body. Nerve ring surrounds muscular esophagus 0.25 (0.24-0.26) mm. from anterior end.

Muscular esophagus 0.43 (0.39-0.49) mm. long; glandular esophagus 5.6 (4.5-6.5) mm. long, maximum width 0.33 (0.26-0.39) mm. Total length of esophagus 6.0 (4.9-7.0) mm. Intestine broad, thin-walled. Rectum and anus atrophied. Vulva conspicuous, slightly salient, opening short distance posterior to junction of two parts of esophagus 0.69 (0.62-0.80) mm. from anterior end of body. Vagina long, 2.93 (2.81-3.11) mm., muscular, broadened posteriorly, dividing into two thin-walled opisthodelphic uteri. Uteri packed with ova, twisted around each other and reflexed posteriorly. Distance from loop of posterior uterus to posterior end varies greatly 1.98 (0.62-2.39) mm. One oviduct reflexes anteriorly 0.41 (0.31-0.49) mm. from anterior end, usually extending anterior to vulva. Eggs 31 by 47 microns, contain developed larvae, shells thick but smooth.

HOST: *Sturnella magna magna* Linnaeus.

LOCATION: Beneath coelomic peritoneum.

LOCALITY: Licking County, Ohio.

TYPE SPECIMENS: U.S.N.M. Helm. Collection No. 60189.

HOLOTYPE: male; Allotype: Female.

DISCUSSION

Hitherto, only one species has been described in this genus, *Quadriplotriaena dolichodemus* Wehr, 1939 (genotype) from the abdominal cavity of the magpie, *Pica pica hudsonia* (Linnaeus) collected in Montana. In his generic diagnosis Wehr (1939) states, "Parasites in the body cavity of Icteridae." Actually magpies are not classified as Icteridae but as Corvidae. The meadowlarks, however, do belong to the family Icteridae. Future work may reveal that members of the genus occur in other birds.

Quadriplotriaena hypsokysta differs from *Q. dolichodemus* in having small cuticular bosses. The manubria of the tridents of the new species differ from *Q. dolichodemus* in that they are not rounded at their tips but appear as short thin oval ridges with a dorsoventral orientation. The tridents of *Q. hypsokysta* are much more weakly cuticularized than those of *Q. dolichodemus*. In the new species these structures were observed only in *en face* preparations which were cleared with lacto-phenol and studied carefully with an oil immersion, phase contrast objective. The teeth of the tridents agree in number with those described for *Q. dolichodemus* but they are much smaller, never exceeding 8 microns in length, in *Q. dolichodemus* the shortest are 15 microns in length.

In contrast with *Q. dolichodemus* the males of *Q. hypsokysta* are shorter and have only 5 pairs of caudal papillae. The spicules are cephalated, more twisted, pointed distally and clear at the tips.

The females of *Q. hypsokysta* differ from the females of *Q. dolichodemus* in having a salient vulva and opisthodelphic uteri which extend to much nearer the posterior end of the body. The oviducts of *Q. hypsokysta* reflex in the anterior end of the body and in 90 percent of the specimens one of them extends anterior to the vulva. The eggs of *Q. hypsokysta* are smaller.

Anderson (1956, 1957) elucidated the major portion of the life history of *Diplotriaenoides translucidus*, a member of the subfamily Diplotriaeninae. He demonstrated that all members of this subfamily have eggs with shells, and probably enter the air sacs of their hosts and that the eggs pass up the tracheae and out with the feces. The air sacs of the meadowlark hosts were carefully examined for nematodes and eggs but none were found. It seems

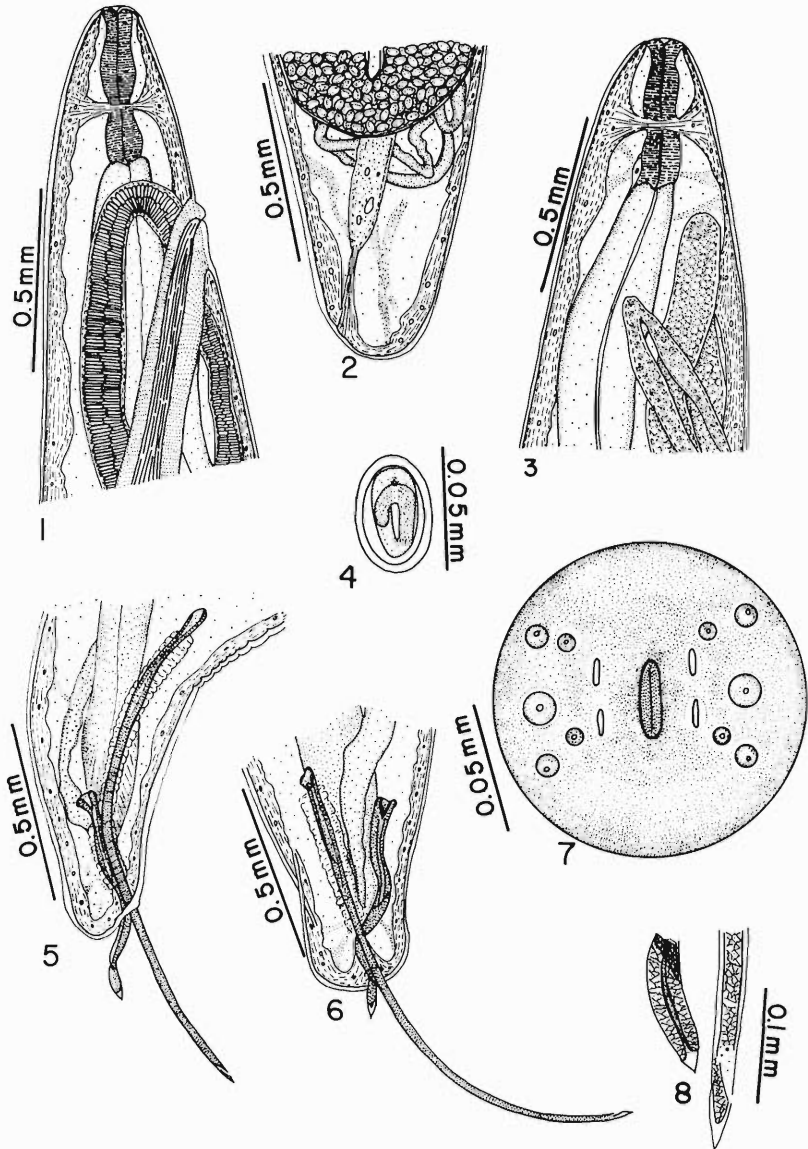


Fig. 1. Female, anterior end, lateral view; Fig. 2. Female, posterior end, lateral view; Fig. 3. Male, anterior end, lateral view; Fig. 4. Embryonated ovum; Fig. 5. Male tail, lateral view; Fig. 6. Male tail, ventral view; Fig. 7. "En face" view; Fig. 8. Distal end of spicules.

probable, however, that at sometime the adult females of this genus do enter the lungs and the life cycle is completed in a manner similar to that demonstrated by Anderson for other diplotriaeids.

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**A New Species of Trematode, *Pycnoporos ramsesi*
(Lecithodendriidae), from Egypt and Notes
on *P. acetabulatus* Looss, 1899***

RALPH W. MACY**

This report represents part of the work on trematodes carried out by the author, at the United States Naval Medical Research Unit No. 3. It is a contribution to the broad program involving the examination of vertebrate animals which may serve as reservoirs of disease. Other recent studies of helminth parasites of bats of Egypt include those of Macy, 1953; Macy, Heyneman, and Kunz, 1961; and Heyneman and Macy, 1962.

Examination of a specimen of Rupell's Pipistrelle, *Pipistrellus ruppelii ruppelii* (Fisher, 1829) collected by a native hunter at Abu Rawash, Giza, January, 1962, revealed two examples of *Pycnoporos acetabulatus* Looss, 1899 and 40 specimens of an undescribed species of the same genus all from the intestine. Specimens of both species were studied alive and then were fixed in Gilson's fluid after which they were stained in borax carmine and prepared as permanent whole mounts.

Measurements are in millimeters followed by minima and maxima in parentheses.

Pycnoporos ramsesi n. sp. (Figs. 1, 2, 3, 4, 5)

SPECIFIC DIAGNOSIS: *Pycnoporos*, as herein emended. Body elongated with anterior end somewhat narrowed; anterior end often invaginated (Fig. 3). Length of 4 largest specimens 0.74 (0.53 to 0.94); maximum width 0.32 (0.28-0.38). Cuticula with fine spines covering body to level of vitellaria. In living specimens layer beneath cuticula irregularly thickened and delimited by an inner membrane (Fig. 4), closely resembling that found in *P. acetabulatus*. Oral sucker subterminal, 0.069 (0.056 to 0.096) long by 0.060 (0.048 to 0.10) wide, mouth opening ventral. Ventral sucker 0.039 (0.032 to 0.044) in diameter, located on anterior margin of testicular zone and anterior to ovary; placed in anterior half of the body. Ventral sucker simple, without larger, non-muscular part characteristic of *P. acetabulatus*.

* The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. Supported by National Science Foundation Grant G-14016, and by Research Subtask LHMR005.09-1450.2, Bureau of Medicine and Surgery, Department of the Navy.

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For facilitating this work in Egypt, special thanks are due to Dr. Donald Heyneman, Dr. Harry Hoogstraal, and Dr. Harry G. Browne.

and several other species of the genus. Pharynx 0.012 in diameter; prepharynx not evident. Esophagus elongate, variable in length depending upon extent of body contraction. Structure of esophagus includes transverse fibers, possibly muscle cells. Ceca short, expanded.

Testes oval, opposite or nearly so, located in anterior half of body near middle, depending upon amount of extension of anterior part of body. Right testis 0.115 (0.096 to 0.152) long by 0.089 (0.05 to 0.116) wide; left testis 0.013 (0.092 to 0.120) long by 0.086 (0.06 to 0.112) wide. Pseudo-cirrus sac elongated and curved around left side of ventral sucker (Fig. 1) or subspherical (Figs. 2, 6), 0.13 (0.10 to 0.16) long by 0.073 (0.06 to 0.092) wide; winding seminal vesicle in posterior half; prostate cells forming a mass immediately anterior to seminal vesicle; ejaculatory duct reaching to genital pore in anterior part.

Ovary between and reaching posterior to testes, located to one side of median body axis, 0.101 (0.080 to 0.122) long by 0.077 (0.060 to 0.092) wide. Vitellaria anterior testes and in paracecal position; consisting of two compact clusters of from six to eight follicles each; follicles 0.024 to 0.036. Common vitelline duct from each side jointed into small vitelline reservoir near anterior margin of ovary. Seminal receptacle small, connected at one side to a short Laurer's canal which overlaps posterior part of pseudo-cirrus sac; last two structures observed only in living specimens. Uterus filling body posterior to ovary, metraterm leads forward around right side of ventral sucker to genital pore. Eggs 0.020 by 0.013; oval, operculum with no lateral lips; smooth distal pole; light brown.

Excretory vesicle large and V-shaped (Fig. 2).

HOST: *Pipistrellus ruppelii ruppelii* (Fisher, 1829)

HABITAT: intestine

LOCALITY: Abu Rawash, Giza, Egypt.

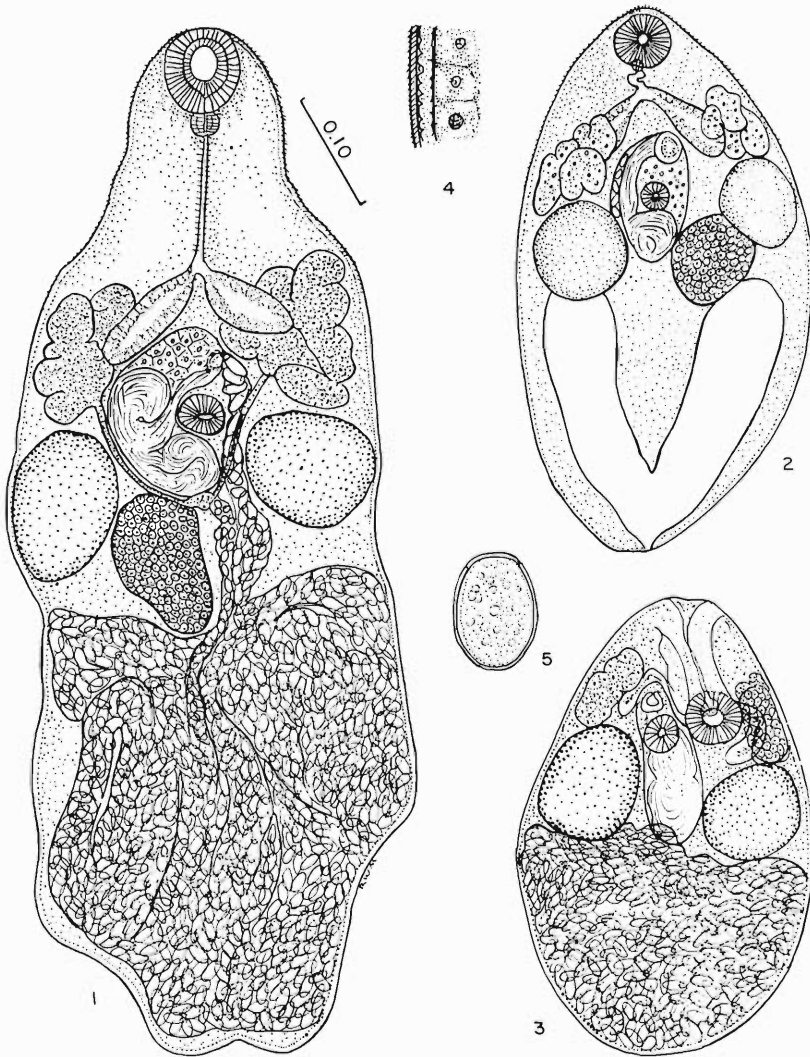
SPECIMEN DEPOSITED: paratype no. 60188, Helm. Coll. U. S. National Museum.

DISCUSSION: *Pycnoporos ramsesi* differs from other species of the genus in the possession of paracecal vitellaria and in having the ventral sucker smaller than the oral sucker. Only two other species, *P. macrolaimus* and *P. indicus*, have the ventral sucker smaller than the oral sucker. In both of these the ovary is definitely anterior to the testes whereas in *P. ramsesi* the center of the ovary is posterior to a line drawn through the centers of the testes.

Pycnoporos acetabulatus Looss, 1899 (Figs. 7, 8)

The two specimens of this species collected from the intestines of the same bat, *Pipistrellus ruppelii ruppelii*, referred to in the first part of this report, were identified as *P. acetabulatus* in spite of certain differences when compared to the original description by Looss. The present study is based upon both specimens, studies alive and also after they were stained and mounted. Measurements are from the better of the mounted specimens; the other was badly damaged in preparation.

Body elongate, narrowed (Fig. 7) or pyriform (Fig. 8). Cuticula covered to the level of the vitellaria with small spines with double layer beneath cuticula; closely resembling that found in *P. ramsesi*. Body 0.96 long by 0.21 wide; anterior region with abundant unicellular glands. Numerous granules scattered through parenchyma, extending posteriorly to the ceca. Oral sucker 0.06 wide by 0.045 long; ventral sucker 0.078 in diameter, the muscular part 0.057 in diameter, located anterior to middle of body. Pharynx 0.018 in diameter followed by elongated esophagus characterized by numerous small transverse fibers of the type also observed in *P. ramsesi*. Ceca short and distended, located some distance anterior to ventral sucker or reaching its anterior margin.



Figures 1 and 7 were drawn with the aid of a camera lucida. The projected scales are in parts of a millimeter. Figures 2, 3 and 8 were drawn to a slightly smaller scale than Figures 1 and 7. Abbreviations: ej, ejaculatory duct; gp, genital pore; met, metraterm; pr, prostate cells; sv, seminal vesicle; vit, vitellaria.

Figure 1. *Pycnoporos ramsesi*, n. sp., from intestine of *Pipistrellus ruppelii*, Egypt. Dorsal aspect; Figure 2. Same, from live specimen. Anterior end contracted. Uterus omitted to show V-shaped excretory vesicle. Ventral aspect; Figure 3. Same, from live specimen to show anterior part invaginated within the body, a condition exhibited by most individuals; Figure 4. Same, small part of body wall, much enlarged. Beneath the spinose cuticula is an additional, somewhat irregular layer with inner limiting membrane. Beneath this are the parenchyma cells, three of which are shown; Figure 5. Same. Living egg.

Testes opposite or nearly so, in posterior half of body and posterior to acetabulum; right testis 0.087 long by 0.066 wide; left testis 0.078 long by 0.060 wide. Pseudo-cirrus sac about twice as long as its maximum width, 0.13 long by 0.063, curved around left side of ventral sucker, filled largely by seminal vesicle. Genital pore lateral to ventral sucker instead of anterior to it as described by Looss.

Ovary on the right side of the ventral sucker, 0.096 long by 0.048 wide. Seminal receptacle 0.051 in diameter, in life changing from spherical to elongate. Vitelline follicles irregularly oval, in two masses on either side immediately anterior to testes.

Uterus largely filling posterior third or more of the body. Eggs 0.018 long by 0.009 wide, light brown in color.

Host: *Pipistrellus ruppelii ruppelii* (Fisher); type host *P. kuhli kuhli* (Natterer, 1819), referred to by Looss as "*Vesperugo kuhli* Keys and Blas."

DISCUSSION: Except for certain differences, "autotypes" collected from the type host at Cairo, Egypt, identified by Looss as *Pycnoporos acetabulatus*, and mounted on a slide, borrowed from U. S. National Museum, correspond closely with descriptions by Looss, by the writer, and by Pande for *P. loossi*. The concentration of unicellular glands in the anterior part of the body was also mentioned by Pande. Looss' specimens are stained lightly, but such unicellular gland can be identified. No such glands were found in *P. ramsesi*. The peculiar structure of the body wall and the minute transverse fibers of the esophagus were plainly seen in some of Looss' autotypes. However, the sucker ratios and location of the genital pore in my specimens varied somewhat from the descriptions of Looss and Pande. These authors found the diameter of the ventral sucker to be about twice that of the oral sucker. In specimens collected by the writer it is less than twice the size of the oral sucker but the difference may be due to the method of fixation. Further, some autotypes of *P. acetabulatus* show that the ventral sucker is elongated dorso-ventrally and when tipped it presents a different appearance. In some cases a non-muscular portion can be seen; in others it appears to be entirely muscular.

The position of the genital pore seems to be variable in *Pycnoporos acetabulatus*. In at least two autotypes it appears to be located at the antero-lateral margin of the ventral sucker. In others, the tipped and elongated cavity of the ventral sucker, or relaxed portions of the ejaculatory duct, could be mistaken for the genital pore. Pande states that the genital pore is median and situated directly in front of the acetabulum, which implies that it is at the posterior part of the pseudo-cirrus sac.

The original generic diagnosis by Looss, 1899, was modified by Pande, 1935. In the present report, the position of the vitellaria lateral to the ceca in *Pycnoprour ramsesi* and the location of the genital pore lateral to the acetabulum in *P. acetabulatus*, as redescribed herein, require further emendation of the genus as follows:

Pycnoporos, EMEDED DIAGNOSIS:

Small Lecithodentriinae; cuticula densely spinose or smooth, oral sucker smaller or larger than acetabulum; pre-pharynx present or absent; pharynx followed by long esophagus; intestinal ceca short, ending in front of acetabulum. Genital pore immediately in front of or lateral to acetabulum. Excretory vesicle V-shaped. Testes reaching level of acetabulum or post-acetabular, symmetrical or asymmetrical; pseudo-cirrus sac subspherical to curved elongate, with large, coiled seminal vesicle, pars prostatica, and ejaculatory duct. Ovary in acetabular zone or posterior to it; vitellaria pretesticular, at level of ceca or post-cecal, lateral to acetabulum; uterus in descending and ascending coils lying mainly post-testicular, filling body behind testes. Eggs 13-23 by 7-11 microns. Parasitic in intestine of insectivorous bats.

Dubois, 1960, in a comprehensive and critical treatment of the genus *Pycnoporos* reduced the number of species to seven and included a key to these. The redescription of *P. acetabulatus* and addition of a new species has required a revised key.

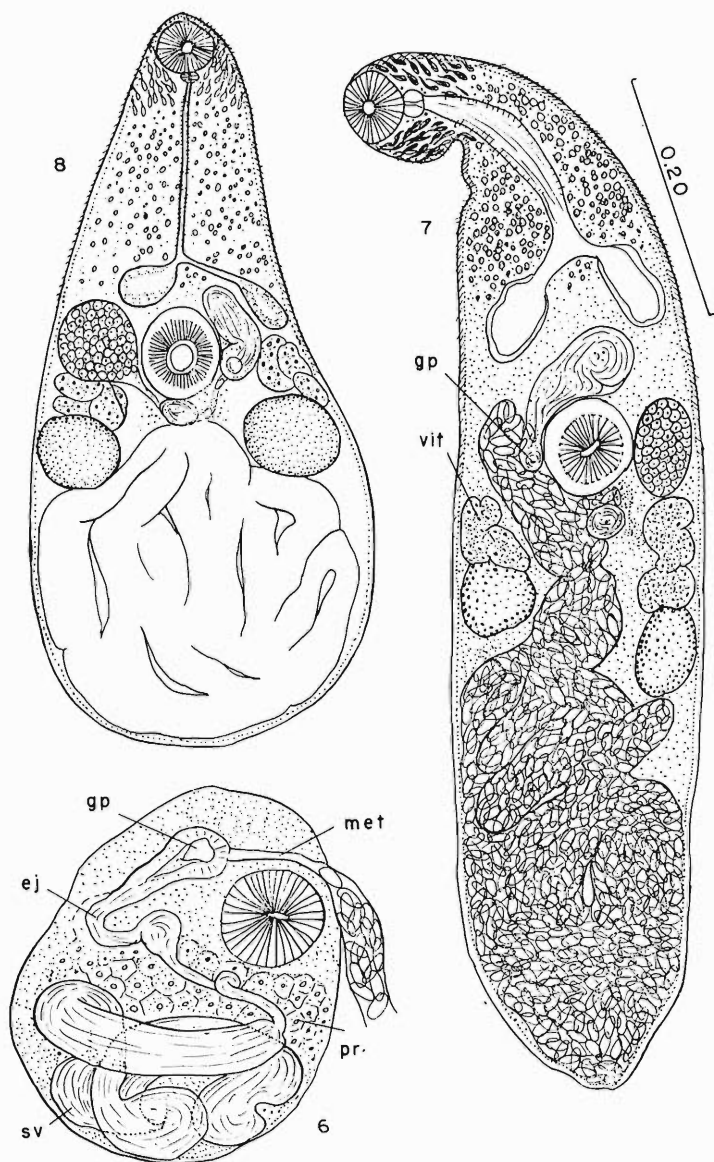


Figure 6. *Pycnoporos ramsesi*. Pseudo-cirrus sac, much enlarged. Dorsal aspect. Acetabulum lies ventral to this structure. Figure 7. *Pycnoporos acetabulatus* Looss, from intestine of *Pipistrellus ruppelii*. Dorsal view. Figure 8. Same, from live specimen.

KEY TO THE SPECIES OF *Pycnopus*

- | | |
|---|------------------------------------|
| 1. Ventral sucker larger than the oral sucker..... | 2 |
| Ventral sucker smaller than the oral sucker..... | 6 |
| 2. Vitellaria overlap zone of intestinal ceca— <i>P. megacotyle</i> (Ogata, 1939) | |
| Vitellaria posterior to ceca; not overlapping the cecal zone..... | 3 |
| 3. Ventral sucker more than twice the size of the oral sucker..... | 4 |
| Ventral sucker less than twice the size of the oral sucker..... | 5 |
| 4. Ventral sucker more than twice the size of the pseudo-cirrus sac..... | |
| <i>P. heteroporus</i> (Dujardin, 1845) | |
| Ventral sucker approximately the same size as the pseudo-cirrus sac..... | |
| <i>P. transversus</i> Ozaki, 1929 | |
| 5. Ovary post-medial to post-sinistral of right testis | <i>P. rhinolophi</i> (Ogata, 1939) |
| Ovary lateral to acetabulum | <i>P. acetabulatus</i> Looss, 1899 |
| 6. Vitellaria lateral to intestinal ceca | <i>P. ramsesi</i> n. sp. |
| Vitellaria posterior to ceca..... | 7 |
| 7. Oral sucker about twice the size of the ventral sucker..... | |
| <i>P. macrolaimus</i> (von Linstow, 1894) | |
| Oral sucker less than twice the size of the ventral sucker..... | |
| <i>P. indicus</i> Pande, 1935 | |

SUMMARY

A new species of trematode, *Pycnopus ramsesi*, from the intestine of an Egyptian bat, *Pipistrellus ruppelii ruppelii*, is described. It is differentiated from other species of the genus by having paracecal vitellaria, and ventral sucker smaller than the oral sucker. Two other specimens of this genus from the same bat were tentatively identified as *P. acetabulatus* Looss. The body wall of both species was found to have an additional layer of non-cellular material separated from the parenchyma cells by a membrane. The esophagus of both species contained numerous, fine, transverse fibers. A new key to *Pycnopus* is presented and the genus is emended.

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MINUTES

Three Hundred Ninety-Seventh—Through the Four Hundred-Fourth Meetings

397th Meeting: Walter Reed Army Medical Center, Washington, D. C., October 18, 1963. Fifty-third Anniversary Meeting. Allen McIntosh and Edna M. Buhner were elected to Life Membership. Program of invited papers on: The application of newer techniques to the study of epidemiology. Papers presented: General procedures in epidemiology, by A. E. Buck, discussion by E. H. Sadun; Epidemiology of *Echinococcus*, by E. L. Schiller, discussion by J. B. Poole; Epidemiology of filariasis, by L. A. Jachowski, discussion by G. F. Otto.

398th Meeting: Log Lodge, Agricultural Research Center, Beltsville, Maryland, November 5, 1963. Officers elected: C. G. Durbin, President; L. A. Jachowski, Vice President; E. L. Schiller, Recording Secretary; E. M. Buhner, Corresponding Secretary-Treasurer. Papers presented: Some observations on strain variation in the response of *Haemonchus contortus* to phenothiazine, by M. Colglazier; Immunization against the cattle lungworm: Oral vaccination with infective *Dictyocaulus filaria* larvae, by J. Lucker, H. Vegors and F. Douvres; Laboratory studies with the tropical horse tick, *Dermacentor nitens* Neumann, by D. Anthony; Helminths of cattle and sheep: Geographical distribution and economic importance, by W. Becklund; Report of first proven case of intestinal capillariasis in man, by M. Chitwood and C. Velasquez.

399th Meeting: School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, December 13, 1963. Papers presented: Extracellular cultivation of the leishmanial bodies of species belonging to the protozoan genus *Leishmania*, by A. Lemma; Excretions and secretions of *Trichinella spiralis* and their role in immunity, by C. Mills; Proteolytic enzymes in adult *Aedes aegypti* and *Culex fatigans*, by R. Gooding; Inhibition of sporogonic development of *Plasmodium gallinaceum* by extracts from adult mosquitoes, by R. Behin; Studies on glycogen metabolism of *Hymenolepis diminuta*, by A. Colucci. Exhibits viewed: Aretic helminthiasis research; Filariasis in India; Filariasis in Mountain Province, Luzon, Philippine Islands. A demonstration of extracellular leishmanial bodies in culture was presented by A. Lemma and E. Schiller.

400th Meeting: National Academy of Science, Washington, D. C., January 24, 1964. Appointments made: Member-at-large of the Executive Committee, D. Price; Representative to Washington Academy of Sciences, M. Farr; Representative to A.S.P., A. Haley; Librarian, J. Humphrey; Archivist, J. Lucker; Auditors, M. Colglazier, E. Lund and K. Kates. President Durbin read a letter report from Editor, G. F. Otto. Program informal, consisting of papers, notes and comments from assembled members and guests: J. Tiner described an apparatus for the collection and maintenance of plant nematodes under sterile conditions; E. Lund announced plans and developments concerning the proposed International Biological Program. Discussion by the members of the feasibility and desirability of a parasitological association with this International Program followed.

401st Meeting: National Institutes of Health, Bethesda, Maryland, February 18, 1964. Auditor's report was read and approved. Papers presented: The incorporation of radio-phosphorous into calcareous corpuscles of *Taenia taeniaeformis*, by E. Weinbach and T. von Brand; Studies on *Angiostrongylus*

lus cantonensis, by C. Richards; Cyst production in cultures of *Entamoeba invadens*, by L. Diamond and I. Bartgis; Preliminary FA studies on bird malaria, by W. Sodenan and G. Jefferey; Immunological studies on sub-strains of *Entamoeba histolytica* segregated on the basis of size, by M. Goldman; Electron microscopy of *Ascaris* intestinal epithelium, by H. Sheffield.

402nd Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, March 25, 1964. The proposed amendment to Article 7, *Section 1* of the By-Laws was read and discussed. Papers presented: A merganser die-off associated with larval *Eustrongylides*, by L. Locke; Parasites of herons and other birds, by E. Boyd; Viability and infectivity of old cultures of a *T. cruzi*-like organism from raccoons, by D. Winslow; Immunological studies on *Trypanosoma cruzi*, by F. Goble; Physiological age structure of a population of *Anopheles quadrimaculatus* at Patuxent Wildlife Research Center, by J. Hitchcock; Prevalence of *Plasmodium* sp. in Canada geese, by C. Herman. Members and guests were invited to view the Center's new parasitological laboratories.

403rd Meeting: Biology Building, Howard University, Washington, D. C., April 30, 1964. A. Foster presented an Editorial Committee report on the status of the July issue of the Proceedings. The Society passed the amendment to Article 7, *Section 1* of the By-Laws by unanimous vote. Also, by unanimous vote, the Society ratified the new schedule of dues recommended by the Executive Committee. Effective, beginning next year, membership dues will be \$6.00 per annum, and subscriptions \$7.00, with a 50-cent surcharge on foreign subscriptions. Papers presented: Immuno-electrophoretic analysis of *Trypanosoma lewisi* antigen, by R. Watkins; Thiamine metabolism in *Trypanosoma lewisi* infections, by J. Shepperson; Serum protein patterns in *Trichinella spiralis* infected rats, by K. Fergusson; Oxygen consumption of two isolates of *Trypanosoma lewisi*, by A. Smith.

404th Meeting: Beltsville Parasitological Laboratory, Agricultural Research Center, Beltsville, Maryland, June 6, 1964. Annual picnic meeting.

The following were elected to membership at the meetings indicated: 397th—H. L. Holloway, L. R. Ash, S. B. Agrawal, S. H. Hopkins; 398th—J. J. Zaccari, A. I. Kamara, A. A. Warsi; 399th—C. H. Briseño, F. M. Nahhas, K. Phifer, S. E. Knapp, M. G. Radke, C. J. Weinmann; 400th—I. Paperna, R. C. Goss, C. E. Dallimore, G. C. Smart, N. G. Mann, A. Hernandez, S. R. Sylk, J. Vargas-Mena; 401st—D. E. Armstrong, G. O. Schmidt, V. H. Owens, I. B. Tarshis; 402nd—H. Ciordia, Z. Hussain, S. H. Khan, J. M. Castillo, W. E. Bizzell, A. D. Acholom, E. A. Teuber, D. J. Doran (re-elected); 403rd—J. L. Crites, R. E. Putz, J. S. Kim; 404th—S. P. Kruger, A. Greichus, L. T. Cannon, J. R. Crook, Q. T. Jackson, K. Rohde.

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