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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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Notes on the Taxonomy of Certain Digenetic Trematodes of South American Freshwater Fishes *

HAROLD W. MANTER

As the trematodes of freshwater fishes of Africa gradually become known, their similarity to those from South American fishes becomes more and more evident. Comparing trematodes of these two continents, I have found some corrections and revisions in the taxonomy of certain species are necessary before consideration of the zoogeography involved.

Trematodes of Brazilian fishes were collected as early as 1824-1832 by Natterer whose collections in the Vienna Museum were studied by Diesing (1836, 1839) and Daday (1907). These authors dealt only with amphistome trematodes. Travassos, Artigas and Pereira (1928) monographed the known helminths of freshwater fishes of Brazil including new collections from the examination of 565 fishes representing 64 species. Their list included 24 species of trematodes in 14 genera. Later studies have been made by Vaz (1932) and deFreitas (1941). At the present time, 41 species of Digenea are known from freshwater fishes of tropical South America. Szidat (1954, 1956) has described about 20 species of trematodes from Argentine freshwater fishes. These trematodes from Argentina are remarkably different from those of tropical South America. The contrast in the two faunas is highlighted by the fact that 12 of the 41 tropical species are amphistomes while no amphistome is known to occur in Argentine fishes.

FAMILY PARAMPHISTOMATIDAE

Two changes should be made regarding the record of the amphistomes of South America. Travassos (1934) reported *Helostomatis helostomatis* (MacCallum, 1905) Travassos, 1934** in a synopsis of the Paramphistomatidae. No reference was made to the only previous records of this trematode (from Sumatra and India). The record from South America is probably an error. *Helostoma,* as an aquarium fish, has been imported into many parts of the world, and it is possible that Travassos collected *Helostomatis* in Brazil. If so, its occurrence there was clearly an introduction from tropical Asia, the native home of *Helostoma.*

The genus *Diplodiscus* is a common amphistome of amphibia in many parts of the Old World including Europe, India, China, and Japan, although in North America it seems to be replaced by *Megalodiscus.* The *"Diplodiscus cornu* (Diesing, 1836) Daday, 1907" from a fish in Brazil does not belong in the genus *Diplodiscus* for reasons noted by Bravo-H. (1941), who did not,

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**_Helostomatis_ was named as a subgenus of *Chiorchis* by Fukui (1929). Travassos (1934) was the first to use it as a generic name.

however, reclassify it. This species resembles *Microrchis* in the rudimentary oral diverticula, oral sphincter muscles, and genital sucker. A cirrus sac was at least implied in the description of each of the two species of *Microrchis* although it does not appear in the figures. *"Diplodiscus cornu"* cannot be placed in the genus *Microrchis* because it has one rather than two testes and lacks the posterior extension of the aperture of the acetabulum. It resembles *Diplodiscus* in having a single testis. The new name *Pseudodiplodiscus* is proposed for it.

GENERIC DIAGNOSIS OF PSEUDODIPLODISCUS: Body cylindrical. Oral sucker with anterior sphincter; oral diverticula rudimentary, embedded in wall of sucker; acetabulum simple, without central papilla, without posterior extension of aperture. Esophagus long; pharynx weakly developed. Genital sucker present. Testis single; cirrus sac weakly developed. Type species: *P. cornu* (Diesing, 1836) n. comb. Synonym: *Diplodiscus cornu* (Diesing, 1836) Daday, 1907.

The geographical distribution of the amphistomes of fishes will be discussed in a separate paper. It might be noted here, however, that no amphistome of freshwater fishes is known from Europe and the only one in North America (*Pisciamphistoma stunkardi* (Holl, 1929)) is of marine origin and not closely related to any of the South American species. Amphistomes do occur in Indian freshwater fishes; one of the nine species of India is of marine origin, the other eight being related to amphistomes of Africa and Brazil. Three species of amphistomes have already been described from African fishes.

FAMILY LEPOCREADIIDAE

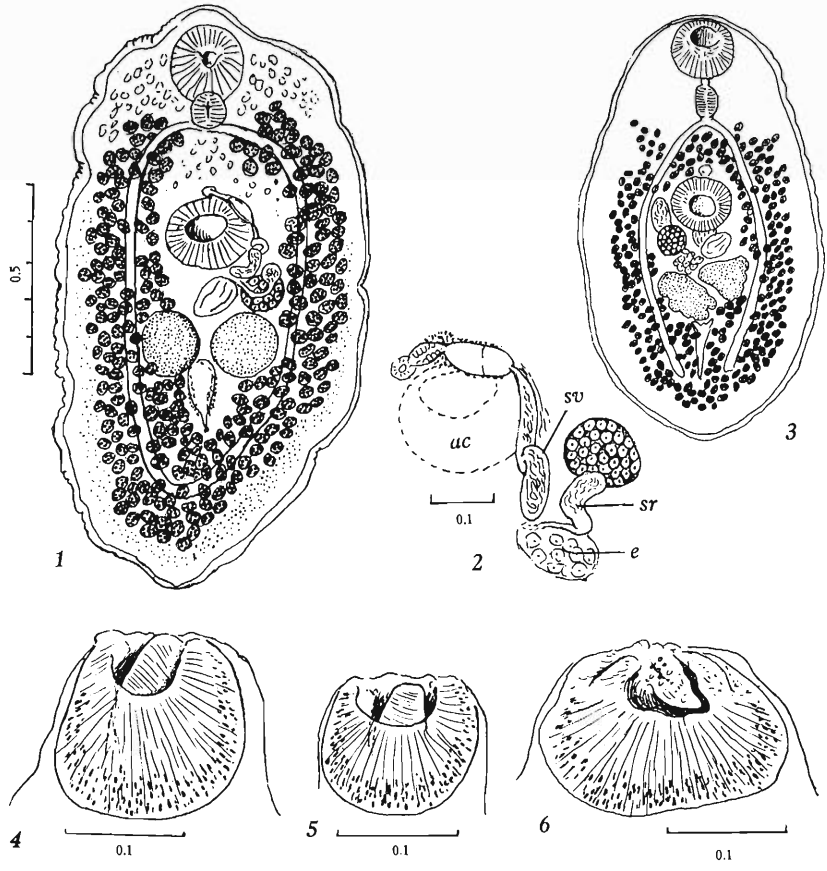
The two species of *"Allocreadium"* described by Pearse (1920) from fishes of Lake Valencia, Venezuela have been shown by Peters (1957) to belong in the genus *Crassicutis* (Lepocreadiidae). The genus *Allocreadium,* widely distributed in the Northern Hemisphere, is not known to occur in South America.

Lepocreadiids are almost entirely parasites of marine fishes. The subfamily Homalometroninae, however, occurs in marine, brackish water, and freshwater fishes. The genus *Microcreadium* Simer, 1929 from *Aplodinotus grunniens,* the freshwater drum or sheepshead, clearly belongs in the Homalometroninae, and the host is a member of a predominantly marine family of fishes. The genus *Crassicutis* is another member of the subfamily Homalometroninae. The genus was named by Manter (1936) for *C. cichlasomae* from *Cichlasoma mayorum* in Yucatan. Other species now known are *C. chuscoi* (Pearse, 1920) Peters, 1957; *C. wallini* (Pearse, 1920) Peters, 1957 from a characid and a cichlid fish respectively in Venezuela; *C. marina* Manter, 1947; and *C. archosargii* Sparks & Thatcher, 1960 from marine fishes in the Gulf of Mexico.

Dollfus (1950) named the genus *Trematobrien* for *T. haplochromios* from a cichlid fish, *Haplochromis,* in the Congo. He named for it a new family, Trematobrienidae, of the Allocreadioidea. This trematode resembled *Crassicutis* so much in general appearance, thick cuticula, and host, that type specimens were borrowed from the Musée Royal de L'Afrique Centrale, by courtesy of Dr. P. L. G. Benoit. An important character to determine was the presence or absence of a cirrus sac, present according to the description but not indicated in the figure of *Trematobrien.* A cirrus sac is definitely lacking (Fig. 2). Accordingly, the genus should be considered related to *Crassicutis* and can be classified in the Family Lepocreadiidae, subfamily Homalometroninae. Its similarities to *Crassicutis* are shown in Fig. 1. It differs in

having united ceca and symmetrical testes. I consider the Family Trematobrienidae a synonym of Lepocreadiidae and Trematobrieninae a synonym of Homalomentroninae.

Records of papillose allocreadiids from Brazil are doubtlessly incorrect. The genus *Creptotrema* Travassos, Artigas, & Pereira, 1928 of Brazil has been assumed to be one of the papillose allocreadiids related to *Crepidostomum* and *Bunodera*. The single pair of outgrowths on the oral sucker are not described in detail, but they are figured more as flaps or folds than as the muscular papillae of *Crepidostomum*. More importantly, the type species, *Creptotrema creptotrema*, is described as *spined*, a character not mentioned by later workers, but which would exclude the species from relationship to *Crepidostomum*. A cirrus sac was mentioned in the description but does not show in the figure. If a cirrus sac is actually lacking, *Creptotrema* is probably



All figures were drawn with the aid of a camera lucida. The scale is in mms. Abbreviations: *ac*, acetabulum; *e*, egg; *sr*, seminal receptacle; *sv*, seminal vesicle.

Fig. 1. *Trematobrien haplochromios*. Dorsal view of a paratype specimen.

Fig. 2. *T. haplochromios*. Enlarged view of terminal sex ducts.

Fig. 3. *Crassicutis cichlasomae*. Ventral view, drawn to same scale as Fig. 1.

Figs. 4-6. Oral suckers of three "co-type" specimens of *"Creptotrema" funduli*, showing the dorsal oral lobe.

related to *Homalometron* (Lepocreadiidae). A marine species, *H. elongatum*, has lateral oral flaps each with three papillae (Bravo-Hollis & Manter, 1957).

Two northern species have been incorrectly assigned to the genus *Creptotrema*. *Creptotrema funduli* Mueller, 1934 is unspined and has a pair of lateral papillae. It seems to be a papillose allocreadiid related to *Crepidostomum*. A slide of ten cotypes of *C. funduli* was kindly loaned by Allen McIntosh. The so-called lateral papillae are often very inconspicuous or lacking. A rather conspicuous finger-like lobe projects inward from the dorsal wall of the oral cavity (Figs. 4-6). The anterior edge of the body was sometimes more or less folded to resemble a pair of papillae but actual presence of such papillae is doubtful. For these reasons, *C. funduli* cannot be retained in the genus *Creptotrema*. It may be more related to *Trematichtys* Vaz, 1932 from a catfish in Brazil. *Trematichtys* was considered a synonym of *Plagioporus*, (subgenus *Caudotestis*) by Yamaguti (1958). The exact generic status of *C. funduli* must await study of new material. This species may be the nearest northern relative of a Brazilian *Plagioporus*. One of its hosts is *Fundulus*, a salt tolerant fish. *Plagioporus* is a very large genus with many marine species.

Creptotrema mülleri Coil & Kuntz, 1960 must fall as a synonym of *Crepidostomum farionis* (O. F. Müller, 1784) Lühe, 1909. Examination of the type and paratype specimens shows that four partially retracted papillae occur on the dorsal surface of the oral sucker. These specimens show all the characteristic features emphasized by Slusarski (1958) for this species. The host (a trout) and locality agree with those of *C. farionis*.

FAMILY CALLODISTOMATIDAE

The Family Callodistomatidae is characterized by an unspined body; I-shaped excretory vesicle; uterus extending to near the posterior end of the body and filling the hindbody; eggs large, thin-shelled, and at least usually embryonated before laying; somewhat restricted vitellaria; ceca of varying length; presence of a pharynx and cirrus sac; occurring in the gall bladder or intestine of freshwater fishes; one species from a turtle.

The type genus is *Callodistomum* Odhner, 1902 with a single species, *C. diaphanum* Odhner, 1902 from a gall bladder of a primitive fish, *Polypterus bichir*, in the White Nile. Odhner compared it with *Anaporrhutum*, and it is evidently related to the Gorgoderidae, differing chiefly in the presence of a cirrus sac.

Yamaguti (1958) included in the Callodistomatidae the following genera: *Callodistomum, Prosthenhystera, Cholepotes, Teratotrema, Braunotrema, Cylindrorchis, Diplangus*, and *Parantorchis*. Manter (1947), Siddiqi & Cable (1960), and others classify *Diplangus* in the Zoogonidae which is evidently a related family. The genus *Paranthorchis* with its spined body and small eggs probably belongs in the Fellodistomatidae where it was first classified by Yamaguti (1934). However, if its excretory vesicle is actually unforked the genus is of uncertain status although still not best classified in the Callodistomatidae. The genus *Cylindrorchis* Southwell, 1913, from the air bladder of *Tetraodon* in Ceylon cannot be classified in the Callodistomatidae, even with a separate subfamily Cylindrorchinae Yamaguti, 1958. Although Yamaguti (1958) states a cirrus sac is present in this genus, none is mentioned or diagramed by Southwell; it is probably lacking. The very thin body and the occurrence in the air bladder of a marine fish suggest the Anaporrhutinae

(family Gorgoderidae). I consider the subfamily Cylindrorchinae to belong in the family Gorgoderidae.

Braunotrema Price, 1930 (syn.: *Thaumatocotyle* Odhner, 1910) is a genus from a turtle (*Podocnemis expansa*) in Brazil and the only member of the family not in fishes. It has a pair of lobes ("pulvinate bolsters") at the anterior end dorsal to the oral sucker. These lobes suggest the genus *Creptotrematina* Yamaguti, 1954 which agrees with the Callodistomatidae in other respects (posterior extent of uterus; large, thin-shelled eggs sometimes embryonated before laid; I-shaped excretory vesicle, symmetrical testes, etc.).

The genus *Creptotrematina* was named by Yamaguti (1954) for *Creptotrema dissimilis* deFreitas, 1941. Although its eggs are relatively larger, I believe *Creptotrema dispar* deFreitas, 1941 should also be transferred to *Creptotrematina,* becoming *Creptotrematina dispar* (deFreitas, 1941) n. comb. The genus is removed from the family Allocreadiidae to the Callodistomatidae.

The genus *Bunoderina* Miller, 1936 from a brook stickleback, *Eucalia inconstans,* in Canada is very unlike the Allocreadiidae in which it was placed and should be included in the Callodistomatidae. The uterus filling the hindbody, the restricted vitellaria, and the large eggs containing active miracidia are some of its callodistomid characteristics. *Creptotrematina* is so similar to *Bunoderina* that it perhaps should be considered a synonym. The chief difference is the presence of a single pair of oral lobes rather than two pairs of papillae. It is possible that these lobes are not homologous in the two genera (they are ventral in *Creptotrematina*), so that the two genera should be retained for the present.

The type species of *Bunoderina, B. eucaliae* Miller, 1936 (synonym: *Bunodera eucaliae* (Miller, 1936) Miller, 1940) occurs also in a partly marine stickleback, *Gasterosteus aculeatus,* in British Columbia (Bangham and Adams, 1954) and in the mud-minnow, *Umbia limi,* in Lake Huron. The other species in the genus *Bunoderina* is *B. sacculata* (VanCleave & Mueller, 1932) from freshwater fishes in New York. This species has more vitellaria and the eggs do not contain fully developed miracidia.

The genus *Megalogonia* Surber, 1928 with its single species, *M. ictaluri,* is more allocreadiid in its restricted uterus and extensive vitellaria. I would retain it in the Allocreadiidae at least for the present.

SUMMARY

The following recommendations are made regarding the Digenea of South American fishes.

1. *Helostomatis helostomatis* (MacCallum, 1905) Travassos, 1934 is a native of tropical Asia, not South America.
2. The new generic name, *Pseudodiplodiscus,* is proposed for *Diplodiscus cornu* (Diesing, 1836) Daday, 1907.
3. *Trematobrien* Dollfus, 1950 lacks a cirrus sac and is transferred to the family Lepocreadiidae, subfamily Homalometroninae (syn.: Trematobrienidae Dollfus, 1950; Trematobrieninae Dollfus, 1950).
4. The genus *Creptotrema* Trav., Artigas & Pereira, 1928 is a member of the family Lepocreadiidae, subfamily Homalometroninae.
5. *Creptotrema funduli* Mueller, 1934 should not be retained in the genus. It is an opecoelid related to *Plagioporus.*
6. *Creptotrema mülleri* Coil & Kuntz, 1960 must fall as a synonym of *Crepidostomum farionis* (O. F. Müller, 1784) Lühe, 1909.

7. The genus *Cylindrorchis* Southwell, 1913 is considered to belong in the family Gorgoderidae rather than the Callodistomatidae.
8. The genus *Creptotrematina* Yamaguti, 1954, including *C. dispar* (de-Freitas, 1941) n. comb. (Syn. *Creptotrema dispar*), and the genus *Bunoderina* Miller, 1936 are removed from the Allocreadiidae to the family Callodistomatidae.

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Some trematodes from *Clarias* spp. in the Rhodesias, including *Allocreadium mazoensis* n.sp. and *Eumasenia bangweulensis* n.sp., and comments on the species of the genus *Orientocreadium* Tubangui, 1931

MARY BEVERLEY-BURTON (Mrs. D. F. Mettrick)*

During the two years 1959 and 1960 a number of specimens of the catfish, *Clarias mossambicus* Peters from Mazoe and Gatooma, S. Rhodesia, were examined for helminths. In August 1960, at the invitation of the Joint Fisheries Research Organisation, an expedition to the Bangweulu Swamps, Northern Rhodesia, was undertaken where *Clarias mellandi* Boulenger as well as *C. mossambicus* was available for examination.

The worms collected from these fish were fixed in cold formal-acetic alcohol under slight coverslip pressure and stained with Kirkpatrick's carmalum. Transverse sections were cut of *Allocreadium mazoensis* n. sp. and *Orientocreadium batrachoides* using the Polyethylene glycol distearate embedding technique (Steedman, 1957). Unless otherwise specified all measurements are in millimeters.

FAMILY PLAGIORCHIIDAE LÜHE, 1901, EMEND. WARD, 1917
SUBFAMILY ASTIOTREMATINAE BAER, 1924.

Astiotrema Looss, 1900.

Astiotrema reniferum (Looss, 1898) Stossich, 1904. (Figs. 1 and 2).

DESCRIPTION: Body lanceolate, 2.19-3.08 long by 0.37-0.60 wide. Living specimens extremely mobile and capable of great elongation and contraction. Cuticle armed anteriorly with numerous spines measuring up to 7 microns long; spines become sparse and smaller in region of ventral sucker. Oral sucker, 0.19-0.23 long by 0.19-0.22 wide, subterminal; prepharynx variable, up to 0.12 long in relaxed specimens; pharynx 0.09-0.12 long by 0.08-0.13 wide; oesophagus 0.09-0.18 long; intestinal caeca extend, posteriorly, to level midway between posterior testis and posterior body margin. Ventral sucker, 0.21-0.24 long by 0.19-0.22 wide, almost equal in size to oral sucker and situated at one third of body length. Testes rounded, tandem, in posterior half of body. Anterior testis, 0.16-0.24 long by 0.12-0.19 wide; posterior testis 0.19-0.31 by 0.15-0.19. External seminal vesicle absent. Cirrus sac elongate, 0.26-0.50 long by 0.09-0.11 in maximum diameter. Cirrus sac curves round to left or right of ventral sucker and contains folded internal seminal vesicle, 0.12-0.26 long by 0.05-0.08 wide, elongate pars prostatica and ejaculatory duct. Common genital pore median or submedian, immediately in front of ventral sucker. Ovary, 0.12-0.18 long by 0.12-0.15 wide, lies on left side of body just behind cirrus sac. Receptaculum seminis, 0.06-0.12 long by 0.06-0.12 in diameter, lies immediately posterior to ovary. Mehlis' gland diffuse, situated to right of ovary and receptaculum seminis. Uterus with descending and ascending limbs, extends to posterior margin of body and,

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in mature specimens fills all available post-testicular space. Vitelline follicles, irregular in outline, up to 0.08 in diameter, extend from level of ventral sucker to level of posterior margin of posterior testis. Excretory vessel, clearly visible in living material, Y shaped. Eggs numerous, operculate, 35-39 microns long by 18-21 microns wide.

HOST: *Clarias mossambicus* Peters.

LOCATION: Posterior region of intestine.

LOCALITY: Mazoe, Southern Rhodesia and Bangweulu Swamps, Northern Rhodesia.

DISCUSSION: After a preliminary examination, with reference to Yamaguti (1958), the material from Mazoe was assigned to two genera; *Astiotrema* Looss, 1900 (Fig. 1) and *Gauhatiana* Dayal and Gupta, 1953 (Fig. 2). Yeh and Fotedar (1958) reviewed the genus *Astiotrema* and synonymised the genus *Gauhatiana* Gupta, 1955* with *Astiotrema* Looss, 1900. The present author is in complete agreement with this proposal.

Yeh and Fotedar (1958) list twenty one species which have been assigned to the genus *Astiotrema* but only accept four of these as valid viz: *A. reniferum* (Looss, 1898), *A. impletum* (Looss, 1899), *A. monticellii* Stossich, 1904 and *A. odhneri* Bhalerao, 1936. Of these *A. reniferum* (sensu Yeh and Fotedar, 1958) and *A. impletum* have been recorded from fish. Yeh and Fotedar (1958) give a revised diagnosis of the genus *Astiotrema* and also a key to the four recognised species. The present material is easily identified as *Astiotrema reniferum* and has similar measurements to the smaller specimens described by Yeh and Fotedar (1958).

A. reniferum has not previously been recorded from *Clarias mossambicus* and is a new record for Southern Africa. As is shown in Table 1, *A. reniferum* has been found frequently in the Mazoe area (from several dams). *C. mossambicus* can travel overland during the rainy season so that it is probable that *A. reniferum* is a widely distributed species.

Table 1. Illustrating the numbers of *Clarias mossambicus* and *C. mellandi* found to be infected with the trematodes described in the present paper.

	Mazoe		*Gatooma*		*Bangweulu Swamps*	
	Total Examined	Infected	Total Examined	Infected	Total Examined	Infected
Astiotrema reniferum						
C. mossambicus	28	21	4	0	11	2
C. mellandi	0	0	0	0	2	0
Allocreadium mazoensis n.sp.						
C. mossambicus	28	2	4	0	11	0
C. mellandi	0	0	0	0	2	0
Orientocreadium batrachoides						
C. mossambicus	28	19	4	4	11	6
C. mellandi	0	0	0	0	2	2
Eumasenia bangweulensis n.sp.						
C. mossambicus	28	0	4	0	11	0
C. mellandi	0	0	0	0	2	1

*According to Helminthological Abstracts Volume 22, Dayal & Gupta (1953) proposed the genus *Gauhatiana* in abstract. Subsequently Gupta (1955) described the type species *Gauhatiana batrachii*, n.g., n.sp. in detail—hence the discrepancy.

FAMILY ALLOCREADIIDAE STOSSICH, 1903.
SUBFAMILY ALLOCREADIINAE LOOSS, 1902.

Allocreadium Looss, 1900.
Allocreadium mazoensis n. sp. (Fig. 3).

DESCRIPTION: Body 1.69-2.62 long by 0.65-0.98 in maximum diameter. Cuticle spinous anteriorly. Oral sucker terminal, rounded, 0.19-0.28 long by 0.22-0.30 wide; prepharynx not apparent in present specimens; pharynx 0.12-0.15 long by 0.09-0.16 wide; oesophagus variable in length, up to 0.27 long; intestinal caeca extend almost to posterior margin of body. Ventral sucker, 0.23-0.32 long by 0.26-0.36 wide, slightly larger than oral sucker, lies at one third of body length. Testes, tandem, in posterior half of body. Anterior testis, 0.22-0.30 long by 0.14-0.30 wide; posterior testis 0.27-0.33 long by 0.17-0.32 wide. External seminal vesicle absent. Cirrus sac oval, 0.26-0.30 long by 0.16-0.19 wide, almost completely pre-acetabular, contains internal seminal vesicle, pars prostatica and ejaculatory duct. Everted cirrus not observed. Genital atrium median or submedian, anterior to level of intestinal bifurcation. Ovary rounded 0.21-0.25 long by 0.19-0.27 wide, situated immediately behind ventral sucker. Mehlis' gland, receptaculum seminis and Laurer's canal only visible in sectioned material. Uterus, with descending and ascending limbs, fills all available space between ovary and anterior testis and extends posteriorly to level midway down posterior testis. Metraterm opens via separate female pore which leads into common genital atrium. Vitelline follicles extend from level of ventral sucker to posterior margin of body. Posterior to testes vitellaria from continuous band of follicles, and measures up to 0.1 wide. Excretory vessel tubular, extends from excretory pore to posterior border of posterior testis. Eggs, thick shelled, operculate, measure 88-95 microns long by 56-60 microns wide.

HOST: *Clarias mossambicus* Peters.

LOCATION: Intestine.

LOCALITY: Mazoe Dam, Mazoe, Southern Rhodesia.

DISCUSSION: The present species is assigned to the genus *Allocreadium* Looss, 1900. The posterior extent of the uterus, which is confined to the pretesticular field in other species of *Allocreadium*, is considered to be of subgeneric importance. Thomas (1957) reviewed the genus *Allocreadium* and listed thirty species of which several are, apparently, either inadequately described or have been incorrectly assigned. Yamaguti (1958) includes twenty two species of *Allocreadium* and lists six more that are tentaively accepted pending re-examination.

Apart from *A. roltanum* Thomas, 1957, none of the species listed by the above two authors have been described from African material. *A. mazoensis* is separated from all the other species of the genus *Allocreadium* on the extent of the uterus to a level midway down the posterior testis. Five specimens of this new species were recovered from two fish taken on the same day from the Mazoe Dam. In spite of repeated fishing in the same locality no other specimens have been recovered.

SUBFAMILY ORIENTOCREADIINAE YAMAGUTI, 1958

Orientocreadium Tubangui, 1931.
Orientocreadium batrachoides Tubangui, 1931. (Figs. 4 and 5).

DESCRIPTION: Body elongate, 1.11-2.58 long by 0.22-0.77 wide. Cuticle armed with spines measuring 11-14 microns long. Oral sucker subterminal,

0.11-0.23 long by 0.11-0.33 in diameter; prepharynx 0.02-0.09 long; pharynx 0.08-0.16 long by 0.09-0.16 wide; oesophagus 0.04-0.12 long; intestinal caeca extend behind uterine coils almost to posterior body margin. Ventral sucker, 0.12-0.24 long by 0.12-0.25 in diameter, lies at about one third of body length. Testes rounded, tandem with anterior testis situated in mid region of body. Anterior testis measures 0.11-0.25 long by 0.12-0.31 wide; posterior testis

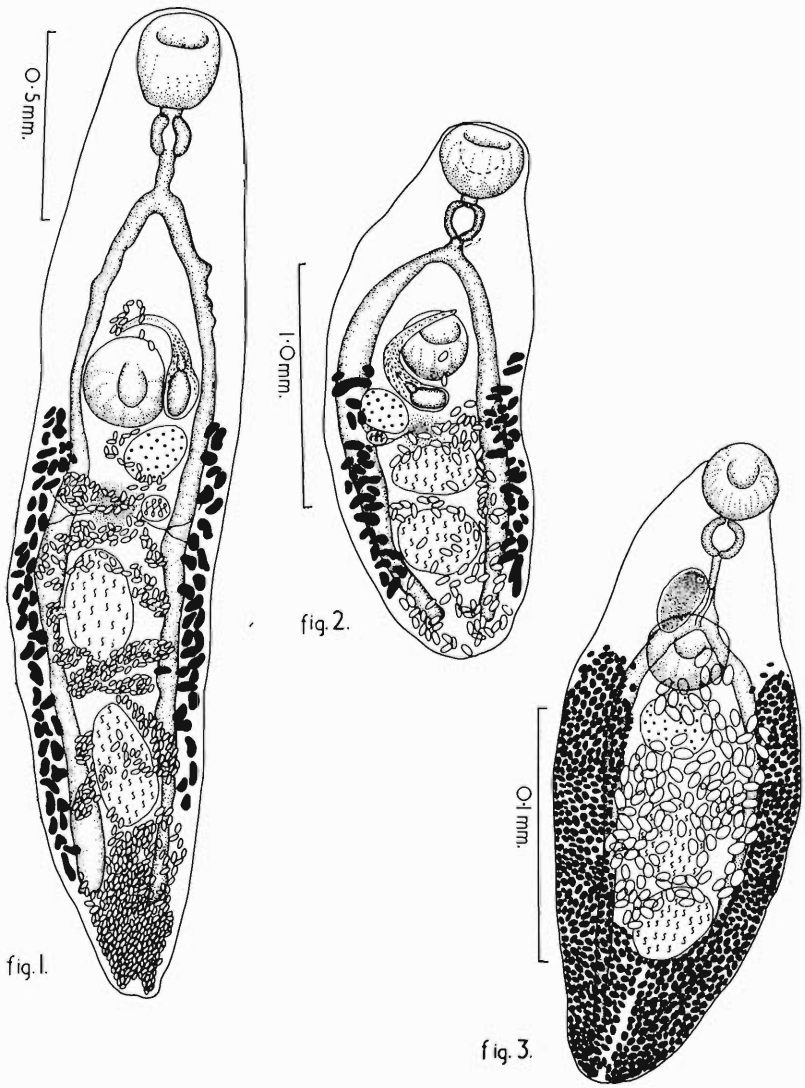

Fig. 1. *Astiotrema reniferum* (Looss, 1898) Stossich, 1904. Entire extended specimen. Ventral view.

Fig. 2. *Astiotrema reniferum* (Looss, 1898) Stossich, 1904. Entire contracted specimen which resembles the material designated by Dayal & Gupta in 1953 as the type of the genus *Gauhatiana* (syn. *Astiotrema*). Ventral view.

Fig. 3. *Allocreadium mazocnsis* **n.** sp. Entire specimen. Ventral view.

0.11-0.30 by 0.10-0.25. External seminal vesicle extremely variable in size, 0.06-0.23 long by 0.04-0.15 wide. Cirrus sac also variable depending on state of contraction, 0.12-0.29 long by 0.06-0.15 in maximum diameter. Cirrus sac may curve round to right or left of ventral sucker (Fig. 4) or may be anterior to it (Fig. 5). Cirrus sac contains bipartite seminal vesicle, 0.06-0.23 long by 0.04-0.13 wide; pars prostatica and ejaculatory duct. Common genital pore median, situated between ventral sucker and bifurcation of intestine. Ovary rounded, 0.13-0.27 long by 0.09-0.27 wide, often separated from ventral sucker by cirrus sac and/or seminal vesicle. Receptaculum seminis not observed either in whole mounts or sectioned material. Mehlis' gland diffuse, immediately behind ovary. Uterus with descending and ascending limbs forms a series of irregular transverse coils. Uterine coils separated from posterior body margin by vitelline follicles. Metraterm 0.04-0.05 wide, with muscular walls passes dorsal to ventral sucker, to open at common genital pore. In young or early mature worms vitelline follicles strap shaped forming a "lattice" (Fig. 4). In fully mature and senescent specimens follicles become rounded or irregular in shape (Fig. 5). Vitellaria lie in 2 extracaecal bands extending from level of ventral sucker to posterior extremity. Excretory vessel tubular, extending from excretory pore to posterior testis. Eggs numerous, operculate, 32-36 microns long by 18-20 microns wide.

HOSTS: *Clarias mossambicus* Peters and *C. mellandi* Boulenger.

LOCATION: Intestine.

LOCALITY: Mazoe, Southern Rhodesia, Gatooma, Southern Rhodesia and Lake Chali, Bangweulu Swamps, Northern Rhodesia.

DISCUSSION: The above data was compared with the description and measurements of *O. batrachoides* given by Tubangui (1931) (Table 2) and found to be very similar. The present material is therefore identified as *O. batrachoides*. This is the first time that *O. batrachoides* has been reported from Africa and thus is a new record for both *Clarias mossambicus* and *C.*

Table 2. Comparing the measurements of *Orientocreadium batrachoides* recovered in the present survey from *Clarias mossambicus* and *C. mellandi* with the measurements quoted by Tubangui, 1931, from material from *C. batrachus*. Unless otherwise stated all measurements are in mm.

	Present material		
	Clarias mossambicus	*C. mellandi*	*C. batrachus* after Tubangui, 1931
Body length	1.93-2.58	1.11-2.11	1.72-2.12
Body width	0.63-0.77	0.22-0.41	0.41-0.54
Oral sucker diameter	0.19-0.33	0.11-0.17	0.17-0.19
Pharynx length	0.12-0.16	0.08-0.12	0.08-0.13
Pharynx diameter	0.11-0.16	0.09-0.12	0.11-0.13
Ventral sucker diameter	0.19-0.25	0.12-0.16	0.16-0.20
Ovary length	0.13-0.27	0.11-0.18	0.16-0.19
Ovary width	0.17-0.27	0.09-0.16	0.12-0.13
Ant. testis length	0.15-0.25	0.11-0.15	0.15-0.20
Ant. testis width	0.20-0.31	0.12-0.18	0.13-0.17
Post. testis length	0.15-0.30	0.11-0.19	0.16-0.26
Post. testis width	0.15-0.25	0.10-0.19	0.11-0.17
C. sac length	0.12-0.29	0.12-0.15	0.30-0.33
C. sac diameter	0.09-0.15	0.06-0.08	0.07-0.09
Eggs length (microns)	32-36	32-35	25-32
Eggs width (microns)	18-20	18	18-20

mellandi. *O. batrachoides* is apparently a common parasite of these fish and has a wide distribution throughout the Rhodesias. Specimens of *C. mossambicus* have been found carrying up to 56 individuals of *O. batrachoides*.

Tabangui (1931) erected the genus *Orientocreadium* to accommodate *O. batrachoides* recovered from *Clarias batrachus* in the Philippines. Subse-

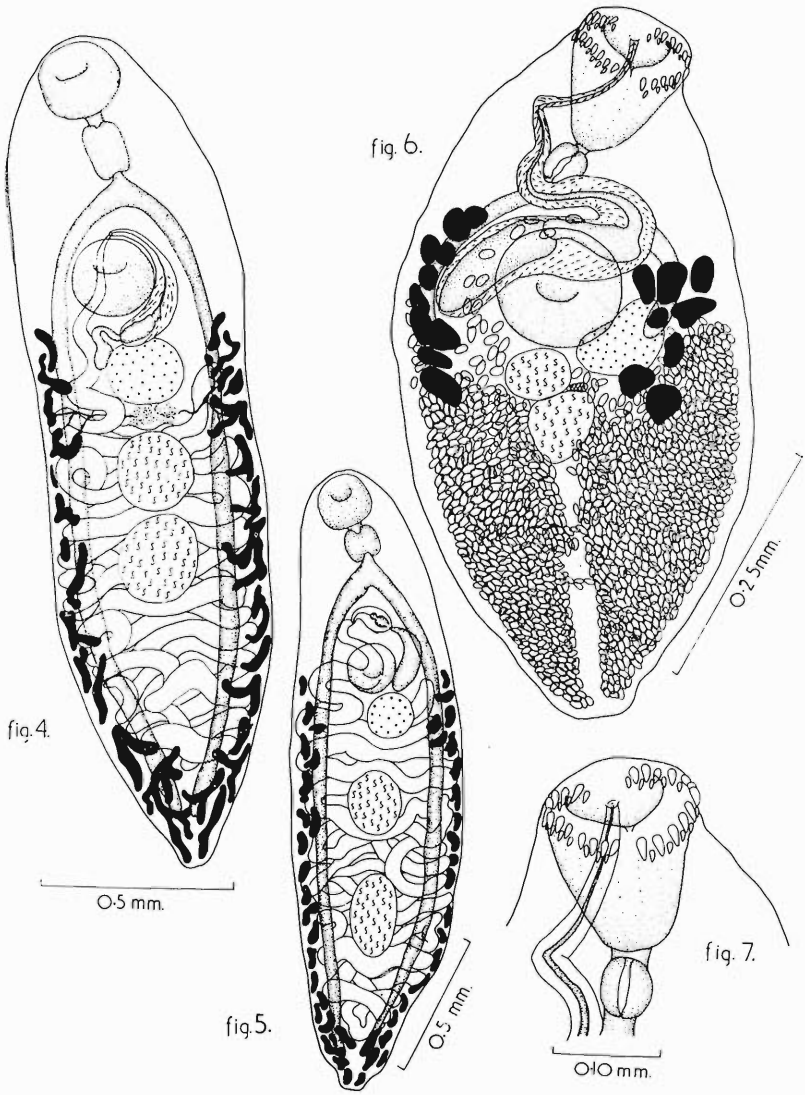

Fig. 4. *Orientocreadium batrachoides* Tubangui, 1931. Entire specimen showing follicular vitellaria. Ventral view.
Fig. 5. *Orientocreadium batrachoides* Tubangui, 1931. Entire specimen showing strap-shaped vitelline follicles. Ventral view.
Fig. 6. *Eumasenia bangweulensis* n. sp. Entire specimen. Ventral view.
Fig. 7. *Eumasenia bangweulensis* n. sp. Details of the circumoral species. Ventral view.

quently, two other species were assigned to this genus viz: *O. indicum* Pande, 1934 and *O. pseudobagri* Yamaguti, 1934.

Yamaguti (1958) lists twelve species in the genus *Orientocreadium*, of which nine have been transferred from other genera, considered by Yamaguti (1958) to be synonymous with *Orientocreadium* viz: *Ganada* Chatterji, 1933, *Neoganada* Dayal, 1938, *Nizamia* Dayal, 1938, *Ganadotrema* Dayal, 1949 and *Paratormopsolus* Dubinina and Bychowsky, 1954. (Table 3). Five of the twelve species listed by Yamaguti (1958) are recorded from *Clarias batrachus* and of these four are Indian. Table 3 also shows that at least four species and two genera have been erected on data from single specimens. In fact Gupta (1951) proposed no less that three new species of *Ganadotrema* (= *Orientocreadium*) based on only 5 worms. Because of this multiplicity of species described from a small host range in a comparatively localised zoogeographical area a comparison was made of ten* of the species listed by Yamaguti (1958)—the measurements are summarised in Table 4.

As a result the following proposals are put forward:—

1. That only three of the ten species of *Orientocreadium* under consideration in Table 4 be recognised as valid, viz: *O. batrachoides* Tubangui, 1931; *O. clariae* (Chatterji, 1933) and *O. pseudobagri* Yamaguti, 1934.

2. That *O. indicum* Pande, 1934; *O. barabankiae* (Dayal, 1938); *O. dayali* Yamaguti, 1958 (= *Ganadotrema indica* Dayal, 1949); *O. secundum* (Dayal, 1949); *O. mahendrai* (Gupta, 1951); *O. philippai* (Gupta, 1951) and *O. vermai* (Gupta, 1951) be regarded as synonyms of *O. batrachoides* Tubangui, 1931.

The validity of each form is discussed below.

O. clariae (Chatterji, 1933).

Chatterji (1933) described *G. clariae* as the type species of his new genus *Ganada* Chatterji, 1933, which is regarded by Yamaguti (1958) as synonymous with *Orientocreadium*. *O. clariae* appears to be, superficially, similar to *O. batrachoides* but from the data given by Chatterji (1933) the eggs of *O. clariae* are considerably smaller than those of *O. batrachoides* (Table 4). *O. clariae* is therefore, provisionally retained as a valid species pending re-examination of the type material.

O. indicum Pande, 1934.

Pande (1934) separated his new species, *O. indicum*, from *O. batrachoides* on a number of extremely variable characters e.g. shape of the ovary—spherical in *O. indicum*, oval in *O. batrachoides;* maximum body width—pre-equatorial in *O. indicum*, post-equatorial in *O. batrachoides;* shape and extent of vitellaria—follicular and more anterior in extent in *O. indicum* than the "latticed" follicles of *O. batrachoides;* etc. Pande (1934) finally gave a key separating the two species based on the fact that the seminal vesicle is wholly outside the cirrus sac in the type species while in *O. indicum* part of the seminal vesicle is internal. In the present material the shape of the ovary is round or oval; the maximum body width may occur almost anywhere along the entire length of the body depending on the state of contraction of the worm at fixation; the vitelline follicles are tubular or round depending on the maturity of the specimen; the anterior level of the vitellaria varies between the anterior and the posterior margin of the ventral sucker;

*Descriptions of *O. hyderabadi* (Dayal, 1938) and *O. siluri* (Dubinina and Bychowsky, 1954) were not available to the present author.

finally the size of internal seminal vesicle is dependant on the sexual state of the individual, and may appear tubular or voluminous. The wide range of morphological variation observed in the present large collection of specimens of *O. batrachoides* embraces all the diagnostic criteria cited by Pande (1934) and it is therefore suggested that *O. indicum* is synonymous with *O. batrachoides.*

O. pseudobagri Yamaguti, 1934.

O. pseudobagri was described from *Pseudobagrus aurantiacus* in Japan by Yamaguti (1934) and is accepted by the present author as a valid form. Yamaguti (1934) separated *O. pseudobagri* from *O batrachoides* on the posterior extent of the vitellaria. In the former the vitellaria extend to a level half way between the posterior testis and the posterior body margin, whereas in *O. batrachoides* the vitellaria extend to the posterior extremity and separate the posterior uterine coils from the posterior body margin.

O. barabankiae (Dayal, 1938)

Dayal (1938) erected the genus *Neoganada* with *barabankiae* as the type species, described from a single specimen. Dayal (1938) separated *N. barabankiae* from *Ganada clariae* Chatterji, 1933 (and hence *Neoganada* from *Ganada*) on the relative sizes of the oral and ventral suckers; shape of the testes and cirrus sac; size and shape of seminal vesicles; extent of vitelline glands; distribution of the uterine coils, etc. All these features are considered to be too variable to support the erection of new taxa. e.g. Dayal (1938)

Table 3. The species assigned to the genus *Orientocreadium* by Yamaguti (1958) with details of their hosts, locality and previous designation (in chronological order).

	Previous designation and author	Host Species	Locality
* *O. batrachoides*	*O. batrachoides* Tubangui, 1931.	*Clarias batrachus*	Philippines
* *O. clariae*	*Ganada clariae* Chatterji, 1933.	*Clarias batrachus*	India
O. indicum	*O. indicum* Pande, 1934.	*Rita buchanani*	India
O. pseudobagri	*O. pseudobagri* Yamaguti, 1934.	*Pseudobagrus aurantiacus*	Japan
* *O. hyderabadi*	*Nizamia hyderabadi* Dayal, 1938.	*Ophiocephalus punctatus*	India
*† *O. barabankiae*	*Neoganada barabankiae* Dayal, 1938.	*Clarias batrachus*	India
*† *O. dayali* nom. nov. Yamaguti, 1958	*Ganadotrema indica* Dayal, 1949.	*Heteropneustes fossilis*	India
O. secundum	*Neoganada secunda* Dayal, 1949.	*Clupisoma garua*	India
† *O. mahendrai*	*Ganadotrema mahendrai* Gupta, 1951.	*Clarias batrachus*	India
† *O. philippai*	*Ganadotrema philippi* Gupta, 1951.	*Ophiocephalus punctatus*	India
O. vermai	*Ganadotrema vermai* Gupta, 1951.	*Clarias batrachus*	India
* *O. siluri*	*Paratormopsolus siluri* Dubinina and Bychowsky, 1954.	*Silurus glanis* and *Glyptosternus reticulatum*	Russia

*Indicates species which were designated as type species of their respective genera.
†Indicates species which have been described from a single specimen.

reports that the oral sucker in *Neoganada barabankiae* was larger than the ventral sucker (0.17mm. and 0.14mm. respectively) while in *Ganada clariae* the oral sucker is smaller than the ventral (according to Chatterji (1933) 0.114-0.164 mm, and 0.133-0.18 mm. respectively). Eight specimens were selected at random from the present material and the variation in the measurements of the oral and ventral suckers are summarised below, Table 5, which illustrates that differences in the size of the oral and ventral suckers of only 0.03mm. cannot be used for specific separation.

As stated above, Yamaguti (1958) proposed *Neoganada* as a synonym of *Orientocreadium*. There is no character quoted by Dayal (1938) that can be used to separate *O. barabankiae* from *O. batrachoides* (Table 4), and it is therefore suggested that *O. barabankiae* should fall as a synonym of *O. batrachoides*.

O. dayali Yamaguti, 1958. (= *Ganadotrema indica* Dayal, 1949).

Dayal (1949) proposed the genus *Ganadotrema* to accommodate his new species *G. indica* which was based on a single specimen. Yamaguti (1958) suggested that *Ganadotrema* was a synonym of *Orientocreadium*. If *O. dayali* is compared with *O. batrachoides* it will be observed that there is no feature which can be used to separate the two forms and they are therefore regarded as synonymous.

O. secundum (Dayal, 1949).

Neoganada secunda was described by Dayal (1949) and was reported to differ from *N. barabankiae* in having rounded testes of equal size; a larger cirrus sac; larger eggs (28-31 microns by 15-17 microns in *N. secunda;* 30-31 microns by 14-16 microns in *N. barabankiae*). Yamaguti (1958) lists *N. secundum* as a valid species in the genus *Orientocreadium* but the "differences" outlined by Dayal (1949) are so small that they cannot be considered as specific criteria. *O. secundum* is therefore synonymised with *O. barabankiae* which is regarded as a synonym of *O. batrachoides*.

O. mahendrai (Gupta, 1951).

Gupta (1951) described *Ganadotrema mahendrai* from a single specimen and separated it from *G. indica* (= *O. dayali*) on the basis that in *G. mahendrai* the anterior testis is larger than the posterior; that the ovary is rounded and the eggs larger. As *O. dayali* has already, in the present work, been suggested as a synonym of *O. batrachoides,* it is proposed that *O. mahendrai* should also fall as a synonym of the type species.

O. philippai (Gupta, 1951)

O. philippai, described as *Ganadotrema philippai* by Gupta (1951) was also compared with *G. indica* (= *O. dayali*). Gupta (1951) separated *G. philippai* from *G. indica* on differences in the anterior extent of the vitellaria; size and shape of the ovary, and the length of the prepharynx—all variable characters. *O. philippai* is therefore synonymised with *O. batrachoides*.

O. vermai (Gupta, 1951).

O. vermai, described by Gupta (1951) and placed in the genus *Ganadotrema* was recovered from the same host species as *G. mahendrai* and was also based on a single specimen. Gupta (1951) gives a key for the separation of the four species assigned to the genus *Ganadotrema* based of the following:

Table 4. Comparing the measurements of the species of *Orientocreadium* under revi

	O. batrachoides Tubangui, 1931	*O. clariae* (Chatterji, 1933)	*O. indicum* Pande, 1934	*O. pseudobag* Yamaguti, 19
Body length	1.72-2.12	1.53 -2.8	1.9 -2.6	1.49
Body width	0.41-0.54	0.3 -0.4	0.56-0.66	0.34
Oral sucker dia.	0.17-0.19	0.114-0.164	0.17-0.21	0.15
Pharynx length	0.08-0.13	...	0.12	0.084
Pharynx dia.	0.11-0.13	0.08 -0.14	0.12-0.14	0.095
Ventral sucker dia.	0.16-0.20	0.133-0.18	0.19-0.23	0.13
Ant. testis length	0.15-0.20	...	0.25-0.37	0.095
Ant. testis width	0.13-0.17	0.137-0.18	0.21-0.31	0.116
Post. testis length	0.16-0.26	...	0.37-0.44	0.13
Post. testis width	0.11-0.17	0.152-0.21	0.21-0.28	0.12
Cirrus sac length	0.30-0.33	0.234-0.4	...	...
Cirrus sac dia.	0.07-0.09	...	...	...
Ovary length	0.16-0.19	...	...	...
Ovary width	0.12-0.13	0.114-0.162	0.19-0.23	0.12
Eggs length (microns)	25-32	18	31	27-33
Eggs width (microns)	18-20	12	18	18-21

relative sizes of the oral and ventral suckers; relative sizes of the anterior and posterior testes; length of prepharynx; shape of the ovary and symmetry of the anterior level of the vitelline follicles. As stated above all these criteria are considered to be too variable for taxonomic separation in the genus *Orientocreadium*. This is accentuated in the case of the "species" described by Gupta (1951) by the paucity of the material available. Consequently, *O. vermai* is also suggested as a synonym of *O. batrachoides*.

FAMILY MASENIIDAE YAMAGUTI, 1954

Eumasenia Srivastava, 1951.

Eumasenia bangweulensis n. sp. (Figs. 6 and 7).

DESCRIPTION: Body small, 0.78-0.81 long by 0.38-0.40 in maximum diameter, at level of ventral sucker. Cuticle armed anteriorly with spines up to 14 microns long; cuticular spines absent in posterior quarter of body. Oral sucker large and funnel shaped, 0.15-0.17 long by 0.12-0.13 wide, surrounded by double row of alternating spines—interrupted dorsally. 48 circumoral spines observed; (Fig. 7.) 24 oral in position, measuring up to 22 microns long by 11 microns wide; 24 aboral, up to 12 microns long by 6 microns wide. Prepharynx, 0.007 long; pharynx 0.05-0.06 long by 0.05 wide; oesophagus short, obscured by cirrus sac; intestinal caeca short, extending to level of posterior margin of ventral sucker. Ventral sucker rounded; 0.13-0.14 in diameter, situated at one third of body length. Testes irregular in outline, oblique, immediately posterior to ventral sucker; anterior testis measures 0.05-0.07 long by 0.08-0.09 wide; posterior testis 0.08 by 0.09-0.10 wide. Cirrus sac large and sinuous measuring up to 0.70 long by 0.08 in basal diameter. Cirrus sac contains large bipartite seminal vesicle, approximately 0.27 long; pars prostatica without lateral diverticulum; and apparently unarmed ejaculatory duct. Common genital pore dorsal to oral sucker just posterior to dorsal gap in circumoral spines. Ovary, 0.08-0.10 long by 0.09 wide lies obliquely to left behind ventral sucker. Receptaculum seminis small, 0.04 long by 0.02 wide, between testes and ovary. Mehlis' gland obscured by developing eggs. Uterus with descending and ascending limbs almost fills posterior half of body. Metraterm, apparently undifferentiated, ascends close

the present paper. Unless otherwise stated all measurements are in millimeters.

O. bara-bankiae Dayal, 1938)	*O. dayali* (*G. indica* Dayal, 1949)	*O. secundum* (Dayal, 1949)	*O. mahendrai* (Gupta, 1951)	*O philippai* (Gupta, 1951)	*O. vermai* (Gupta, 1951)
1.6	4.08	1.3	2.78	1.97	3.18
0.37	0.85	0.31	0.64	0.43	0.56
0.17	0.35	0.15	0.24	0.18	0.23
0.08	0.22	0.07	0.12	0.09	0.12
0.09	0.15	0.06	0.17	0.13	0.18
0.14	0.29	0.12	0.21	0.14	0.20
0.11	0.33	0.11	0.25	0.17	0.22
0.15	0.3	0.14	0.27	0.18	0.21
0.18	0.43	0.14	0.19	0.22	0.27
0.15	0.36	0.11	0.17	0.21	0.24
0.26	0.52	0.26	0.44	0.32	0.44
0.07	0.17	0.05	0.12	0.11	0.12
0.11	0.16	0.06	0.18	0.18	0.16
0.09	0.31	0.08	0.19	0.13	0.18
30-31	32-34	28-31	27-36	32-36	23-30
14-16	17-18	15-17	16-18	16-18	13-16

to cirrus sac to open at common genital pore. Vitelline follicles, in 2 lateral bands, extend from a level just anterior to ventral sucker to that of testes. Excretory vessel tubular extending from excretory pore to posterior margin of posterior testis. Eggs numerous, operculate, 23-26 microns long by 12-14 microns wide.

HOST: *Clarias mellandi* Boulenger.

LOCATION: Intestine.

LOCALITY: Lake Chali, Bangweulu Swamps, Northern Rhodesia.

DISCUSSION: Chatterji (1933) proposed the genus *Masenia* for a new species, *M. collata,* recovered from *Clarias batrachus* in Burma. Because of the funnel shaped oral sucker surrounded by spines, Chatterji (1933) assigned *Masenia* to the family Acanthostomidae. One of the characters of the family Acanthostomidae is the absence, or weak development, of the cirrus sac. In *Masenia* the cirrus sac is prominent. Srivastava (1951) erected the genus *Eumasenia* which was assigned, together with *Masenia,* to the Plagiorchiidae. According to Yamaguti (1958) both the above genera were removed to the family Maseniidae Yamaguti 1954.

The genus *Eumasenia* Srivastava, 1951 is separated from the closely related genus *Masenia* because of the dorsal interruption in the circumoral crown of spines and the presence of a lateral diverticulum associated with the pars prostatica in the former. The only previously described species in the genus *Eumasenia* is the type, *E. moradabadensis* from *Heteropneustes fossilis* in India. As *E. bangweulensis* does not appear to have a lateral prostatic diverticulum, the generic diagnosis given by Yamaguti (1958) is emended and the presence or absence of the lateral diverticulum is regarded as a specific

Table 5. To compare the diameters of the oral and ventral suckers from specimens of *O. batrachoides* collected during the present survey.

	1	2	3	4	5	6	7	8
Body length	2.11	2.23	2.11	2.58	2.04	2.45	2.31	2.39
Oral sucker	0.17	0.22	0.23	0.22	0.22	0.24	0.20	0.19
Ventral sucker	0.16	0.22	0.23	0.25	0.21	0.24	0.21	0.19
	O.>V.	O.=V.	O.=V.	O.<V.	O.>V.	O.=V.	O.<V.	O.=V.

All measurements in mm.

character. *E. bangweulensis* is separated from *E. moradabadensis,* as described by Srivastava (1951), on the size and number of oral spines (26 + 26), circumoral spines measuring 11-12 microns in the type and on the absence of a lateral prostatic diverticulum in the present species. *E. bangweulensis* is the first record of a maseniid from Africa and constitutes a new host record for *Clarias mellandi.* Three specimens of *E. bangweulensis* were recovered from a single *C. mellandi.* Subsequently several specimens of this fish, taken in Lake Chali, were examined by Mr. D. Harding of J.F.R.O. but were found to be free from trematode infection.

While looking at the descriptions of *Masenia dayali* and *M. fossilis* given by Gupta (1955) the present author was struck by the similarity between these forms and *Oudhia horai* Gupta, 1955. *O. horai* is the type and only species of the genus *Oudhia* Gupta, 1955, described from a single specimen, recovered from the same host, *Heteropneustes fossilis,* as *M. fossilis.* It is suggested that the type specimen should be re-examined to determine the shape of the excretory bladder; exact position of the genital pore and if traces of a circumoral crown are at all evident. Additional material might reveal that oral spines are present and re-examination could result in the suppression of the genus *Oudhia* and the transfer of *O. horai* to the genus *Masenia* or even synonymy with a previously described species already assigned to the genus *Masenia.*

GEOGRAPHICAL DISTRIBUTION

Members of the family Clariidae are found throughout the Afro-Oriental tropical belt. The trematode fauna of the Asian clariids has been described by various authors including those mentioned above. It is of interest that two of the species in the present collection are reported for the first time from Africa viz: *E. bangweulensis* n. sp. and *O. batrachoides* Tubangui, 1931, and belong to genera originally described from Oriental hosts. An examination of Silurids from other parts of tropical Africa may illustrate other similarities between the trematode fauna of the two geographical regions.

SUMMARY

Allocreadium mazoensis n. sp., (Allocreadiidae Stossich, 1903) and *Eumasenia bangweulensis* n. sp., (Maseniidae Yamaguti, 1954) are described.

Astiotrema reniferum (Looss, 1898) Stossich, 1904 and *Orientocreadium batrachoides* Tubangui, 1931 are redescribed from new material.

The species assigned to the genus *Orientocreadium* Tubangui, 1931 by Yamaguti (1958) are reviewed and an extensive synonymy is suggested.

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Further Observations on the Life Cycle of *Echinostoma nudicaudatum* Nasir, 1960 (Echinostomatidae: Trematoda)

P. NASIR*

A detailed description of miracidium, redia, cercaria and adult of *Echinostoma nudicaudatum,* as bred out in pigeons from cercarial stage found in *Lymnaea stagnalis,* has already been published (Nasir, 1960A). Some additional observations were made which are presented in this paper.

TAXONOMIC IMPORTANCE OF MIRACIDIUM

From time to time attempts have been made by various workers e.g. Stunkard (1923), LaRue (1926; 1938), Price (1931), Hunter and Hunter (1935), and Beaver (1939), to use the morphological characters of the miracidium such as the size and contour, eye-spots, number and arrangement of epidermal plates and the excretory system, in assessing taxonomic relationships. Of all those features, the number and arrangement of the epidermal plates has been employed most extensively. Thus Price remarked that "the similarity in both the number and arrangement of the ciliated epidermal cells in the miracidia of these three trematodes, (*Strigea tarda, Diplostomum flexicaudum, Schistosomatium douthitti*), appears to be another fact in the support of the claim for relationship between the families strigeidae and schistosomatidae."

On the other hand, Bennett (1936) is more cautious when saying that the number and arrangement of the plates may be of value for establishing "relationships within the families and there is some evidence that it may be of value in establishing relationships between families." Lynch (1933) found individual variations in the epidermal plates of the miracidia of *Heronimus*

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chelydrae and therefore regarded the number and arrangement of plates of "dubious value" for the determination of "family relationships or symmetry," and so did Dobrovolny (1939). Thomas (1883) observed a variable number of epidermal plates in the first two tiers of the miracidium of *Fasciola hepatica*. Pearson (1956) has also found variations in the epidermal plates of the miracidium of *Alaria arisaemoides*.

Then, since the number of epidermal plates sometimes varies in individuals of the same species, and moreover since in some unrelated families the number and arrangement of epidermal plates may be similar, e.g. the miracidia of *Plagioporous sinitsini* Mueller, 1934 as reported by Dobrovolny (1939), of *Allocreadium ictaluri* Pearse, 1924 as reported by Seitner (1951) of the family Allocreadiidae, and that of *Parorchis acanthus* belonging to the family Philophthalmidae also have 19 epidermal plates, whereas the miracidia of *Psilostomum ondatrae* Price, 1931, as reported by Beaver (1939A) of *Psilostomatidae, Crepidostomum ictaluri* (Surber, 1928) Van Cleave, and Mueller, 1934, as reported by Seitner (1951), *Allocreadium neotenicum* Peters, 1957, as reported by Peters (1955; 1957), *Allocreadium alloneotenicum* Wootton, 1957, as reported by Wootton, 1957 of Allocreadiidae as well as that of *Vasotrema robustum* Stunkard, 1928 as reported by Wall (1951) of the family Spirorchiidae, and that of the genus *Echinostoma* having 18 epidermal plates, the taxonomic value of epidermal plates is severely limited. This, however, does not detract from the value of such observations as those of Najarian (1954) who stated that the "lateral condition of the two plates of the fourth tier is probably a characteristic of the genus *Echinoparyphium*," e.g. the miracidia of *Echinoparyphium recurvatum* and *E. flexum*.

In the allied genus *Echinostoma*, the two plates of the last tier lie one above the other as is illustrated by the arrangement of the epidermal plates of the miracidia of *Echinostoma revolutum* and *Echinostoma nudicaudatum*. Thus there is some evidence to suggest that it is not only the number but the exact arrangement of the epidermal plates in a particular tier (last tier in this case) which is of taxonomic importance.

CONTENTS OF REDIAL GUT

Mutilated rediae were present in the lumen of the intestine of the two mother rediae of *Echinostoma nudicaudatum* enclosing daughter rediae and in thirteen mature rediae containing only cercariae. The intestine in all those cases was also filled with a yellowish material. The possibility that the daughter rediae while inside the mother rediae might have found their way into the intestine of the mother is excluded altogether since the intestine was intact. Wesenberg-Lund (1934) seems to be the only other worker who has "often seen daughter rediae in the mother rediae devouring either other daughter rediae or young cercariae where the rediae or young cercariae are present simultaneously in the same redia." In present case, the situation is slightly different since all these thirteen rediae must have devoured the other rediae while free in the liver of the host. That the ingestion of the rediae was merely accidental could be granted if it had been a rare occurrence. The only possible explanation that can be offered is that the rediae were feeding, at least in part, on each other. It must be mentioned here that these thirteen rediae were recovered from the snails which were heavily parasitized. It is probable that this cannibalistic habit of the rediae is not the normal way of securing food. Mathias (1925) thought that the intestinal contents of the rediae of *Hypoderaeum conoideum* were derived from liver tissue of the host

which, indeed, could be gradually eaten completely by the parasite. Rees, W. J. (1936) observed that the rediae of the cercaria of *Himasthla secunda* (Nicoll, 1906) fed on lymph as well as on the liver of the host, *Littorina littorea,* and the intestine of the redia was described as being "full of eggs and yolk sucked from the ovarian tubules by its powerful muscular pharynx." Rees, F. G. (1932) described the intestine of the rediae of *Cercaria limbifera* Seifert, 1926 and *Cercaria Z* of Rees to be filled with food material "derived from the liver cells of the host." Johnston and Angel (1941) stated that the pale orange rediae of *Cercaria gigantura* were packed with the liver of the snail.

It is probable that the cannibalism observed here in the rediae of *Echinostoma nudicaudatum* is facultative, and practiced only when the host livers have been eroded to the point of forming insufficient food supply for the parasites.

COMPARISON WITH REDIAE OF OTHER SPECIES

In all the three different species of echinostome larvae, cercaria of *Echinostoma nudicaudatum* Nasir, cercaria of *E. pinnicaudatum* Nasir, 1961, and the cercaria of *Hypoderaeum conoideum* (Bloch), which I have studied the rediae display such a close resemblance in color, shape, size, presence or absence of locomotory processes, presence or absence of collar, pharyngeal diameter and the extent of the intestine under different conditions of contraction of the redia that I have been unable to separate one from the other. The posterior extremity of most of the rediae of *Echinostoma pinnicaudatum* and *Hypoderaeum conoideum* is produced into a swelling and this might well appear as a distinguishing feature. However, some rediae of *E. nudicaudatum* (usually having a smoothly rounded posterior end) may have a knob-like swelling at the posterior end, thus this character cannot be used as a diagnostic feature in separating rediae of these three species. Mathias (1925) found the intestine in mother rediae to be relatively longer than in cercariae-producing rediae. Wesenberg-Lund (1934) made similar observations on the rediae of *Cercaria echinata;* he also remarked that "many of the characters which we use to distinguish the rediae are subject to great variations; this is the case with the intestine." Tubangui (1932) could not distinguish between the rediae of *E. revolutum* and *Euparyphium murinum.* Beaver (1937) found it difficult to describe the rediae of *E. revolutum* since they were so variable in size and shape; moreover due to "very close similarity between all the species of the genus none of the descriptions made thus far differentiate it from that of the closely related species."

It seems to be an extremely difficult task to distinguish the rediae in question from those of the other echinostomes of which the life cycles have been worked out, e.g. *Echinostoma revolutum, E. pinnicaudatum, Echinoparyphium recurvatum, E. flexum, Euparyphium murinum, E. ilocanum, Echinochasmus donaldsoni, Hypoderaeum conoideum,* as well as from *Parorchis acanthus,* belonging to the closely related family, Philophthalmidae. However, Beaver (1941) has introduced the use of a new diagnostic character, namely the state of the collar; in some rediae the collar is subdivided into dorsal, ventral and two lateral lobes, e.g. *Euparyphium melis, Petasiger nitidus* and *Psilostomum ondatrae.* At the same time I have come across the rediae of two different species of giant-tailed echinostome cercariae, soon to be described where collar is subdivided into four lobes but I have been unable to distinguish between the rediae of these two species of cercariae and that of *E. melis, P. nitidus* and *P. ondatrae.*

EXTRA-REDIAL DEVELOPMENT

Cort (1915) stated that *Cercaria trivolvis* "completes its development before leaving the redia. Therefore, very few cercariae were found free in the liver of the host." Since that time a number of workers on larval trematodes, e.g. Beaver (1930; 1939; 1941A). McCoy (1930), Stunkard (1930), Horsfall (1931), Wesenberg-Lund (1934), Miller, E. L. (1936), Johnston and Angel (1941), Etges (1956) and Tandon (1957), have demonstrated that the various cercariae leave the sporocysts or rediae, as the case may be, while still incompletely developed, and complete their development in the tissues of the snail, during which process some structural changes are brought about. Some larval organs called "escape glands" were shown to be present in the cercariae of *Schistosomatium douthitti* by Price, H. F. (1931) and organs called "posterior locomotor organs" in *Cercaria wesenberg-lundi* by Etges (1956); these organs are present in cercariae dissected from the snail but can no longer be found in cercariae which emerge naturally from their hosts.

In the present study 87 rediae of *Echinostoma nudicaudatum* containing apparently mature cercariae have been studied on different occasions in order to examine the structural details of the contained cercariae, and from most of them the cercariae were dissected out and examined while they were still alive. All of these cercariae which may be called immature, were poor swimmers, the collar and cuticular spines were poorly developed, the caudal branches of the excretory duct opened by definite pores, and the body was relatively clear since the cystogenous gland cells were not filled with cyst material. Stunkard (1930) and Wesenberg-Lund (1934) have already noticed that the cercariae of *Cryptocotyle lingua* and *Cercaria echinata* leave their respective rediae before their gland cells are filled with secretory products. Wesenberg-Lund observed this phenomenon in a dead snail where the cercariae had left the rediae and were lying freely in the tissue of the snail but no encystation had taken place, undoubtedly due to incomplete development of the cystogenous apparatus.

I have not dissected any dead snails to confirm the observation of Wesenberg-Lund, but in many living snails harboring the larvae of *E. nudicaudatum*, the numbers of such immature cercariae were vastly in excess of the normal daily emission of cercariae, thus indicating the occurrence of an extra-redial phase of development in the snail prior to the emergence of the cercariae from the host. The period of extra-redial development in the snail is not known, but it is certain that these cercariae wander about inside the snail for some time.

Unfavorable conditions, such as extremes of cold or the starvation of the host seem to prolong, probably to an abnormal extent, the period required for the maturation of the cercariae during their sojourn in the organs of the host.

EMERGENCE OF CERCACIAE FROM SNAIL HOST

The cercariae of *Echinostoma nudicaudatum* are liberated from the snail hosts, *Lymnaea stagnalis,* in relatively fewer numbers than the cercariae of *Cotylurus brevis* Dubois and Rausch, 1950 as reported by Nasir (1960B) and *Cercaria edgbastonensis* Nasir, 1960, as reported by Nasir (1960A) and the greatest number of larvae emerges, under laboratory conditions 19°-24° C, between 10:00 AM and noon.

Very often the field material when brought into the laboratory in the afternoon, and studied as usual, yields a fair supply of these cercariae. This might be explained if it is assumed that a sudden change in temperature,

addition of fresh water and perhaps rough handling of the snails acts as a stimulant to effect their release.

During the first three to five weeks of the stay of snails in laboratory the number of cercariae which appear in examination tubes daily, varies between 300-700; after that there is a gradual increase up to 800-900 per day but eventually the number falls to nil.

OCCURRENCE OF CYSTS IN SECONDARY INTERMEDIATE HOST

The cercariae of *Echinostoma nudicaudatum* in order to be infective are obliged, as will be shown later, to leave the first intermediate host, *Lymnaea stagnalis,* and to spend a short free-living period before entering and encysting in another host. This second intermediate host may be another specimen of the same species as the first intermediate host or the same individual specimen from which the cercariae emerged, or it may belong to a different species of *Lymnaea* or to other unrelated genera or even a tadpole. Organisms other than molluscs are known to serve for the encystment of cercariae of other species belonging to the genus *Echinostoma,* e.g. Planarians (Johnson, 1920; Macy, 1942); Leeches, Turbellarians, Batracia (Caballero and Larios, 1940); Fish (Beaver, 1937) and Tadpoles (Lutz, 1924; Tsuchimochi, 1926; Fallis, 1934; Beaver, 1937; Johnston and Angel, 1941A).

Whether or not secondary-host preference is exhibited by echinostome cercariae is not known. However, Beaver (1937) has shown that the most "natural" hosts for the penetration of *Cercaria echinostomi revoluti* are *"Physa gyrina, Helisoma trivolvis,* and *Rana pipiens,* named in the order of frequency of natural infection." (*Helisoma trivolvis* serves as the primary intermediate host as well).

During the course of repeated observations extending over a period of over two years, free-swimming cercariae of *E. nudicaudatum* were never seen to encyst in open water. Similarly, no encystment of the cercariae was observed in the life cycle of *E. revolutum* by Johnson (1920), Tsuchimochi (1924), Tubangui (1932), Fallis (1934), Beaver (1937), Johnston and Angel (1941), and Churchill (1951); Sandground and Bonne (1940) did not observe any tendency on the part of the cercariae to encyst in the open. On the other hand Wesenberg-Lund (1934) on dissecting a recently dead snail, observed cyst formation in *Cercaria echinata,* and again Caballero and Larios (1940) mentioned the encystment of the cercaria of *E. revolutum* "on immersed objects in water." Thus there are conflicting observations upon the occurrence of encystment in cercaria of *E. revolutum* but the present study permits of no doubt with regard to cercaria of *E. nudicaudatum*: encystment does not take place outside living organisms.

There does exist some evidence in the literature that certain echinostome cercariae encyst without leaving their primary intermediate host. This is so in *Cercaria biflexa* Faust, 1917 and *C. chekiensis* Faust 1924 as reported by Faust (1917; 1924) which have been seen to encyst immediately after escaping through the birth pore of the redia. Johnson (1920) believes that the encystment of the cercariae within the host immediately upon breaking through the redia is "an obvious and probable shortening of the process" but "it does take place quite often with the cercaria of *E. revolutum"* and the cercariae often escape from the snail only to re-enter the same specimen or a different one. Tubangui (1932) stated that "the cercaria of *Euparyphium murinum,* like that of *E. revolutum* and related echinostomes, seems to lead, if at all, only a very brief free-swimming existence. for it is capable of

encysting within its redia or in the tissues of its snail host immediately after issuing from its redia." Harper, (1929) who experimentally demonstrated the life cycle of *Echinoparyphium recurvatum,* stated that its cercaria normally encysts in the host specimen harboring the rediae, but that the cercariae are able to migrate from the first host, as is proved by the occurrence of encysted stages in molluscs free from the rediae. Wesenberg-Lund (1934) observed that the encystation may take place in the snail without a free-swimming period, especially under unfavorable conditions, when the snail is almost exhausted or at low temperatures. On the other hand, he found evidence that echinostome cercariae normally leave the host and encyst in a second one.

Chatterji (1933) in opposition to Johnson (1920) believed that the echinostome *Cercaria palustris* emerges from the tissues of its host and re-enters fresh snails to encyst.

Krull (1935) remarked that the cercariae of *Echinostoma coalitum* are "never shed in large numbers and soon re-enter the snail and encyst as metacercariae." Miller (1936) observed that the specimens of *Cercaria trivolvis* "after swimming about for some time enter their snail host again to encyst, or other snails if they are available."

Experiments were carried out on the cercariae of *Echinostoma nudicaudatum* to investigate whether or not a short sojourn outside the snail is obligatory before encystment will take place. On September 5th, 1956, thirty snails were obtained from the same locality in the pond; when brought into the laboratory twenty-two of them were found to be discharging the cercariae of *E. nudicaudatum.* In the afternoon of the same day, four of these twenty-two snails were dissected and only a few cysts were found in the renal organ of each. Four of the remaining eighteen snails which appeared the most active at that time were selected and after marking them A, B, C, and D with waterproof ink, they were treated as follows:

Snails A and B were isolated individually and kept under conditions permitting maximum opportunity for re-entry of the cercariae, i.e. the water was changed only when the health of the snail made it necessary—this proved to be about every two days. Snail B was dissected on September 11th, 1956, i.e. after six days and Snail A on September 19th, 1956, i.e. after fourteen days.

Snails C and D were also put into separate jars and the water of these jars was observed at frequent intervals (every few minutes) over a period of maximum emergency of cercariae (i.e. 10:00 AM to noon) and as soon as cercariae appeared in the water, the snails were removed, and after they were dried with blotting paper they were transferred to new jars; thus the cercariae which escaped from these snails (usually about 100-300 between 10:00 AM and noon) were given a minimal opportunity to re-enter. Both of these snails at the beginning of the experiment appeared to be quite healthy but despite the fact that they were well tended six days later (on September 11, 1956) Snail C was found to be dying, and was dissected (together with Snail B for comparison), at 11:00 AM. Snail D was allowed to live up to September 19, 1956, when it was dissected, this time with Snail A for comparison.

The renal organ of the Snail A was found to be infected with over 1000 cysts of *E. nudicaudatum* and in addition forty-three cysts were recovered from the liver. From the renal organ and liver, respectively, of Snail B, 659 and 27 cysts were recovered. On the other hand the renal organ of the Snail C

contained 69 cysts and the liver 11 cysts, and the renal organ of Snail D had only 17 cysts whereas no cysts were found in its liver.

The inference from these results is that the cercariae given the opportunity re-entered the snail to encyst; when opportunities for re-entry were minimized, the numbers of cysts present were correspondingly very small. That even these small numbers of cysts were present could be explained in a number of ways:

(1) The cysts might have been already present before the snails were brought into the laboratory;

(2) Some of the cercariae could have re-entered during the period of maximum emergence (from 10:00 AM to noon), in spite of the fact that the snails were immediately segregated as soon as the cercariae appeared in water;

(3) Re-entry of some of the cercariae, during the early morning hours and at night; and

(4) The cysts were formed by cercariae which did not leave the snails, either in the pond or in the laboratory.

It is highly probable that at least some of the cysts were due to each of the conditions, 1, 2, and 3 above. Thus the number encysting without emergence and re-entering (i.e. fourth condition given above) must be exceedingly small, and, indeed, as seems to me most likely, may be none at all.

The remaining fourteen snails were left with the laboratory bred specimens for the experimental infestation when hundreds of cysts have been recovered from both lots for the feeding purposes.

Further evidence supporting "this belief" was obtained as follows: of about 1400 specimens of *L. stagnalis* brought into the laboratory, between January and August 1957, over 1000 were found to be infected with various stages of *E. nudicaudatum*. All of these 1000 snails were found to contain redial and cercarial stages in the liver, but only 35% of them contained metacercarial cysts; these cysts occurred almost exclusively in the renal organ; each cyst-bearing snail had usually several hundreds and sometimes over a thousand such cysts in the renal organ, and a much smaller number, usually 10-30 in the liver.

A sample of 20 *L. stagnalis* known (by separating the individual snails into glass tubes as usual) to be emitting cercariae of *E. nudicaudatum* was divided into two batches. The first batch of ten was dissected immediately and, as before, only about 35% were found to contain cysts almost exclusively in the renal organ. The second batch was confined for ten days in a small tank with 25 snails known to be harboring cercariae of *E. nudicaudatum*, and all the snails were dissected. Without exception the renal organ of each snail was found to contain hundreds of cysts, each enclosing a moving (and therefore freshly encysted) metacercaria; small numbers (less than 20) of cysts were found also in the liver, and there were usually several hundreds of tail-less cercariae in the mantle cavity.

There is no predilection for penetration into the same snail in which the cercariae developed; if other snails are available, then they stand an equal chance of being carriers for the encysted cercariae.

From repeated experiments both with laboratory-bred and naturally infected hosts, the impression has been gained that once having entered the secondary intermediate host, the cercariae of *Echinostoma nudicaudatum* exhibit a marked preference for encystment in the renal organ than in any other organs; the results of these experiments are shown below:

Secondary Host	*Preferential Organ*	*No. of Cysts*
Lymnaea auricularia	Renal Organ	Several Hundreds
L. pereger	Renal Organ	Several Hundreds
L. stagnalis	Renal Organ	Commonly over 1000
Bithynia tentaculata	Wall of mantle near renal organ and mucous gland	Several Hundreds
Paludina contecta	Renal Organ	Several Hundreds
Planorbis corneus	Renal Organ	Several Hundreds
P. carinatus	Renal Organ	Several Hundreds
Rana tempararia	Renal Organ	137-200

In *Lymnaea stagnalis* never more than 50 cysts were found outside the renal organ, these latter cysts occurring almost exclusively in the liver; only on one occasion cysts were recovered from pericardium in addition to renal organ. In some specimens of *Planorbis carinatus* cysts were enclosed in a thin transparent sac at the base of the albumen gland near the hermaphrodite duct. A number of workers, namely Filippi (1854), Sewell (1922), Mathias (1925), Brown (1926), McCoy (1929), Wesenberg-Lund (1934), Ono (1935), and Johnston and Angel (1941) have previously noted the occurrence of the encysted echinostome cercariae in particular organs of the secondary intermediate hosts, e.g. the cysts of *Cercaria echinatoides* were found in the auricle of *Paludina,* those of *Cercaria indicae XXII* in the renal organ of *Limnaea acuminata,* and those of *Cercaria echinata* were recovered from the salivary gland and esophagus of *L. stagnalis* and *L. pereger.*

INTRA-REDIAL ENCYSTMENT

From time to time during the present study, encysted cercariae of *Echinostoma nudicaudatum* have been observed within rediae of the same species. In over 2000 snails for which records were kept, 79 cases of cysts within rediae were observed; usually the number of cysts per redia was 2-5, but on one occasion eleven cysts were observed in a single redia. Similar observations have been made by others, e.g. Johnson (1920), Mathias (1927), and Wesenberg-Lund (1934).

There have been conflicting interpretations of the occurrence of cysts within rediae. Johnson thought that the cercariae of *Echinostoma revolutum* got encysted inside the rediae "without emerging through the birthpore; in fact that one cercaria should penetrate a redia and encyst is quite impossible." Wesenberg-Lund doubtfully regards the echinostome cysts as belonging to the cercariae which developed in the redia and did not enter the rediae from without, while Mathias (1925) in contradiction to Johnson believed that the cysts present inside the snails, in several hundreds, and even inside the rediae were formed by the cercariae which penetrated the snail from outside and their occurrence in the rediae is accidental and not a normal fact. In the present investigation it has been noted that the cystogenous glands of the cercariae of *E. nudicaudatum* do not usually develop fully, until the cercariae leave the rediae. Thus even if the cercaria itself did not leave the snail host, a period of extra-redial life appears to be necessary for the complete development of the cercaria. It is unlikely therefore that encystment would take place while the cercaria is still enclosed in the redia. In fact it is very probable that the cercaria leaves not only the redia but also leaves the snail host before re-entering (possibly a different host specimen) to encyst, and that very occasionally it

penetrates a redia before encysting. Moreover, the penetration into a larval stage of a trematode before encystment is not confined to rediae of *E. nudicaudatum*: sporocysts of *Cotylurus brevis* and *Cercaria edgbastonensis* are equally subject to invasion by cercariae of *E. nudicaudatum*.

VIABILITY OF CYSTS

It was discovered from feeding experiments (Nasir, 1960A) that it is not until the fifth day of the penetration of the cercariae of *E. nudicaudatum* into the snails that the resulting metacercariae are capable of further development upon transfer to a definitive host.

Cysts remain viable for a considerable time: some cysts known to have developed in laboratory-bred snails at least fourteen months previously were fed to pigeons, and yielded corresponding adult echinostosomes.

When cysts containing active metacercariae were removed from living snails and allowed to remain at a temperature of 19°-24° C in fresh water, renewed daily, they died within seven days. Similarly, cysts recovered from snails that had been dead for some time also perished within seven days. If, however, the cysts were allowed to remain within dead snails kept in water, then the cysts remained viable for much longer periods. Some preliminary experiments showed that cysts in dead snails usually remained alive (criterion of life = beating of ciliary patches in secondary excretory tubules and activity of metacercaria) for five weeks, but died after about seven weeks.

It is possible that the ability of metacercariae to survive for considerable periods in dead hosts may be an important factor in the maintenance of the life cycle of *E. nudicaudatum*: seasonal changes in water level of Edgbaston Pool could cause the stranding and consequent death of the snails, when they would form ready prey for birds.

Johnson (1920) observed that the cyst of *E. revolutum* was "probably absorbing moisture all the time and possibly a small amount of food." Krull (1935) stated that some of the metacercariae of *E. coalitum* "may grow slightly" and cysts a month or more old average 140 microns in diameter in contrast with newly formed cysts which average 126 microns. My observations have shown that no such increase in size of the cysts occurs in *E. nudicaudatum*, even in experimentally-bred cysts of more than fourteen months old.

SPECIFIC DIAGNOSIS OF ADULT

GENUS: *Echinostoma Rudolphi,* 1809 *sensu* Dietz (1909; 1910) emend. Mendheim (1943).

SPECIES: *Echinostoma nudicaudatum* Nasir, 1960.

Head collar with 37 spines, length of spines 0.052-0.081 mm, arranged in (3 + 2) + 7 + (6 + 7) + 7 + (3 + 2) fashion; five corner spines on each side; innermost oral corner spine 0.055-0.066, middle oral corner spine 0.061-0.070, lateral oral corner spine 0.059-0.072, innermost aboral corner spine 0.061-0.066 and lateral aboral corner spine 0.072-0.081; seven unalternating lateral spines 0.063-0.077; oral spines of dorsal series 0.052-0.059 and aboral spines of dorsal series 0.065-0.072; oral spines of dorsal series shortest whole series of thirty-seven spines and lateral aboral spine of corner group longest of thirty-seven collar spines. Testes unlobed. Cercaria without a fin-fold; number and arrangement of collar spines similar to that of adult.

DISCUSSION

In the previous paper (Nasir, 1960A) all stages in the life cycle of

Echinostoma nudicaudatum, excepting, sporocyst, have been described. That a sporocyst stage does in fact occur has been inferred from the work of other helminthologists on related species. The total number of the species of the genus *Echinostoma* for which more or less complete life cycles are known is hereby increased from four to five; the four previously known being *Echinostoma revolutum, E. lindoensis, E. macrorchis* and *E. pinnicaudatum* Nasir, 1961.

It is probable that Macy's (1942) account of *E. collawayensis* constitutes a sixth species of which the life cycle is known in part, but in this respect it must be pointed out that Beaver (1937) had regarded *E. collawayensis* as a synonym of *E. revolutum,* and that Macy's paper was undocumented so that it is not known whether Macy was aware of Beaver's work.

Some evidence was gathered from the arrangement of the epidermal plates of miracidium for separation of the two closely allied genera, *Echinostoma* and *Echinoparyphium;* in the genus *Echinoparyphium* the epidermal plates of the last tier are lateral to each other while in the genus *Echinostoma* as shown in the miracidium of *E. revolutum* by Beaver and the present study of *E. nudicaudatum* the two plates of the same tier lie one above the other.

In the life cycle of *E. nudicaudatum* there are at least four successive generations of rediae interpolated between the sporocyst and the cercarial stages.

The previous study (see Nasir, 1960A) has drawn attention to the importance of larval characters in determining the taxonomic identity of the adult; in particular the absence of a fin fold from the tail of the cercaria of *E. nudicaudatum* in contradiction to the presence of fin fold in the cercariae of *E. revolutum, E. lindoensis,* and *E. pinnicaudatum* to distinguish *E. nudicaudatum* from *E. revolutum, E. lindoensis* and *E. pinnicaudatum* when all the three adults, excepting *E. lindoensis* where testes are lobed, are so closely similar to each other that the only distinguishing feature lies in relative differences of the collar-spination. It is noteworthy that the number and arrangement of the collar spines of the cercaria of *E. nudicaudatum* is carried through to the adult without any alteration, but the question of a possible increase in the size of spines during the adult period of the parasite remains to be investigated. A further point of special interest in regard to the cercaria of *E. nudicaudatum* is that a period of extra-radial development has been shown to be necessary.

Probably the most difficult task in connection with the elucidation of the life cycle of *Echinostoma nudicaudatum* has been the identification of the adult. The taxonomy of the adults of 37- spined species of *Echinostoma* was thoroughly reviewed by Beaver who concluded that the form of cephalic spination, the relative proportion of the muscular organs, and the length of the uterus were the most useful diagnostic characters. The present study has shown that of those characters only that of cephalic spination is likely to be in anyway reliable. Similar view has been expressed by Sandground and Bonne (1940) and by Bras, Bonne and Joe (1953). The present study has shown, however, that even the character of the cephalic spination is sometimes insufficient to distinguish between some adults known to have developed from clearly distinguishable cercariae. In such cases then it is necessary to employ characters of larval morphology in specific diagnosis. At the same time, it must be borne in mind that Beaver demonstrated the occurrence of variability in characters of adults of the same species when reared in different definitive hosts; it is conceivable then that larval development in different intermediate hosts might produce corresponding differences in larval morphology. However

in the case of *E. nudicaudatum* and *E. pinnicaudatum* the cercariae happen to employ the same species of the first intermediate host, *L. stagnalis,* and at the same time, the differences between the larvae are greatly pronounced and yet the adults are indistinguishable. It is concluded therefore that in regarding the species of 37- spined echinostomes, larval characters must be taken into consideration. Eventually, of course, more detailed morphological studies of the adult might reveal differences that have so far escaped notice. In this respect it is noteworthy that chromosome studies (in *Parorchis acanthus* as studied by Rees, 1939 and in *E. revolutum* as studied by Churchill, 1951, the diploid number of chromosomes is 22) have not so far yielded useful results.

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Elaphonema mirabile n. gen., n. sp. (Rhabditida), a remarkable new mematode from South Africa*

JUAN HEYNS

During the course of a preliminary survey of the nematode fauna of South Africa, numerous specimens were encountered of a remarkable new nematode, a description of which is here given. Most of the specimens were recovered from cultivated fields, mainly on fairly dry, light sandy soils. Both sexes were equally abundant. Although nothing is known about its feeding habits, it is most likely saprophagous. The nematode seems to be widely distributed in South Africa, having been collected in Hartebeesfontein, Lichtenburg, Ottosdal, Rustenburg, Sannieshof, Ventersdorp and Wolhüterskop in the Transvaal, and Bothaville, Odendaalsrus and Viljoenskroon in the Orange Free State.

It was necessary to erect a genus and new subfamily to accommodate this new species, because of the following exceptional characters: the downward directed head, the fin-like structures, the unusual appearance of the stomatorhabdions, and a female gonad which corresponds with Acrobelinae in the presence of a spermatheca and with Panagrolaiminae in the absence of additional flexures.

ELAPHONEMATINAE n. subf.: Cephalobidae. Mouth directed downwards, bordered by 2 membranous fins. Esophagus cephaloboid. Stomatorhabdions transformed into series of rasp-like denticles. Ovary single, prodelphic, reflexed back past vulva without additional flexures. Spermatheca present near flexure. Male tail cephaloboid, without a bursa.

TYPE GENUS: *Elaphonema* n. gen. with characters of the subfamily.

Elaphonema mirabile n. sp. (Fig. 1)

10 females: 0.47 (0.35 — 0.57) mm; a= 16.6 (14.0 — 21.1); b= 3.5 (3.1 — 4.0) c= 15.6 (13.2 — 17.1); V= $^{19\ (10\ -\ 24)}_{10\ (4\ -\ 17)}$ 62 (58 — 66)%

10 males: 0.45 (0.33 — 0.65) mm; a= 16.9 (13.7 — 20.6); b= 3.6 (3.2 — 4.4; c= 12.8 (11.4 — 15.0); T= 51 (44 — 58)%

FEMALE: Body robust, tapering gradually from about the middle towards both extremities, but more so posteriorly. Cuticle coarsely annulated, the annules 2.0 — 2.5 microns wide around the middle of the body, becoming wider towards the head. Cuticle further ornamented by fine longitudinal lines (somewhat exaggerated in Fig. 1D). Lateral field about 1/10 as wide as body, marked by 3 incisures, the outer ones of which have crenations coinciding with the annules. Anteriorly the center line ends at the deirid, the outer lines near the head, and posteriorly the lines fade out near the terminus. Head a complicated structure with homology of parts difficult to interpret. The "face" is directed downwards. On either side of the oral opening is a delicate membranous appendage, resembling the fin of a fish. Each of these is strengthened by 3 ribs, one on either side, and a central one which curves distally towards the anterior corner. These fins are at first narrow and directed outwards, but at about their middle they broaden out and curve ventrally, and end in a more or less truncate edge. Dorsal to the

*Adapted from a thesis done under the supervision of Professor Gerald Thorne, and presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

oral opening is another narrower, more sturdy truncate appendage, which in a lateral view looks like a forward and then downward projecting finger-like process. On either side between the dorsal and lateral fins is a complicated, subdivided lobe. Several papillae are visible on these lobes, which probably represent the lateral and/or subdorsal lips. Ventral to the oral opening are 2 smaller, roundish lobes, each with a single papilla. These probably represent the submedian lips. Amphids and sensillae not observed. Corpus of esophagus of uniform thickness, broad, with strong radial musculature, and made up of 3 sections of about equal length. Lumen in distal section narrow, apparently triquetrous, and each of the 3 inner ridges minutely denticulate, these denticles apparently representing the highly transformed rhabdions. Lumen in middle section much broader, then again quite narrow in basal section, which is separated from the isthmus by 3 or 4 conspicuous transverse markings. Nerve ring surrounding isthmus in its anterior half. Hemizonid absent. Excetory pore situated opposite basal section of corpus. Deirids located opposite nerve ring. Basal bulb of esophagus with well-developed valve, terminating in a distinct cardia. Intestine about 4 cells in circumference, the wall with sparsely scattered refractive granules. Ventral body wall anterior to the anus extending into a flap overlapping the anus. Tail conoid, the terminus usually acute but sometimes minutely obtuse. Phasmid conspicuous, protruding, situated in lateral field just beyond level of anus.

Vulva a transverse slit, the lips usually somewhat elevated above the body contour. Rudimentary posterior uterine branch less than 1 body width in length. Ovary reflexed back past vulva, without any further flexures. Sac-like spermatheca present at anterior flexure, usually containing several spermatozoa. Ovary consisting of a single row of oöcytes for most of its length. No eggs observed although numerous females were examined.

MALE: Anterior portion of body similar to that of female. Tail slightly longer and more robust than in female, bearing 5 pairs of conspicuous protruding papillae. Three of these situated subdorsally, submedially and almost ventrally, close to the terminus, the other 2 at about the middle of the tail, submedially and almost ventrally, close together. Phasmid conspicuous, protruding, situated near middle of tail. Three pairs of submedian papillae present before anus, the 1st adanal, the others each about 2 spicule lengths apart. Cuticle with a prominent elevation just anterior to anus. Spicula somewhat arcuate, 20 to 28 microns long. Gubernaculum about ½ as long and closely approximated to spicula. Testis single, with multiple row of cells almost filling body cavity, reflexed, the narrower reflexed portion 2 to 3 body widths in length.

TYPE LOCALITY AND HABITAT: Cultivated soil, Waterkloof, 5 miles east of Rustenburg, Transvaal.

HOLOTYPE (female): Slide *Elaphonema* 1, collection of the Division of Entomology, Pretoria, South Africa.

ALLOTYPE (male): Slide *Elaphonema* 1a, same data as above.

PARATYPES: Slides *Elaphonema* 1b, 1c and 1d, same data as above. Also slides *Elaphonema* 1 and 1a, deposited in the collection of the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, U.S.A.

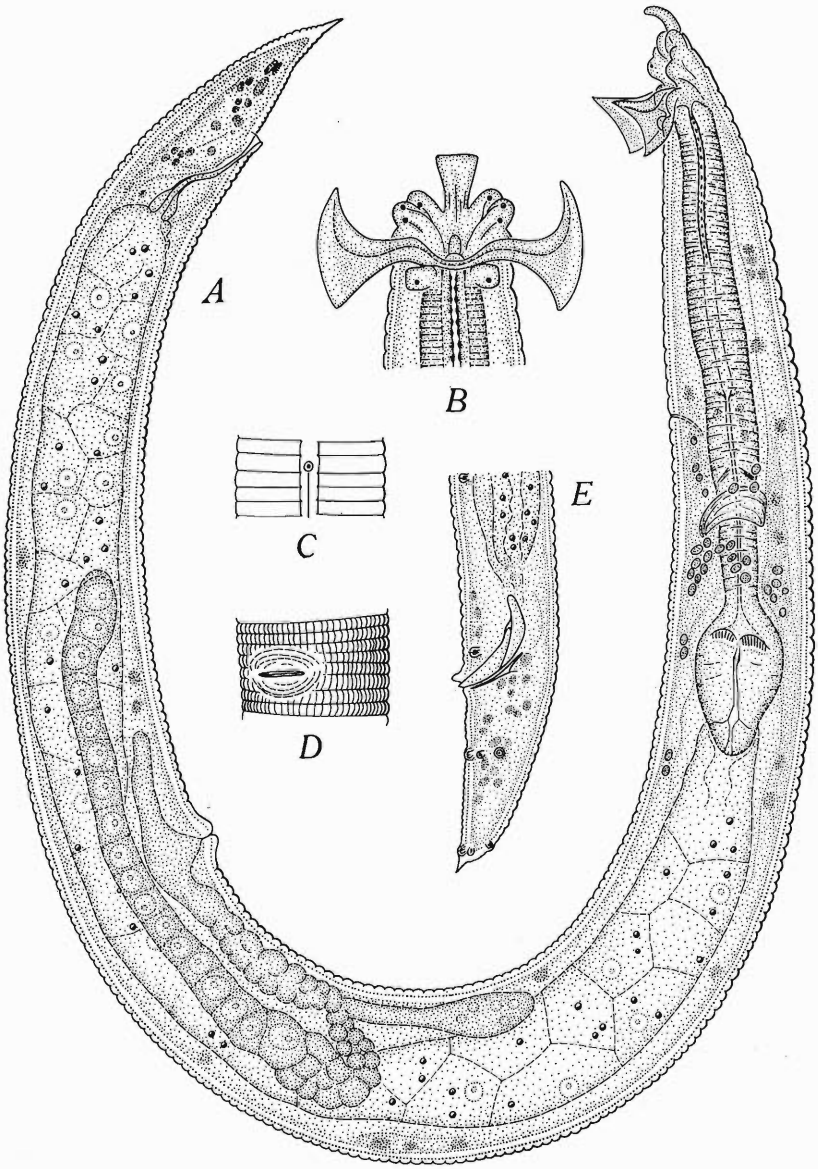

Figure 1. *Elaphonema mirabile* n. gen., n. sp.
A. Female; B. Face view; C. Surface view showing lateral field and deirid; D. Surface view showing vulva; E. Male tail.

Studies on in Vitro Survival of Acanthocephala*

T. T. DUNAGAN

Although von Brand (1940) was unable to keep *Macracanthorhynchus hirudinaceus* alive *in vitro* more than a few hours, Gettier (1942) reported that *Neoechinorhynchus emydis* lived a maximum of 25 days (av. 20.3 days) in 0.5 % NaCl and 0.02% $CaCl_2$. Van Cleave and Ross (1944) found that *N. emydis* survived 15 days in 0.85% NaCl, without swelling for 13 days; in 0.7% NaCl few lived more than 10 days and all were turgid in 3 to 4 days; in 0.8% NaCl the worms became turgid in 6 days and died in 10 to 14 days. No attempt was made to devise media in which the life of the worms might be prolonged under aseptic conditions. Moreover, it is not certain that N. *emydis* or even a single species was used as it was not recognized until later that turtles harbor five species of *Neoechinorhynchus* (Cable and Hopp, 1954; Fisher, 1960; and Cable and Fisher, 1961).

In the present study, *N. emydis* and *N. pseudemydis* were maintained *in vitro* aseptically under a variety of conditions for an extended period of time during which worms copulated and eggs were produced.

MATERIALS AND METHODS

Pseudemydis scripta was used throughout as a source of turtle serum for culture media. That is the species commercial suppliers usually provide and it may harbor three of the five species of *Neoechinorhynchus*. The others have been found only in *Graptemys geographica* and *G. pseudogeographica,* which were available in limited numbers; *G. geographica* was trapped locally where it harbors only *N. emydis* and was conserved as a source of that acanthocephalan. Turtles were bled aseptically, either once and sacrificed to obtain worms or at weekly intervals by puncture of the ventricle after a hole was drilled through the plastron. At such intervals, 10 to 15 ml of blood could be removed from a 10-inch turtle and the hole in the plastron closed with a collodion coated cork stopper. Serum not immediately used was stored at —20° C.

On removal from the host, worms were washed three times in 0.7% NaCl by shaking in a test tube, and transferred to a petri dish where they were separated according to sex and species. Specimens that failed to evert the proboscis actively were discarded to avoid contaminating organisms that could be retained in the retracted proboscis. After further separation into presumed immature, mature, and old categories on the basis of size and color, mature specimens were used in survival studies. Preliminary experiments indicated that immature and old worms did not survive as well as mature worms. Mature specimens were passed through three changes of sterile Tyrodes balanced salt solution (T-BSS) with a NaCl concentration of 0.9%. The first and second contained 200 units /ml of Penicillin G potassium and 1 mg/ml of Streptomycin sulfate. After the third washing, worms were placed in lots of four each in culture tubes of two types. One was a modification of that used by Smyth (1946, 1955) and contained dialyzing tubing (24 angstrom diameter holes) within which the worms were placed along with T-BSS. The other was a screw cap 8 ml test tube in which worms were in direct contact with 2 ml of media. Standards observed in preparation of tissue culture explants were maintained.

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Nutrient solutions of buffered T-BSS containing 0.9% NaCl and various amounts of sugars and turtle serum are given in Tables 2-4. For studies at pH 7.0-8.4, 10 microliters of 0.1% phenol red/liter of medium served as an indicator but at lower pH values, no indicator was used. The preparations were maintained at 22-23° C. without agitation except for a brief shaking prior to daily examination. The shaking seemed to stimulate movement of the worms which could then be observed through the wall of the tube without removing them and risking contamination.

Each test series consisted of three experimental tubes and one control. The control tube always contained only T-BSS buffered at pH 8.0. At first, the medium was changed at one, three, seven, or twenty-eight day intervals with no apparent difference in survival rate. Thereafter, it was changed each seventh day unless otherwise noted in the data. When any unusual shift in pH occurred in a tube, a sample of its contents was streaked on nutrient agar. The same test for contamination was also made at the end of each experiment. The end point was the first of two successive days in which the worms failed to move within ten minutes when shaken and observed. At that time, however, worms often were still alive as they responded to pricking or puncture. Failure to do so could have been used as an end point had sufficient serum been available to prepare a large number of tubes in each series.

RESULTS AND DISCUSSION

Table 1 summarizes observations on the effects of pH. It is evident that the worms can survive at a rather wide range of hydrogen ion concentration, but for a longer time on the alkaline side of neutrality with no significant

Table 1. Effects of pH on survival of *Neoechinorhynchus emydis* (1) and *N. pseudemydis* (2), 4 worms per tube, in buffered T-BSS containing 0.9% NaCl changed at seven day intervals.

Species	Number of worms	pH	Days of Motility	
			Range	Average
2	32	10.7	5-16	9
2	120	8.2	14-26	21
2	120	8.0	16-25	20
1	72	8.0	15-24	19
2	180	7.8	12-24	20
2	96	7.0	16-23	19
1	48	6.0	12-18	14
1	36	5.0	5-16	9
1	48	3.0	1-2	1.1
1	48	2.0	1-2	1.3

difference at pH 7.0 to 8.2. On the acid side there is progressive shortening of the period of motility with decrease in pH until it reaches a value approximating the maximum acidity of the stomach. Response of both *N. emydis* and *N. pseudemydis* was essentially the same. Furthermore, omitting the dialyzing tubing and leaving the T-BSS unchanged at pH 8.0 gave the same results as those included in Table 1 for the test at that pH. Location of the parasites in the host is reflected by the data on pH effects. In all species studied, most of the worms were attached to the duodenal wall in the segment between the entry of the bile duct and the tail of the adjacent pancreas with the number decreasing posterior to that level. However, under abnormal dietary conditions, exclusively freshwater mussels, *N. emydis* occasionally occurred only in the stomach. At these times, there may have been an alteration in pH as well as other physiological changes.

Among other helminths, toleration of a wide range of pH has been reported for nematodes but cestodes apparently are more susceptible to changes in hydrogen ion concentration. According to Smyth (1955), a harmful drop in pH develops adjacent to plerocercoids of *Schistocephalus solidus* in culture unless they are continuously agitated, even though the buffered medium does not change in pH. This does not appear to be true of acanthocephala maintained under the conditions of these experiments.

In vitro survival of turtle acanthocephala is prolonged by placing them in serum alone or added to T-BSS and various chemically defined media as shown in Table 2. Worms remained motile more than twice as long in 100% turtle serum or Eagle's HeLa plus 20% calf serum as in T-BSS alone at the same pH and over three times as long when glucose was added to T-BSS and serum. Omitting the dialyzing tube in experiments with 100% turtle serum did not affect the results. It thus seems that motility is prolonged by substances of small molecular weight and that one such substance is certain carbohydrates. However, 0.1% glucose was as effective as 1.0%. As no

Table 2. Effects of serum on survival of *N. emydis* (1) and *N. pseudemydis* (2) in various media. Dialyzing tube used and media changed each seven days unless otherwise noted.

Species	Nnmber of worms	Experimental Variables	Days of Motility Range	Average
2	36	T-BSS, pH 8.2, 1.0% glucose, 11% Turtle serum	62-80	71
2	48	T-BSS, pH 8.0, 0.1% glucose, 11% Turtle serum	65-88	75
1	36	100% Turtle serum, No dialyzing tube, serum changed at 10 day intervals, Worms out of media 12-18 hours per day	17-66	51
1	36	100% Turtle serum, No dialyzing tube, serum not changed, Tubes not rotated	44-71	55
2	4	TC 199, pH 7.8, 3.0% Turtle serum, Media changed only on 18th day	54-90	64
1	24	TC 199, pH 8.0, 3% Turtle serum	23-42	33
1	32	Eagle's HeLa, pH 8.0, 20% calf serum	26-96	61

advantage was gained by changing the nutrient solution, metabolites of the worms either are not toxic or did not reach harmful concentrations before death from other causes. This is unusual since most cells are sensitive to their own waste products and require frequent changes of nutrient solutions.

As glucose prolonged the period of motility significantly, further studies on the effects and utilization of sugars were made. From Table 3, it is evident that galactose, maltose, and trehalose, as well as glucose increased the period of survival over that in T-BSS alone. It is also apparent that concentration of glucose is not important in the amounts used and is more effective than the other carbohydrates tested. Certain aspects of the carbo-

hydrate metabolism of these worms will be covered in another report. However, it should be pointed out here that *N. emydis* and *N. pseudemydis* are unable to use exogenous glucose to maintain their original glycogen level. The presence of glucose merely slows the rate at which the glycogen is depleted.

Table 3. Effects of various carbohydrate sources on survival of *N. pseudemydis* in T-BSS at pH 8.2 using dialyzing tubes and changing media at seven day intervals.

Carbohydrate and Concentration	Number of Worms	Days of Motility Range	Average
None	120	14-26	21
0.1% glucose	48	34-42	39
1.0% glucose	72	33-46	38
0.1% galactose	72	25-39	30
0.1% maltose	36	30-39	32
0.1% trehalose	36	23-35	29

The effect of certain carbohydrates in prolonging motility of these parasites suggests that they possess enzymes that catalyze reactions with those substances. Although Bullock (1949, 1958) was unable to find histochemical evidence for the presence of alkaline phosphatase activity in *Neoechinorhynchus* spp., it seems obvious from the results presented here that glucose is utilized. However, Warburg experiments failed to indicate the uptake of oxygen or the liberation of carbon dioxide by these worms in the presence of glucose.

Another unusual observation concerning the *in vitro* survival of these parasites is presented in Table 4. Although the data are not extensive, it is

Table 4. *In vitro* survival of *N. emydis* in the presence of phlorizin. Medium consisted of T-BSS buffered at pH 7.8 using no dialyzing tube and with or without added sugars

Experimental Variable	Number of Worms	Days of Motility Range	Average
Phlorizin-1,500 microg./ml	36	17-36	26
Phlorizin-3,000 microg./ml	24	23-29	27
Phlorizin-3,000 microg./ml 0.1% galactose	24	24-30	26
Phlorizin-3,000 microg./ml 0.1% glucose	24	23-32	26

obvious that phlorizin, a plant glucoside, prolongs motility of the worms over that observed in T-BSS alone. Moreover, in the presence of phlorizin galactose and glucose exert no effect. This is to be expected if phlorizin acts on these acanthocephala in a manner similar to that observed by Laurie (1957) for *Moniliformis dubius* and Phifer (1960) for *Hymenolepis diminuta*. Yet this fails to explain the mode of action of phlorizin in prolonging the motility of these worms.

Throughout this study no indication of growth was observed, but copulation and egg maturation occurred. Female worms examined on the 51st to 73rd day of survival contained motile sperm attached to developing ovarian balls and also eggs with no visible abnormalities. Their viability was not tested by feeding them to ostracods, which serve as the intermediate host of at least two species of *Neoechinorhynchus*.

Regardless of the length of time that worms remained motile, they were adversely affected in about the same manner in all of the media used. The process seemed to be a gradual swelling leading to immobilization. By that time, the cuticle was opaque and scattered blisters appeared on the body surface. Although some substances prolonged survival, it is clear that essential nutrients and perhaps physical requirements normally provided in the duodenum of the turtle were not met by any of the media used. Their inadequacy is further indicated by failure of juveniles of *N. emydis* to survive as long as mature specimens even when the juveniles were removed from the snail which turtles eat to become infected. Such young worms would be expected to survive even longer than the older specimens if the requirements were adequately met.

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Syncoelium priacanthi n. sp. (Digenea:Syncoeliidae) from the Atlantic Coast of Virginia*

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During the course of a study of digenetic trematodes of deepwater marine fishes of the North American Atlantic Coast, a single specimen of an undescribed species of the Genus *Syncoelium* was recovered from a fish of the family Priacanthidae. Branchial material was placed in a saturated

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solution of Chloretone and subsequently transferred to alcohol-formalin-acetic acid fixative. A single worm was recovered from the branchial material and stained in hematein. Figures 1 and 2 were drawn with the aid of a microprojector and Figure 3 with the aid of a camera lucida. Some details of the uterus in Figure 2 were drawn freehand.

All egg and papillae measurements are in microns, and all other measurements are in millimeters.

Syncoelium priacanthi n. sp. (Figs. 1, 2, and 3)

DESCRIPTION: Body, Y-shaped; total length 11.96; forebody somewhat shorter than hindbody; hindbody 5.77 in length; cuticle of forebody with small papillae on both dorsal and ventral surfaces; fully extended papillae 23 to 33 long. Acetabulum borne subterminally on peduncle; peduncle slightly less than one third length of forebody; greatest width, .95 at level of middle of hindbody; hindbody tapered towards posterior end. Subcuticular musculature underlain by large vacuolate cells abundant from posterior margin of pharynx to tip of hindbody; vacuolate cells somewhat less abundant in acetabular peduncle; peduncle with broadly crenulate margins; smaller, denser cells aggregated in base of both acetabulum and oral sucker. Preoral lip present and well developed. Large bundle of extrinsic muscle fibers extends from base of acetabulum through peduncle and divides at its base into two smaller fiber bundles, one of which extends to base of oral sucker and pharynx and other ventral to gonads and nearly reaches end of hindbody. Acetabulum bowl-shaped, .34 long and .73 wide. Oral sucker .56 long and .48 deep. Pharynx .35 long and .26 deep. Esophagus .30 long; ceca variable in width, slightly sinuous, united just in front of posterior extremity.

Testes rounded, fifteen, in two rows with exception of anterior three in tandem, extending from slightly behind acetabular peduncle to approximately middle of hindbody; testes .30 to .39 in length by .42 to .50 in depth. Vesicula seminalis convoluted, intercecal, extending from a point 1.13 anterior to peduncle base to a point well forward in anterior forebody, tapering anteriorly into slender, straight, ejaculatory duct; ejaculatory duct with circular muscle fibers. Pars prostatica and prostate cells absent. No true cirrus. Hermaphroditic duct slender, straight, slightly muscular, .85 long by .04 wide. Hermaphroditic pouch .23 wide at base, with base ventral to pharynx. Ovaries posttesticular, five, rounded, in two rows, two on right, three on left. Vitellaria consisting of six small lobes, anteriormost lobe opposite posterior end of Mehlis gland. Laurer's canal not observed. Anterior part of uterus functions as receptaculum seminis, extending to posterior end of body then directed forward, forming transverse loops across body as far forward as base of peduncle; uterus crosses body at level of peduncle and extends forward as a slightly convoluted tube to its juncture with ejaculatory duct at base of hermaphroditic pouch. Eggs numerous, small, light brown in anterior part of uterus, colorless in posterior uterus, nearly oval, 14.4 to 19.2 long by 10 to 14.4 wide.

Excretory vesicle simple, tubular; excretory arms not traced with certainty but appear to unite above the pharynx.

HOST: *Priacanthus boops* Block and Schneider
LOCATION IN HOST: Gill Raker
LOCALITY: Atlantic Ocean, 50 miles east of Virginia, 100-125 fathoms
TYPE: U.S. National Museum Helminthological Collection 47300.

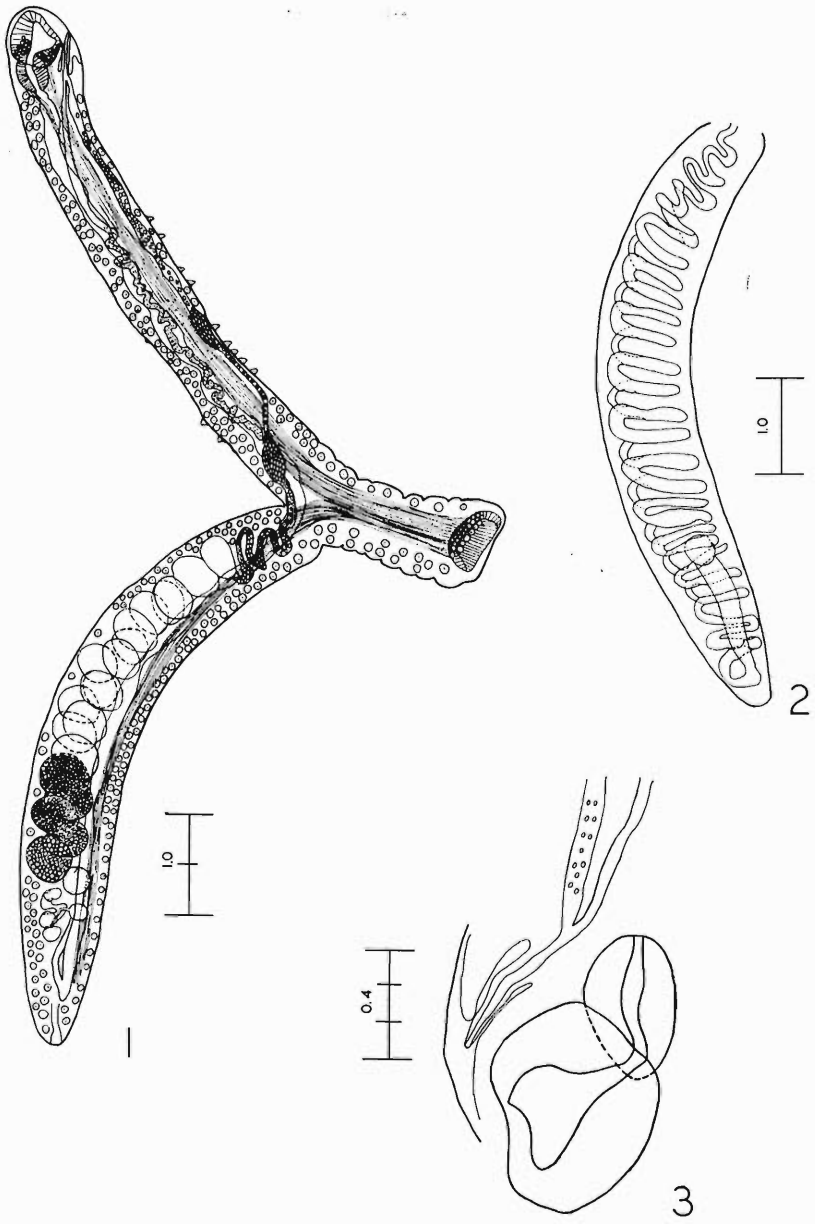

Fig. 1. Syncoelium priacanthi, holotype; right lateral view, portion of uterus omitted

Fig. 2. Syncoelium priacanthi, holotype; right lateral view of hindbody showing course of uterus

Fig. 3. Syncoelium priacanthi, holotype; terminal genital ducts

DISCUSSION: Looss (1899) established the Genus *Syncoelium* and the type, *S. ragazzii* (Setti), was described more adequately by him. A species of *Syncoelium* was reported by Lloyd and Guberlet (1936) from the Pacific salmons, *Oncorhynchus gorbuscha* and *O. nerka* in Puget Sound, Washington. They identified their specimens as *S. filiferum* (Sars), described by Sars (1885) and Leuckart (1889) as *Distomum filiferum* from immature specimens collected by Sars from the body cavity of Schizopods (*Thysanoessa gregaria* and *Nematoscelis megalops*) in the South Atlantic. Leuckart noted the locality simply as "South of the Cape." Odhner (1911) transferred *D. filiferum* to *Syncoelium* and distinguished it form the type, *S. ragazzii* on the basis of a stalked ventral sucker and the possession of 18 rather than 11 testes.

Crowcroft (1948) described *Copiatestes thyrsitae* from the gills of *Thyrsites atun* (Euphrasen) from Tasmania. Yamaguti (1953) considered *Copiatestes* to be a synonym of *Syncoelium*. Manter (1954) also reported *S. thrysitae* from the gills of the same host at Wellington and Portobello, New Zealand. A fourth species is *S. katuwo* Yamaguti. 1938, from the gills of *Euthynnus pelamys* from the Japanese Pacific. Manter considered that Lloyd and Guberlet probably were unjustified in identifying their material as *S. filiferum* (Sars) and thought that their species was *S. katuwo*. He therefore considered the North Pacific species as *S. katuwo* with the Puget Sound *"S. filiferum"* a synonym. Manter suggested that Crowcroft may have been dealing with *S. filiferum* but retained the name, *S. thrysitae,* on the basis of its geographical location and the immaturity of Sars' specimens.

S. priacanthi differs from all other species in the genus on the basis of its 15 testes. *S. ragazzii* has 11 testes, and all other species characteristically have 18. *S. priacanthi* further differs from *S. ragazzii* in having a pedunculate ventral sucker. It also differs from *S. thrysitae* in the absence of pars prostatica and prostate gland cells. It may be distinguished from all other members of the genus on the basis of its extremely small eggs which reach a maximum length below the minimum of that of the other species. The occurrence of *S. priacanthi* off the coast of Virginia extends the range of this peculiar genus into the North Atlantic. Syncoelium now consists of five known species.

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The Production of the Gelatinous Matrix and its Taxonomic Significance in *Tylenchulus* (Nematoda: Tylenchulinae)

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The gelatinous matrix in *Meloidogyne* is a product of the rectal glands and is discharged through the anal opening (Maggenti and Allen 1960). In *Heterodera cruciferae* a similar matrix is apparently produced by the cells of the uterine wall (Mackintosh, 1960). The present paper is concerned with the production of the matrix by three species of Tylenchulinae.

The three species: *Tylenchulus semipenetrans* Cobb, 1913, *Tylenchulus mangenoti* Luc, 1957 and *Trophotylenchulus floridensis* Raski, 1957 are closely related and are here considered to be congeneric. *Trophotylenchulus* becomes a synonym of *Tylenchulus* and *Tylenchulus floridensis* (Raski, 1957) n. comb. is proposed.

METHODS AND MATERIALS

Observations were made on glycerine prepared totomounts of adults and juveniles of the three concerned species of Tylenchulinae; living specimens and stained serial sections of *T. semipenetrans* and stained serial sections of *T. floridensis*.

Living specimens of *T. semipenetrans* were used to study the production of the gelatinous matrix by mature females. Specimens were prepared for observation by placing small pieces of roots in water and dissecting away the gelatinous matrix to expose the posterior portion of the females. Roots and females prepared in this manner were then placed on a slide in water and protected with a coverslip supported on three glass rods about the same diameter as the root. Water level in the preparation was maintained by adding from a pipette at variable intervals.

Specimens of *T. semipenetrans* to be sectioned were killed by gentle heating over an alcohol lamp and then fixed in F.A.A. for at least 24 hours. *T. floridensis* had been previously fixed in 5% formalin.

Dehydration was accomplished by a dripping and stirring apparatus. This system consists of a 95% alcohol reservoir which supplies alcohol through a micropipette at the rate of .0023 ml/sec. The alcohol is dripped into a vial containing the specimens in 5.0 cc of water. The nematodes are kept in suspension by continuous agitation with a magnetic stirrer. After three hours they are allowed to settle and all but 5 ml of the supernatant solution is then decanted. The process of dehydration is then continued for another three hours. Specimens are brought to 91% alcohol slowly and in a reproducible manner by this method. Changes to 95% and absolute alcohol are carried out in a well slide by substitution of single drops until the exchange is completed. This latter technique is also applied to the xylene substitutions.

Paraffin infiltration was accomplished by adding paraffin chips (m.p. 53-55°C) to the final xylene change. Specimens were left in an infiltration chamber for 24 hours. After removal from the infiltration chamber specimens were placed in a vacuum oven (53°C at 625 mm of mercury) for one-half hour.

Embedding and staining procedures were as outlined in Maggenti and Allen, 1960. Sections were cut at 6 microns.

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OBSERVATIONS

An anal opening or rectum was not observed in *Tylenchulus semipenetrans* either in totomounts or serial sections. Therefore the source of the gelatinous matrix was limited to the excretory system or the reproductive system. A conclusive means of verifying the source is by observing the matrix actually being produced. Using the technique previously described it was possible to determine that the excretory cell is a source of the gelatinous matrix. The gelatinous matrix emanated from the excretory pore (Fig. 1). This phenomenon was observed under a compound microscope using a high dry objective with incident and transmitted light. The matrix in *T. semipenetrans* exudes from the excretory pore as an ever expanding globule. This globule does not maintain itself as a discrete mass but rather it begins to spread over and enclose the posterior end of the female. As the matrix continues to flow it joins and fuses with the matrices of other females in the immediate area. Eventually a "colony" of females becomes covered by one mass from the combined matrices of several females. This differs from *Meloidogyne* where the matrix emanates from the anal opening as a thread and has a fibrous appearance (Chitwood, 1938 and Maggenti and Allen, 1960). Also in *Meloidogyne* the matrix maintains itself as a more or less discrete mass at the posterior end of the mature female.

In serial sections (frontal, sagittal and transverse) the excretory cell of *Tylenchulus* occupies approximately 30% of the body cavity volume (Figs. 2 and 3). Collecting tubules were not observed extending from the renette cell. However, since all the body organs including the intestinal syncytium are in intimate contact with or in the immediate area of this cell and since the volume occupied by the cell is so great collecting tubules would not appear to be necessary.

The position of the excretory cell within the body varies among the species

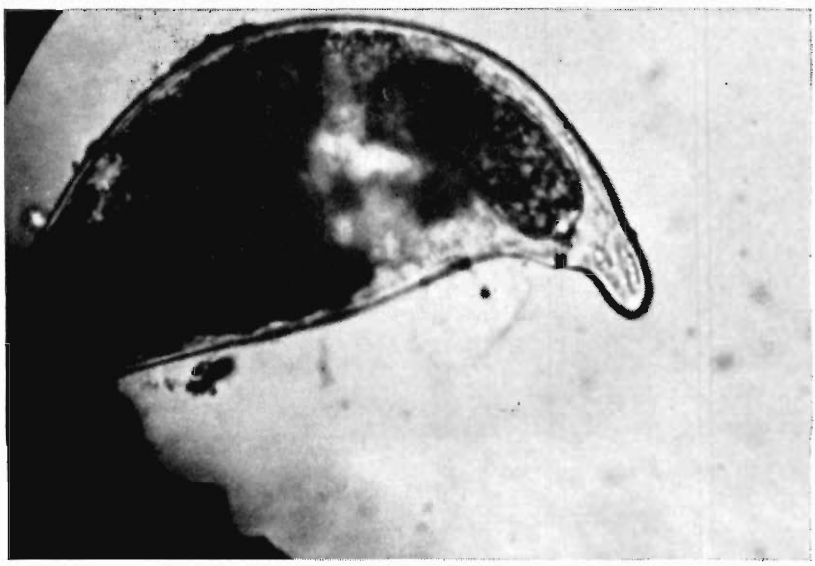

Fig. 1. *Tylenchulus semipenetrans*. Gelatinous matrix emanating from excretory pore.

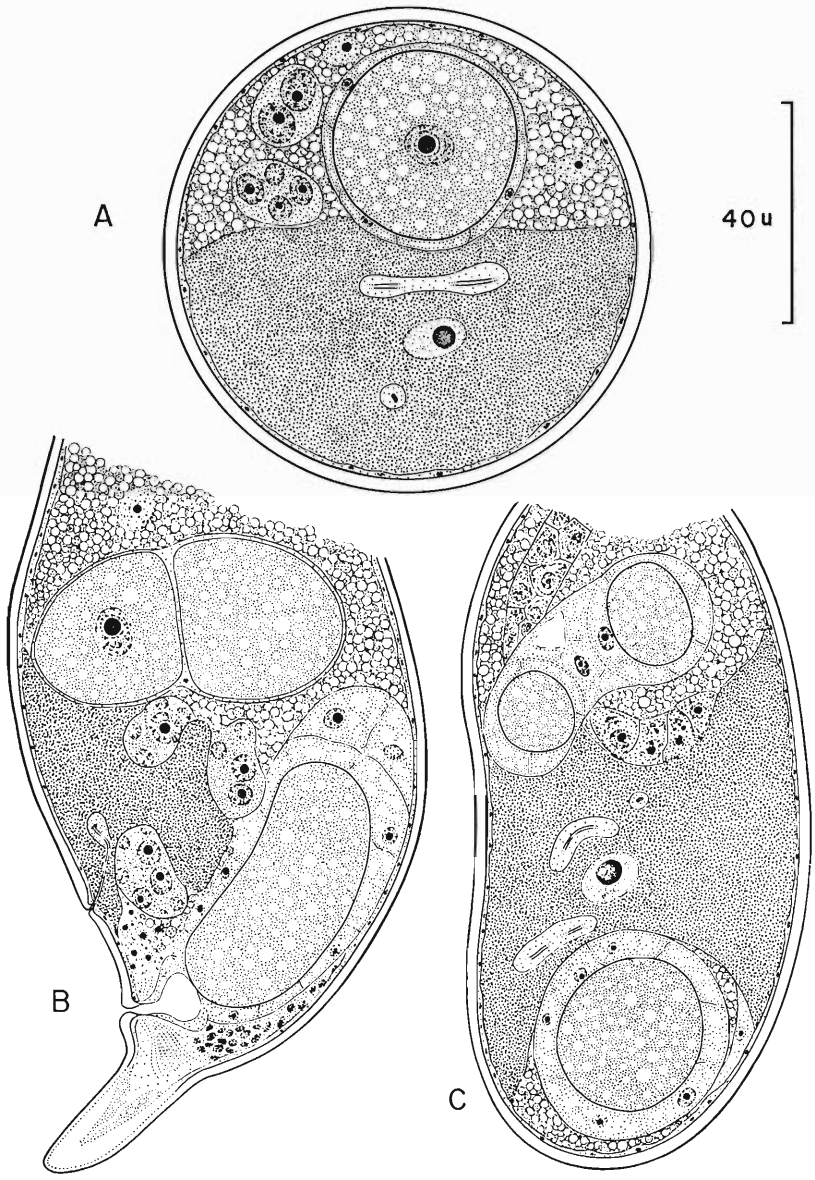

Fig. 2. *Tylenchulus semipenetrans*. Excretory system and associated structures. A. Transverse, B. Sagittal, C. Frontal. The excretory cell is indicated by the densely shaded area.

studied. In *T. semipenetrans* the cell occupies the ventral sector (Fig. 2A) while in *T. floridensis* it is located in the left lateral sector (Fig. 3B). It appears from totomount studies that the location of the renette cell in *T. mangenoti* is similar to *T. floridensis*. In the three species the cell is located just post-equatorial to the main body mass (Figs. 2 and 3). The excretory duct enters the anterior end of the excretory cell in *T. floridensis* and remains close to the internal surface of the cell and has few flexures (Fig. 3). The duct enters at the posterior end of the renette cell in *T. semipenetrans* and remains more centrally located and has many flexures throughout its length (Fig. 2). The differences in cell configuration are illustrated in Figs. 2 and 3.

The optical density of the intestinal mass and the numerous flexures in the reproductive system make it difficult to determine the extent and configuration of the renette cell when examining totomounts. However, its presence is usually discernible by the large nucleus and nucleolus. The renette cell nucleus is centrally located within the cell and is the most prominent nucleus found in the body of the mature female of Tylenchulinae. The excretory cell nucleus varies in size among the species and among specimens: *T. semipenetrans*, nucleus 9-13 × 7-8 microns, nucleolus 4-6 microns; *T. floridensis*, nucleus 10-18 × 8-14 microns, nucleolus 4-8 microns; *T. mangenoti* nucleus 11-14 × 7-9 microns, nucleolus 5-7 microns.

The intestine of Tylenchulinae lacks a lumen. The intestine is syncytial as in *Meloidogyne* (Chitwood, 1951, Elsea, 1951 and Maggenti and Allen, 1960). A posterior tubular intestine or rectum were not observed in *T. semipenetrans* or *T. floridensis* as was illustrated for *T. mangenoti* by Luc, 1957. Specimens of *T. mangenoti* were studied only in totomount; however, their internal morphology appears very similar to *T. floridensis*. In all three species large discrete intestinal nuclei are found scattered throughout the intestinal mass (Figs. 2 and 3). Protruding into and embedded in this syncytial mesenteron are the body organs (reproductive system, excretory system and the posterior portion of the esophagus). At the base of the esophagus, in the region of the esophago-intestinal valve an intestinal lumen was not visible. The hypodermis in *Tylenchulus* is also syncytial and no evidence of somatic musculature could be discerned.

In a critical study of the general gross morphology of these animals it was noted that there are certain characters common to these species that have not been previously reported. The larvae of the three species possess two incisures in the lateral fields (noted for *T. mangenoti* by Luc, 1957). A deirid was observed just posterior to the level of the nerve ring in each of the species. *T. floridensis* has the same degree of anal development as *T. mangenoti*. That is, the anus appears pore-like and without a rectum or posterior tubular intestine being visible. An anal opening or rectum was not observed in *T. semipenetrans* in either totomounts or serial sections. Internally, there is the syncytial mesenteron and the unique excretory system. It appears from studies of serial sections that a spermatheca is always present in Tylenchulinae. It is upon the recognition of these additional features and the already known characters that the synonmy of *Trophotylenchulus* with *Tylenchulus* is based.

DISCUSSION

Luc (1957) in discussing the question of the "functional vs. nonfunctional" anus reasoned that the quantity of food intake necessary for the nutrition of the species of Tylenchulidae necessitates a functional anus. The amount of

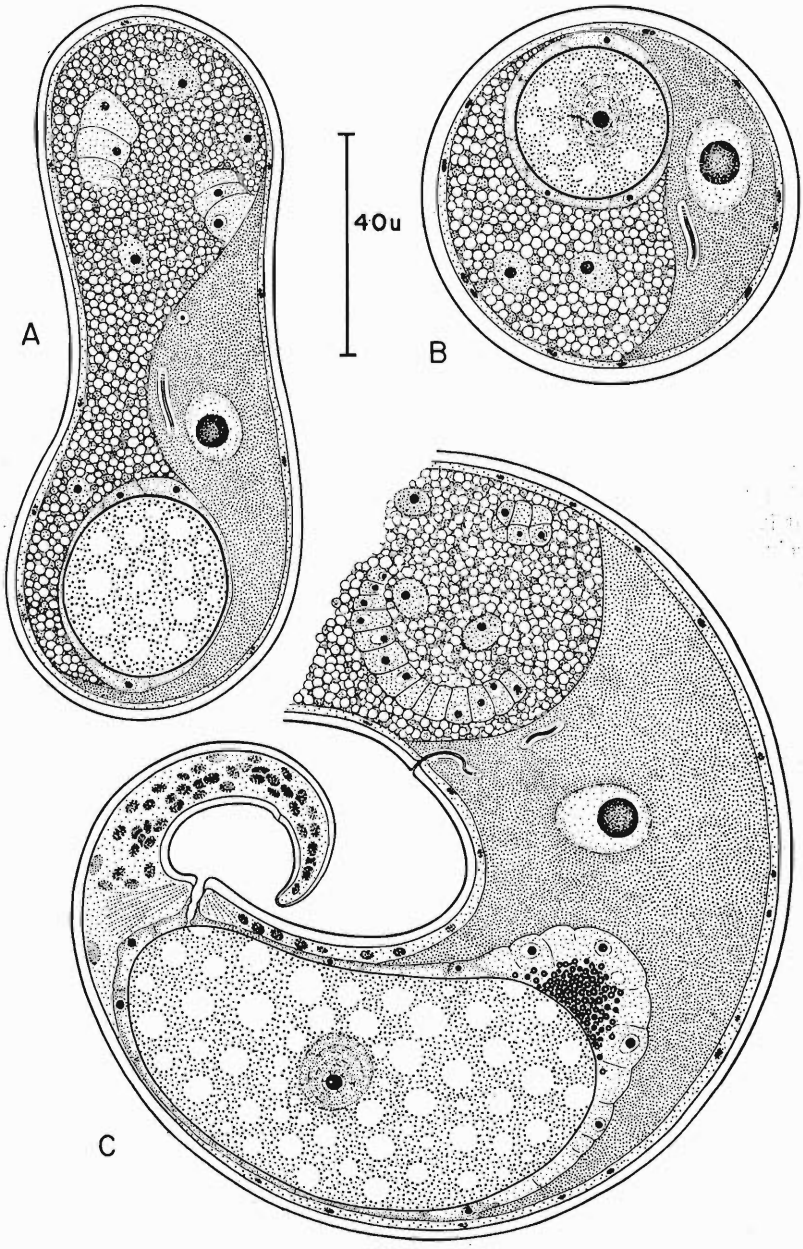

Fig. 3. *Tylenchulus floridensis.* Excretory system and associated structures. A. Frontal, B. Transverse, C. Sagittal. The excretory cell is indicated by the densely shaded area.

food intake was correlated by Luc to the number of eggs produced. There is no information available as to the amount of food ingested by these animals. It should be recognized that the degree of anal function is dependent upon the physiology of the animal concerned and the type of food ingested (diet) rather than quantity alone.

That the anal function may be reduced in *T. mangenoti* and *T. floridensis* and completely absent in *T. semipenetrans* is supported by observations made on the external and internal morphology of adult females: the syncytial intestine without a lumen and the apparent lack of a rectum. If it is true that the anal function is decreased or completely absent then certain compenstory factors must be operative within Tylenchulinae. The diet consists entirely of liquids taken from plant cells. The chance of ingesting any solid materials or food in a form such as to leave a non-digestible residue is reduced by the mechanics of their feeding. That is, the spear lumen is of such a size (approximately 0.3 microns) as to prohibit the passage of any but the most minute solid particles. Since the amount of non-digestible solid particles ingested must be very small and the life span relatively short, it is conceivable that non-digestible solid particles could be stored in the intestinal mass without impairment of body functions. Soluble metabolic waste materials as well as other unneeded soluble products are eliminated by the excretory cell rather than by the rectum or anus. Study of the excretory system showed that it is of sufficient capacity to eliminate metabolic waste and other soluble products. That it is capable of exuding large volumes of material is shown by the production of the gelatinous matrix.

SUMMARY

The excretory system of Tylenchulinae is described from hematoxylin stained serial sections and glycerine prepared totomounts. In *T. semipenetrans* the role of the excretory system in the production of the gelatinous matrix is described.

The intestine of *Tylenchulus* is described as a syncytium without discrete cells. This and associated compensatory factors are discussed in relation to the functional vs. non-functional anus.

The nominal genus *Trophotylenchulus* Raski, 1957 is synonomized with the genus *Tylenchulus* Cobb, 1913. *Tylenchulus floridensis* (Raski, 1957) n. comb. is proposed.

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Occurrence of the Nematodes, *Trichostrongylus longispicularis*, *Ostertagia lyrata*, and *Cooperia spatulata* in Ruminants in Mississippi

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Small numbers of *Trichostrongylus longispicularis* Gordon, 1933; *Ostertagia lyrata* Sjoberg, 1926; and *Cooperia spatulata* Baylis, 1938; were recovered during post-mortem examinations of cattle and sheep in Mississippi. These species have not previously been reported from this state. *Trichostrongylus longispicularis*, and *O. lyrata* have been reported in Louisiana by Andrews (1934), and Dikmans (1929), respectively; in Florida by Andrews (1935), and in Georgia by Becklund (1958b). *Cooperia spatulata* has been reported from Georgia and Florida by Becklund (1958a, 1961). The cattle examined were ones that had been involved in parasite studies in Mississippi. They were slaughtered at an abattoir at the termination of the studies, and the viscera removed to the laboratory where post-mortem procedures described by Porter (1942) were used. Only the males of the three species discussed in this report were identified and counted. The writer's encounters with these parasites, and some observatitons on their morphology are reported.

Trichostrongylus longispicularis: This species was recovered from 2 of 8 yearling steers raised at Poplarville, Mississippi, approximately 60 miles from the Gulf of Mexico, and 30 miles from Louisiana. Along with other species of nematodes, 3 male *T. longispicularis* were found in the dilution sample representing a total of 120 individuals, which comprised les than 1% of the entire worm populations. In another group of steers raised near Hillhouse, Mississippi, located on the Mississippi River approximately 90 miles south of Memphis, Tennessee, 3 of 9 steers examined were found harboring *T. longispicularis*, also in numbers representing less than 1% of the total numbers of worms. Specimens were also found in 3 of 20 yearling animals purchased at a public livestock auction in Mississippi. The origin of these cattle is unknown but is presumed to be Mississippi. Of the total number of worms recovered from each of these 3 animals, from 1-7% were *T. longispicularis* males; 840 specimens were present in all. Another group of 16 yearling steers was purchased at a public livestock auction in Louisiana, and moved to Poplarville, Mississippi, where they were placed on pastures. After 7 months on Mississippi pastures these animals were slaughtered and post-mortem examinations for parasites were made. Two steers harbored a total of 160 male *T. longispicularis*, which was less than 1% of all the worms present.

Measurements were made of 7 specimens in good condition and the means agreed very closely with those reported by Becklund (1958b): body length, 5.1 mm.; right spicule, 161 microns; left spicule, 175 microns; gubernaculum, 90 microns. The spicules were hooked as described by Andrews (1934), and noted by Becklund (*loc. cit.*)

Ostertagia lyrata: This species appears to be more common and more widely distributed over Mississippi than the other two species discussed in this report. Specimens were recovered from yearling cattle raised at (1) Mississippi State University, where 3 of 21 animals examined were infected;

* Mississippi Experiment Station Journal No. 975.

(2) Brooksville, located approximately 30 miles from Mississippi State University, where 2 of 6 animals examined were infected; and (3) Poplarville, where 3 of 8 animals examined were infected.

In the group of 20 yearling steers purchased at public livestock auction in Mississippi, 7 (35%) were positive for this parasite in numbers less than 1% of the total worm loads. Thirteen of the 16 head of cattle originating in Louisiana were infected with *O. lyrata* in numbers representing 1-3% of the total worm loads.

The specimens obtained from Mississippi agreed morphologically with Dikmans' (1931) redescription of this species from specimens from cattle in Louisiana, and those described by Becklund (1958b). The dorsal ray has a single stem that bifurcates into two branches, each of which divides again distally. The length of the branches varied in Mississippi specimens, at times reaching up to one-third of the length of the ray. Measurements of 13 specimens were similar to those reported by Becklund (*loc. cit.*): length, 5.1-7.0 mm.; spicules, 202-245 microns; dorsal ray, 98-139 microns.

Cooperia spatulata: Specimens of *Cooperia spatulata* were recovered from a lamb raised at Mississippi State University. Eleven males were found in the dilution sample, representing a total of 440 individuals, or less than 1% of the total worm populations; no females were identified. The lamb had been grazing in a pasture with 23 other lambs and several head of variously aged cattle acquired from unknown areas. Other species of *Cooperia* found were: *C. punctata, C. oncophora,* and *C. curticei.*

Two steers from the group originating in Louisiana were also infected with *C. spatulata* in numbers representing 1-2% of the total number of worms. One steer harbored approximately 2,000 male specimens of this species; the other had 40 specimens.

Measurements were made of 13 male specimens. Body length averaged 6.7 mm.; the spicules ranged from 202-226 microns and averaged 210 microns. This range of spicule lengths is smaller than those recorded by Baylis (1938), 230-290 microns, and Becklund (1958a), 224-270 microns.

W. W. Becklund of the Animal Disease and Parasite Research Division, Beltsville, Maryland, confirmed identification of 4 of the male specimens as *C. spatulata.* They were deposited in the U. S. National Museum Helminthological Collection and assigned No. 57114. This report extends the range of *C. spatulata* to Mississippi although the incidence of infection is not known.

The three species of nematodes new to Mississippi ruminants, namely *T. longispicularis, O. lyrata,* and *C. spatulata,* were also found in cattle seven months after shipment from Louisiana. It is not known whether or not these animals were infected before shipment to Mississippi.

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A Note on *Mesocestoides lineatus* (Goeze, 1782) from Dogs in Colorado

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There are two groups of slides in the collection of parasites, Agricultural Experiment Station, University of Wyoming, which have been identified as *Mesocestoides lineatus*. Each group consists of six slides with one or more pieces of cestode on each. The slides are unnumbered, but otherwise fully labeled. The dates of collection and a study of the fragments show that they represent two cestodes at least, and that the two (or more) cestodes belong to the same species. Group 1. Host: Dog. Greeley, Colorado. Collected 3-4-'19. Identified by D. R. S. (cott), 1-22-'27. A scolex, attached to about 3.5 cm of strobila, is present. It is 0.51 mm. in diameter, with well developed suckers which are not strongly notched, but they do appear to open anteriorly. The testes develop in four groups, two on either side of the lateral excretory vessels, but this arrangement is later lost to a large extent. The testes number between 85 and 96 in mature proglottids. GROUP 2. Host: Dog. Greeley, Colorado. Collected by L. A. Adams, 3-16-'19. Identified by J. W. S. (cott). There is no scolex, but there is a neck region on one of the fragments. The testes number between 80 and 95 in mature proglottids.

The location and development of the paruterine body is the same for both groups. This structure is first seen near the posterior margin of the proglottid, and as the proglottid matures it drifts anteriorly to an almost central location.

A comparison of the diameter of the scolex, and the number of testes with data given by M. Voge (1955: Univ. of California Pub. in Zoo. 59(5): 125-156.) shows that these cestodes could belong to either of two species; *M. latus* Mueller, 1927 or *M. kirbyi* Chandler, 1944. The large number of testes and the position of the paruterine body indicates they are not *M. carnivoricolus* Grundmann, 1956 (Grundmann, A. W. 1956. Proc. Helm. Soc. Wash. 23(1):26-28). Since there is no feature of these cestodes that would exclude them from belonging to the species *lineatus*, and since G. R. Coatney (1936. J. Parasit, 22:409) has reported *M. linetus* as occurring in a dog at Garland, Nebraska, it is thought the identification as *Mesocestoides lineatus* should stand. Also, it is not known where the Greeley dogs obtained the infection and there is a remote possibility these dogs came from Europe. My knowledge of the collection extends back for 31 years. All of the slides have been deposited in the Helminthological Collection of the U.S. National Museum; numbers 39094 and 39095.

Raillietina (R.) trinitatae (Cameron and Reesal, 1951), Baer and Sandars, 1956 (Cestoda) from a Peruvian Primate

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A single intact tapeworm, conforming to the genus and subgenus *Raillietina*, was recovered from the small intestine of an adult male South American monkey, *Callicebus cupreus* (Spix, 1823), which died on February 20, 1961 while en route to the United States by air from Peru. The animal was collected in the Amazon forest lowlands of Loreto Province in eastern Peru earlier the same month. Through the courtesy of Dr. William Greer of Asiatic Animal Imports, Inc. the writer was given the opportunity to dissect the animal soon after its death. In addition to the single tapeworm specimen, a single mature acanthocephalan, *Prosthenorchis elegans* (Diesing, 1851), was removed from the small intestine. No other intestinal or extraintestinal helminths were found. The cestode specimen was preserved in 10 per cent formalin and subsequently stained with Grenacher's borax-carmine and mounted on a series of slides which are now in the helminthological collection of the Division of Parasitology, School of Medicine, University of California, San Francisco Medical Center.

Human *Raillietina* infections have been recorded infrequently in the Western Hemisphere; worms of this genus have also been collected from howler monkeys (*Alouatta* sp.) on several occasions. There are, however, no records for *Raillietina* (*Raillietina*) in other Neotropical primates; indeed all other recorded mammalian hosts in this hemisphere have been rodents. Most of those who have worked with the New World *Raillietina* (*R.*) now believe that these cestodes are naturally parasitic in rodents, and accidental in man and monkeys. The present specimen is of interest inasmuch as it comes from a primate host and appears to be referable to a species, *R.* (*R.*) *trinitatae* (Cameron and Reesal, 1951), Baer and Sandars, 1956, which has hitherto been recorded only from hystricomorph rodents. The *Raillietina* show considerable morphologic variation within species; the lines between species are not clearly drawn. (Discussed by Chandler and Pradatsundarasar, 1957.) Because of the fluid taxonomic situation in this genus and subgenus the tapeworm recovered from *Callicebus cupreus* is described in detail as follows:

DESCRIPTION. (Measurements in microns unless otherwise stated) Scolex and mature strobila with about 1250 proglottids. Length about 319 mm. Maximum width 2.7 mm. near proglottid no. 1100. Proglottid measurements (width vs. length): no. 400, 450 x 60; no. 600, 1100 x 75; no. 700, 1470 x 150; no. 850, 1860 x 210; no. 900, 2190 x 270; no. 970, 2400 x 300; no. 1025, 2600 x 390; no. 1100, 2700 x 660; no. 1150, 2400 x 900; no. 1200, 2100 x 1200; no. 1240, 1800 x 1500; no. 1250, 1500 x 1650. Scolex 297 in diameter. Minimum width of neck 180. Rostellum 84 in diameter armed with about 160 hammer-shaped hooks arranged in a double crown. Heads of anterior hooks project 3 above heads of posterior hooks. Tails of posterior hooks project 1 below tails of anterior hooks. Anterior hooks 14 long; posterior hooks 12. Suckers 86-96 in maximum diameter, with about 8 rows of small spines 6-8 in length.

First definite segmentation 990 microns from base of scolex. First fully developed testes at about proglottid no. 600, 34 mm. from anterior end.

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Mature proglottids nos. 700-850, 45-70 mm. from anterior end; terminal proglottids slightly longer than they are wide. Egg capsules well defined at about proglottid no. 1025, 132.5 mm. from anterior end. Genital pores unilateral, on right side, with one exception, and situated about one-quarter to one-third the length of the proglottid from its anterior border. Short

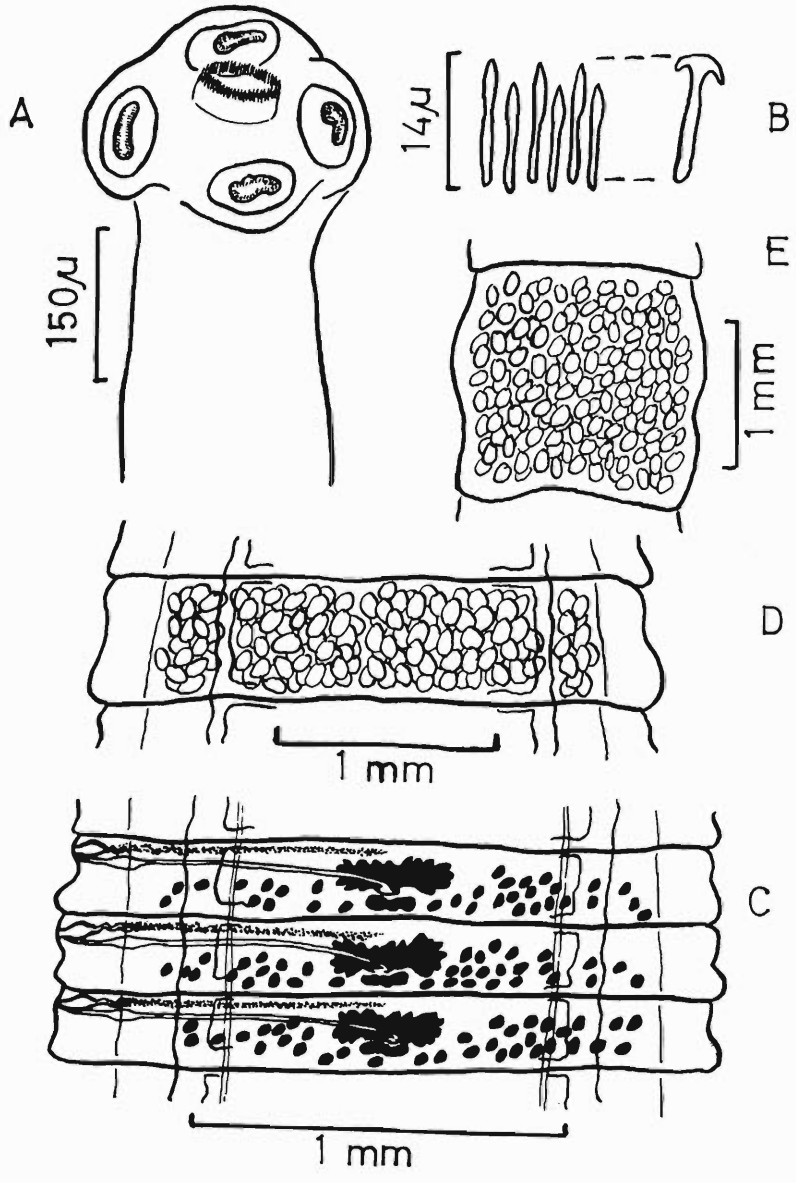

Figure 1. *Raillietina* (*R.*) *trinitatae* from *Callicebus cupreus*. A. Scolex and neck; B. Rostellar hooks; C. Mature proglottids; D. Incompletely gravid subterminal proglottid; E. Fully gravid terminal proglottid.

genital atrium into which the cirrus sac and vagina open. Cirrus sac, gourd-shaped, measuring 90-120 by 44-50, opening in atrium anterior to the vagina. Internal vas deferens with several loops in proximal portion of cirrus sac; external vas deferens convoluted and surrounded by prostatic cells, extending to middle of proglottid anterior to ovary. Testes number 27-40, 8-16 poral and 16-22 aporal; often 2-3 posterior to ovary and vitelline gland; usually absent anterior to genital ducts but a few reach or extend beyond the ventral excretory ducts; mature testes measure from 25 to 30 in width by 30-38 in length.

Distal portion of vagina dilated to a diameter of 20 for about 80-120. Vagina passes between dorsal and ventral excretory ducts and continues without convolutions to mid-proglottid where it bends toward the vitelline gland. Shell gland, about 50 long, is situated anterior to the vitelline gland. Vitelline gland, slightly lobulated, 40-45 in length by 100-117 in lateral width. Ovary located at mid-proglottid anterior to vitelline and shell glands. Ovary, with about 15 small lobes, measures 100-125 in length by 270-290 in lateral width.

Egg capsules in gravid proglottids (nos. 1025-1250) vary in number from about 140 to 220. The oval capsules vary from 95 to 126 in width and from 126 to 180 in length. Each capsule contains 5 to 9 eggs, usually oval and measuring 22-25 in greatest diameter.

DISCUSSION

Baer and Sandars (1956) reduced the species of Neotropical mammalian *Raillietina* to three, and discussed the synonomy of these species in detail. The first of these, *Raillietina* (*R.*) *demerariensis* (Daniels, 1895), Joyeux and Baer, 1929, has been recorded in man, the red howler monkey, *Alouatta seniculus* (L.) and a hystricomorph rodent of Cuba, *Capromys pilorides* Say. (Joyeux and Baer, 1949; Perez-Vigueras, 1943). The second, *R.* (*R.*) *alouattae* Baylis, 1947 (see Perkins, 1950) has been reported only from the red howler monkey, *A. seniculus,* and one of its subspecies (Hill 1960), *A. seniculus macconnelli* Elliot, 1910. The third species, *R.* (*R.*) *trinitatae,* originally described as *R.* (*R.*) *demerariensis* var. *trinitatae,* was found in two species of hystricomorph rodents of Trinidad, *Dasyprocta agouti* (L.) and *Cuniculus paca* (L.). Baer and Sandars (1956) elevated this variety to species level, and also assigned to *R.* (*R.*) *trinitatae* a tapeworm described by Stunkard (1953) as *R.* (*R.*) *demerariensis* from another hystricomorph, *Proechimys cayennensis* (Desm.) of Venezuela. In 1957 Lopez-Neyra and Diaz-Ungria recorded another Venezuelan hystricomorph rodent host, *Coendou melanurus,* for a variety of *R.* (*R.*) *demerariensis,* var. *venezolanensis.* Thus the Neotropical mammalian *Raillietina* have been recorded to date from man, one lower primate species (and a subspecies), and five species of hystricomorph rodents. Cameron and Reesal (1951) suggested that man and the monkey are accidental hosts of New World *Raillietina* and that, as in the Old World, the natural hosts are rodents. Stunkard (1953) also considered the native hystricomorph rodents of the Neotropical region to be the natural hosts of *R.* (*R.*) *demerariensis.*

If the Neotropical *Raillietina* are indeed normally parasites of hystricomorphs, the present infection of *Callicebus cupreus* must be considered accidental. However, information on cestodes of New World primates is at present limited and the true status of the recorded lower primate hosts of *Raillietina* (as incidental or maintaining hosts) remains to be determined.

Evidence so far available certainly suggests that rodents are natural or maintaining hosts, but the South American lower primates may also be more than mere incidental or accidental hosts. In any case, whatever the host status of the lower primates may be, there seems little doubt that man is only an accidental host for Neotropical *Raillietina*.

The present specimen closely resembles that described by Stunkard (1953). The worms differ appreciably only in measurements of total length and sucker diameters. Morphologically and in most mensural respects the present tapeworm agrees with the description by Cameron and Reesal (1951). Such dimensional differences as there are seem to fall within the usual range of infraspecific variation in this genus. Some of the more critical measurements and characters of the *Callicebus* cestode are compared with those for the other described specimens of this species in the accompanying table (Table 1).

Table I. *Raillietina* (*R.*) *trinitatae:* Comparison of some critical characters and dimensions of specimens assigned to this species. Measurements are in microns unless otherwise indicated.

	Cameron & Reesal (1951)	Stunkard (1953)	Specimen from *Callicebus cupreus*
Scolex diameter	370	270–340	297
Sucker diameters	88–100	130–140	86–96
Rostellum diameter	132	60–90	84
No. of hooks	170	175	160
Length of hooks	11	11	12–14
No. of testes	28–32	26–46	27–40
Length of cirrus sac	140–200	120–180	90–120
No. of egg capsules	50–70	40–243	140–220
Length of eggs	10	25–31	22–25
Total length (mm.)	60	100	319
Maximum width (mm.)	1.3	2.7	2.7

SUMMARY

A cestode recovered from a South American primate, *Callicebus cupreus* (Spix, 1823), is described in detail. The specimen is assigned to the species, *Raillietina* (*R.*) *trinitatae* (Cameron and Reesal, 1951), which has hitherto been recorded only from hystricomorph rodents. The tapeworm is compared with other specimens assigned to this species, and the mammalian hosts of Neotropical *Raillietina* are briefly discussed. *Callicebus cupreus* is the third known New World lower primate host for cestodes of the genus and subgenus *Raillietina*.

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Testing Alfalfa Seedlings for Resistance to the Stem Nematode *Ditylenchus dipsaci* (Kühn) Filipjev*

F. J. GRUNDBACHER**

In several alfalfa producing areas of California the stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, represents an increasing hazard to the cultivation of this crop. The most efficient control of this parasite is by means of resistant alfalfa varieties. However, before initiating a breeding program, a dependable technique for testing the resistance of the plants had to be developed. Initial experiments utilizing the techniques of inoculation and the criteria of classification for resistance proposed by other investigators (Bingefors 1957, Burkart 1937, Dijkstra 1956, Frandsen 1951, Smith 1955), gave unsatisfactory results.

SEEDLING INOCULATION TECHNIQUE

The seed was germinated in moist vermiculite contained in paper cups, which were kept in the greenhouse. Three or 4 days after sowing the seedlings, consisting of the cotyledons and radicles, were approximately 4 to 6 centimeters long. At this stage they were transferred to a 22-1/2 x 4-7/16 inch strip of moistened filter paper (No. 3). Ten or 12 plants were laid on the filter paper so that the cotyledons of the seedlings extended about 1 cm. above the edge. The paper was rolled into a cylinder around a vial (21 x 70 mm.), care being taken that the plants were evenly distributed, and did not touch each other. A small rubber band was used to hold the roll in place (Fig. 1).

Groups of 4 rolls were tied together by rubber bands and put into a 1000 ml. beaker, filled to one-third capacity with tap water or highly diluted Hoagland's solution. In either medium it was possible to grow the plants for several months at a temperature of 52° F. However, it was easier to suppress the growth of algae in tap water. The seedlings of adjacent rolls were kept apart by small wooden sticks inserted between the rolls. After the

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plants were placed in the beakers, they were covered with plastic overnight in order to assure the regaining of full turgor pressure before the root hairs had established sufficient contact with the moist filter paper. The beakers were kept in growth chambers.

The nematodes for inoculation were obtained by collecting heavily infested tops of alfalfa plants in the field in early spring. The infested material was spread out to dry in a warm room, or out-of-doors in the shade. The nematodes were extracted by a modification of the apparatus designed by Seinhorst (1945). A plastic covered frame was built to hold several large funnels. The infested plant material was put on muslin cloth in the funnels and subjected overnight to a fine water spray. The nematodes were revived by the water, moved down, and were collected in Petri dishes. The suspension was cleared by repeated decantation, and the nematodes concentrated.

The nematodes to be used for inoculation were suspended in a 1 per cent "Hercules Cellulose Gum" solution.† This solution is clear and has a high viscosity, which retarded the swarming and downward movement of the nematodes, resulting in a uniform suspension.

A Klett-Summerson photoelectric colorimeter was used in the preparation of nematode suspension of desired concentration. A dark-blue filter, approximate range 4000-4650 angstroms, was employed, since in this range the colorimeter displayed the greatest sensitivity. A test tube containing 5 ml. of gum solution was inserted into the colorimeter and the reading adjusted to zero. A highly concentrated suspension of nematodes was transferred into the test tube, and the tube shaken slightly until the suspension was uniformly distributed. The concentration was then measured, after which it was diluted

†Sodium carboxymethylcellulose, manufactured by Hercules Powder Company, Wilmington 99, Delaware.

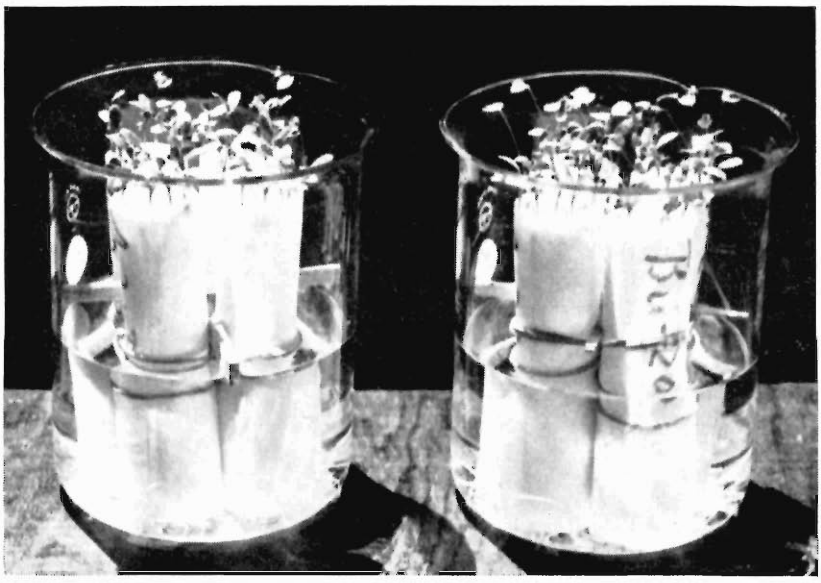

Figure 1. Stem nematode inoculated alfalfa seedlings growing on filter paper rolls in beakers.

with Hercules Cellulose Gum until a concentration having an optical density of 0.16, corresponding to about 5400 nematodes per ml. of suspension was obtained. Following preparation the nematode suspension was transferred to a small beaker kept in ice water to further reduce the downward movement and swarming of the nematodes.

The seedlings were inoculated by applying a droplet of the suspension between the cotyledons with a small bore dropper directly above the cotyledonary node. Care was taken that the inoculum extended down to the region of the shoot apex, and that it remained in this area. The inoculation was repeated after two or three days.

The beakers were covered with plastic for five days after the first inoculation to assure high humidity, but complete saturation was undesirable, for if the cotyledons became wet the inoculum tended to flow away from the apical region. Therefore a small hole was left in the plastic to permit some exchange of air.

For five days following inoculation the plants were kept under continuous light, thereby preventing the closing of the cotyledons, which occurs in darkness. In continuous light the inoculum remained around the shoot apex region much better than in alternating dark and light periods. Furthermore, comparisons between continuous and alternating light revealed that in continuous light the nematodes remained on the outside of the alfalfa stems, whereas when kept in dark, they entered into the stems. Similar results were found with cotyledons. When the seedlings were kept in continuous light, fewer nematodes entered the cotyledonary leaves and relatively greater numbers concentrated in the shoot apex region.

The stage of development of the seedlings was important for inoculation. Good results were obtained by inoculating at the time of emergence of the unifoliate leaf, specifically, when its tip became visible in the cotyledonary node. When the plants were kept at 52°F. they usually were ready for inoculation 2 to 3 days after the transfer to filter paper, about 6 days after sowing of the seed. Earlier inoculation frequently resulted in plants becoming "blind," i.e., the shoot apex ceased growth. Plants inoculated too late were difficult to classify, as described below.

The plants were kept under controlled environment with 13 hours of fluorescent light in each 24-hour period, except for the 5-day period immediately following inoculation. Most of the experiments were carried out at 52°F. At this temperature it was possible to maintain a high relative humidity without artificial application of moisture, but at 60° or 70°F. the humidity was increased by spraying with an atomizer.

At 52°F. the plants were grown for a period of one month after inoculation, at 60°F. for three weeks, and then classified for resistance. At the end of these periods the susceptible plants showed a distinct swelling in the shoot apex region. Also, the lower region of the petiole of the unifoliate leaf was especially heavily swollen, and generally the length of this petiole was much reduced. The resistant seedlings usually exhibited little swelling. Due to the presence of plants with intermediate types of symptoms, however, an accurate classification based on visible symptoms alone was not always possible in segregating populations. A more critical evaluation of resistance based on the amount of nematode reproduction became necessary which required staining of the nematodes and eggs within the plant tissue.

For staining of the nematodes, the method of McBeth, et al. (1941) was modified. The seedlings were removed from the filter paper rolls by severing the plants at a point just above the filter paper and put into small beakers. A boiling solution of 0.05 per cent acid fuchsin lactophenol was poured over the plants. The lactophenol consisted of glycerine, phenol, lactic acid, and distilled water in a ratio of 2:1:1:1. The beakers were transferred to a preheated hot-plate where they were kept until the stain had boiled for 2½ minutes. The time of boiling was increased to 4 minutes for tissues of older plants. The samples were left in the stain until cool (about 1 hour), after which the stain was poured off and the material rinsed clean with water. The plant tissue was cleared by covering with clear lactophenol for one or more days.

For examination the seedlings were transferred to glass slides and covered with 22 x 44 mm. cover slips. Using a stereoscopic microscope, the swelling of the plants in the shoot apex region was rated on a scale ranging from zero to four. Then the seedlings were squashed and the numbers of nematodes, eggs, and larvae counted. In cases of extraordinary large numbers an estimate was made. Usually it was not difficult to detect the nematodes, larvae, and eggs, since their striking red color was in sharp contrast to the cleared plant tissue. However, if there was any doubt as to the presence of eggs, the plant tissue was squashed again and then re-examined. Moreover, by applying interrupted slight pressure on the cover glass by means of a pencil, the plant tissue could be shifted slightly. This made the detection of the oval-shaped eggs easier.

In the course of the experiment, it became obvious that the stem nematode could reproduce in the hypocotyl, the cotyledons, and petioles of susceptible as well as resistant seedlings. Only the shoot apex region (the region above the cotyledons) gave a critical evaluation of resistance. The susceptible plants usually contained adult nematodes, eggs, and some small larvae in the

Figure 2. Bu-20 susceptible alfalfa seedlings exhibiting swelling in the shoot apex region. A-14 resistant seedlings.

shoot apex when the plants were stained after a growing period of one month at 52°F. In this region of the resistant plants, however, the nematodes were smaller, and no or very few eggs were produced, indicating an inhibition of nematode development. Presumably, the inoculum used consisted almost exclusively of fourth-stage (pre-adult) larvae. This assumption is based on the observation made by Sherman (1934) that especially the fourth-stage larvae survived desiccation of the plant tissue by becoming dormant.

Usually, plants in which eggs were produced in the shoot apex also exhibited swelling in this area (Figures 2-4). Therefore, in the latter phase of the experiments, when large samples of plants seemed to segregate in a wide genetic ratio, only those plants with some swelling in the shoot apex were stained and examined. The group with intermediate degrees of swelling included both resistant and susceptible plants. The resistant plants that displayed swelling in the apical region generally contained relatively large numbers of nematodes. This swelling might be attributed to mechanical irritation by the nematodes.

DISCUSSION

The most significant step toward the development of a satisfactory technique for determining resistance was the use of a controlled environment with continuous light for a period of time after inoculation. With these measures a uniform infection was achieved. Although a growing temperature of 52°F. is considered low for alfalfa, this temperature gave satisfactory results in the present study, and it had certain advantages over higher temperatures. At the low temperature it was easier to maintain a constant high air humidity, the damage by fungi was reduced, and the plants remained relatively small, facilitating staining and examination procedures under the stereoscopic microscope.

The nematodes could reproduce in the mesophyll of the cotyledons, in the cortex of the hypocotyl, and the cotyledonary petioles of susceptible as well as resistant alfalfa seedlings. This fact caused considerable difficulty in the early stages of the search to find a reliable criterion for resistance to the stem nematode. For critical evaluation of resistance a tissue had to be observed which reacted to nematode attack differently in resistant and susceptible plants. The meristematic tissue of the shoot apex with its emerging unifoliate leaf and first true leaves fulfilled the requirement. Susceptible plants reacted by swelling of the meristematic tissue, and large numbers of eggs were produced by the nematodes. Resistant plants most frequently exhibited little swelling in the apical region, and generally nematode reproduction did not occur. The unifoliate leaf and its petiole usually developed normally, but the first true leaves and the primary shoot often remained rudimentary, i.e., growth was inhibited. In later phases of development the resistant plants often formed a secondary shoot and overcame in this way the nematode attack.

From the above observations it may be concluded that the nematodes exerted a grow-inhibiting effect on the meristems of resistant plants. Possibly, inhibition was the result of hypersensitivity similar to that occurring with some virus infections. However, brown necrotic spots, as they were described for red clover (Dijkstra 1956, Goodey 1950, Seinhorst 1956), were not a critical expression of resistance, since such spots appeared irregularly on resistant as well as on susceptible alfalfa seedlings.

Goodey (1937) used the reproduction of nematodes as an indication of susceptibility of oats. For alfalfa seedlings the criterion of nematode reproduction has to be restricted to the meristematic shoot apex region. Nematodes other than *Ditylenchus dipsaci,* such as *Meloidogyne* spp. and *Heterodera,* spp., are more critical in selecting meristematic tissue of the host; they invade the host in the meristematic region of the root tip. In general, the reaction of the attacked apices of the root and the shoot of susceptible plants appear to be the same. However, the galling on the roots is more distinctly visible than the swelling in the shoot apex region because the shoot apex is enclosed by other structures such as petioles and leaf primordia. The latter possess meristematic tissues which may react to stem nematode attacks. Furthermore, the shoot apex is more exposed to more variable external influences than the root apex.

The most critical step in the completion of the life cycle within alfalfa plants appeared to be egg production. The development from eggs to larvae seemed not to be critical because at later stages larvae of different sizes usually were present when eggs were found in a plant.

The seedling inoculation technique has possibilities of application in practical plant breeding. However, in screening for resistance, the plants would have to be kept alive; otherwise the method could only be used for progeny testing. For breeding purposes the staining procedure may be omitted, and

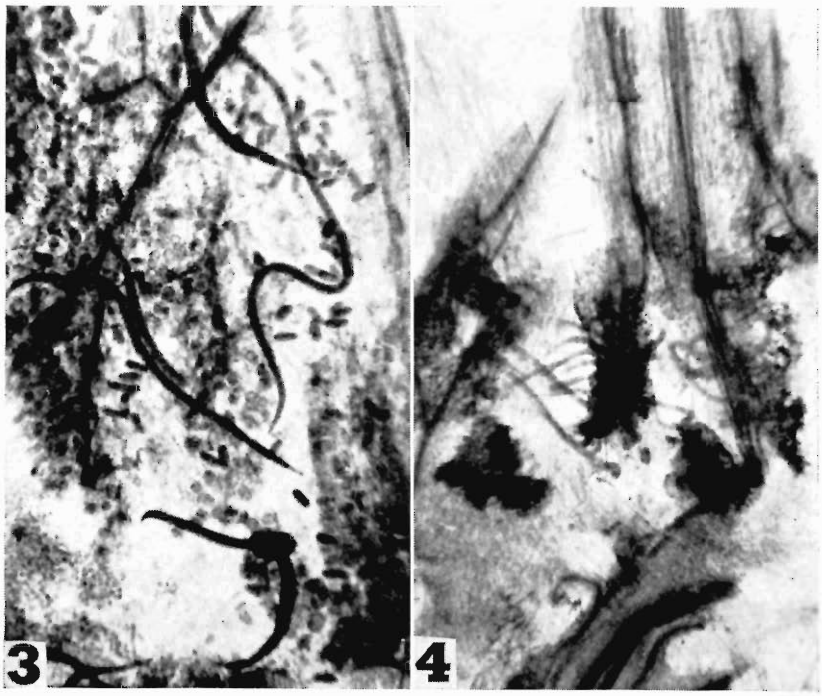

Figure 3. Part of the apical region of a susceptible alfalfa seedling showing eggs and large nematodes.

Figure 4. Shoot apex region of a resistant alfalfa seedling with no eggs and smaller nematodes than in susceptible plant (Figures 3 and 4 are of the same magnification).

the plants classified on their visible symptoms alone. This appears practical, since all the plants with an intermediate type of reaction may be discarded even though this may result in sacrificing some resistant plants. If classification for resistance were to be based on visible symptoms alone, a temperature between 55° and 60°F. may be more satisfactory, since in this range the visible symptoms of susceptibility are expressed somewhat more strongly than at lower temperatures. It would still be possible to maintain a relative high air humidity without inducing too much damage by pathogenic fungi, which becomes a hazard at humid higher temperatures.

The reaction of older alfalfa plants in other studies was in good agreement with the reaction of seedlings. However, when testing older plants in flats, some of the susceptible check plants escaped infection, indicating that the seedling test is more reliable than the test of older plants. This is in agreement with observations by Frandsen (1951), who obtained a lower percentage of resistant red clover plants in seedling tests than he did with older plants under field conditions.

The seedling testing method developed in this study has the advantage of yielding results in a relatively short time. It can also be conducted throughout the year independent of climatic conditions. Various sources of alfalfa were evaluated for their resistance to the stem nematode and the inheritance of resistance was studied. The results of these studies will be reported elsewhere.

SUMMARY

A technique for testing alfalfa seedlings for resistance to the stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, was developed. Plants were grown on filter paper rolls in a controlled environment. For measuring the degree of resistance the nematodes and eggs within the plants were stained and their numbers determined. The amount of nematode reproduction in the shoot apex region was a reliable criterion for resistance of the plants. Some interpretations of the nature of resistance to the stem nematode are discussed.

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Some Internal Parasites of the Cotton-Topped Pinche (Columbian Marmoset), *Oedipomidas oedipus*, with a Note on the Survival, *in vitro*, of Microfilariae of One of the Parasites

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Two Pinché marmosets, *Oedipomidas oedipus* were referred to one of us (TKS) by Mr. W. Jahnes, Division of Biologics Standards, National Institutes of Health, Bethesda, Md., after viable microfilariae were observed in kidney tissue clutures prepared from the animals. Parasitological examination provided the following species: *Athesmia foxi* (Trematoda:Dicrocoeliidae), from the bile ducts; *Prosthenorchis elegans* (Acanthocephala), from the ileum; and several unidentified female filariids (Nematoda:Dipetalonematidae), from between the lumbar muscles. *A. foxi* has been found in the Cebidae, (Goldberger and Crane, 1911; Stunkard, 1924; Caballero, Grocott, and Zerecero, 1952), but has not been reported previously from the Callitrichidae. *P. elegans* has been collected from *Oedipomidas* (Stiles and Nolan, 1929; Benirschke and Richart, 1960). Adult filariids have not been reported from this host although microfilariae were found but not described by Plimmer, (1912).

This report is presented to record *O. oedipus* as a new host for *A. foxi* and for an unidentified filariid, and to describe changes in measurements of microfilariae during maintenance in kidney tissue cultures.

METHODS

Two marmosets, frozen after removal of the kidneys, were thawed and skinned at the time of dissection. Examination of the skin, connective tissues, and superficial muscle fascia under direct light failed to reveal tissue parasites. However, after the fascia began to dry and wrinkle, small bubbles formed along the mid-dorsal region of the body. Depression of the bubbles with a teasing needle displaced tightly coiled adult worms from between the muscles; these were preserved in AFA fixative. Examination of the intestines and bile ducts provided the other species which were fixed in 10% buffered formalin. Histologic sections, stained with hematoxylin and eosin were prepared from various tissues and organs.

The microfilariae were maintained in stoppered test tubes previously inoculated with trypsinized marmoset kidney cells. The cultures, received from the Division of Biologics Standards, N.I.H., contained 1.0 ml of tissue culture medium 199 (Morgan, Morton, and Parker, 1950), supplemented with 2.0% calf serum. At intervals of 2, 8, and 22 days the media from 5 tubes were pooled and centrifuged, and the sediment stained with 1:1000 methylene blue (Newton and Wright, 1956) for the study of internal morphology. Measurements were made with a calibrated ocular micrometer.

RESULTS

Measurements of the microfilariae during the 22-day culture period are given in Table No. 1. In two-day old cultures microfilariae measured 266 to

281 by 3.6 microns and were structurally similar to microfilariae of *Tetrapetalonema marmosetae* from the Panamanian marmosets described by Faust (1935). The microfilariae of both Columbian and Panamanian marmosets were similar in the following respects: cephalic space devoid of nuclei; 1, 2-3-4 G-cell pattern; blunt tail; and absence of a sheath. Measurements were essentially unchanged after 8 days in culture. After 22 days the microfilariae varied from 274 to 358 microns long by 2.9 to 4.7 microns wide; however, no change in cellular morphology was noted. Adult female filariids measured 68.0 to 100.0 mm in length (average, 83.0 mm); adult males were not recovered. Subsequent examination of blood from two other marmosets was negative for microfilariae and post-mortem examination did not provide adult worms. Examination of tissues of eleven other cotton-topped Pinché's from the N.I.H. primate colony by Drs. A. M. Allen and R. F. Kinard, Comparative Pathology Section, Laboratory Aids Branch, Division of Research Services, N.I.H. revealed that 6 harbored microfilariae in the blood. These animals died of various diseases during 1960 and were submitted for routine post-mortem examination. These data showed that approximately half of the marmosets (8 of 15 animals) were parasitized by filariid nematodes.

Examination of the extraphepatic bile ducts revealed numerous trematodes approximately 1.0 cm in length. In histologic sections the worms nearly occluded moderately dilated branches of proximal intrahepatic bile ducts. In one animal acute and chronic inflammatory cells surrounded bile duct branches and portal venules. Whole specimens removed from the ducts and stained with Harris' hematoxylin were identified as *A. foxi.*

Numerous specimens of *P. elegans* were found in both animals. Worms were lying free in the ileal lumen or partially imbedded in the wall of the ileum. Imbedded specimens were surrounded by large areas of fibrosis and inflammation which extended to the serosa. Marked mesenteric lymphadenopathy was present in both marmosets.

TABLE NO. 1

Changes in Measurement (in microns) of Microfilariae During 22 Days' Maintenance *in vitro*

Days in Culture	Length	Width	Average
2	266	3.60	
	281	3.60	
	281	3.60	
	266	3.60	274 x 3.6
8	266	3.60	
	266	3.60	
	281	3.60	
	304	3.60	
	281	3.60	
	289	3.60	276 x 3.6
22	334	3.60	
	358	2.90	
	319	2.90	
	327	2.90	
	304	2.90	
	319	2.90	
	274	4.70	305 x 3.3

DISCUSSION

Only two groups of mammals are known hosts for the genus *Athesmia*: primates which harbor *A. foxi,* and a bat *Artibeus jamaicensis parvipes,* the host for *A. parkeri,* (Vigueras, 1942). The two species are easily separated by the ratio of length to width (approx. 10:1 for *A. foxi,* and 4:1 for *A. parkeri*), and by differences in testicular lobation. Goldberger and Crane (1911) originally separated *A. foxi* from the type species *A. heterolecithodes* (Braun, 1899) Looss, 1899, in part, because in dorsal aspect the former was right-handed and the latter left-handed with respect to the unilateral vitelline glands. Subsequently Stunkard (1924) studied 25 specimens of *A. foxi* from *Cebus apella* and in 4 worms found complete reversal in the arrangement of internal organs. A more recent report of *A. foxi* from *C. capucinus* by Caballero et al (1952) described specimens which were lefthanded in dorsal view, agreeing with the *situs inversus* reported by Stunkard (1924). The specimens discussed in the present report are right-handed in dorsal aspect and agree with descriptions of Goldberger and Crane (1911), and Stunkard (1924). The right-handed or left-handed character of the unilateral vitelline glands, in dorsal view, appears to be subject to variation within this species. The only report of *A. parkeri* (Vigueras, 1942), describes left-handed vitellaria. In contrast to the extensive variations among the Athesmiinae from birds (Travassos, 1944), all of the specimens of *A. foxi* are characterized by the following: ovary always opposite the vitellaria, testes and ovary deeply lobed, and cirrus sac posterior to the intestinal bifurcation. With the exception of the position of the vitellaria, internal morphology and overall measurements are in good agreement in all reports of *A. foxi.*

Positive identification of the adult filariids was not possible since male worms were not recovered. Preliminary studies by Mrs. Chitwood, Agricultural Research Center, USDA, Beltsville, Md., indicate that the female worms are members of the subfamily Dipetalonematinae (Wehr, 1935), and are tentatively identified as *Tetrapetalonema* sp. The microfilariae from kidney tissue cultures, although of the dipetalonemid-type, could not be definitely associated with the adult worms. A multiple infection could not be ruled out since the animals were received frozen and fresh blood was not obtained. The 25% increase in length of some of the microfilariae is considered to be an abnormal response to culture conditions as suggested by Taylor (1960). Two-fold increases in length by *Mf. immitis* has been reported by Wellman and Johns (1911), and Joyeux and Sautet (1937).

The recovery of *P. elegans* is reported here to extend the observation of Benirschke and Richart (1960), that this parasite is encountered frequently among captive marmosets. The heavy infection and extensive damage to the ileum by this acanthocephalid may contribute to the high mortality rate of these primates during captivity. The non-specific hepatic portal inflamation may be related to the *P. elegans* infection rather than to *A. foxi.*

SUMMARY

The cotton-topped Pinché, *Oedipomidas oedipus,* has been reported as a new host for the dicrocoeliid trematode *Athesmia foxi,* and adult filarial worms have been recovered from the same host for the first time.

A description of microfilariae is given and their increase in length under *in vitro* conditions is discussed.

Variations in the position of vitellaria and other internal organs of *A. foxi* are reviewed and further substantiated.

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The Development of *Aphelenchus avenae* Bastian, 1865 in Fungus Culture

HELEN CAROL HECHLER*

Aphelenchus avenae Bastian, 1865 feeding on fungi in agar has been used by the writer as prey to feed various predaceous nematodes. The following is a description of *A. avenae* and a brief report on its development biology in an artificial environment.

DESCRIPTION

Nematodes similar to *A. avenae* have been described under many different names, although Goodey (1960) synonymized most of these with *A. avenae.* To clarify the taxonomic position of the nematodes used in these studies they are described and illustrated.

The nematodes were from three different sources. One population, from a source not recorded, was isolated and established in culture by M. B. Linford in about 1953. Another was collected from a field of parsnips near Downers

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Grove, Illinois in December 1959, and the third was from soil around roots of henbit deadnettle (*Lamium amplexicaule* L.), collected near Urbana, Illinois in August 1960. Progeny of single females from each of the sources showed all the variations given in the following description. All the populations were morphologically similar but the Urbana population had a higher proportion of males.

100 females: L = 0.70 mm. (0.55-1.05); a = 32.1 (24.8-43.0); b = 8.5 (7.2-9.6); c = 29 (18-35); V = 76.5 (74.3-78.4)%.

10 males: L = 0.75 mm (0.63-0.90); a = 38 (32-43); b = 8.4 (7.2-9.6); c = 30 (27-32).

FEMALES: (Fig. 1: A, B. C, D, E. F) Body tapering slightly at head, narrowing abruptly behind vulva, usually a slight constriction just in front of anus as in description of *Metaphelenchus rhopalocercus* (Steiner, 1943), occasionally no constriction, or rarely, constriction occurs behind anus. Tail rounded, rarely slightly flattened at terminus. Cuticular striations about 1 micron apart near middle of body. Lateral fields about 42% of body width at widest part, crenate in cross section, with 2 incisures two stylet lengths behind lips, 4 at level of median bulb, 6 between bulb and deirid. Immediately behind deirid the two outer incisures divide, increasing total to 8, then to 10 near end of dorsal gland, and to 12-14 at middle of body (Fig. 2, D). Incisures decrease to 11 just in front of vulva by joining of inner lines. Just behind vulva lateral field narrows sharply to 8 incisures, it constricts slightly just in front of anus, then spaces between incisures widen until the 8 incisures fade near curvature of tail. Phasmids nearly terminal.

Head rounded, not offset, no annules on lips. Lips six, papillae on four submedian lips, amphids on lateral lips. Excretory pore opposite nerve ring, hemizonid and deirids 3-4 annules behind pore, hemizonion 1 bulb length behind hemizonid. Anterior cephalid ½ stylet length from lips, posterior cephalid ½ stylet length behind stylet.

Stylet 12-15 microns long, knobless, junction conical and cylindrical parts at slightly less than ½ its length. Precorpus cylindrical, median bulb round to slightly elongate, crescentic valve plates central in bulb, muscle fibers occupying all but extreme anterior and posterior sectors. Nerve ring just behind bulb. Narrow esophageal lumen widens into intestine about 1½ bulb lengths behind bulb. Dorsal gland overlapping intestine, usually on left side, as seen under the compound microscope about 65 microns long. Intestine tesselated, rectum about 12 microns long, straight, prominent. Anus a transverse slit ⅓ body width, anterior lip protruding slightly.

Vulva oval, vagina walls thick. Ovary single, anterior, outstretched, reflexed in well fed specimens, oocytes in single or double row. Anterior end of ovary between middle of body and nerve ring. Postvulvar uterine branch wide near vulva, with a narrow appendage reaching to ½ distance from vulva to anus as described for *A. macrobolbus* (Steiner, 1942). Wide part proportionately longer in well fed specimens.

MALES: (Fig. 1: G, H) Anterior end similar to female except only 10-12 incisures in lateral fields (Fig. 2, F). Stylet 12-14 microns long. Spicules 24-29 microns long, cephalated. Gubernaculum 11-12 microns long, narrow at anterior end, widening abruptly, tapering to anus. Bursa arising near head of spicules, enveloping terminus, supported by 1 pair of papillae arising just in front of anus, 3 pairs near terminus. Males rare, less than 1:100,000 females; 1:10,000 in Urbana population.

METHODS

MAINTENANCE: Populations were maintained at 28° C. in Petri dishes in the same manner as described by Hechler (1962) for *Neotylenchus linfordi,* except full strength potato dextrose agar was used. The fungus used was *Pyrenochaeta terrestris* (Hansen) Gorenz, J. C. Walker and Larson.

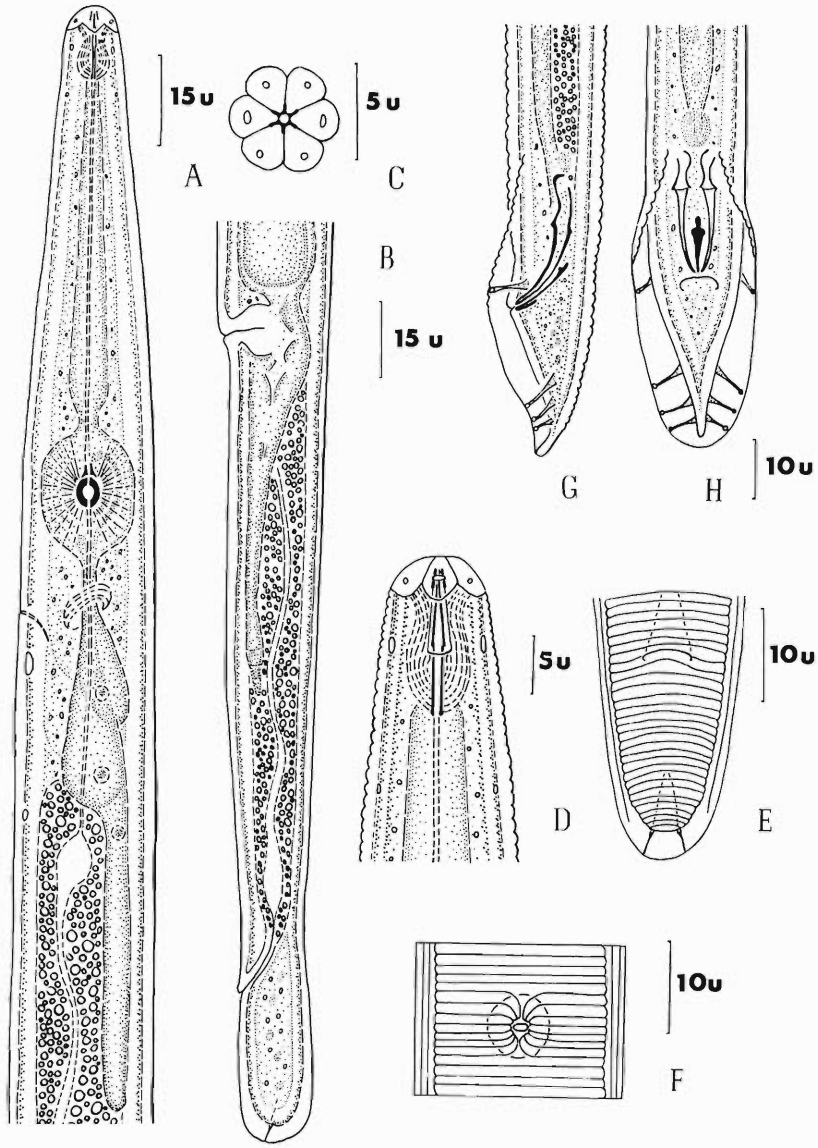

Fig. 1. *Aphelenchus avenae:* A. Anterior of female; B. Posterior of female; C. *En face* view; D. Female head; E. Ventral view female tail; F. Ventral view male tail.

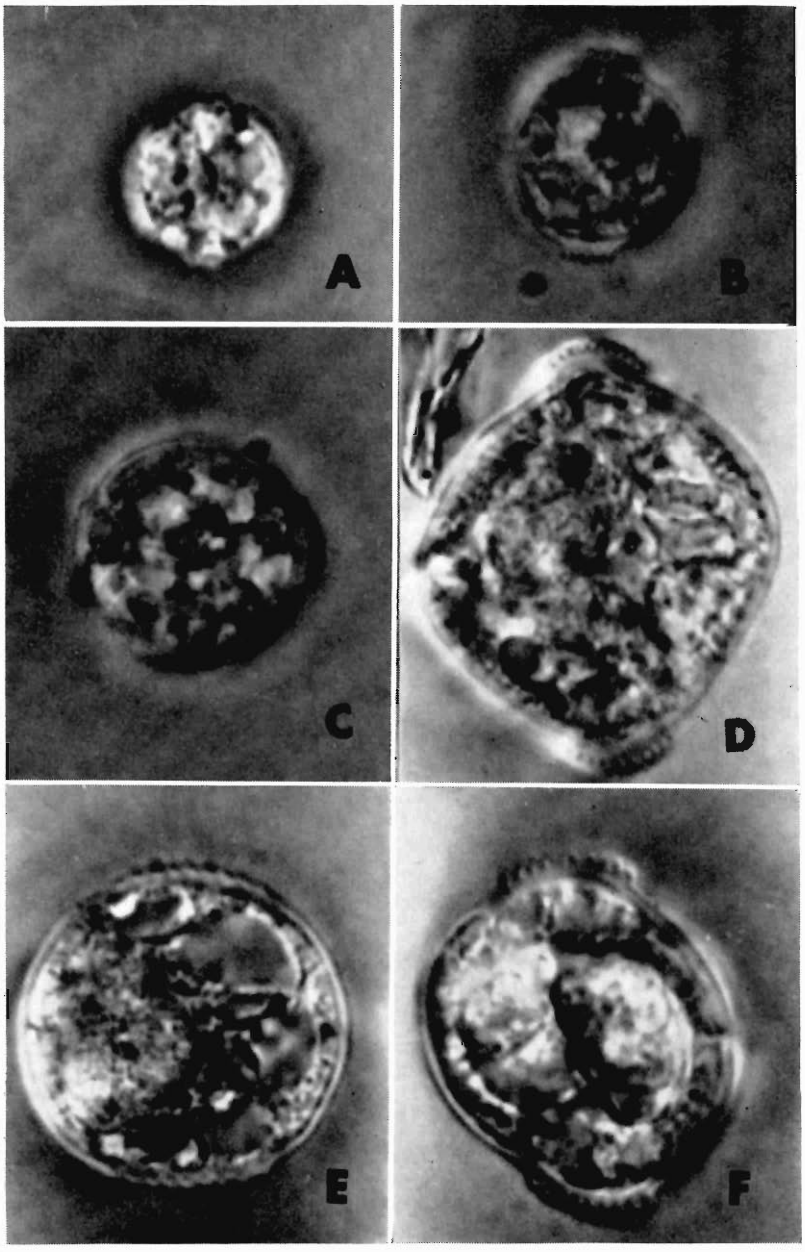

Fig. 2. Cross section through middle of body: A. Second stage; B. Third stage; C. Fourth stage; D. Female; E. Aged enlarged female; F. Male.

EGG DEVELOPMENT STUDIES: To determine the incubation period eggs were obtained by placing gravid females in separate sterilized small Syracuse dishes filled with sterilized water. After an egg was laid the female was removed and the time recorded. The eggs were held at 28°C. in Petri dishes until hatched. Other eggs were observed with a 40X water immersion objective to learn the details of embryo development. To learn the rate of egg production, individual gravid females were placed in separate dishes for 24 hours, and the total number of eggs in each dish was counted. Accurate counts on egg production of well fed females could not be obtained because hyphal growth obstructed the view of many eggs.

LARVAL DEVELOPMENT STUDIES: To study larval development nematodes were reared a few at a time in special cells prepared by attaching 2 cm. diameter glass rings to 1 x 3 inch glass slides with thickened Canada balsam. The cells were placed in Petri dishes and sterilized in an oven at 320°F. for 90 minutes. The heat dried the balsam enough to prevent separation of the two parts. About 0.8 ml. of melted potato dexrose agar was put in a cell with a sterlized medicine dropper, and a few hyphal strands of the fungus were added using a flamed wire. After two days the fungus colony was large enough to feed a limited number of nematodes adequately. A few newly hatched larvae were added and observed periodically.

LIFE HISTORY

EGG DEVELOPMENT: Eggs are 70-80 microns long and 25-35 microns wide, ranging from 2 to 3 times as long as wide. The shell appears smooth after hatching. Eggs are unsegmented when laid. The protoplasm rounds up and cell division begins in a few minutes, with the first two divisions perpendicular to the long axis of the egg. The vermiform shape of the embryo becomes apparent after 3-4 hours, with the anterior part more hyaline. Motion begins when the embryo reaches 1½ times the length of the egg. It continues to grow, always moving forward and backward around the egg until it is about three times as long as the egg. A faint stylet is then visible but no muscular bulb can be seen. At this time an obvious molt occurs. The second stage larvae possess a well formed stylet and muscular bulb. Just before hatching the embryo is 3½ to 4 times as long as the egg.

After the molt, motion resumes and as hatching time nears the contours of the shell change to correspond to the shape of the embryo as it moves, with the most prominent bulge always at the position of the head. The valve in the muscular bulb pulsates, often so violently that the stylet vibrates in unison with it. Nothing can be seen entering the stylet or intestine during these periods of pulsation although it is possible that the nematode is ingesting fluid from around itself. Twenty to forty minutes before hatching when the nematode fills the shell so snugly it moves with difficulty, it repeatedly presses with its head against one end of the egg, stretching the shell. The stylet is protruded and withdrawn rapidly while the head is at the end of the egg although a stylet was never seen to penetrate the shell. Eventually the shell ruptures at one end and the larva emerges.

The development of the egg from laying to hatching occupies 40-44 hours at 28°C.

SECOND STAGE LARVAE: L = 0.24 mm. (0.22-0.31); a = 18.9 (16.0-21.9); b = 4.4 (3.9-5.2); c = 17.7 (15.0-20.4). Stylet = 6-9 microns.

Lateral field marked by 3 incisures 2 stylet lengths behind lips, 4 at latitude of bulb. The 4 incisures, the 2 inner ones more shallow, continue throughout length of nematode until they fade at curvature of tail (Fig. 2, A). Genital primordium about four cells, appearing as a hyaline area at about 77% of body length.

The larvae begin to feed immediately after emerging from the egg and must feed before the second molt.

THIRD STAGE LARVAE: L = 0.35 mm. (0.26-0.44); a = 22.8 (19.5-25.7); b = 5.4 (4.4-6.3); c = 21.1 (15.7-27.5). Stylet = 8-11 microns.

Lateral fields with 2 incisures two stylet lengths behind lips, 3 at latitude of bulb, 4 between bulb and deirid, 6 between deirid and tail, where they fade over the terminus (Fig. 2, B).

Genital primordium with about 6 cells reaching from about 75% of body length to ½ distance from future vulva to end of dorsal gland. Future vulva occasionally marked by faint hyaline area.

FOURTH STAGE LARVAE: L = 0.53 mm. (0.44-0.66); a = 25.9 (22.5-34.2); b = 6.8 (5.7-8.0); c = 25.0 (22.2-30.0). Stylet = 10-12 microns.

Lateral field as in third stage from lips to deirid, 6 incisures deirid to middle of body, 8 incisures to about ½ distance future vulva to anus, where two inner pairs join to form 6 (Fig. 2, C). Just in front of anus a constriction occurs as in adult, then distance between incisures widens. Incisures fade at curvature of tail.

Genital primordium reaches ½ distance future vulva to end of dorsal gland, vulva visible as a tube at about 76% of body length.

ADULTS—Females begin to lay eggs a few hours after emerging from the final molt. In water at 28°C. they lay about 12 eggs the first 24 hours away from the fungus, only 2 or 3 in the second 24 hours.

Aged enlarged females occur occasionally. As they increase in width the lateral fields are stretched and flattened in cross section. (Fig. 2, E).

Females isolated while in a larval stage produced many progeny, and although an occasional male occurs they are not necessary for reproduction.

POPULATION DEVELOPMENT: The generation time at 28°C. is about 6 days. Petri dish cultures started with about 100 nematodes contained 75,000 to 100,000 of their progeny in 14-16 days.

SUMMARY

Aphelenchus avenae Bastian 1865 is described. In its life history there are 4 molts, one in the egg. The first stage larva has a stylet but no median bulb. Each molt is accompanied by an increase in the incisures in the lateral field, each cuticle is cast separately, and the nematode feeds after each molt. The generation time at 28°C. is about 6 days.

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Heterodera cyperi (Heteroderidae), a new species of cyst-forming nematode

A. MORGAN GOLDEN,* GEORGE J. RAU* and GRACE S. COBB*

During the past 8 years on several occasions small numbers of cysts evidently representing an undescribed species of *Heterodera* were received for identification. The first specimens were obtained from 2 soil samples collected in 1954 at Fort Myers, Florida by J. A. West of Plant Pest Control Division, Agricultural Research Service, United States Department of Agriculture. In 1956 similar cysts were received from soil from Wilmington, North Carolina, in 1957 from Sanford, Florida, and in 1959 from Pender County, North Carolina. Later additional cysts of the same nematode were obtained from soil collected in other areas of Florida, including Hastings and Bunnel, and also Osceola, Arkansas. In all these cases however, the specimens were unsuitable for critical study. In 1960, by making additional collections at Sanford, Florida, where one of us (Rau) had found *Heterodera* males in association with nut-grass in 1955, sufficient specimens were obtained for urther examination. A description of this new species of *Heterodera* is presented.

Heterodera cyperi, n. sp.

MEASUREMENTS: 20 females (Figs. 1A and 3D)—Length 0.526 mm (0.459-0.663); width 0.275 mm. (0.228-0.345); stylet 22.4 microns (22.0-22.4).

Holotype (female)—Length 0.535 mm.; width 0.280 mm; stylet 22.4 microns.

Body pearl-white in appearance and swollen, basically lemon-shaped with protruding neck and vulva. Cuticle thick, measuring several microns at middle of body and exhibiting a zig-zag pattern externally. Head distinctly set-off from neck, bearing two prominent annules; second annule larger than first and generally disc-shaped, appearing as illustrated in Fig. 1A. Cephalic sclerotization weak and indistinct. Stylet rather delicate, with generally slight dorsal curvature and with distinct knobs sloping posteriorly. Outlet of dorsal esophageal gland 4.9 microns (4.5-5.6) from base of stylet. Valvated median bulb particularly large. Esophageal glands apparently contained in a single lobe, variable in size and shape but generally appearing about as shown in Fig. 1A. Excretory pore distinct, located at a level posterior to the end of the esophageal glands, usually by a distance of several microns. Ovaries 2, convoluted, becoming indistinguishable as the body eventually fills with eggs. Vulva prominent, protruding posteriorly, often seen surrounded by large gelatinous sac containing many eggs. Anal area conspicuous, with clear anus usually having a definite cuticular pattern surrounding it similar to that shown for cysts.

20 males (Fig. 1, B and C)—Length 0.964 mm. (0.874-1.159); a = 38.6 (32.9-42.0); b = 5.5 (4.5-6.7); c = 196.0 (90.5-351.4); stylet 22.1 microns (21.2-22.9).

Allotype (male)—Length 0.964 mm.; a = 41.0; b = 5.3; c = 247.0; stylet 22.4 microns.

Body cylindrical, elongate and tapering gradually at each end. Head offset from body, bearing about 4 or 5 indistinct annules. Cuticular annulation

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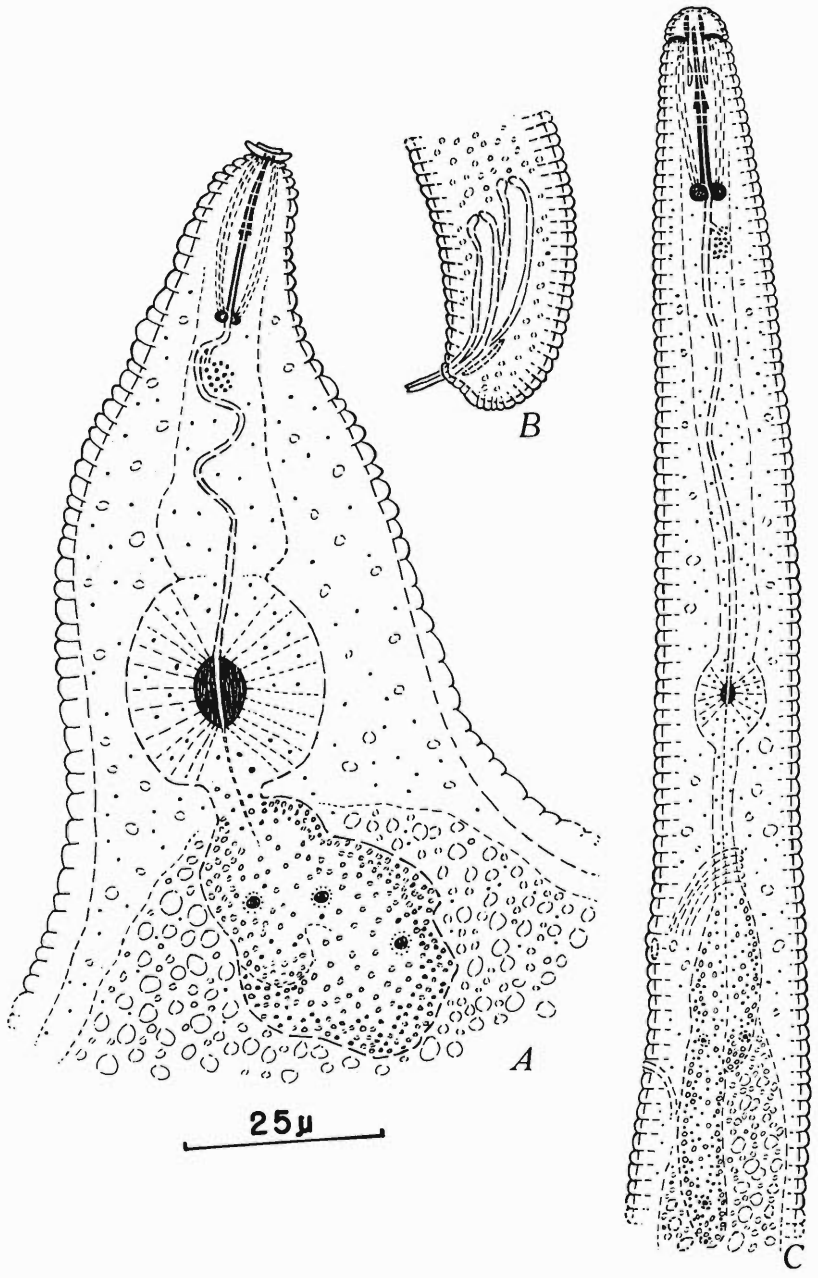

Figure 1. Drawings of *Heterodera cyperi*, n. sp. A. Anterior portion of female. B. Posterior portion of male. C. Anterior portion of male.

of body distinct, the annules measuring approximately 1.8 microns each, subcuticle apparently with about twice as many annules, each measuring a little less than 1 micron. Lateral field about 1/6 body width, not aerolated; appearing sometimes as 3 lines but in many cases as 4 in which case the 2 center lines are much closer than the outer ones, forming a center band considerably narrower than the outer two. Cephalic framework heavily sclerotized. Stylet strong, with rounded basal knobs. Outlet of dorsal esophageal glands 3.9 microns (3.3-4.5) from base of stylet. Valvated median bulb distinct and usually appears about as shown. Nerve ring encircling a portion of isthmus. Esophageal glands variable in size and shape but generally appearing as illustrated. Hemizonid prominent, located 15-20 microns anterior to the clearly visible excretory pore, the latter being 12-14% of body length from anterior end. Testis 1, outstretched anteriorly. Spicules arcuate, 28.8 microns (28.0-30.8) long, singly notched at tips. Gubernaculum 8.9 microns (8.0-10.0) in length. Tail very short, somewhat inexact shape. Phasmid near terminus.

20 second-stage larvae (Fig. 2)—Length 0.441 mm. (0.414-0.465); a = 25.1 (24.3-26.0); b = 2.8 (2.7-3.0); c = 7.3 (7.0-7.8); stylet 20.0 microns (19.2-20.7).

Body cylindrical, elongate, tapering markedly toward posterior end. Head well offset from body, bearing about 4-5 very faint annules. "Head cap" averaging 3.3 x 7.9 microns. Cuticular annulation of body distinct, the annules measuring approximately 1.5 microns at middle of specimen. Lateral field about 1/6 body width, appearing as 3 lines forming 2 bands. Cephalic framework heavily sclerotized. Stylet strong, with well developed knobs shaped usually as illustrated in Fig. 2 A, B. Outlet of dorsal esophageal glands 5.2 microns (4.8-6.1) from base of stylet. Valvated median bulb, nerve ring and esophageal glands appearing usually as in Fig. 2. Hemizonid distinct, located slightly anterior to the clearly visible excretory pore, the latter being located about 20% of the body length from anterior end. Tail 60 microns (56-63) in length. Hyaline portion of tail variable, averaging 24.7 microns (18.0-31.0) in length. Terminus bluntly rounded. Phasmids indistinct but seem after staining with aniline blue to be located very slightly anterior to the middle of the tail.

20 cysts (Fig. 3A, B and C)—Length 0.64 mm. (0.459-0.742); width 0.325 mm. (0.229-0.382).

Cysts light to dark brown, lemon-shaped, with protruding neck and vulva No bullae in vulval cone. External pattern on cysts clearly zig-zag but often becoming rather indistinct in middle portion. Anal area conspicuous; anus prominent, usually surrounded by a definite pattern and located roughly 10% of cyst length from posterior end.

20 eggs—Length 0.104 mm. (0.095-0.110); Width 0.040 mm. (0.038-0.042); Length/width ratio = 2.6.

Egg shell hyaline, without visible markings.

DIAGNOSIS: *Heterodera* differing from closely related described species (*H. humuli, H. fici,* and others of the *"göttingiana* group") particularly by (1) presence of only 3 lines in lateral field of the larvae; (2) highly conspicuous anal area usually with definite pattern around anus of mature females and especially cysts; and (3) generally indistinct external pattern on cyst in middle portion.

HOLOTYPE—Female: Collected by A. Morgan Golden on August 22, 1960, at Central Florida Experiment Station, Sanford, Florida. Slide T-22-t,

United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

ALLOTYPE—Male: Same data as for holotype. Slide T-23-t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

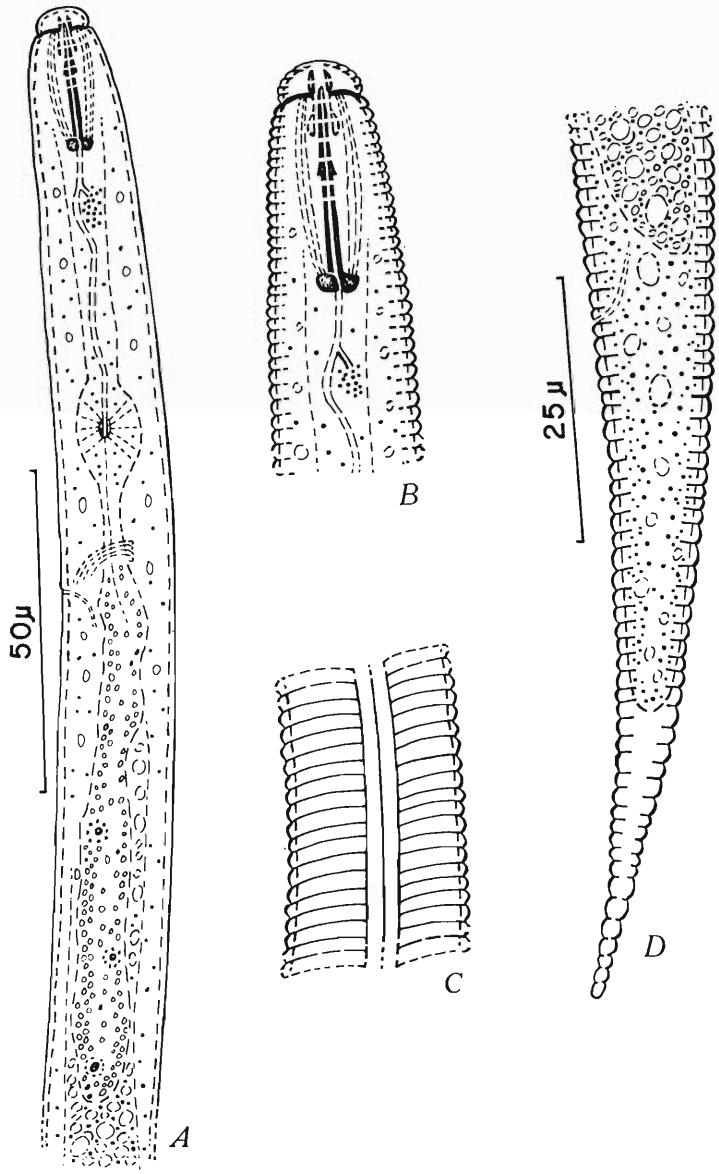

Figure 2. Drawings of larvae of *H. cyperi*, n. sp. A. Anterior portion. B. Enlarged anterior portion. C. Lateral field in middle of body. D. Posterior portion. (B, C and D same scale).

PARATYPES—Males, females, cysts, larvae and eggs: United States Department of Agriculture Nematode Collection, Beltsville, Maryland; and University of California Nematode Survey Collection.

TYPE HABITAT, HOST AND LOCALITY: Roots and tuber ("nuts") of *Cyperus esculentus* L. (yellow nut-grass) in field immediately behind the present office and laboratory building of the Central Florida Experiment Station, Sanford, Florida.

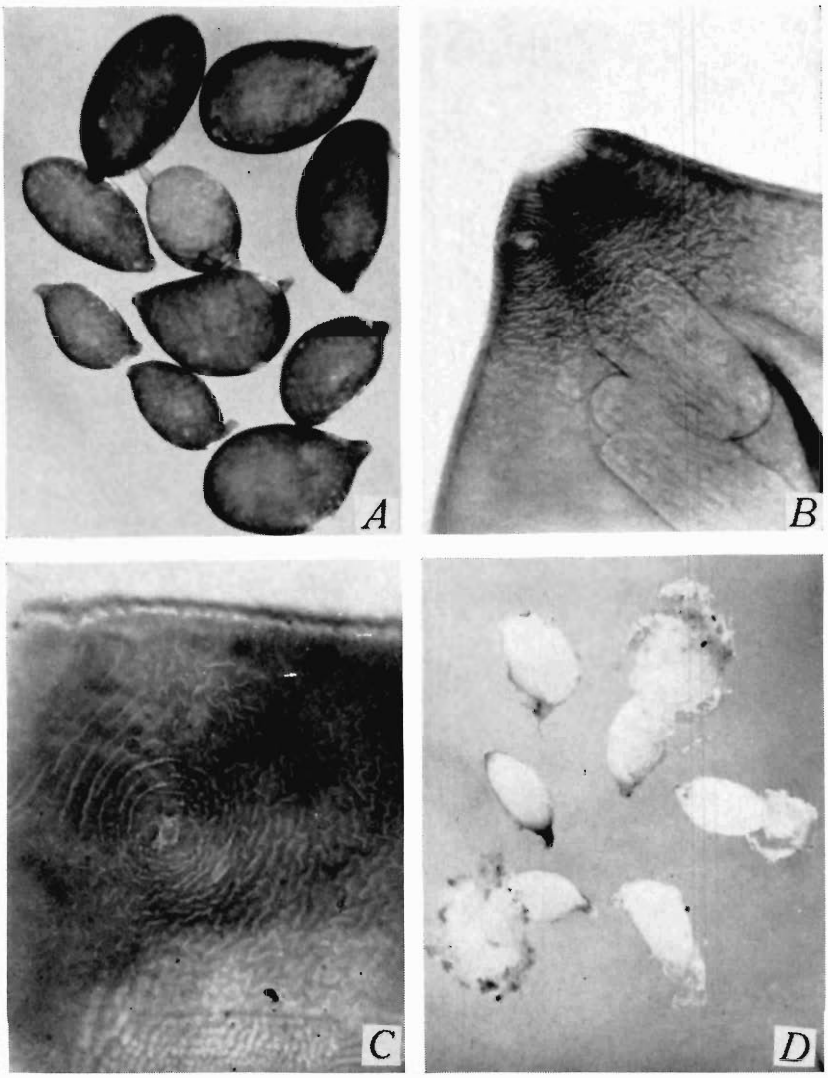

Figure 3. Photomicrographs at various magnifications of *H. cyperi*, n. sp. A. Whole cysts. B. Posterior portion of cyst. (Note conspicuous anal area). C. Enlarged view of anal area of cysts. D. White females. (Note large egg sac on some specimens).

In culture many females and cysts were found to have developed on the hard tubers. Also, although most adult females apparently ruptured through the root epidermis and could be seen on the surface, in many cases some females and especially cysts filled with eggs were noted to have developed completely under the epidermis of the root.

Gonad Development in *Pratylenchus crenatus* Loof and Observations on the Female Genital Structures of *P. penetrans**

OTTIE J. DICKERSON**

Pratylenchus crenatus Loof, 1960 is one of the monosexual species of the genus recognized as either parthenogenetic or hermaphroditic. To determine which method of reproduction occurred in *P. crenatus,* a study was made of the development of the reproductive system.

Zimmerman (1898) described and illustrated stages in the life cycle of the bisexual species *P. coffeae* (Zimmerman, 1898) Filipjev and Stekhoven, 1941. However, he did not delineate the development of the gonads. An illustration by Chambers (Cobb, 1917) of *P. scribneri* Steiner, 1943 gave the first morphological details of the genus. This illustration and those by Thorne (1949) and Sher and Allen (1953) were of adult nematodes only and no mention was made of the morphology of immature stages.

The structure of the posterior uterine branch has been a controversial question. Sher and Allen (1953) found that *P. vulnus* Allen and Jensen, 1950 had a long posterior uterine branch composed of a short extension of the uterus and several vestigial ovarian cells. Taylor and Jenkins (1957) observed similar structures in *P. subpenetrans* which they used as diagnostic characters to separate this species from *P. penetrans.* Loof (1960) found the posterior uterine branch of *P. coffeae* variable in length sometimes reaching 50 microns. Rudimentary posterior ovaries occurred in approximately 20% of the females examined and Loof inferred that these ovaries were more prevalent in older specimens. Species other than *P. vulnus* and *P. coffeae* have been described as having undifferentiated posterior uterine branches.

Maupas (1900) discussed reproduction and listed several protandrous hermaphroditic genera and species of which 4 genera were plant parasites. Cobb (1918) discussed hermaphroditism and observed functional sperm in the gonads of certain species in which males were either unknown or rare. In every case but one the females he examined were syngonic (sperm and ova produced in the same gonad). *Monochulus ventralis* Cobb, 1918 produced functional sperm in a small posterior gonad and Cobb termed this type of arrangement as digonic (sperm and ova produced in different gonads of the same individual). Perry (1959) described a structure which

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he called a spermagonium producing sperm in certain species of *Helicotylenchus* Steiner, 1945. This spermagonium was a differentiated cell of the oviduct. Yuksel (1960) illustrated and described gonad development of the bisexual nematode *Ditylenchus dipsaci* (Kűhn, 1857) Filipjev, 1936.

MATERIALS AND METHODS

Live as well as fixed specimens were utilized. Specimens relaxed with gentle heat were fixed in 5% formaldehyde (40% by volume) or FAA plus a trace of picric acid. Some specimens were permanently mounted in anhydrous glycerin. Occasionally mounts were made by transferring fixed specimens directly to a small drop of polyvinyl alcohol. When nematodes were mounted in this medium, only the sclerotized portions were visible.

RESULTS

Gonad development in *Pratylenchus crenatus:* Primordial germ cells were not observed in the first and second stage larvae. However, the entire intestinal region was filled with dense granules obscuring the first genital primordia in these stages.

Gonad development in the third stage larvae was at first amphidelphic (Fig. 1-A). Both branches developed in a similar manner briefly. Posterior development ceased before the third molt and not more than 9 cells were observed in this branch. The anterior genital branch continued to develop in a normal manner. During the latter part of the third stage the vagina became defined as a refractive line extending outward from the gonad to the hypodermis. Two distinct, finely granular cells were present on each side of the vagina (Fig. 1-B). The anterior branch of the reproductive system continued to develop and increase in length between the third and fourth molts. The development of the uterus, spermagonium, valvular cells, and ovary were not complete until after the fourth molt. The cells of the genital tract immediately posterior and anterior to the vagina probably coalesced to form the uterus; only 3 cells remained in the posterior uterine branch. Each of 5 cells anterior to the uterus divided twice, giving rise to 20 cells which became arranged in 4 alternate rows of 5 cells. These cells became slightly rectangular with age and correspond to the 16-celled quadricolumella of *Ditylenchus destructor* (Wu, 1958).

The spermagonium first appeared as a large, thick walled, doughnut-shaped cell of the oviduct. This cell developed into a spheroid organ composed of several nucleated cells. An oblong protrusion developed from the anterior end as a separate cell which connected the spermagonium to the lumen of the oviduct. As the genital system developed, the spermagonium was disassociated from the protruding cell and the cell then became a small spermatheca. The spermagonium then moved to a dorsal position above the valvular cells and remained attached to the spermatheca by a duct (Fig. 1-E).

Details of the nature of spermatogenesis were not determined. However, spermatozoa were observed in cells of the spermagonium, in the lumen connected to the spermatheca, and in the spermatheca.

The valvular section was composed of 5 sets of cells: 1) small muscular cells at the base of the spermagonial protrusion; 2) 4 relatively large crescent-shaped cells which surrounded the protruding cell and at maturity surrounded the spermatheca; 3) 2 cells lying 90° to the axis; 4) 4 cells which may or

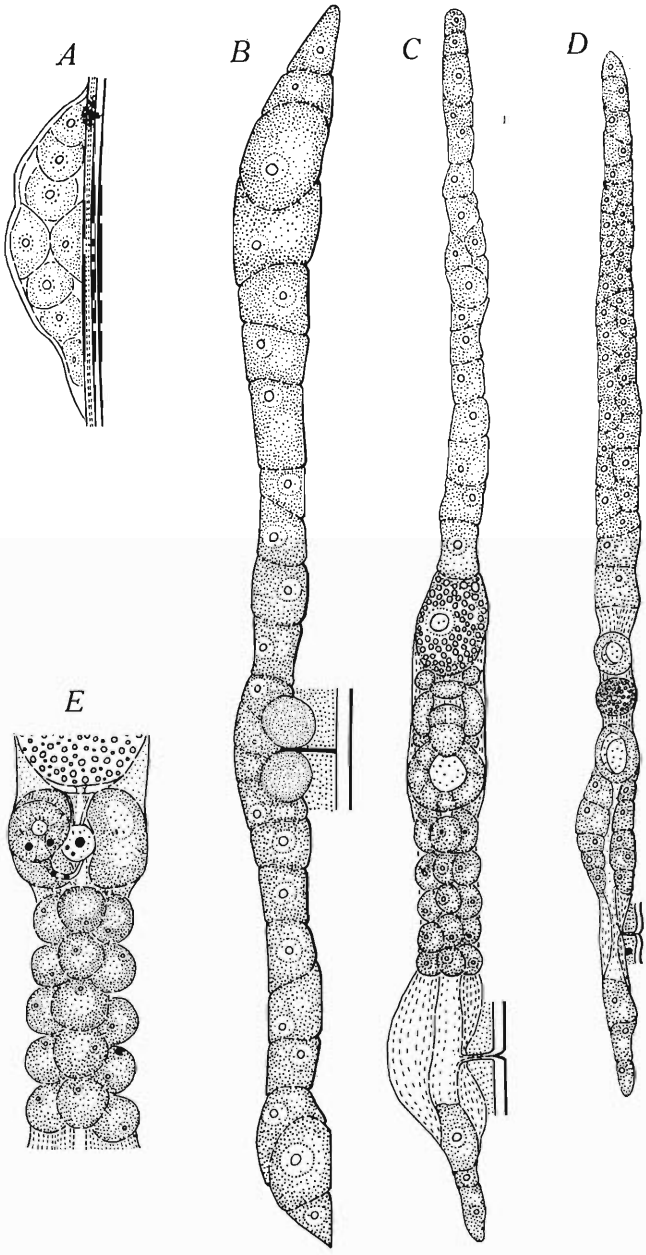

Fig. 1. A—Gonad of *P. crenatus* soon after second molt; X 1400. B—Gonad of *P. crenatus* just prior to third molt; X 1400. C—Gonad of *P. crenatus* after final molt; X 800. D—Gonad of *P. penetrans* after final molt; X 800. E—Portion of *P. crenatus* gonad including cells surrounding portion of oviduct, part of the valvular cells, spermagonium, sperm, and spermatheca; X 700.

may not be crescent-shaped; 5) small muscular cells at the junction of the oviduct and ovary (Fig. 1-C). When an oocyte began to move into the oviduct, the valvular cells enlarged to the point where they almost filled the reproductive tract.

The ovary consisted of a single row of oocytes except a region more often near the anterior end, 6-8 cells in length, where division occurred.

Observations on *Pratylenchus penetrans* female genital structures: Early gonad development in *P. penetrans* followed approximately the same pattern as it did in *P. crenatus*. A vestigial posterior ovary about 3 cells long was present in most specimens but sometimes became indefinite in older specimens.

The spermatheca began as a somewhat oval structure with a thick wall and remained as such until filled with spermatozoa. It then became a spheroid chamber as illustrated by Sher and Allen (1953). A dense structure just anterior to the spermatheca appeared as a group of cells or as a large cell with large translucent granules. This structure was probably a cement gland.

Anterior to the cement gland, another thick walled hollow cell appeared. It was morphologically similar to the spermatheca but smaller. Its function was not determined.

Maturing oocytes passed through all the structures described above (Fig. 1-D). The ovary developed a double row of oocytes except at each end where the oocytes were in a single row as described by Sher and Allen (1953).

SUMMARY

Pratylenchus crenatus, a monosexual nematode, was found to be a digonic hermaprhodite. Genital development was amphidelphic for a short period but only the anterior gonad developed into a functional organ. Early female genital development of *P. penetrans* was similar to that of *P. crenatus*.

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Studies on the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea), with descriptions of three new species

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The name *Longidorus* was first proposed as a sub-generic name by Micoletzky (1922) to differentiate the species, *Dorylaimus elongatus* de Man, 1876, from other dorylaims. In 1936, Thorne and Swanger gave it generic status. In his monograph on Dorylaimoidea, Thorne (1939) recognized four species under this genus and placed it in the sub-family Longidorinae Thorne, 1935. The genera *Xiphinema* Cobb, 1913, and *Longidorella* Thorne, 1939, were also included in it. A fourth genus, which was originally reported as *Taprobanus* by Loos (1949) and later (1950) re-named by him as *Xiphinemella*, was also added to this group.

Chitwood (1957) noted the similarities between *Xiphinemella* and *Longidorella* on the one hand and the genera of the Tylencholaiminae Filipjev, 1934, on the other and consequently synonymised the Longidorinae with the Tylencholaiminae. Clark (1961) also held a similar view when he proposed a re-classification of the order Enoplida. The present writer, however, agrees with the opinion expressed by Allen (1960) that in order to decide about the final positions of the nematodes of these groups it is safer and more appropriate to wait for further observations and findings. Very recently, Meyl (1960) gave Longidorinae a family rank and this view has been held by Thorne (1961) as well.

In 1959, the present author published comments on the validity of the genus *Longidorus*. He described two species of *Xiphinema* viz., *X. brevicaudatum* Schuurmans Stekhoven, 1951, and *X. citri* Siddiqi, 1959, which had many characters of *Longidorus* but which were placed in this genus mainly because of the presence of the basal swellings on the spear extension. Further studies on these and some other related species were conducted and the results are presented in the present paper. These include some useful comments on the important diagnostic characters, an amended diagnosis, a key to the various species of the genus and descriptions of three new species. On the basis of the present definition of the genus, *X. brevicaudatum* and *X. citri* belong in the genus *Longidorus* as has also been suggested by Thorne (1961).

Longidorus multipapillatus Schuurmans Stekhoven et Teunissen, 1938, is apparently a *Longidorella* species and therefore becomes *Longidorella multipapillata* (Schuurmans Stekhoven et Teunissen, 1938) n. comb. *Longidorus menthasolanus* Conicek and Jensen, 1961, is very similar to *L. elongatus* (de Man, 1876) Thorne and Swanger, 1936. The writer has studied some specimens of *L. menthasolanus* from mint soil, Talbot, Oregon and from bent turf soil, Adamsville, Rhode Island and also some specimens of *L. elongatus*, including de Man's slide numbered H-7, from the Netherlands. There are not enough differences to make it possible to separate these two species from each other and hence *L. menthasolanus* is considered as a synonym of *L. elongatus*.

Closest to *Longidorus* is the genus *Xiphinema* Cobb, 1913, from which it is mainly distinguished by the size and shape of the basal swellings of the

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spear extension and the structure and location of the spear guiding ring. The exceptionally large size of the amphids and their very fine, pore- or slit-like apertures in *Longidorus* also help in separating it from *Xiphinema*.

THE SPEAR AND ITS EXTENSION: In *Longidorus,* the spear or onchiostyle is cylindrical and much attenuated. Usually its proximal end does not carry short, pointed processes at the base as is usual in *Xiphinema*. In the larvae, in addition to the functioning spear, a developing spear is found in the left subventral portion of the anterior slender part of the oesophagus.

In the adults, the anterior slender portion of the oesophagus, sometimes, has a clear space containing a short, sclerotized structure resembling the spear tip, named 'mucro' by Andrássy (1960). In the light of the present study, the size and the location of the mucro appear to be fairly constant within a species and can well be used as a specific character. However, in many species the mucro may be indistinct and difficult to be located.

The spear extension is elongate and tripartite. Its base is usually swollen but the swellings are often slight and may easily be overlooked; these, however, are open posteriorly and never take the form of powerful, knobbed flanges as in *Xiphinema*. The outermost cuticularized membrane of the spear extension is shed along with the spear at each moult. Chitwood and Chitwood (1950) regarded the spear extension as a continuation and modification of the oesophageal tissue.

THE SPEAR GUIDING RING: The guiding ring of the spear in *Longidorus* is said to be single and located near the apex of the spear in contrast to the 'double' ring of *Xiphinema* which is usually located near the spear base. In the latter genus the spear guiding ring gives out anteriorly a fine, extensible, tubular sheath whose anterior end gives the appearance of a second ring in optical view; while in the former it does not form a sheath anteriorly and never appears to be double. The spear guiding ring in *Longidorus* is usually in the form of a broad, cup-shaped structure with the main ring forming its anterior edge (Fig. 1, D).

In *Longidorus,* the spear guiding ring is located around the anterior half of the spear, usually at two to four labial-widths from anterior end. The distance from the anterior end of the body to the spear guiding ring represents the length of the stoma and is fairly constant in a particular species and also in the larvae of a particular stage and hence can serve as a useful diagnostic character.

THE AMPHIDS: The amphids in *Longidorus* are characteristically enlarged into pouch-like structures almost occupying the whole width of the body at that level. Coiled, nervous, fibrillar terminals are seen in the amphidial pouches. The peripheral portion of the base of the amphid pouch sometimes extends backward thus giving it a bilobed appearance in lateral view. This serves as a useful character in species differentiation.

The amphid apertures are usually very fine, pore- or slit-like, situated at the base of the lateral lips. Each amphid is continued posteriorly with its corresponding amphidial duct, which, after running for a short distance, swells up to form a sensillar sac containing nervous sensillae. The amphidial ducts appear to be continued with the amphidial glands which are ampulla-like and usually lie much behind the nerve ring near the level of the middle of the anterior slender portion of the oesophagus.

THE GENUS *Longidorus* Micoletzky, 1922

DIAGNOSIS (amended): Longidorinae: Body elongate, over 2 mm. long;

slender (a = over 50). Body cuticle thick, smooth, marked with very fine transverse striations. Lateral body pores usually in two rows, leading into clear, pouch-like areas in hypodermal chords; in addition, a series of dorsal and ventral body pores are sometimes seen, especially on the extremities. Lip region smoothly rounded, continuous with or set off from the body, with six amalgamated lips bearing sixteen distinct papillae arranged in two circlets, six in inner and ten in outer. Amphids abnormally large, pouch-like, with very fine, pore- or slit-like apertures situated at the base of the lateral lips. Stoma tubular, without sclerotization, less than five cephalic-widths in length.

Buccal spear elongate, cylindrical, much attenuated, not bearing pointed processes at base for attachment with its extension. Spear extension elongate, with or without basal swellings. Basal swellings on spear extension, if present, never forming distinct knobbed flanges. Spear guiding ring single, usually appearing cup-shaped in optical view, not extending into a sheath anteriorly, located around the anterior half of the spear usually at 2 to 4 labial-widths from anterior end. Oesophageal bulb elongate-cylindroid, not distinctly set off from anterior slender portion of oesophagus, with inner walls forming an elongate, valvular apparatus. Nuclei of the dorsal and the anterior pair of the sub-ventral oesophageal glands usually distinct, located near the anterior end and the middle of the oesophageal bulb respectively. Cardia present.

Vulva transverse, near middle of body. Ovaries usually paired, reflexed. No sexual dimorphism. Males, when present, with paired heavily re-enforced spicules and supplements in the form of an ad-anal pair and a ventro-median series. Gubernaculum absent. Lateral guiding pieces for spicules present. Tails of both sexes similar, hemi-speroidal to elongate-conoid.

TYPE SPECIES: *Longidorus elongatus* (de Man, 1876) Thorne and Swanger, 1936.

Key to species of Longidorus (Based on females)

1. Ovary single ..... *monohystera* Altherr, 1953
   Ovaries paired 2
2. Lip region set off by a deep constriction 3
   Lip region not set off by a deep constriction 5
3. Body-length over 8 mm ..... *maximus* (Bütschli, 1874) Thorne and Swanger, 1936
   Body-length under 8 mm 4
4. Tail longer than anal-body-width ..... *citri* (Siddiqi, 1959) Thorne, 1961
   Tail shorter than anal-body-width ..... *georgiensis* Tulaganov, 1937
5. Spear guiding ring at 4 labial-widths from anterior end 6
   Spear guiding ring at less than 3 labial-widths from anterior end 8
6. Tail longer than anal-body-width ..... *brevicaudatus* (Schuurmans Stekhoven, 1951) Thorne, 1961
   Tail shorter than anal-body-width 7
7. Lip region expanded; mucro 5.5-8 microns long ..... *macromucronatus* n. sp.
   Lip region not expanded, mucro 2-2.5 microns long ..... *jonesi* n. sp.
8. Tail about one anal-body-width or longer 9
   Tail shorter than anal-body-width 17
9. Body-length about 3 mm or shorter 10
   Body-length about 4 mm or longer 12

10. Vulva at 60 per cent of body length ..... *pachtaicus* Tulaganov, 1938
    Vulva at less than 50 per cent of body length 11
11. Tail about 3 × anal-body-width long ..... *longicaudatus* n. sp.
    Tail less than 2 × anal-body-width long ..... *laevicapitatus* Williams, 1959
12. C = 36-37 ..... *striola* Merzheevskaya, 1951
    C = over 80 13
13. Spear 58-68 microns long ..... *leptocephalus* Hooper, 1961
    Spear over 75 microns long 14
14 Amphid base prolonged into distinct elongated lobes 15
    Amphid base not prolonged into distinct elongated lobes 16
15. Lip region expanded ..... *attenuatus* Hooper, 1961
    Lip region not expanded ..... *goodeyi* Hooper, 1961
16. Tail less than 1½ × anal-body-width, tail tip broadly rounded; males present ..... *elongatus* (de Man, 1876) Thorne and Swanger, 1936
    Tail more than 1½ × anal-body-width, tail tip narrowed; males absent ..... *sylphus* Thorne, 1939
17. Body-length 3.7-4 mm; lip region slightly set off ..... *nudus* Kirjanova, 1951
    Body-length over 4.5 mm; lip region not set off 18
18. Body-length 8-11 mm ..... *macrosomus* Hooper, 1961
    Body-length under 8 mm 19
19. Lip region cone-shaped; a = 60-82 ..... *caespiticola* Hooper, 1961
    Lip region not cone-shaped; a = 100-105 ..... *tardicauda* Merzheevskaya, 1951

*Longidorus jonesi** n. sp. (Fig. 1, A-J)

MEASUREMENTS: 20 females.—Length = 3.17-3.8 mm (3.43 mm); a = 61-75 (66); b' = 8-9.3 (8.6); c = 140-185 (167); V = 50-52.4% (50.8%); spear = 107-120 microns (113 microns); spear extension' = 66-73 microns (68.5 microns); total length of spear = 174-192 microns (182.7 microns); spear guiding ring = 57-66 microns (61.5 microns) from anterior end; mucro = 2-2.5 microns; tail' = 0.6-0.87 (0.75) times anal-body-width.

LARVAE: I group. 8 larvae.—Length = 1.12-1.45 mm (1.25 mm); a = 46-52 (49.2); b' = 4.4-5 (4.6); c = 39-47 (44); spear = 65-71 microns (68.2 microns); developing spear = 77-81 microns (78.4 microns); spear guiding ring' = 25-31 microns (29 microns) from anterior end; tail = 1.25-1.65 (1.5) times anal-body-width.

II group. 8 larvae.—Length = 1.6-1.9 mm (1.8 mm); a = 50-57 (53.6); b' = 5.2-6 (5.5); c = 68-80 (75); spear = 77-80 microns (78 microns); spear extension' = 51-58 microns (55 microns); developing spear = 90-96 microns (94 microns); spear guiding ring = 35-43 microns (39.8 microns) from anterior end; tail' = 0.8-1 (0.9) times anal-body-width.

III group. 6 larvae (fourth-stage larvae).—Length = 2.2-2.5 mm (2.36 mm); a = 54-64 (59); b' = 6.5-7.5) (7); c = 100-120 (109); spear = 91-97 microns (93.8 microns); spear extension = 59-64 microns (63 microns); developing spear = 110-118 microns (112.6 microns); spear guiding ring' = 46-49 microns (47.6 microns) from anterior end; tail = 0.65-0.8 (0.68) times anal-body-width.

FEMALE (Holotype): Length = 3.37 mm; a = 63; b' = 9; c = 168; V = $^{14.6}-50.8\%-^{17.3}$

Named after Mr. F. G. W. Jones, Head, Nematology Department, Rothamsted Experimental Station, Harpenden, England, to mark his visit to Aligarh (India) in October, 1960.

Body almost cylindrical, gradually tapering anteriorly from neck base to the smoothly rounded lip region which is approximately one-fourth of body-diameter in width, assuming an arcuate position when relaxed by gentle heating. Body cuticle plain, with very fine, uninterrupted transverse striations; uniformly thick throughout entire length of body except on caudal terminus where it is thicker and radially striated; appearing double layered in optical view. Lateral hypodermal chords appearing as broad bands, about

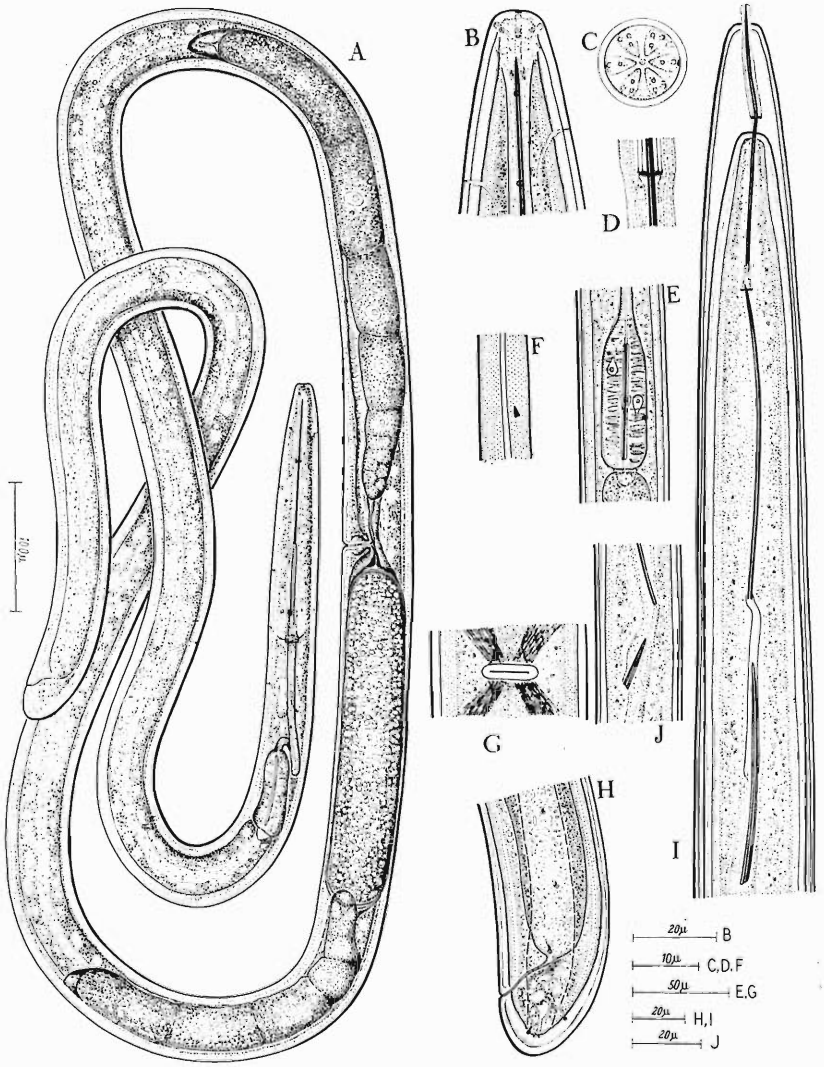

Figure 1. *Longidorus jonesi.* A. Female. B. Head end of female, lateral view. C. *En face* view. D. Spear guiding ring. E. Oesophageal bulb, lateral view. F. Mucro in oesophageal wall. G. Vulva, ventral view. H. Tail end of female. I. & J. Spear-case-like structure in oesophageal tissue of moulting larvae.

one-fourth as wide as body diameter, presenting a reticulate pattern in surface view; in some paratypes, these chords appearing to have two rows of cells enclosing inter-cellular spaces which appear as clear, pouch-like areas into which lead the lateral body pores. Each chord begins a little behind lip region in the form of a narrow streak which gradually broadens to occupy a uniform width posterior to the cervical region. Lateral body pores at first arranged in a single file, beginning about 20 microns behind lip region and extending up to neck base, then irregularly arranged over entire body-length. A single dorsal body pore seen a little behind lip region; ventral body pores seen on anteriormost portion of body, arranged as illustrated (Fig. 1, A). Two ventral body pores near tail end were also seen in one of the paratype females.

Lip region continuous with body contour, not set off in any manner. An *en face* view showing six narrow, perfectly amalgamated lips bordering a depressed oral opening, bearing 16 labial papillae arranged in two circlets as in figure 1, C. Amphids broad, with thick walls, occupying about 80 per cent of body width, opening through small, pore-like apertures at base of lateral lips; amphidial glands extending up to middle of anterior slender part of oesophagus. Stoma a cylindrical tube, without sclerotization, ending posteriorly in a spear guiding ring; its thick wall forming a cylindrical guiding tube for the spear.

Spear or onchiostyle attenuated, 118 microns in length, with a smooth, bluntly rounded proximal end. Spear extension 66 microns long, with a slightly swollen base; swellings not rounded posteriorly, not set off from oesophageal contour. Spear guiding ring single; appearing cup-shaped in optical section, with the main ring forming its anterior edge; not extending into a sheath anteriorly; located at 62 microns or approximately 4.7 labial-body-widths from anterior end.

Oesophagus with an anterior slender portion, 165 microns long by 8 microns wide, and a posterior cylindrical bulb measuring 68 microns long by 21 microns wide. Nerve ring encircling anterior part of oesophagus at about half body-width behind spear extension, giving out a pair of nerves to the hypodermis from the dorsolateral aspect of its posterior margin in a transverse direction. 'Mucro' in the form of a triangular, cuticularized piece resembling spear tip, 2 microns long, located in the left sub-ventral sector of anterior portion of oesophagus slightly posterior to its middle. Oesophageal bulb containing three oesophageal glands; a dorsal gland located near its anterior end and opening into its lumen anteriad, and two sub-ventral glands located near its middle with their ducts continued posteriad (Fig. 1, E). Inner walls of oesophageal bulb sclerotized, forming an elongated, valvular apparatus. Cardia well developed, conoid-rounded. Intestine with pronounced lumen; its cells containing small, refractive granules.

Vulva a transverse slit with thick labia, about one-fourth of vulvar-body-width long, leading at right angles to ventral body surface into vagina which extends about half-way into body. Powerful dilator muscles attached to the vulva and walls of vagina vera (Fig. 1, G); vaginal walls supplied with strong, circular muscles forming a globular mass around it. Uteri with thick, muscular walls, connected with long, tubular oviducts through a poorly developed sphincter valve (sphincter Z of Luc, 1961). Ovaries paired, roughly symmetrical, with oöcytes arranged in a single file except in multiplication zone near cap-cell. A single egg in posterior uterus, 275 microns long by 48

microns broad. Both sets of reproductive organs lying on left side of intestine.

Pre-rectum distinctly marked off from intestine by an inner valvular thickening and reduction in diameter, about 11 per cent of total body-length. Rectum about one anal-body-width long, opening through a distinct anus. Tail conoid, obtusely-rounded, 0.64 times anal-body-width in length, with two pairs of caudal pores.

MALE: Not found.

LARVAE: Body similar to that of female. Lip region not set off from body contour in any manner. Anterior slender portion of oesophagus always with a developing spear. On the basis of the length of the body and the size of the functioning and developing spears, the present specimens can easily be divided into three groups. Tail in the larvae of first group is elongate-conoid with rounded terminus, while in the larvae of third group (fourth-stage larvae) it is much like that of female. In three different moulting larvae, a sclerotized spear-case-like structure was found with its anterior end thrust into the spear extension when the developing spear had almost replaced the functional one (Fig. 1, I, J.). In all of these larvae this structure was of different size but was always with a greater width and lumen than those of the spear. During each moult body cuticle including stoma wall, spear guiding ring and rectal lining, spear, and outlines of spear extension are shed off and replaced.

HOLOTYPE: Female specimen isolated from soil sample collected in January, 1959, tube no. PN/D/3-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

PARATYPES: 19 females and many larvae, other data same as for holotype.

TYPE HOST: Collected from soil around roots of apricot, *Prunus armeniaca* L.

TYPE LOCALITY: Simla (Elevation 7,100 feet), Punjab State, North India.

DIAGNOSIS AND RELATIONSHIP: *Longidorus* with the above measurements and general description. It is distinguished by the following characters: Body robust, a = 61-75; lip region continuous with body contour; buccal spear 107-120 microns long; spear guiding ring at 4-5 labial-widths from anterior end; mucro triangular, 2-2.5 microns in length; vulva at about 50-53 per cent of body length from anterior end; tail conoid, obtusely-rounded, 0.6-0.87 times anal-body-width, bearing two pairs of caudal pores.

Longidorus jonesi n. sp. is closest to *L. macromucronatus* n. sp., from which it differs in having a smaller body-size, a less slender body, a continuous lip region which is not set off in any manner, a triangular mucro, and a more posteriorly located vulva. From all the other known species of the genus, *L. jonesi* can be distinguished by having a far posteriorly located spear guiding ring, except from *L. brevicaudatus* (Schuurmans Stekhoven, 1951), Thorne, 1961, from which it can be separated by having a continuous lip region (expanded, knob-like in *L. brevicaudatus*) and a shorter tail.

Longidorus macromucronatus n. sp. (Fig. 2, A-K)

MEASUREMENTS: 25 females: Length = 4-4.9 mm (4.34 mm); a = 94-105 (98); b = 7.6-10.5 (9.2); c = 190-230 (204); V = 43-47.8% (46.3%); spear = 117-128 microns (121 microns); spear extension = 67-77 microns (71.6 microns); total length of spear = 185-200 microns (192.4 microns); spear guiding ring = 58-68 microns (62.5 microns) from anterior end; mucro = 5.5-8 microns (6.3 microns); tail = 0.63-0.8 (0.72) times anal-body-width.

LARVAE: 5 I-stage larvae: Length = 1.15-1.3 mm (1.2 mm); a = 53-59 (55.6); b = 5-5.3 (5.1); c = 35-39 (37); spear = 59-61 microns (60 microns); spear extension = 47-53 microns (49 microns); developing spear = 71-72 microns (71.8 microns); spear guiding ring = 28-32 microns (29.6 microns) from anterior end; tail = 1.9-2.1 (2) times anal-body-width.

4 II-stage larvae: Length = 1.6-1.8 mm (1.7 mm); a = 60-65 (61.5); b = 5.6-6.2 (5.8); c = 56-60 (58); spear = 72-73 microns (72.7 microns); spear extension = 54-56 microns (55 microns); developing spear = 87-90 microns (88 microns); spear guiding ring = 37-40 microns (38 microns) from anterior end; tail = 0.9-1 (1) times anal-body-width.

5 III-stage larvae: Length = 2.16-2.5 mm (2.36 mm); a = 60-74 (67); b = 6.4-7.3 (6.6); c = 80-91 (85); spear = 85-94 microns (89 microns); spear extension = 57-71 microns (62.7 microns); developing spear = 100-106 microns (103.6 microns); spear guiding ring = 43-47 microns (45.4 microns) from anterior end; tail = 0.99-1.1 (1) times anal-body-width.

6 IV-stage larvae: Length = 3-3.3 mm (3.15 mm); a = 82-90 (85.5); b = 8-9 (8.45); c = 130-137 (134); spear = 97-107 microns (102 microns); spear extension = 63-68 microns (65.4 microns); developing spear = 114-120 microns (117.6 microns); spear guiding ring = 50-55 microns (53 microns) from anterior end; tail = 0.79-0.86 (0.83) times anal-body-width.

FEMALE (Holotype): Length = 4.6 mm; a = 100; b = 10.5; c = 220; V = ^{5}46.5%$^{-5}$.

Body much attenuated, almost cylindrical, with gradual tapering on both extremities, more so on anterior end; assuming a spiral 'C' form on death. Cuticle appearing in two layers, marked with very fine, continuous, transverse striations. Lateral hypodermal chords about one-fourth as wide as body on mid-body. Lateral body pores not in definite lines, leading into clear, pouch-like areas of hypodermis. Three dorsal body pores seen on the anterior end; ventral body pores traceable to a greater distance on neck region. Amphids as described for *L. jonesi;* coiled nerve terminals in amphidial pouches distinct.

Lip region slightly swollen, semi-knob-like, anteriorly flattened, giving the anterior end a rather truncated appearance, about one-third as wide as maximum-body-width. An *en face* view showing six indistinct lips with 16 labial papillae arranged in two circlets; the papillae of the outer circlet lie in two different levels (Fig. 2, C). Spear 120 microns long, with simple, smooth base, not bearing any pointed projections on the point of its attachment with its extension. Spear extension 73 microns long, with proximal end slightly swollen; swellings about one-sixth as wide as corresponding body diameter, not rounded posteriorly. Spear guiding ring single, cup-shaped, at 68 microns or 4 cephalic diameters from anterior end.

Anterior slender part of oesophagus 133 microns long by 6.5 microns wide, enveloped by nerve ring at its anterior end just behind spear extension. A pair of stout dorso-lateral nerves given out from nerve ring to hypodermis anteriorly in an oblique direction. These nerves help in easily locating the nerve ring which is sometime difficult to distinguish from the surrounding tissues. A pair of sub-ventral nerves also given out from nerve ring to hypodermis (Fig. 2, G). 'Mucro' resembling spear tip, elongate, 6 microns long, lodged in the left sub-ventral sector of anterior part of oesophagus, at 38 per cent of its length. Oesophageal bulb 76 microns long by 19 microns wide; oesophageal glands as described for *L. jonesi.* Cardia distinct, conoid-rounded.

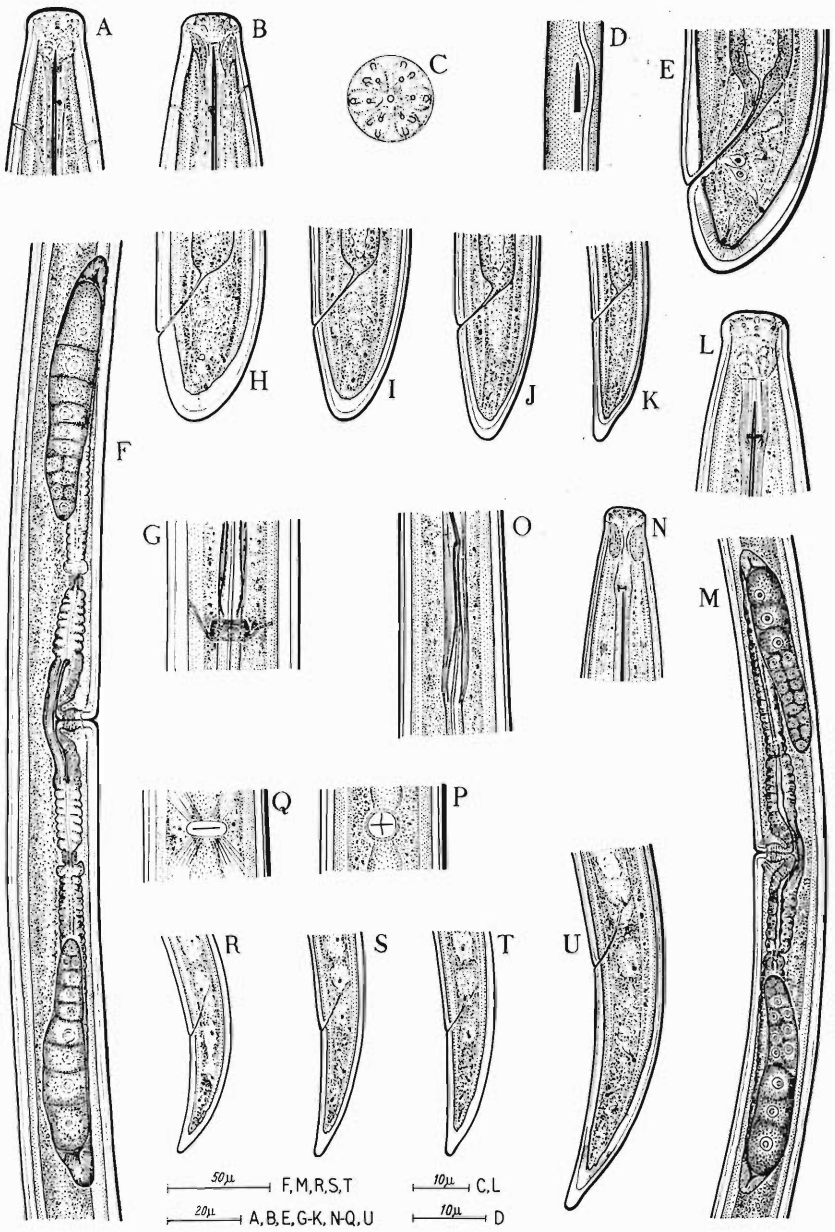

Figure 2. A-K—*Longidorus macromucronatus*. A. Head end of female, lateral view. B. Head end of female, ventral view. C. *En face* view. D. Mucro in oesophageal wall. E. Female tail. F. Reproductive organs. G. Base of spear extension and nerve ring. H-K. Larval tails; H, IV-stage; I, III-stage; J, II-stage; K, I-stage. L-U—*Longidorus longicaudatus*. L. Head end of female, lateral view. M. Reproductive organs. N. Head end of female, ventral view. O. Spear extension. P. Vagina, ventral view. Q. Vulva, ventral view. R-U. Female tails.

Vulva a transverse slit, 11 microns in length. Vagina and its musculature as in *L. jonesi*. Uteri thick-walled, highly extensile. Each oviduct elongated, joined to the uterus through a muscular sphincter Z. Ovaries paired, symmetrically reflexed.

Pre-rectum 13.6 per cent of total body-length (10.4-16 per cent in paratypes). Rectum slightly longer than anal-body-width. Hypodermal chords bulging out at level of anus, then narrowing down to end a little before caudal terminus. Two pairs of caudal pores displaced on tail as in figure 2, E. Tail bluntly rounded, slightly tapering, dorsally convex, 0.7 times anal-body-width; caudal terminus with thick, radially striated cuticle.

MALE: Not found.

LARVAE: Four distinct groups of larvae, perhaps the larval stages of the worm, are easily recognized. From larvae of I-stage to those of IV-stage, various values of body measurements show an ascending grade. All the larvae have a swollen and slightly set off lip region. Tails of larvae of various groups vary in length and shape (Fig. 2, H-K).

HOLOTYPE: Female isolated from soil sample collected in February, 1961; tube no. PN/D/3-002, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

PARATYPES: 20 females and many larvae; other data same as for holotype.

TYPE HOST: Collected from soil around roots of apple tree, *Malus sylvestris* (L.) Mill.

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

DIAGNOSIS AND RELATIONSHIP: *Longidorus* with the above measurements and general description. Distinctive because of a much slender body, averaging 4.34 mm in length; a slightly set off, semi-knob-like region; buccal spear measuring 117-128 microns in length; spear guiding ring located at 4 lip-region-widths from anterior end; mucro elongate, measuring 5.5-8 microns long, located anteriorly to middle of anterior slender portion of oesophagus; vulva pre-equatorial, at 43-48 per cent of body length; a conoid-rounded tail, averaging 0.72 times anal-body-width long, bearing two pairs of caudal pores.

Longidorus macromucronatus n. sp. is very similar to *L. jonesi* n. sp. in many structural details, but can easily be differentiated by having a longer and more slender body, a slightly set off, semi-knob-like head, an elongate mucro, a more anteriorly located vulva (post-equatorial in *L. jonesi*) and in the shape of the tail. Furthermore, its larvae can easily be distinguished from those of *L. jonesi* by having a longer body (the IV-stage larvae of *L. jonesi* are about the length of the III-stage larvae of *L. macromucronatus* and the IV-stage larvae of *L. macromucronatus* are about the length of the females of *L. jonesi*), and by slightly swollen and set off lip region.

It is also related to *L. nudus* Kirjanova, 1951, and *L. tardicauda* Merzheevskaya, 1951. From the former it can be distinguished by having a more slender body (a = 56-83 in *L. nudus*), a shorter tail (c = 94.3-111.6 in *L. nudus*), and a far posteriorly located spear guiding ring; from the latter by having slightly set-off, semi-knob-like lip region (continuous in *L. tardicauda*), a shorter tail (c = 154.8-160.7 in *L. tardicauda*), and a more posteriorly located spear guiding ring.

Longidorus longicaudatus n. sp. (Fig. 2, L-U)

MEASUREMENTS: 5 females: Length = 2.25-3 mm (2.64 mm); a (slightly pressed) = 73-80 (77); b = 7-8.2 (7.7); c = 40-50 (44.6); V = 44-47.6%

(46.5%); spear = 92-100 microns (95 microns); spear extension = 46-51 microns (49 microns); spear guiding ring = 21-24 microns (22.5 microns) from anterior end; tail = 2.8-3.2 (3) times anal-body-width.

FEMALE (Holotype): Length = 2.75 mm; a = 78.5; b = 7.8; c = 41; V = $^{5.4\text{-}47}\%^{\text{-}6}$.

Body cylindrical, with gradual taperings on either extremities, in the form of an open spiral in which posterior half has a greater curvature. Cuticle with fine, faint transverse striations. Lateral hypodermal chords one-third as wide as body-width, with large, pouch-like clear areas into which lead the lateral body pores. First lateral body pore from anterior end near level of spear guiding ring. Dorsal and ventral series of body pores not located.

Lip region swollen, smoothly rounded, knob-like, one-third as wide as maximum body-width. Lips with usual number of distinct papillae. Amphids large, with thick walls enclosing a narrow lumen, with coiled nerve terminals, occupying about 90 per cent of body-width, opening through pore-like apertures at base of lateral lips. Amphidial tube continued posteriad to form poorly developed sensillar pouch at 3½ labial-widths from anterior end; amphidial glands reaching up to level of middle of anterior slender portion of oesophagus.

Spear elongate, 95 microns long, with a smooth, undifferentiated proximal end. Spear extension 47 microns in length, gradually expanding in its posterior half to have basal swellings, 4.5 microns thick at their widest, not rounded posteriorly or set off from oesophageal contour. Spear guiding ring cup-shaped, located at 23 microns or about two cephalic-widths from anterior end. Anterior slender portion of oesophagus a cylindrical tube, 125 microns long by 8 microns wide, crossed by nerve ring just behind base of spear extension. Basal oesophageal bulb cylindroid, 68 microns long by 15.5 microns in diameter, with one dorsal and two sub-ventral glands. Cardia small, conoid.

Vulva a transverse slit-like aperture, located slightly anterior to middle of body, leading into a thick-walled vagina extending about 2/5 of body-width; musculature of vulva and vagina as described for *L. jonesi*. Uteri thick-walled, with distal portion much folded, each joined with its oviduct through a small-sized sphincter Z immediately behind which the oviduct is swollen into a spermatheca-like pouch, devoid of any spermatozoa. Each oviduct elongate, about the length of the ovary. Ovaries paired, almost symmetrically reflexed.

Pre-rectum not differentiated. Rectum not as distinctly marked as in preceding species, opening outside through anus which is sometimes difficult to locate. Tail elongate-conoid, slightly ventrally arcuate, 3.2 times anal-body-width long, with three pairs of caudal pores of which the first pair from anterior end is ad-anal in position while others as illustrated (Fig. 2, U).

MALE: Not found.

HOLOTYPE: Female collected by Dr. Q. L. Holdeman; tube no. PN/D/3-003, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U.P.), India.

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

HOST: Not known.

LOCALITY: Edmunds, South Carolina, U. S. A.

DIAGNOSIS AND RELATIONSHIP: *Longidorus* with the above description and measurements. It can easily be recognized by its small body-size (2.25-3) mm long), a swollen, knob-like lip region, 92-100 microns long spear, shape and size of spear extension, spear guiding ring located at about 2 cephalic-

widths from anterior end, vulva at 44-48 per cent of body, and an elongate-conoid tail.

Longidorus longicaudatus n. sp. resembles *L. pachtaicus* Tulaganov, 1938, and *L. brevicaudatus* (Schuurmans Stekhoven, 1951) n. comb. From the former it can be differentiated by its longer and more slender body (body 1.61 mm long in *L. pachtaicus*) and more anteriorly located vulva (vulva at 60 per cent in *L. pachtaicus*), and from the latter in having a longer buccal spear and a more elongate-conoid tail (c = 87.5-107.4 in *L. brevicaudatus*).

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Paratylenchidae n. fam. with Descriptions of Five New Species of *Gracilacus* n. g. and an Emendation of *Cacopaurus* Thorne, 1943, *Paratylenchus* Micoletzky, 1922 and Criconematidae Thorne, 1943

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The Paratylenchinae is comprised of two genera, *Cacopaurus* Thorne, 1943 and *Paratylenchus* Micoletzky, 1922. In recent years a number of species of *Paratylenchus* have been described with an elongated stylet 48 microns or longer. A comparison of type specimens and new collections of many nominal species with specimens representing new species has been made possible from material from the University of California Nematode Survey Collection at Davis.

After reviewing the characters used to distinguish these genera and species it was apparent that the short blunt tail and ornate cuticular markings of *C. pestis* are unique among all the Paratylenchinae. Since this is the type species of the genus and the new species described below are closely related to *C. epacris*, the logical change is to place *C. epacris* in a new genus with the new species and certain species now assigned to *Paratylenchus*.

The relationships of the two subfamilies of the Criconematidae were considered. The relation between these two groups is found in the swollen and amalgamated pro- and metacorpus, the degenerate males, the stylet with a disproportionately long prorhabdion, and the single prodelphic ovary. However, it is also clear that several basic differences exist between these two groups. In Paratylenchinae the isthmus is slender and quite distinct, the annulation fine, stylet slender and caudal alae very reduced or lacking. On the other hand in Criconematinae the isthmus is short and broad, very reduced or almost lacking, the female stylet very robust, and annulation is very strong, often retrorse, sometimes with scales or spines. In three genera of Criconematinae the adult female bears a fifth cuticle. Finally the caudal alae of males are most strongly developed. These differences are judged distinct enough to warrant a separate family status for the Paratylenchinae and it is hereby proposed.

PARATYLENCHIDAE n. fam.

DIAGNOSIS: Tylenchoidea. Procorpus and metacorpus swollen and amalgamated into a large valvated bulb; isthmus slender and distinct. Stylet of females well developed, prorhabdion much longer than meso-metarhabdions; male degenerate, stylet reduced or lacking. Cuticle finely annulated. Vulva near posterior end. Ovary single. Postuterine branch usually lacking, rarely present, very reduced. Caudal alae lacking or at most slight evaginations of the cuticle.

TYPE GENUS: *Paratylenchus* Micoletzky, 1922
OTHER GENERA: *Cacopaurus* Thorne, 1943
Gracilacus n. g.

KEY TO THE GENERA OF PARATYLENCHIDAE

1. Female obese with cuticular ornamentations, with short very blunt tail, excretory pore in region of valve of metacorpus or further anterior ..... *Cacopaurus*

Female slender to obese, without cuticular ornamentations, tail short and conical to elongate—slender, excretory pore variable from bulbar region to metacorpus 2

2. Female with short stylet (36 microns or less), young larvae with small, delicate stylet, non-feeding preadult with stylet degenerate or lacking, excretory pore always near nerve ring or posterior ..... *Paratylenchus*

Female with elongate stylet (48-119 microns), young larvae with strong well-developed stylet, preadult with strong stylet (*G. peraticus* excepted), excretory pore generally near metacorpus or further anterior but near nerve ring in some species ..... *Gracilacus*

KEY TO THE SPECIES OF *Gracilacus*

1. Lateral field 2 or 3 lines 2
   Lateral field 4 lines 5
2. Lateral field 2 lines, stylet 110-119 microns ..... *elegans* n. sp.
   Lateral field 3 lines, stylet 54-88 microns 3
3. Female stylet 87-88 microns, vulvar flap small ..... *idalimus* n. sp.
   Female stylet <69 microns, vulvar flap lacking 4
4. Female stylet 54-62 microns, tail broad ..... *aculentus* (Brown)
   Female stylet 61-69 microns, tail slender ..... *aciculus* (Brown)
5. Spermatheca absent, males unknown 6
   Spermatheca present 7
6. Stylet ave. = 89 microns (83-92 microns); swollen to obese females common ..... *intermedius* n. sp.
   Stylet ave. = 77 microns (70-85 microns); only slender females known ..... *mirus* n. sp.
7. Female stylet 85-119 microns 8
   Female stylet 48-71 microns 10
8. Lips set off ..... *peraticus* n. sp.
   Lips rounded 9
9. Tail elongate, conoid; males unknown ..... *anceps* (Cobb)
   Tail short, blunt; males common ..... *epacris* (Allen & Jensen)
10. Female ave.L. = .48 mm., male with stylet ..... *goodeyi* (Oostenbrink)
    Female ave.L. = .39 mm., male unknown or without stylet 11
11. Tail claw-like; female ave.L. = .34 mm. ..... *audriellus* (Brown)
    Tail not claw-like, conical with rounded tip 12
12. Female stylet ave. =53 microns (54-60 microns); male without stylet ..... *sarissus* (Tarjan)
    Female stylet ave. = 67 microns (63-71 microns); male unknown 13
13. Female ave.L. = .28 mm. (.24.-.31 mm.); excretory pore near base of stylet ..... *steineri* (Golden)
    Female ave.L. = .39 mm. (.34-.42 mm.); excretory pore near nerve ring ..... *marylandicus* (Jenkins)

GENUS *Gracilacus* n. g.

DIAGNOSIS: Paratylenchidae. Small species less than 0.50 mm. Most larvae with elongate stylet. Female slender to obese with a stylet 48-119 microns in length. Body posterior to vulva elongate. Cuticle finely annulated, without ornamentation. Excretory pore generally in region of metacorpus near the valve or further anterior but may be near nerve ring. Male slender,

active, stylet absent or much reduced. Caudal alae represented by thickened, cuticular evaginations. Ovary single. Testis one.

TYPE SPECIES: *Gracilacus epacris* (Allen & Jensen, 1950) n. comb.

Syn.: *Cacopaurus epacris* Allen & Jensen, 1950

OTHER SPECIES INCLUDE:

Gracilacus aciculus (Brown, 1959) n. comb.
syn. *Paratylenchus aciculus* Brown, 1959
Gracilacus aculentus (Brown, 1959) n. comb.
syn. *Paratylenchus aculentus* Brown, 1959
Gracilacus anceps (Cobb, 1923) n. comb.
syn. *Paratylenchus anceps* Cobb, 1923
Gracilacus audriellus (Brown, 1959) n. comb.
syn. *Paratylenchus audriellus* Brown, 1959
Gracilacus goodeyi (Oostenbrink, 1953) n. comb.
syn. *Paratylenchus goodeyi* Oostenbrink, 1953
Gracilacus marylandicus (Jenkins, 1960) n. comb.
syn. *Paratylenchus marylandicus* Jenkins, 1960
Gracilacus sarissus (Tarjan, 1960) n. comb.
syn. *Paratylenchus sarissus* Tarjan, 1960
Gracilacus steineri (Golden, 1961) n. comb.
syn. *Paratylenchus steineri* Golden, 1961

Gracilacus is derived from the Latin *gracilis,* slender, and *acus,* pin.

The genus *Gracilacus* differs from *Cacopaurus* in the absence of cuticular ornamentation and in the elongate, slender body shape posterior to the vulva. It differs from *Paratylenchus* in the well-developed stylet of the younger larval stages and the elongate stylet of the female (48-119 microns in *Gracilacus*, <36 microns in *Paratylenchus*). In *Gracilacus* the excretory pore is generally near the metacorpus or further anterior (exceptions being *G. marylandicus, G. audriellus, G. sarissus, G. goodeyi* in which the pore is located near the nerve ring).

Gracilacus epacris (Allen & Jensen, 1950)

LARVA (SECOND-STAGE?): (7) 0.25 mm. (.23-.28 mm); a = 22.2 (20.0-24.1); b = 3.2 (2.6-4.1); c = ?; stylet = 43 microns (39-47 microns).

Slender larva with rounded head. Stylet guide prominent. Esophagus well developed. Excretory pore at level of posterior end of valve in median bulb. Tail curves slightly tapering to a rounded tip.

LARVA (THIRD-STAGE?): (7) 0.29 mm. (.25-.32 mm.); a = 18.0 (15.2-20.2); b = 2.8 (2.4-3.7); c = ?; stylet = 63 microns (60-70 microns).

Similar to larva (second stage) with much stronger, longer stylet. Esophagus well developed. Excreory pore at level of anterior end of valve in median bulb. Tail slightly more conoid and narrower at rounded tip.

LARVA (FOURTH-STAGE FEMALE?): (2) 0.28 mm. (.27-.29 mm.); a = 15.0 (13.5-16.5); b = 2.9; c = ?; stylet-lacking.

Robust larva inside cuticle of previous stage with molted prorhabdion 53 and 55 microns respectively. Body tapers anteriorly and posteriorly to conoid tail. Head region similar to adult female. Esophagus well developed, elongate. Stylet rudimentary, located midway in procorpus. Prorhabdion short; basal knobs indistinct.

LARVA (FOURTH-STAGE MALE?): (1) 0.29 mm.; a = 17.6; b = 3.9; c = ?; stylet-lacking.

Head smoothly rounded. Esophagus very short and degenerate. Testis and developing spicules observed. Tail conical with rounded tip.

FEMALE: (5) 0.31 mm. (.25-.35 mm.); a = 14.5 (12.4-15.6); b = 2.3. (1.8-2.6); c = 14.1; V = 84% (82-86%); stylet = 111 microns (99-119 microns).

In the original description of *Gracilacus epacris* the excretory pore was shown anterior to the metacorpus in the young female and near the posterior bulb in the senile, obese female. This latter may be the result of shortening of the esophagus when the stylet is extruded during feeding.

The degree of thickening of the cuticle in *G. epacris* is quite variable even in the type material. Other collections show similar variations and some populations had virtually no special thickening of the cuticle. It is not known whether this variation reflects an influence of different host plants, time of year or age of specimens but in all other respects the populations are similar.

LECTOTYPE: Female. 0.28 mm.; a = 8.8; b = 2.4; c = ?; V = 82%; stylet =105 microns.

The lectotype was selected from original material collected from roots of California black walnut, *Juglans hindsii* Jepson, near Visalia, Tulare County, California in April, 1949 and used by Allen and Jensen in the description of this species. Catalogue number 207 University of California, Nematode Survey Collection, Davis.

OTHER HOSTS: Oak, *Quercus* sp., 2 mi. north of Woodland, Yolo County; California laurel, *Umbellularia californica* Nutt., bank of Russian River, 5 miles east of Jenner, Sonoma County, and Berkeley, Alameda County.

Gracilacus anceps (Cobb, 1923) (Plate II C, D, Plate VI, C)

LARVA (SECOND-STAGE?): (8) 0.21 mm. (.21-.23 mm.); a = 22.2 (19.0-26.1); b = 2.6 (2.4-3.1); c = ?; stylet = 42 microns (39-43 microns).

Slender larvae with rounded head. Stylet stout, esophagus well developed and strong median bulb with valve. Tail tapering to bluntly rounded tip.

LARVA[2] (THIRD-STAGE?): (5) 0.27 mm. (.26-.28 mm.); a = 21.4 (19.1-22.5); b = 3.0 (2.5-3.5); c = ?; stylet = 52 microns (50-54 microns).

Body stout, rounded head. Esophagus and stylet strongly developed. Tail more bluntly conical with rounded terminus.

FEMALE: (7) 0.31 mm. (.28-.34 mm.); a = 21.6 (19.1-23.3); b = 2.2 (2.1-2.3); c = 13.8; V = 82% (78-86%); stylet = 97 microns (91-103 microns).

NEOTYPE. Female. 0.34 mm.; a = 23.2; b = 2.2; c = 15.0; V = 81%; stylet = 102 microns.

Slender female, annulation fine becoming obscure on rounded head region. Sclerotization delicate, stylet guide prominent. Excretory pore anterior to knobs of stylet, about 92 microns from anterior end. Stylet knobs very strongly developed. Lateral field marked by 4 lines, the outer two slightly more prominent than the inner two. Vulvar lips protrude slightly. Vulvar flaps moderately developed. Vagina sclerotized, prominent. Uterus contains what appear to be minute spermatozoa possibly produced by specialized cells at anterior end of uterus. Tail slender conoid, taper to fine, almost pointed terminus.

DIAGNOSIS (EMENDED): *Gracilacus anceps* most closely resembles *G. intermedius* and *G. mirus* but differs in the presence of minute spermatozoa produced apparently by a modified cell at the anterior end of the uterus. The

longer stylet of *anceps* (91-103 microns) further differentiates it from *mirus* (70-85 microns). This is also reflected in a difference in esophageal length. It is differentiated also by its shorter length (ave. .31 mm.) from *G. intermedius* (ave. .38 mm.). The esophageal length further distinguishes these two species.

NEOTYPE: Female collected January 6, 1959 by the author from soil about the roots of California laurel, *Umbellularia californica* Nutt., in White Park, Riverside, California. Catalogue number 208 University of California, Nematode Survey Collection, Davis.

OTHER HOSTS: *Platanus racemosa,* Santa Barbara, California.

California laurel is not known to grow in non-cultivated areas in the environs of Riverside. The only known trees that could be found were growing in White Park in the center of town. The larval specimens collected about the roots of these trees fit very closely the description by Cobb. The identification of two larval stages also clarifies the apparent inconsistencies discussed by Tarjan (1960).

Samples were obtained on three different occasions and a special effort made to recover senile forms from the roots and soil but the only specimens found were slender females not developed to the stage of oviposition.

Gracilacus marylandicus (Jenkins, 1960)

A slide containing two female paratypes was sent to the University of California, Davis by Jenkins for deposit in the Nematode Survey Collection.

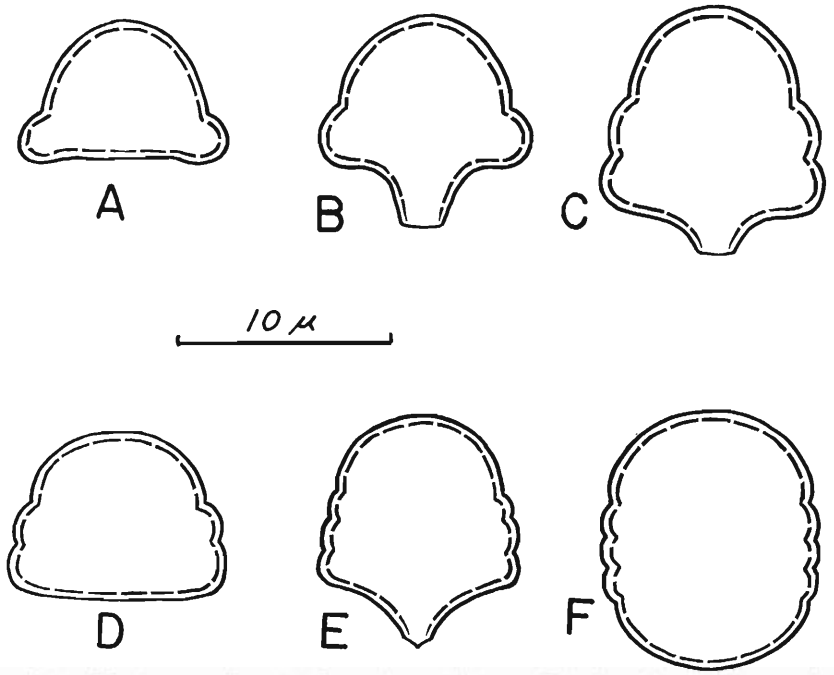

Plate I. Cross sections in the region of the caudal alae. *Cacopaurus pestis,* A-B; *Gracilacus epacris,* C; *Paratylenchus hamatus,* D-F.

On the same slide were two females and one male of a species which keys to *G. aciculus*. The males described as *marylandicus* were evidently specimens of *aciculus* which it fits very closely in size, tail shape and lines in lateral field. Pending the collection of additional material the males of *marylandicus* must be considered unknown.

Gracilacus aciculus (Brown, 1959)

The identification of specimens in the type collection of *G. marylandicus* extends the range of *G. aciculus* to Maryland about the roots of *Pinus virginiana*.

Additional specimens have also been collected by the author 9 May 1955 about the roots of grass and *Mesembryanthemum* sp. at Dillon Beach, California.

Gracilacus sarissus (Tarjan, 1960)

LARVA (SECOND-STAGE?): (2) 0.20 mm. (.19-.20 mm.); a = 19.8 (19.5-20.1); b = 2.4; c = ?; stylet = 36 microns.

LARVA (FOURTH-STAGE): (6) 0.29 mm. (.25-.33 mm.); a = 22.3 (19.6-25.2); b = 3.3 (2.9-3.8); c = ?; stylet = 45 microns (43-46 microns).

FEMALE: (19) 0.37 mm. (.32-.42 mm.); a = 23.9 (16.0-30.0); b = 3.7 (2.9-4.4); c = 13.8 (11.9-15.0); V = 82% (80-84%); stylet = 53 microns (48-61 microns).

MALE: (3) 0.34 mm. (.33-.38 mm.); a = 33.7 (26.0-38.3); b = ?; c = 13.6 (12.2-15.0); T = 22% (15-28%); spicules = 20 microns (19-20 microns); gubernaculum = 4 microns (2-4 microns).

The males of *Gracilacus sarissus* were described as lacking an anal sheath. These specimens have a prominent sheath extending from the cloacal opening. The posterior edge extends as a rodlike projection.

These records extend slightly the specific limits of *G. sarissus* and identify two larval stages.

This species is quite common in California and has been collected in soil about the roots of the following: oak, Mt. Diablo, Alameda County; black sage, Badger Canyon; California laurel, near Fairfield, Solano County; Willets, Mendocino County; and University of California campus, Berkeley; manzanita, near Monticello, Solano County.

Gracilacus mirus n. sp. (Plate III A, B and Plate VI D)

LARVA (SECOND-STAGE?): (2) 0.25 mm. (.24-.27 mm.); a = 18.6; b = 2.8 (2.7-2.9); c = ?; stylet = 48 microns (45-51 microns).

Slender larva with rounded head. Annulation fine, well pronounced. Excretory pore opposite nerve ring. Stylet long, slender, prorhabdion especially strong.

LARVA (THIRD-STAGE?): (2) 0.31 mm. (.29-.33 mm.); a = 21.3 (19.9-23.8); b = 3.3 (3.3-3.4); c = ?; stylet = 41 microns (39-44 microns).

Slender larva with rounded head. Esophagus weakly developed, slender and degenerate. Stylet slender, knobs weak, backwardly directed. Tail conoid, almost pointed.

FEMALE: (8) 0.33 mm. (.28-.38 mm.); a = 21.4 (17.1-25.4); b = 2.8 (2.5-2.9); c = 19.8 (15.1-20.5); V = 83% (80-86%); stylet 77 microns (70-85 microns).

HOLOTYPE: Female. 0.35 mm.; a = 20.4; b = 2.6; c = 16.8; V = 83%; stylet = 80 microns.

Female slender with rounded lip region. Body annulation fine, visible on lip region. Sclerotization delicate, stylet guide prominent. Hemizonid two annules wide, anterior to excretory pore at level of stylet knobs. Lateral field with four very fine lines. Vulvar lips do not protrude. Vulvar flaps moderate in size. Anus obscure. Tail slender, conoid almost pointed at tip.

DIAGNOSIS: This species is most closely related to *G. anceps* and *G. intermedius* and is differentiated from the former as discussed under that species.

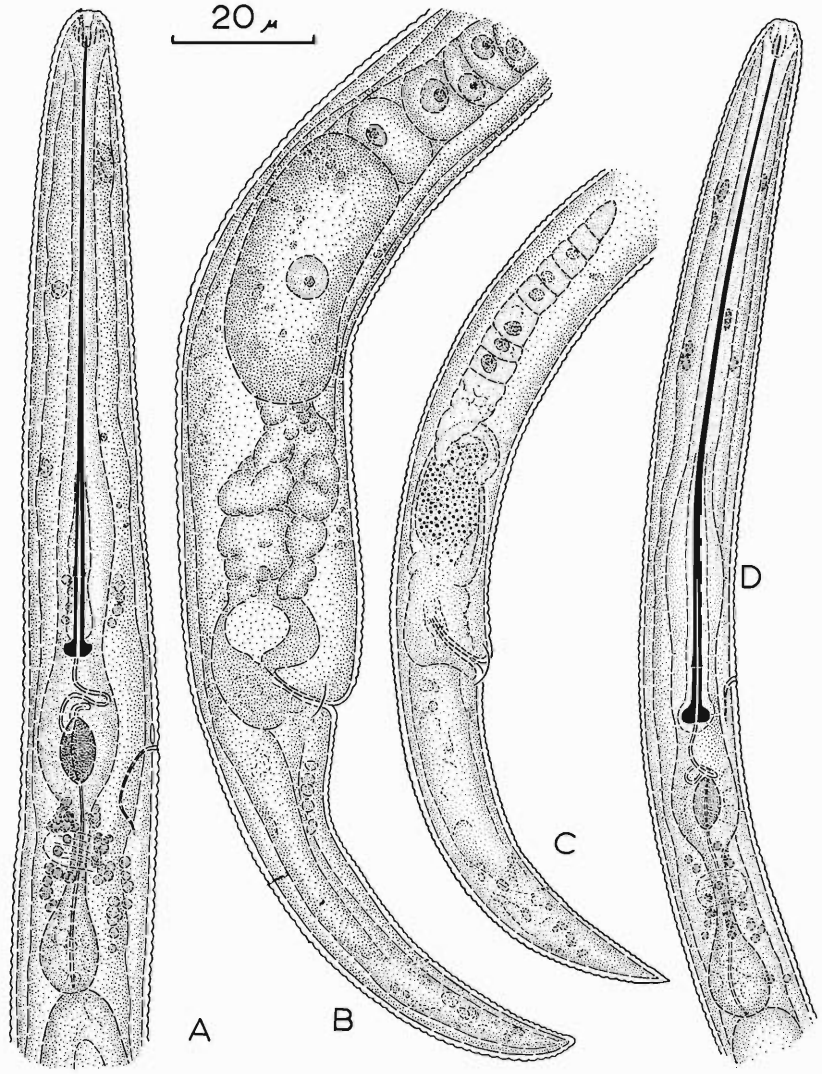

Plate II. *Gracilacus intermedius*, head region—A, tail region—B. *Gracilacus anceps*, tail region—C, head region—D.

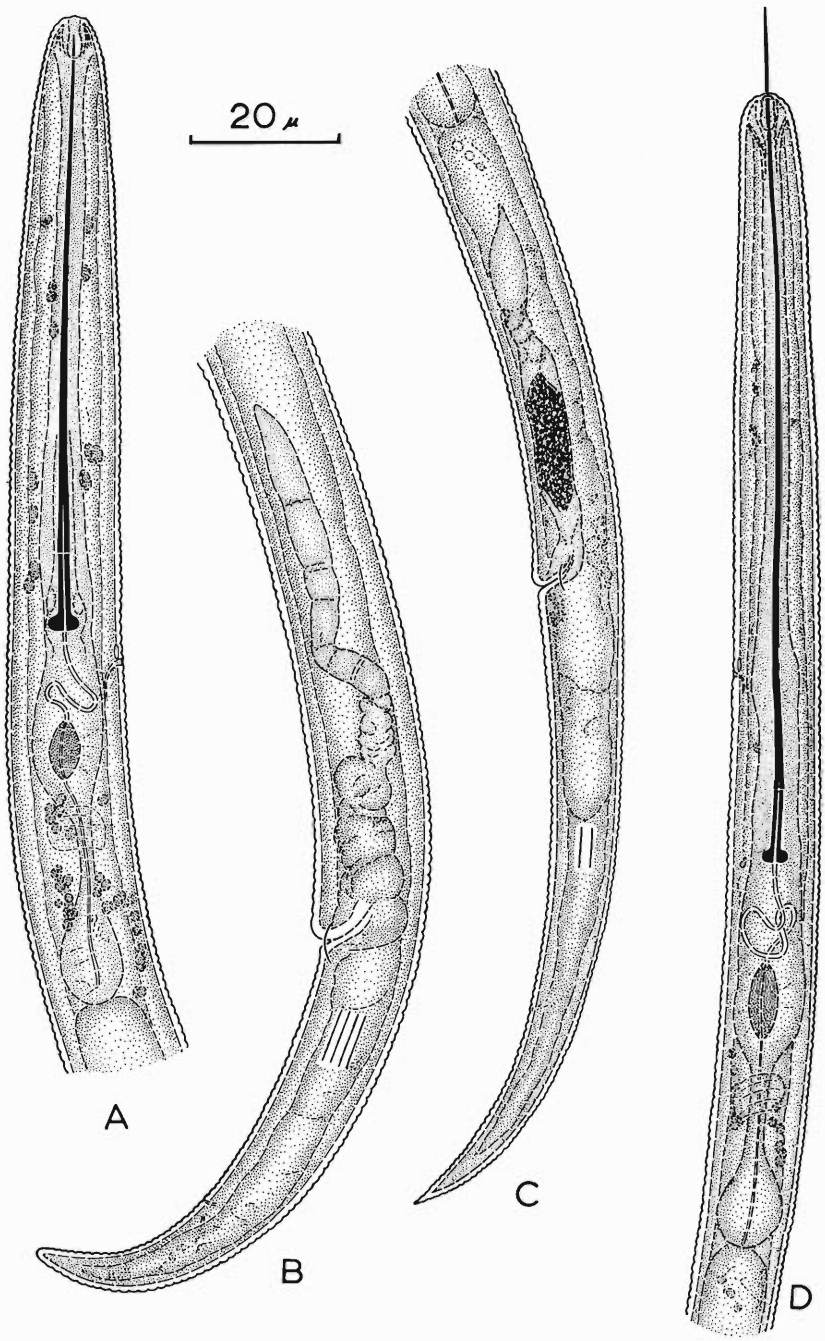

Plate III. *Gracilacus mirus*, head region—A, tail region—B. *Gracilacus elegans*, tail region—C, head region—D.

It differs from *G. intermedius* in the shorter stylet (70-85 microns): (83-92 microns) of *mirus* vs. that of *G. intermedius* (83-92 microns). The larger size of *intermedius* (.32-.44 mm. vs. .28-.38 mm. for *mirus*) is another distinguishing character.

HOLOTYPE: Female collected February 27, 1961, by the author, Catalogue number 209 University of California, Nematode Survey Collection, Davis.

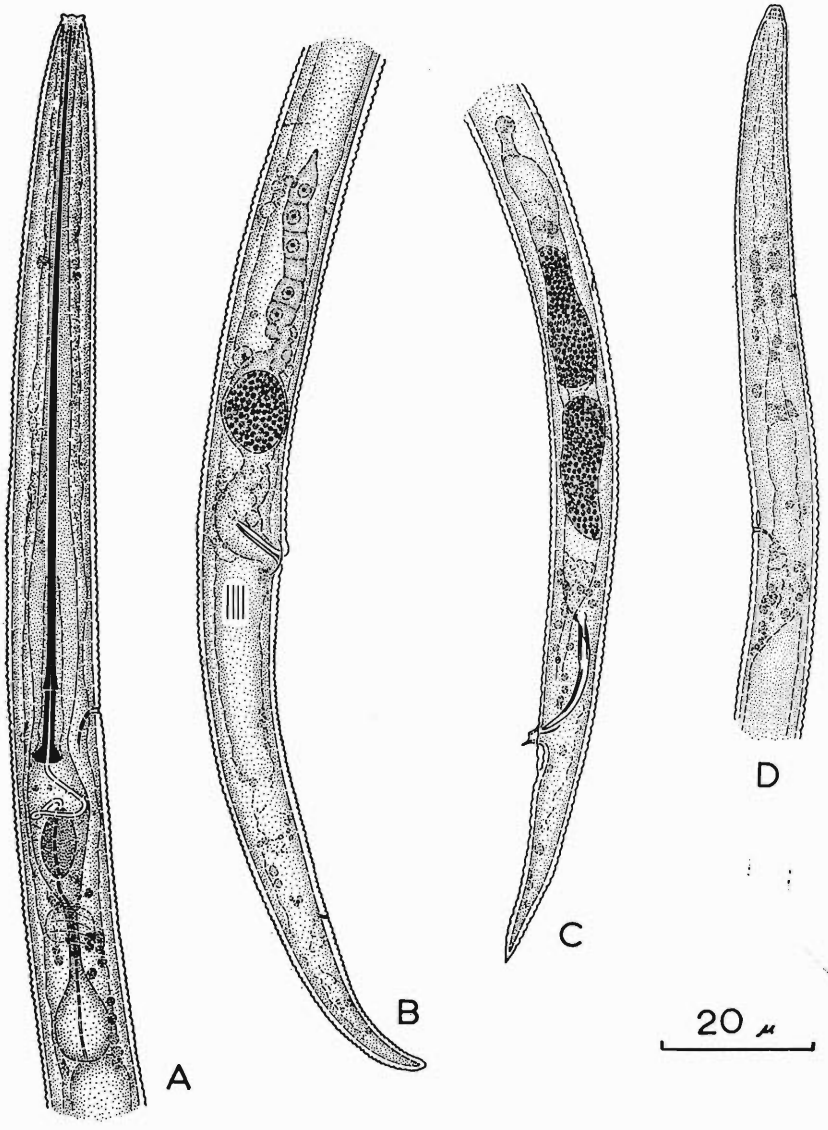

Plate IV. *Gracilacus idalimus*, female head region—A, female tail region—B, male tail region—C, male head region—D.

PARATYPES: Five females, same data as holotype, deposited in University of California, Nematode Survey Collection, Davis; two females, same data as holotype, deposited one each in United States Department of Agriculture Nematode Collection, Beltsville, Maryland and Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

TYPE HABIT: Soil about roots of cultivated grapevines, *Vitis vinifera* L., grown on St. George rootstocks.

TYPE LOCALITY: Wente Bros. commercial vineyard near Livermore, California.

OTHER LOCALITY: Two females and one larva of *G. mirus* were also collected in soil from a vineyard in Redwood Valley, Mendocino County, California.

Gracilacus intermedius n. sp. (Plate II A-B, Plate VI A-B, E-G)

LARVA (FOURTH-STAGE?): (5) 0.42 mm. (.40-.45 mm.); a = 26.8 (22.9-32.2); b = 4.2 (4.0-4.4); c = ?; stylet = 51 microns (49-52 microns).

FEMALE: (7) .38 mm. (.32-.44 mm.); a = 24.8 (16.7-30.8); b = 3.0 (2.8-3.2); c = ?; V = 82% (79-86%); stylet = 89 microns (83-92 microns).

HOLOTYPE: Female. .40 mm.; a = 26.3; b = 2.8; c = ?; V = 79%; stylet = 92 microns.

Slender female with fine but definite annulation extending onto rounded head region. Sclerotization weak, stylet guide prominent. Dierids and phasmids not observed. Excretory pore at level of stylet knobs. Hemizonid two annules wide anterior to excretory pore, separated from pore by about two annules. Stylet strong, slightly curved, knobs set off, not backwardly directed. Spermatheca lacking. Lips of vulva protrude slightly. Vulvar flaps moderately developed. Anus not visible. Tail conoid, tapering slightly, strongly annulated to rounded tip.

DIAGNOSIS: As mentioned earlier *Gracilacus intermedius* is most closely related to *G. mirus* and *G. anceps* and are distinguished from them in the diagnosis of each.

HOLOTYPE: Female collected March 23, 1952 by the author. Catalogue number 210 University of California, Nematode Survey Collection, Davis.

PARATYPES: 51 females, same data as holotype, deposited in University of California, Nematode Survey Collection, Davis; four females, same data as holotype, two each deposited in United States Department of Agriculture Nematode Collection, Beltsville, Maryland and Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

TYPE HABITAT: Soil about the roots of Monterey cypress, *Cupressus macrocarpa* Hartweg.

TYPE LOCALITY: Two miles northeast of Bolinas, Marin County, California.

OTHER HABITATS: Soil about roots of California laurel near Napa, California; *Arctostaphylos manzanita,* highway 37, 10 miles south of Monticello, Napa County and *Arctostaphylos* sp., Moraga Ridge, Contra Costa County.

Gracilacus elegans n. sp. (Plate III C, D, Plate VI, L)

FEMALE: (9) 0.31 mm. (.27-.34 mm.); a = 24.0 (21.1-28.0); b = 2.0 (1.9-2.1); c = 9.8 (8.6-13.2); V = 72% (70-74%); stylet-= 114 microns (110-119 microns).

HOLOTYPE: Female. 0.34 mm.; a = 28.2; b = 2.1; c = 12.1; V = 73%; stylet = 107 microns.

Female slender with fine body annulation faintly visible on bluntly rounded head region. Sclerotization delicate, stylet guide prominent. Hemizonid about

two body annules wide anterior to excretory pore which is situated one body width anterior to knobs of stylet. Ovary rudimentary, spermatheca prominent. Lips of vulva protrude very slightly. Cuticular vulvar flaps very small. Anus obscure. Tail elongate, tapering, ending in an acute, almost pointed, terminus.

DIAGNOSIS: *Gracilacus elegans* differs from all other species of this genus by having two lines in the lateral field area and a long slender body posterior

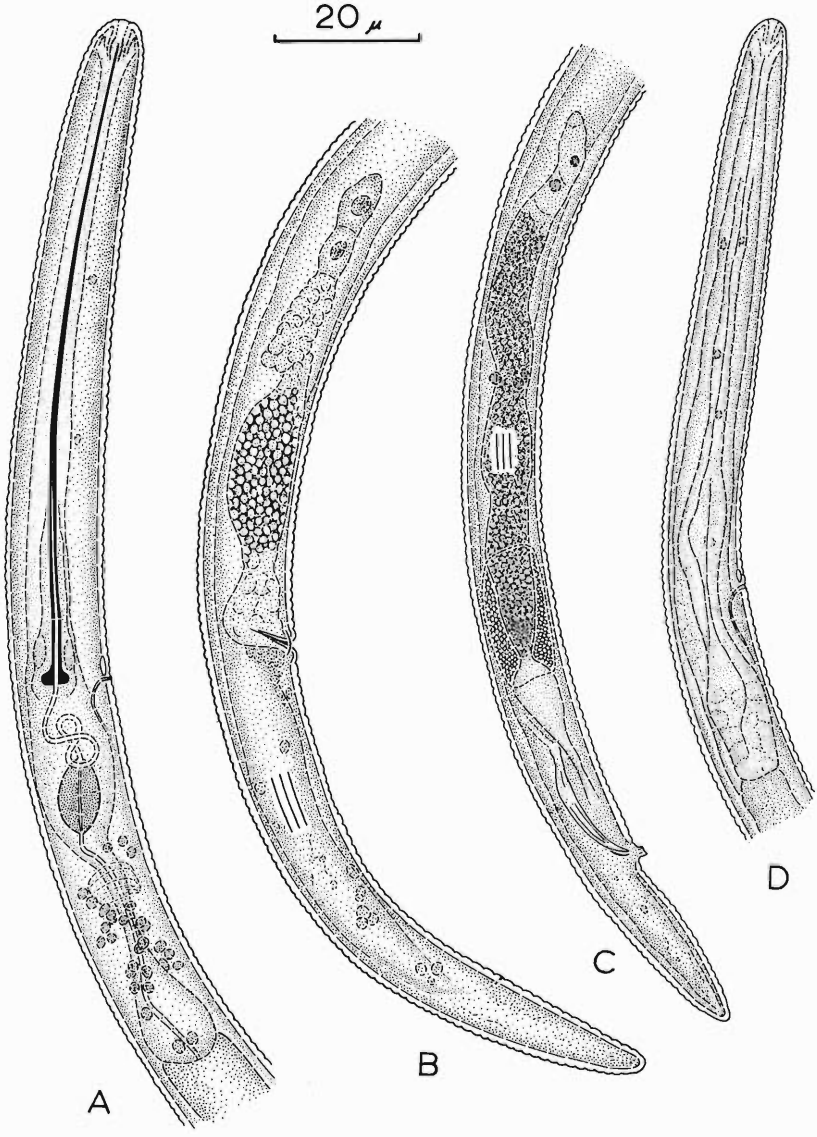

Plate V. *Gracilacus peraticus,* female head region—A, female tail region—B, male tail region—C, male head region—D.

to the vulva (V = 72%). The long slender stylet (ave. = 114 microns) and esophagus (ave. a = 2.0) further distinguish it from other species.

HOLOTYPE: Female collected November 20, 1955 by the author. Catalogue number 211 University of California, Nematode Survey Collection, Davis.

PARATYPES: Eight females, same data as holotype, deposited in University of California, Nematode Survey Collection, Davis; two females, same data as holotype, one each deposited in United States Department of Agriculture Nematode Collection, Beltsville, Maryland and Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

TYPE HABITAT: Soil about roots of magnolia.

TYPE LOCALITY: Along marshy shore of lake on west side of highway near south end of Winter Haven, Florida.

Gracilacus peraticus n. sp. (Plate IV, A-D, Plate VI, H-1)

LARVA (SECOND-STAGE?): (11) 0.22 mm. (.20-.24 mm.); a = 22.6 (20.2-24.0); b = 2.6 (2.4-2.8); c = ?; stylet = 47 microns (44-51 microns).

Head rounded, lips protrude, slightly set off. Esophagus elongate, median bulb and valve moderately developed. Excretory pore opposite nerve ring. Tail elongate conical, terminus digitate or hook-like occasionally simply rounded.

LARVA (THIRD-STAGE?): 0.25 mm.; a = 22.0; b = 2.7; c = ?; stylet = 56 microns.

Lips definitely set off. Esophagus well developed. Tail elongate conical with rounded terminus.

LARVA (FOURTH-STAGE FEMALE?): (2) 0.28 mm. (.27-.28 mm); a = 25.4 (22.4-28.3); b = 3.3 (3.2-3.3); c = 10.6; stylet = lacking.

Lips definitely set off but not so conspicuous as those of adult female. Esophagus elongate, moderately developed; median bulb with a valve; isthmus elongate. Sclerotization of lumen observable in anterior part of esophagus but no definable stylet present. Excretory pore near posterior bulb. Genital primordium forming the vagina at 72-82%. Tail digitate to hooked.

LARVA (FOURTH-STAGE MALE?): (2) 0.28 mm. (.25-.30 mm.); a = 28.4 (27.1-29.7); b = ?; c = 10.3 (9.7-10.9); stylet=lacking.

Slender larva. Lips rounded, not set off. Esophagus very slender, degenerate, median bulb and valve lacking. Developing spicules at 90-91%. Tail tapering, digitate to hooked.

FEMALE: (6) 0.31 mm. (.28-.34 mm.); a = 24.5 (14.1-30.1); b = 2.4 (2.3-2.6); c = 13.8 (12.4-16.4); V = 78% (77-79%); stylet = 92 microns (86-95 microns).

MALE: (2) 0.32 mm. (.30-.33 mm.); a = 33.4 (30.0-36.8); b = 3.6 (3.6-3.7); c = 10.4 (9.7-11.0); T = 22% (25-28%); stylet = lacking; spicules = 17 microns (17-18 microns); gubernaculum = 3 microns (3-4 microns).

HOLOTYPE: Female. 0.29 mm.; a = 23.2; b = 2.3; c = 12.6; V = 78%; stylet = 78%; stylet = 90 microns.

Female slender. Lips protrude, distinctively set off. Sclerotization delicate. Lateral field with four, very delicate lines. Dierids and phasmids not observed. Excretory pore slightly anterior to knobs of stylet. Spermatheca well set off at anterior end of uterus. Lips of vulva protrude slightly, vulvar flap small and distinct. Anus obscure, rectum and prerectum not discernible. Tail elongate conoid with rounded tip.

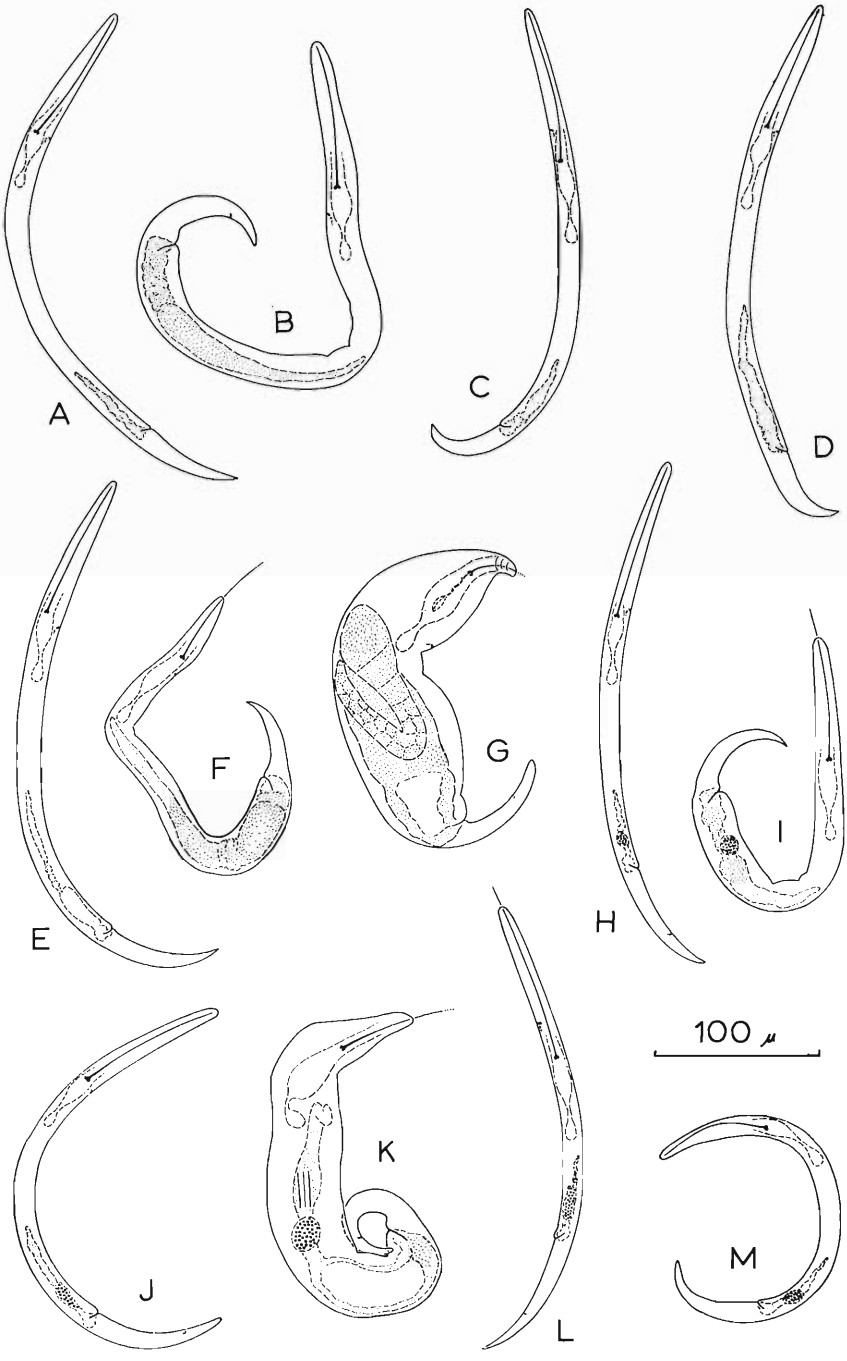

Plate VI. *Gracilacus* spp. A-B, E-G, *intermedius;* C, *anceps;* D, *mirus;* H-I, *peraticus;* J-K, *idalimus;* L, *elegans;* M, *steineri.*

ALLOTYPE: 0.30 mm.; a = 30.0; b = ?; c = 9.7; T = 28%; stylet = lacking; spicules = 18 microns; gubernaculum = 3 microns.

Small, very slender male with rounded head region, lips not set off. Sclerotization very delicate. Annulation fine, not discernible on head region. Esophagus degenerate, stylet lacking. Excretory pore 70 microns from anterior end. Spicules slender, curved. Gubernaculum simple rod-like. Caudal alae very short, distinct. Tail slender, conoid with rounded terminus.

DIAGNOSIS: *Gracilacus peraticus* is uniquely distinctive from other species in this genus by the protruding lips which are set off in both the larvae and adult female. It is most closely related to *G. epacris* but differs in the longer, more slender body shape posterior to the vulva in *peraticus*.

HOLOTYPE: Female collected June 25, 1959 by B. Weischer, Biologische Bundestalt für Land- und Forstwirtschaft, Münster, Germany. Catalogue number 212 University of California, Nematode Survey Collection, Davis.

ALLOTYPE: Male, same data as holotype. Catalogue number 213 University of California, Nematode Survey Collection, Davis.

PARATYPES: Four females, same data as holotype, deposited in University of California, Nematode Survey Collection, Davis; one female, one male, same data as holotype, deposited in Nematode Collection, Münster, Germany.

TYPE HABITAT: Soil about 30-year old grapevines.

TYPE LOCALITY: A slope of the valley of the Mosel at Rehlinger Trausch (near Nittle/Mosel), Germany.

Gracilacus idalimus n. sp. (Plate V, A-D, Plate VI, J-K)

LARVA (SECOND-STAGE?): (1) 0.21 mm.; a = 17.2; b = 2.8; c = ?; stylet = 30 microns.

Slender larva with bluntly rounded head. Annulation moderately strong, extending onto lip region. Sclerotization delicate, stylet guide prominent. Stylet and esophagus well developed. Excretory pore near posterior bulb. Tail tapers slightly with annulation almost to tip. Terminus bluntly rounded. Genital primordium very small, four-celled.

LARVA (FOURTH-STAGE?): (4) 0.26 mm. (.25-.28 mm.); a = 18.7 (16.3-22.1); b = 3.0 (2.7-3.2); c = 11.8; stylet = 40 microns (38-43 microns).

Slender larva with bluntly rounded head. Esophagus strongly developed Stylet strong with knobs well set off. Excretory pore posterior to nerve ring. Tail slender, tapering to rounded terminus.

Specimens of both a female and a male observed inside cast cuticle with molted prorhabdions of 34 and 37 microns respectively. This corresponds to the larval stylet measurements given above which indicates it would be the fourth-stage preadult.

Two specimens of larvae inside cast cuticles with a molted prorhabdion of 24 microns were observed also. This corresponds closely with the first larva and suggests these specimens are the third stage. In both cases the esophagus was extremely reduced and degenerate with no stylet visible. However it is possible this condition represents the extreme change during molt and the fully developed larval stage is not present and could be quite different in structure.

FEMALES: (22) 0.32 mm. (.31-.46 mm.); a = 17.7 (4.6-28.0); b = 2.5 (2.4-2.6); c = 14.3 (14.1-14.5); V = 73% (71-76%); stylet = 83 microns (75-88 microns).

MALE: (6) 0.35 mm. (.29-.39 mm.); a = 24.0 (19.6-26.7); b = ?; c = 12.5 (10.3-13.5); T = 37% (35-41%); stylet = lacking; spicules = 18-20 microns; gubernaculum = 3-4 microns.

HOLOTYPE: Female. .35 mm.; a = 22.8; b = 2.4; c = ?; V = 74%; stylet = 85 microns.

Body tapering slightly anteriorly to a bluntly rounded head. Annules moderately strong extending faintly onto head region. Sclerotization weak, stylet guide prominent. Lateral field with three prominent lines. Hemizonid obscure, anterior to excretory pore which is opposite valve of median bulb. Lips of vulva not protruding. Cuticular flaps of vulva very reduced, indistinct. Spermatheca prominent. Anus obscure. Tail elongate conoid with bluntly rounded terminus.

ALLOTYPE: L. = 32 mm.; a = 26.7; b = ?; c = 12.0; T = 34%; stylet = lacking; spicules = 20 microns; gubernaculum = 4 microns.

Body slightly tapering anteriorly, ending in bluntly rounded head. Sclerotization delicate. Body annulation fine to moderately strong. Hemizonid prominent anteriorly adjacent to excretory pore, 79 microns from anterior end. Testis appears to be composed of two parts, the posterior portion completely surrounded by large granular gland. Spicules cephalated, slightly curved. Gubernaculum simple, rod shaped. Spicule sheath protrudes without hook-like process on posterior edge. Conoid tail narrows slightly posterior to spicules, forms conoid rounded terminus. Strong annulation almost to terminus.

DIAGNOSIS: *Gracilacus idalimus* is most closely related to *G. aculentus* and *G. aciculus*, differing from both in the longer stylet of *G. idalismus* (ave. 83, 58, and 67 microns respectively. It also varies in the obese females found in *G. idalimus* with an elongate, rounded tail and a prominent lateral field.

HOLOTYPE: Female collected November 12, 1958 by S. D. Van Gundy. Catalogue number 214 University of California Nematode Survey Collection, Davis.

ALLOTYPE: Same data as holotype. Catalogue number 215 University of California, Nematode Survey Collection, Davis.

PARATYPES: 24 females, 19 males, same data as holotype deposited in University of California, Nematode Survey Collection, Davis; two females, two males, same data as holotype, one each deposited in United States Department of Agriculture Nematode Collection, Beltsville, Maryland and Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

TYPE HABITAT: Soil about the roots of *Ericameria palmeri* (Gray) Hall.

TYPE LOCALITY: University of California, Riverside, California.

CRICONEMATIDAE Thorne, 1943

DIAGNOSIS (EMENDED): Tylenchoidea. Procorpus and metacorpus swollen and amalgamated into a very large valvated bulb; isthmus short and broad, very reduced or almost lacking. Stylet of females very strongly developed, prorhabdion much longer than meso-metarhabdions; male degenerate, stylet lacking. Cuticle heavily annulated, often retrorse, sometimes with scales or spines. Three genera bear a fifth cuticle on adult female (*Criconema, Hemicycliophora, Hemiriconemoides*). Vulva near posterior end. Ovary single. Postuterine branch lacking. Caudal alae present, well developed.

GENERA: *Criconema* Hofmänner & Menzel, 1914

Criconemoides Taylor, 1936
Hemicycliophora de Man, 1921
Hemicriconemoides Chitwood & Birchfield, 1957

GENUS *Paratylenchus* Micoletzky, 1922

DIAGNOSIS (EMENDED): Paratylenchidae. Small species, .5 mm. or less. Female slender, stylet short <36 microns. Body posterior to vulva elongate. Cuticle finely annulated, without ornamentation. Excretory pore at level of nerve ring or further posterior. Male slender, active, stylet absent or reduced. Caudal alae absent or at most a ventral flattening in anal region. Ovary single. Testis one.

GENUS *Cacopaurus* Thorne, 1943

DIAGNOSIS (EMENDED): Paratylenchidae. Small species, .3 mm. or less. Larvae with strong, elongate stylet. Female short and stout to obese with stylet about 100 microns in length. Cuticle ornamented with minute, refractive elements. Excretory pore in the region of the metacorpus near the valve or further anterior. Body posterior to vulva very short and bluntly rounded. Male slender, active, stylet absent. Caudal alae represented by thickened, cuticular evaginations. Ovary single. Testis one.

TYPE SPECIES: *Cacopaurus pestis* Thorne, 1943

LARVA (SECOND-STAGE?): (7) 0.27 mm. (.24-.30 mm.); a = 25.7 (23.2-30.0); b = 3.2 (3.0-3.5); c = ?; stylet = 42 microns (39-45 microns).

Head rounded, lightly sclerotized. Stylet knobs small, delicate, backwardly directed. Esophagus slender, moderately developed. Excretory pore near or posterior to nerve ring. Tail elongate conoid with bluntly rounded tip.

LARVA (FOURTH-STAGE?): (6) 0.23 mm. (.21-.25 mm.); a = 12.8 (8.0-16.5); b = 2.3 (2.1-2.5); c = ?; stylet = 60 microns (55-66 microns).

Head rounded. Esophagus stout, strongly developed. Excretory pore opposite valve in median bulb.

LECTOTYPE: Female. 0.21 mm.; a = 6.9; b = 2.2; c = ?; V = 93% stylet = 92 microns.

The lectotype was selected from original material collected from walnut, *Juglans regia* Linn., variety Mayette, near Santa Clara, California, May 12, 1942, and used by Thorne in the description of this species. Slide T-21t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

DISCUSSION: The illustrations in the original description distinguish two larval stages which were figured separately by Thorne but in the written description both stages were included in the range of size, shape and stylet length.

The molted prorhabdion of the fourth-stage larval stylet was observed on two specimens of newly molted adult females. These measured 50 and 58 microns respectively. The specimens (larva) most closely fitting this figure bear a stylet of 55-66 microns with a prorhabdion 48-56 microns in length.

The stylet of the adult female was described as 40-110 microns. The lower range is undoubtedly from specimens in which the stylet was broken during collection. Four specimens with the stylet intact showed an average stylet size of 97 microns (92-102 microns).

The lateral field was described as "three lines extending from near the middle of the neck to the terminus, each line composed of two rows of minute

elements". These are broader than lines and more like bands, and in disposition actually present the appearance of four lines setting off these three bands.

DISCUSSION

The work reported here has included detailed studies of certain characters which merit special emphasis. First is the STYLET development in larval forms. The original description of *C. pestis* emphasized the well developed stylet of the larvae. It further suggested to the authors of *C. pestis* that *G. anceps* might be a *Cacopaurus* because of the large, robust stylet found in the larvae of that species. The larvae of *G. epacris* also were described with stylets similar to *C. pestis*.

The larvae of *Paratylenchus* were not known to be so well endowed and were characterized by Thorne (1943) as frequently found living free in the soil and did not possess a well-developed spear. Thorne and Allen (1950) further reported the recovery of immature forms of *P. hamatus* lying dormant in the soil. Rhoades and Linford (1959) specifically identified this stage as the nonfeeding, preadult larvae in their studies on *P. projectus* and *P. dianthus*.

It is true that the predominant larval stage of *Paratylenchus* found in most soil samples is the preadult fourth-stage in which the stylet is very degenerate or lacking. However, in collections of at least two species, *P. hamatus* and *P. elachistus*, younger larval stages have been found which possess a stylet approximately 13-16 microns long. The preadult larvae of these two species have a degenerate stylet which is apparently non-functional. The larvae of *Cacopaurus* and *Gracilacus* show that the preadult stage is variable with regard to the development of the esophagus and stylet. In *C. epacris* and *G. peraticus* the preadult is a dormant, nonfeeding stage without a stylet. Adult females and males of *C. pestis, G. sarissus* and *G. idalimus*, however, have been observed inside molted cuticles containing the prorhabdion of the previous stage. These stylet remnants have been very prominent indicating a strong stylet in the preadult. More information is needed on the life cycles in these genera to be able to compare and judge these differences in larval structures. Until more precise studies under controlled conditions are made with single specimens, such information is only attainable by interpretation of field samples. Unfortunately specimens usually occur in low numbers in natural habitats and the evidence is incomplete. Mixtures of species and/or genera also are common. Larval males and females are not always easy to distinguish. Specimens in the process of molting may present further difficulties when one tries to decide whether the specimens are lacking a stylet or have a degenerate structure. The male stylet is completely lacking in eight species of *Gracilacus* but is present as a reduced organ in males of *goodeyi*.

The use of the OBESE FEMALE body shape as a taxonomic character will depend on more complete collections in the future. The degree to which some of these species enlarge seems to vary considerably. Also the specimens may be rare in some soil samples and abundant in others. Because of the sluggish to immobile condition of such specimens special attention must be given to their recovery from soil by use of coarse screens and the examination of heavy sedimented materials. Collection in this manner led to the discovery of the extreme size in *G. intermedius* (Plate VI, G) even though swollen specimens of the same species (Plate VI, B and F) were relatively common.

The occurrence of obese females in *C. pestis, G. epacris,* etc., appears to be associated with the elongated stylet in the female. It is possible that the obesity of these species derives from their evolution as a sessile type parasite which no longer migrates in search of food once feeding is started. In any event, more information is needed to understand the nature and taxonomic importance of the swollen female stage of these various species.

The first reference to the EXCRETORY PORE as an indication of relationship of species of *Paratylenchus* was by Tarjan (1960). In discussing *Hemicycliophora strenzkei* he pointed out the position of the excretory pore near the metacorpus suggested that species was related to *Cacopaurus.* It has been found that the position of the excretory pore is variable among the species described. In *Paratylenchus* the pore is located near the nerve ring, midway in the isthmus, or as far back as the posterior bulb.

In *C. pestis* and most species of *Gracilacus* the pore is located near the metacorpus or further forward but in four species, *G. marylandicus, G. audriellus, G. sarissus* and *G. goodeyi,* it is near the nerve ring. The basic position of the excretory pore is best observed in the slender females before feeding begins. When the stylet is extruded the esophagus appears to have shortened and the pore located further posterior relative to the esophagus.

Th description of SPERMATHECA in the females of three species (*G. steineri, G. gracilis,* and *G. anceps*) for which males are unknown suggests this structure may be more correctly identified as a spermagonium. Unfortunately there has been only a limited number of specimens of these species collected so far. It is quite possible males are present in natural field populations.

However the specimens available for study are young slender females recently molted. The ovary is rudimentary yet the spermatozoa are found in considerable numbers. In *G. gracilis* the uterus is constricted near the vagina then swells anteriorly full of spermatozoa. The anterior end is a blind sac and the ovary continues anteriorly from the lateral side of the uterus. The same is true for *G. anceps* but differs in that the spermatozoa appear to be much smaller. In both species there is apparently a specialized cell at the anterior end of the uterine wall which produces the spermatozoa.

The extension of the cuticle into VULVAR 'FLAPS' at the lateral margins of the vulva is typical of most females of *Paratylenchus* and has been described for *C. epacris.* However it is lacking in *C. pestis* and *G. steineri* and is very reduced in other species. This character is variable and may be of limited use in specific diagnosis but is not considered a generic differentiation.

The CAUDAL ALAE of *C. pestis* were described by Thorne (1943) as 'small, obscure, appearing to be only a slight evagination of the cuticle.' He also compared this structure with that of a male of *Paratylenchus hamatus* which he described as similar but more obscure. Plate I, A-F illustrates these structures in cross section and shows the simple development of the caudal alae of these species. *G. epacris* appears to be intermediate between *C. pestis* and *P. hamatus.*

SUMMARY

A new family Paratylenchidae is proposed to include the following genera: *Paratylenchus* Micoletzky, 1922, *Cacopaurus* Thorne, 1943, *Gracilacus* n. g.

The new genus *Gracilacus* is proposed to include five new species, *G. elegans, G. mirus, G. intermedius, G. peraticus* and *G. idalimus.*

A redescription of *Paratylenchus anceps* is presented and a neotype designated. The following species are transferred to *Gracilacus*: *Paratylenchus*

anceps, P. goodeyi, P. aciculus, P. aculentus, P. audriellus, P. sarissus, P. marylandicus, P. steineri; Cacopaurus epacris.

Emended diagnoses are presented for the family Criconematidae and for the genera *Paratylenchus* and *Cacopaurus.*

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Paracephalobus (Nematoda: Cephalobidae) A New Genus of Soil-Inhabiting Nematodes*

S. A. AKHTAR

Among the nematodes collected in a soil sample taken in November 1960, from around the roots of sugarcane, *Saccharum officinarum* L., cultivated in plots within the laboratories area, were two female nematodes belonging to the subfamily *Cephalobinae* Filipjev, 1934. The subfamily now contains 3 genera, viz., *Cephalobus* Bastian, 1865, *Eucephalobus* Steiner, 1936, and *Heterocephalobus* Brzeski, 1960. Because the females show certain characters

*From West Regional Laboratories, Soil Zoology, Lahore.
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differing from these genera, they are considered to belong to an undescribed genus. The genus and the species are described here and given the name *Paracephalobus*,n.g., and *Paracephalobus litoralis*,n.sp., respectively.

Nothing is known of their feeding habits but it is presumed that they feed upon the rotten pieces of sugarcane used for planting.

Paracephalobus,n.g.

DIAGNOSIS: *Cephalobinae*: Annules 2 microns or more wide, lateral fields with four incisures extending behind anus but not reaching phasmids, lip-region without probolae, pharyngeal armature cephaloboid, ovary single with double flexure behind vulva; spermatheca near anterior flexure of the ovary, tail conoid with blunt and rounded apex.

TYPE-SPECIES: *Paracephalobus litoralis*,n.sp.

Paracephalobus litoralis n. sp.

DIMENSIONS: Female, (Holotype): L = 0.688 mm. a = 23.0; b = 5.4, c = 17.6. V = 63 per cent. Female, (Paratype): L = 0.800 mm. a = 23.2; b = 6.0; c = 17.7. V = 64.5 per cent.

Body cylindrical tapering towards both ends. Cuticular striations 2.2—2.4 microns apart. Lateral fields distinct, with four incisures beginning a little distance (equal to pharynx length) behind pharynx and extending posteriorly to about midway between anus and phasmids.

Lip-region lightly sclerotized, rounded, rather flattened in front, offset from the body by a slight constriction. Lips duplex, with inner circlet of papillae located on outer contour of the lips. Pharynx distinct, 8-14 microns long, cephaloboid, somewhat funnel-shaped and surrounded by muscles. It consists of cheilostom, slanting downwards and inwards along the inner surface of the lip and narrower protostom and telostom. Prorhabdion, mesorhabdion and metarhabdions not fused, telorhabdion forming the beginning of oesophageal lumen.

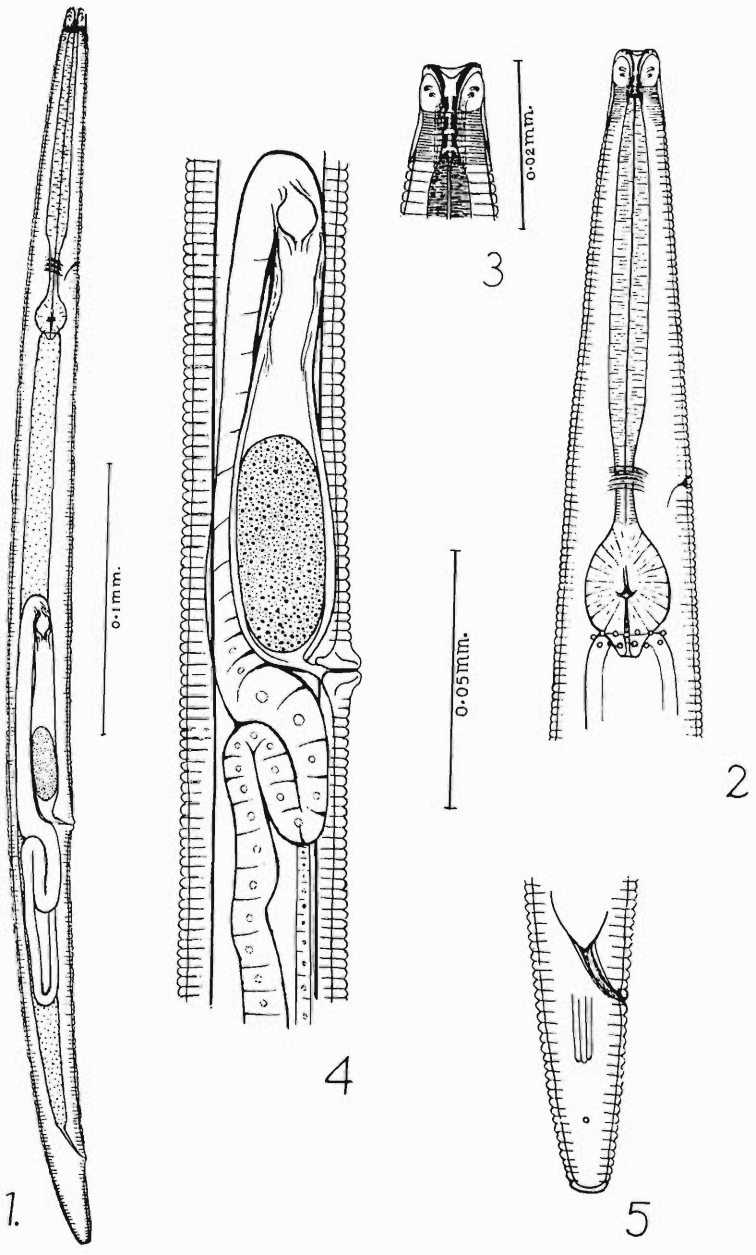
Oesophagus almost cylindrical, precorpus and corpus blending tapering behind gradually to isthmus and ending in a bulb, 13-25 x 18-20 microns, with valvular apparatus. Nerve ring crossing isthmus about midway of its length. Excretory pore conspicuous and ventral to nerve ring. Tail conoid, bluntly rounded, length 39-43 microns, 2.2.-2.5 times anal body diameter. Phasmids pore-like, a little behind the middle of the tail. Ovary single, vulva a transverse slit with prominent lips, situated behind the middle of the body. Vagina short, directed anteriorly. Uterus large with an ovum, 17 x 42 microns, with shell. Anterior flexure of gonad 100 microns in front of vulva and the posterior one 33 microns behind it. Double flexure of the ovary posterior to vulva. Oocytes in a single row.

HOLOTYPE: Female, collected in November, 1960, by S. A. Akhtar. Slide No. 515, Soil Zoology, nematode collections, West Regional Laboratories, Lahore, West Pakistan. Male: Unknown.

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

TYPE LOCALITY: Cultivated plot lying in West Regional Laboratories area, Lahore.

DISCUSSION: The female nematode described above has cuticular striations 2 microns apart, lip region without probolae. There is single ovary with double flexure behind vulva and spermatheca near the anterior flexure of the ovary. Therefore it belongs to the subfamily *Cephalobinae* Filipjev, 1934.



Paracephalobus litoralis n.sp (Female)

Fig. 1. Entire body, lateral view.
Fig. 2. Anterior end, lateral view.
Fig. 3. Anterior end, more enlarged.
Fig. 4. Vulvar region, lateral view.
Fig. 5. Tail, lateral view.

The subfamily at present contains 3 genera, viz. Cephalobus Bastian, 1865 *Eucephalobus* Steiner, 1936, and *Heterocephalobus* Brzeski, 1960, distinguished from each other by shape of the tail and the extensions of lateral fields. The tail of the present female is conoid with blunt and rounded apex which are characters of the genus *Cephalobus*. But it differs from the genus in that the lateral fields do not reach the tail apex. In this character it resembles the genus *Eucephalobus*, but the tail in this genus is pointed. *Heterocephalobus* combines the pointed tail with the lateral fields entending to the end of the tail. Therefore the female belongs to none of the described genera and is given the name *Paracephalobus* n.g.

Key for identification of 4 genera of the subfamily *Cephalobinae* Filipjev, 1934:

I. Wings or lateral fields of female extending to caudal terminus II.
Wings or lateral fields of female not extending posterior to phasmid III.
II. Tail pointed ..... *Heterocephalobus*
Tail blunt and rounded ..... *Cephalobus*
III. Tail attenuated, usually pointed ..... *Eucephalobus*
Tail blunt and rounded ..... *Paracephalobus*

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The Interaction of Concurrent Infections of the Abomasal Nematodes, *Haemonchus contortus*, *Ostertagia circumcincta*, and *Trichostrongylus axei* (Trichostrongylidae), in Lambs*

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Most investigations of the effects of the common gastrointestinal nematodes on sheep have been made with experimental infections of a single species. However, pastured sheep usually acquire multiple-species infections and each species may make its special contribution to pathogenesis. Many complex internal, as well as external, factors may influence the development of parasitism in such sheep. Considerable evidence exists that interaction of the many possible combinations of the different species is an important internal factor in establishment of the worms and their clinical effect.

Stewart (1955) stated that intake of adequate numbers of *Haemonchus contortus* larvae by parasitized sheep caused elimination, *i.e.*, "self-cure," of adult worms of the same species. He further reported that nonhomologous "self-cures" occurred as follows: (1) intake of *H. contortus* larvae caused elimination of two other species of abomasal worms, *Ostertagia circumcincta* and *Trichostrongylus axei;* (2) intake of larvae of the latter two species caused elimination of *H. contortus* and of the intestinal nematode, *T. colubri-*

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formis; (3) intake of larvae of *T. colubriformis* caused the elimination of the species of nematodes located farther down the digestive tract, but not of species in the abomasum. Shumard, Bolin, and Eveleth (1957) reported variable development of *H. contortus, T. colubriformis,* and *Nematodirus spathiger* in lambs after simultaneous administration of larvae of these parasites. Greater numbers of *T. colubriformis* became established than of the other two species.

According to several reports, the pathogenic effects of certain species of nematodes in more or less simultaneously acquired mixed infections may be additive, and possibly accentuated. Turner and Colgazier (1954) showed that infections of *H. contortus* reversed the normal self-limiting course of *N. spathiger* infections, as well as causing exacerbation and prolongation of the nematodirosis. Kates and Turner (1960a) further demonstrated experimentally that the combined effects of these two parasites were additive and the characteristic symptoms of haemonchosis and nematodirosis were generally accentuated. They (1953) had previously demonstrated experimentally that the two intestinal nematodes, *T. colubriformis* and *N. spathiger,* exert additive pathogenic effects in mixed infections.

To investigate further the interaction of roundworms of sheep, an experiment was carried out with simultaneous infection of lambs with three species of abomasal nematodes, *H. contortus, O. circumcincta,* and *T. axei,* in all possible combinations. The species usually associated with parasitic anemia of sheep is *H. contortus,* whereas the other two species are more commonly associated with parasitic gastritis; however, each species can cause both of these clinical conditions. Some of the results of this experiment have been reported by Kates, Wilson and Turner (1957) in abstract; the purpose of the present paper is to give a more complete and comparative account of the results.

MATERIALS AND METHODS

Twelve Shropshire and eight Columbia-Southdale lambs about three months old and averaging 45 pounds in weight were divided into four groups (Groups I to IV; Table 1). In previous work (unpublished data), these breeds were found to be comparable in rate of gain and susceptibility to infection during the first six months of life. Group I consisted of three pairs of lambs; each pair received infective larvae of a different one of the aforementioned three species; Group II also consisted of three pairs; each pair received infective larvae of two of the three species in a different one of the three possible dual-species combinations; Group III consisted of four lambs; each received infective larvae of all three of the species; Group IV consisted of four uninfected control lambs. In Groups I and II each pair consisted of one lamb of each breed, but Groups III and IV each consisted of one Columbia-Southdale and three Shropshire lambs.

The infective larvae administered were obtained from charcoal-feces cultures. Those of each species were taken from a single uniform concentrated suspension. All infective larvae were given *per os* to the lambs within one hour. The number of infective larvae given to each lamb in all infections was the same for each species of parasite, *viz., H. contortus,* 10,000; *O. circumcincta,* 25,000; and *T. axei,* 25,000. It was known from previous experience that the quantity of larvae given in each single-species infection was well below the lethal number for lambs under conditions similar to those of this experiment.

Throughout the experiment the lambs were maintained in concrete-floored

pens with outside runways. Two pens were used for each group of infected lambs. These lambs were moved to alternate clean pens daily to minimize extraneous parasitic infections. Group IV was kept in one pen only, and this pen was cleaned daily. All lambs were rack-fed good grade alfalfa hay in excess and one-half pound mixed grain daily per lamb during the course of the test.

Body weight, blood hemoglobin, and number of worm eggs passed per gram of feces were determined weekly over the experimental period of slightly less than three months. When the experiment was terminated the lambs were necropsied and the nematodes present were recovered and counted. The washed abomasums were digested by pepsin and hydrochloric acid to recover any nematodes retained in the tissues. The contents of the small intestine were also examined for parasites.

RESULTS

One of the lambs given a pure *T. axei* infection died from coccidiosis 3 days before the end of the experiment; all others survived. The development of fatal coccidiosis under the sanitary precautions taken in this experiment is most unusual. Coccidial and *Strongyloides papillosus* infections were present in all the other lambs, including the controls, but were kept at insignificant levels by these precautions.

EGG COUNTS: There was the usual variation in the numbers of eggs produced by infections of each of the three species (Table 1). The egg count data suggested that *H. contortus* definitely became established in greatest numbers in single-species infections, and on the average, *T. axei* probably did also; however, these data suggested that *O. circumcincta* became established in greatest numbers in the triple-species infections.

PARASITE RECOVERY AT NECROPSY: Only adult worms were recovered from the contents and tissues of the abomasums (Table 2). Less than 1% of the total recovery was from the abomasal tissue digestions and from the contents of the small intestine. Percentage recovery of *H. contortus* was much higher from the lambs with pure infections than from the lambs that had received *H. contortus* larvae in combination with other species; only one *H. contortus* was recovered from four lambs given triple-species infections and only 56 from one of two lambs which were given *H. contortus* and *T. axei*, whereas a total of 6,688 *H. contortus* were recovered from two lambs with pure infections. Conversely, more *T. axei* were recovered on the average from the lambs given either dual- or triple-species infections, than from lambs infected with *T. axei* only. *Ostertagia circumcincta* were recovered from all the lambs given larvae of this species. Average recovery of *O. circumcincta* was greater from lambs given pure infections of this nematode than from lambs given other species in combination with it. Relatively few were recovered from some individuals of Groups II and III, but one lamb of Group III yielded more than either lamb given this species only.

WEIGHTS: The average weight gain of the four lambs with triple-species infections was only about half that of the controls and a similarly marked depression in average gain occurred in the pair infected with *H. contortus* and *T. axei* (Table 3). The gain of the pair infected with *H. contortus* only and of the pair infected with *O. circumcincta* and *T. axei* also was somewhat depressed. The pure infections of *O. circumcincta* at the levels established apparently had no effect upon weight gains.

HEMOGLOBIN: An anemia, with blood hemoglobin values depressed to 40 to 50% of normal, developed in the two lambs infected with *H. contortus*

only (Table 3). The average minimum hemoglobin level of this pair occurred approximately eight weeks post-infection. Otherwise, the average minimums suggested a slight depression from the normal where *H. contortus* was administered in combination with either or both of the other species and no depression in the pure *O. circumcincta* or *T. axei* infections.

DISCUSSION

Many workers have shown that pure infections of adequate numbers of *H. contortus* produce a severe, often fatal, anemia in sheep. Much of the work on the pathogenesis of this parasite has been summarized by Andrews (1942) and Lucker (1952). Marked weight loss and anorexia are not generally seen in uncomplicated haemonchosis, except terminally in chronic cases. The fact that pure infections of *T. axei* have a high potential for pathogenesis in ruminants is well known (see Kates and Turner, 1960b). The typical clinical effects of acute trichostrongylosis (*T. axei*) are anorexia, loss of weight, physical weakness, and a terminal polycythemia; marked anemia is not usually associated with this infection, except terminally in some chronic cases. Regarding the pathogenic potential of *O. circumcincta* in sheep, Threlkeld and Downing (1936) reported that only the administration of large numbers of larvae (one-fourth to one million) produced any appreciable clinical signs; the major manifestations of clinical ostertagiasis were loss of weight, eosinophilia, and reduction of hemoglobin.

In the present experiment, the lambs of Group I reacted as expected to the sub-lethal larval doses of each parasite used in the single infections. The lambs given *H. contortus* alone developed haemonchosis accompanied by the expected degree of anemia; their weight gain was somewhat reduced. The animals exposed to *O. circumcincta* or *T. axei* alone did not suffer any obvious ill effects from these parasites. One lamb of the pair receiving *T. axei* alone died near the end of the experiment. However, its death was due to acute coccidiosis, an unusual and unexplained occurrence under the conditions of this experiment.

Table 1. Maximum parasite eggs per gram of feces per lamb; uninfected controls omitted.

	In Single Infections (2 lambs)	In Dual Infections (2 lambs in ea. combination)		In Triple Infections (4 lambs)
H. contortus		with *O. c.*	with *T. a.*	with *O. c.* & *T. a.*
Egg Counts	20,670	360	645	270
	32,100	7,490	773	375
				570
				638
O. circumcincta		*H. c.*	*T. a.*	*H. c.* & *T. a.*
Egg Counts	765	465	188	1,455
	765	990	608	2,153
				2,775
				2,865
T. axei		*H. c.*	*O. c.*	*H. c* & *O. c.*
Egg Counts	1,245	900	578	1,045
	4,080*	1,418	915	1,073
				1,620
				1,672

*Lamb died—see text. Also see text for larvae given.

A somewhat different result was obtained in the lambs of Group II with dual-species infections. In those given *H. contortus* in combination with either *T. axei* or *O. circumcincta,* average hemoglobin levels were only slightly depressed as was consistent with the low level of infection with *H. contortus* at necropsy. In this group, the lowest average weight gain was in the pair given *H. contortus* and *T. axei,* but the effects were primarily those of mild trichostrongylosis with slight anemia as was consistent with the recovery of a total of only 56 *H. contortus* at necropsy and the low peak *Haemonchus* egg counts during infection. The lambs given *O. circumcincta* and *T. axei* also showed some retardation in weight gain.

Apparently *T. axei* did not interfere as much with the establishment of *O. circumcincta* as with the establishment of *H. contortus.* The lambs given both *H. contortus* and *O. circumcincta* did not exhibit any effects of parasitism, other than slight anemia in one, and compared favorably in rate of gain with the uninfected controls throughout the experiment. In the dual infections *O. circumcincta* also apparently interfered with the development of *H. contortus* as the average number of this species recovered was about 27% of the average number obtained from the lambs with pure infections of *H. contortus.*

In the four lambs of Group III that received all three species, the poor establishment of *H. contortus* was very striking. This was apparently due partly to the presence of *T. axei* and partly to that of *O. circumcincta.* Only one *H. contortus* was recovered from the four lambs of this group, or less than 0.01% of the number of larvae given. The average numbers of *O. cir-*

Table 2. Worms recovered per lamb at necropsy; uninfected controls omitted.

	In Single Infections (2 lambs)	In Dual Infections (2 lambs in ea. combination)		In Triple Infections (4 lambs)
H. contortus		with *O. c.*	with *T. a.*	with *O. c.* & *T. a.*
Worm Counts	2,166	25	0	0
	4,522	1,760	56	0
				0
				1
Avg. % of Larvae Given	33	9	<1	<1
O. circumcincta		*H. c.*	*T. a.*	*H. c.* & *T. a.*
Worm Counts	3,750	1,778	541	180
	4,330	3,498	2,200	240
				1,400
				5,630
Avg. % of Larvae Given	16	11	14	7
T. axei		*H. c.*	*O. c.*	*H. c.* & *O. c.*
Worm Counts	7,868*	12,605	9,492	12,083
	10,165	16,664	12,765	13,875
				13,965
				14,887
Avg. % of Larvae Given	36	59	45	55

*Lamb died—see text. Also see text for larvae given.

cumcincta were reduced in this group, although one lamb retained more of these parasites than the lambs given this species only. In relation to larvae administered, the average percentages of *H. contortus, O. circumcincta,* and *T. axei* recovered from lambs with the pure infections were 33.4, 16.2 and 36.0, respectively, whereas the average percentages of these species recovered from lambs with dual and triple infections were 3.1, 10.5, and 52.6, respectively. Apparently only *T. axei* benefited, by increased establishment and/or retention in the host, from simultaneous association with one or both of the other two species.

It is not clear why infections of *H. contortus* were not normally established or retained in the lambs with mixed infections. Perhaps the abomasum was rendered physiologically unsuitable as a habitat for this species because of some activity, secretion, or excretion of the other nematodes or of the abomasum itself. Whatever the reason, the typical clinical effects of haemonchosis did not appear in the presence of the other two species. Marked depression in weight gain occurred in some of these lambs. Possibly it was an effect of the mixture of species or of *T. axei,* which was present in somewhat greater numbers than in the *T. axei*-infected controls. In summary, simultaneous infections with all three species interacted to the detriment of *H. contortus* and probably *O. circumcincta,* but somewhat to the advantage of *T. axei;* the infections of *H. contortus* were almost completely eliminated, and those of *O. circumcincta* were moderately reduced, whereas those of *T. axei* persisted at normal or higher than normal levels.

Our findings agree with those of Stewart (1955) in that infections of *H. contortus* were adversely affected by *T. axei* infections. Obviously, the phenomenon of poor establishment of *H. contortus* subsequent to simultaneous initial exposure to *H. contortus* and *T. axei* larvae in our experiment is to

Table 3. Body weight gain (lb) and minimum hemoglobin (GM/100 ml blood) per lamb.

	Group I Single Infections (2 lambs each)			Group II Dual Infections (2 lambs each)			Group III Triple Infections (4 lambs)	Group IV Uninfected Controls (4 lambs)
	H. c.	*O. c.*	*T. a.*	*H. c.* & *O. c.*	*H. c.* & *T. a.*	*O. c.* & *T. a.*	*H. c., O. c.* & *T. a.*	
Weight Gains	15	29	22*	20	3	15	8	20
	21	30		29	19	21	8	25
							18	27
							21	30
Avg.	18	29	22*	24	11	18	13	25
Minimum Hb	4.4	11.2	11.1	8.3	9.2	10.4	9.9	11.9
	6.6	12.2	12.5**	11.9	9.5	11.1	10.5	12.0
							11.3	12.2
							11.6	12.5
Avg.	5.5	11.7	11.9	10.4	9.4	10.7	10.8	12.2

*One lamb only; the other died of coccidiosis prior to necropsy of others and suffered a weight loss of 24 lb.
**The coccidiosis fatality.

be differentiated from the "self-cure" phenomenon in which an established *H. contortus* infection is eliminated by subsequent exposure to homologous or non-homologous larvae. Nevertheless both may depend upon a similar basic mechanism.

SUMMARY

A small-scale experiment involving 20 lambs was conducted to ascertain the effect of simultaneous infections of two and three species of abomasal nematodes on the host and on establishment of the separate species. The species used were *Haemonchus contortus, Ostertagia circumcincta*, and *Trichostrongylus axei*. To obtain comparative data, three pairs of lambs each were infected with a different one of these species, three additional pairs of lambs each were given two species in a different one of the three possible combinations of these species, and four lambs were given all three species; four lambs were used as uninfected controls. In all single-, dual-, and triple-species infections the following larval doses were used: *H. contortus*—10,000; *O. circumcincta*—25,000; *T. axei*—25,000.

Clinical and parasitological data obtained from the dual- and triple-species infections, compared with those from the single-species infections, indicated (a) that *H. contortus* infections were adversely affected to a marked extent quantitatively and, therefore, in clinical effect by simultaneous infections of one or both of the other two species, (b) that *O. circumcincta* infections tended to be moderately adversely affected by infections of one or both of the other two species, and (c) that *T. axei* infections were not adversely affected by infections of one or both of the other two species and may have been slightly enhanced by them.

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The Status of the Acanthocephalan Genera *Floridosentis* Ward, 1953, and *Atactorhynchus* Chandler, 1935

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Floridosentis elongatus was described as a new genus and new species by Ward (1953) on the basis of twelve worms recovered from the intestines of five of ten striped mullet (*Mugil cephalus*). The fish were collected from Biscayne Bay, Miami, Florida. *F. elongatus* has since been reported from *M. cephalus* by Bullock (1957) in southern Texas and Bullock (1960) from the southwestern coast of Florida. Cable and Quick (1954) identified this parasite from white mullet (*M. curema*) collected in Puerto Rico.

On the basis of these collections the genus *Floridosentis* appears to be well characterized as: Neoechinorhynchidae with a long, slender body of nearly uniform diameter; the cylindrical proboscis is armed with eight diagonal rows of hooks with about seven hooks in each row.

The genus *Atactorhynchus* was erected by Chandler (1935) for the new species, *A. verecundus*. This parasite was collected from *Cyprinodon variegatus* in Galveston Bay, Texas. The species has since been reported from a major portion of the Texas coast (Bullock, 1957) and from the west coast of Florida (Bullock, 1960). In addition, I have recently collected *A. verecundus* from Jone's Beach, Long Island, N.Y. This extends the range of this acanthocephalan almost to the northern limit of the range of the host.

The genus *Atactorhynchus* is characterized as: Neoechinorhynchidae with a small, stout body with greatest diameter behind the middle; the very small proboscis is armed with eight diagonal rows of hooks; these rows have about eight hooks to a row in the anterior half of the proboscis but the number is increased posteriorly so that the basal row has 16 hooks per row.

From these characterizations it would appear that these two genera can be easily separated on the basis of the following key:

Neoechinorhynchidae with long, slender body of nearly uniform diameter; proboscis armed with 8 diagonal rows of about 7 hooks each . . . *Floridosentis* Ward 1953.

Neoechinorhynchidae with short, stout body and maximum diameter posterior to the middle of the body; proboscis armed with 8 diagonal rows of hooks, the anterior rows have about 8 hooks each, the posterior rows have nearly twice as many . . . *Atactorhynchus* Chandler 1935.

Machado Filho (1951) described *Atactorhynchus mugilis* n. sp. from *Mugil plantanus* and *Mugil* sp. These fish were collected at Rio de Janeiro, Brazil. Both the description and the accompanying figures of *A. mugilis* clearly agree with the diagnosis of the genus *Floridosentis*. It would appear, therefore, that these parasites from the Brazilian mullet should become *Floridosentis mugilis* (Machado Filho, 1951). Although Machado Filho correctly attributes the genus *Atactorhynchus* to Chandler (1935) in his text he erroneously indicated in the title of his paper that Van Cleave was the originator of the genus.

The relationship between *Floridosentis mugilis* (Machado Filho, 1951) and *F. elongatus* Ward, 1953, cannot be definitely established without a careful

study of the specimens involved in the original descriptions. However, comparison of a number of dimensions, as indicated by the following table, would suggest that *F. elongatus* Ward, 1953 is a synonym of *F. mugilis* (Machado Filho, 1951). All measurements are taken from Machado Filho (1951) and Ward (1953).

TABLE I.

Comparison of *Floridosentis mugilis* (Machado Filho, 1951) and *F. elongatus* Ward, 1953. All measurements in millimeters

	F. mugilis	*F. elongatus*
Body length, female	20-25	up to 25
Body width, female	1.5-2	1 (maximum)
Body length, male	10-15	up to 18
Body width, male	1-1.5	1 (maximum)
Proboscis length	.365	.30-.40
Proboscis diameter	.016*	.18-.20
Anterior hooks	.046	.040-.050
Middle hooks	.029	.034
Basal hooks	.021	.013-.020
Proboscis receptacle	2.49 x .41	.60 x .18 (males .2 x .20 (females)
Lemnisci	½ length of body .025-.029	½ length of body .034 x .006
Embryos	.008-.012	(immature)

*This measurement is in error. Fig. 2 in Machado Filho's paper indicates that it should be 0.16 mm.

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Report of the Brayton H. Ransom Memorial Trust Fund

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DISBURSEMENTS: Grant to Helminthological Society of Washington	10.00
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A. O. FOSTER
Secretary-Treasurer

MINUTES

Three Hundred Eighty-First—Through the Three Hundred Eighty-Seventh Meetings

381st Meeting: Officer's Club, Walter Reed Army Medical Center, 20 October 1961. 51st Anniversary with 72 members and guests in attendance. Mildred Doss received Anniversary Award. Program of invited papers: Eosiniphilic Menigitis in the South Pacific Area, by L. Rosen; and Why Study Systematics, by G. Wharton.

382nd Meeting: Wilson Hall, National Institutes of Health, Bethesda, 17 November 1961. Society offered moral support to the 2nd Int. Symposium on trichinosis to be held at Ann Arbor, Michigan in August, 1963. Papers presented: Variation in susceptibility of inbred mice to *Cysticercus fasciolaris,* by Cheever; Effect of dead worms on development and progress of experimental hepato-splenic mansoni schistosomiasis in mice by Warren; *In vitro* studies on maintenance and early development of *Diroflaria immitis* microfilariae by Sawyer and Weinstein; Nutritional status of the host in relation to experimental malaria chemotherapy by R. Jacobs; Metastic calcification in rats due to Hytakerol as modified by infection with *Plasmodium berghei,* by Mercado.

383rd Meeting: Hurst Hall, American University, 15 December 1961. Plans made for joint meeting of Helminthological Society and American Society of Parasitologists in Washington in June, 1962. L. Oliver and J. Haley appointed members of Editorial Board of Proceedings. Officers elected: C. M. Herman, President; F. Tromba, Vice-president; V. Dropkin, Recording Secretary; Edna Buhrer, Corr. Sec'y-Treasurer. Papers presented: British parasitology and the International Meeting of Protozoologists at Prague, by Shorb and Shorb; Stability of drug resistance by *Eimeria tenella,* by McLoughlin and Gardiner; Schizogony of *Leucocytozoon smithi* in the turkey, by Wehr; Serum protein changes in Targee lambs exposed to different levels of parasitic infestation on pasture, by Turner and Wilson; *Plasmodium* of Anatidae, by Herman.

384th Meeting: McCort-Ward Hall, Catholic University. 19 January 1962. M. Sarles led the group on a tour of the new biology building. Donation of $25 was approved to the Joint Board of Science Education of Greater Washington. G. Durbin appointed member-at-large of Executive Committee; D. Shorb—representative to Washington Academy of Science; J. Haley—representative to the Council of American Society of Parasitologists; Papers presented "Schizogonic forms of *Plasmodium falciparum* in the peripheral blood, by Young; Floor plan for laboratory to facilitate sterile culture work, by Tiner; Report on malaria in chickens by Herman.

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Presentation

1962 ANNIVERSARY AWARD OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON
388th Meeting June 15, 1962*

RECIPIENT: DR. ALLEN MCINTOSH—Elected to membership in the Helminthological Society, October 20, 1930; Vice-President 1936; Recording Secretary 1938; President 1939.

ACADEMIC AND PROFESSIONAL RECORD: B. S. Mississippi A. and M. College, 1920; M. S. 1927, University of Minnesota. Taught zoology and parasitology from 1920 to 1930 at Mississippi A. and M. College; University of Minnesota; University of Wyoming; University of Michigan Biological Station; University of Miami (Coral Gables). From 1930 to the present Dr. McIntosh has been associated with the Department of Agriculture in the Agricultural Research Service, Animal Disease and Parasite Research Division, Beltsville, Maryland, where he presently holds the position of Leader, Parasite Classification and Distribution Investigations.

Dr. McIntosh is a charter member of the American Society of Parasitologists, American Society of Systematic Zoologists and the *American* Institute of Biological Sciences. He has been member of the Council and Vice-President of the American Society of Parasitologists, and for many years was an assistant editor of the Journal of Parasitology. Although he was formally a member of the Editorial Committee of the Proceedings

*Meeting of the Helminthological Society of Washington held jointly with the Annual Meeting of the American Society of Parasitologists at the Hotel Mayflower, Washington, D. C., June 15, 1962.

of the Helminthological Society of Washington only one year, the Editor of the Proceedings reports that Allen McIntosh has continued to render exceptionally effective editorial advice and service to the editorial staff of and contributors to the Proceedings. Dr. McIntosh has been a Member of the Joint FAO/OIE Expert Panel on Tick-Borne Diseases of Livestock; Collaborator, and Honorary Research Associate of the Smithsonian Institution; and has been invited to attend Congressional hearings as an expert on ticks. In 1959, he received a Honorary Doctor of Science Degree from the University of Miami, Coral Gables, Florida.

AWARD CITATION: For outstanding work for more than 30 years in the systematics and identification of parasitic organisms; for providing to parasitologists, physicians, teachers, zoologists, veterinarian, and other specialists in the United States and foreign countries invaluable advice and information relating to these problems; and for his invaluable services to and involvement in the activities of the Helminthological Society and other parasitological and zoological societies of this country.

CAREER HIGHLIGHTS: In his many studies and in his role as the Chairman of the Committee on Nomenclature of the American Society of Parasitologists and as a participating member of the International Colloquium on Zoological Nomenclature and the International Congress of Zoology, Dr. McIntosh has helped resolve many problems involving taxonomy and nomenclature of parasitic organisms.

For approximately 30 years he has been responsible for identifying external parasites submitted to the Department of Agriculture from domestic animals and poultry, game birds and wildlife. One very important part of this work has been the identification of all ticks forwarded by inspectors in the eradication of the cattle fever tick. Accurate determinations were essential in order to prevent the tick from becoming re-established in tick-free areas. Today the cattle fever tick has been eradicated from the United States, and Dr. McIntosh played an important part in this accomplishment. During the course of this work he published the description of two new species of ticks, and new distribution records.

Dr. McIntosh has performed an invaluable service for countless parasitologists and other specialists in authoritatively identifying trematodes, nematodes, cestodes and acanthocephalids which they have collected from man, domestic animals, and many other types of hosts. Based on these studies he has named and described 39 new species of trematodes, five new species of nematodes and four new species of cestodes. His publications on these and many other subjects now number over 80.

(*Continued from page 219*)

385th Meeting: Log Lodge, Agricultural Research Center, Beltsville, 16 February 1962. Following appointments announced: J. Lucker—Archivist; Judith Humphrey—Librarian; Marian Farr—Member of the Awards Committee which includes P. Weinstein, Chairman, and A. Taylor, member. Executive Committee reported that it voted to increase the July issue to 128 pages. Principal guest of the evening was Dr. Vladimir Ershov, Director of the All-Union Scientific Research Institute of Helminthology in Moscow and Vice-President of the All-Union Helminthology Society. Dr. Ershov reviewed the work in helminthology in Russia. He was elected to honorary membership. A film on the life cycle of coccidia was presented by Doran and exhibits of the work at the Beltsville Parasitological Laboratory were displayed.

386th Meeting: Biology Building, Howard University, 21 March 1962. Papers presented: Life cycle of *Uncinaria lucasi,* the hookworm of the seal, by Herman; Films on the growth and development of *Plasmodium vivax* in the erythrocyte through the schizogonous cycle and the gametogenous cycle of *Plasmodium falciparum* in the mosquito, by Young; Film of fat-tailed gerbils eating the snail host of *Schistosoma mansoni,* by Luttermoser; slides of a trip to Central America, by Lincicome.

387th Meeting: Naval Medical Research Institute, 26 April 1962. Motion to suspend the May meeting was passed. Papers presented: Sandground's hypothesis of age resistance in helminth infections, by Haley; Alteration in resistance of soybeans to root-knot nematodes by temperature, by Dropkin; Genetics of susceptibility to malaria and preliminary studies on cultivation of *Leishmania* in tissues, by Ward; Report of attendance at Annual Meeting of the Parasitology Group at the Institute of Biology in Great Britain, by Lincicome.

The following were elected to membership at the meetings indicated: **381st**—F. L. Dunn, D. S. Havertz, J. Heyns, F. A. Hopper, D. P. Limber, M. Luc, J. S. McDaniel, T. M. Ritchie, J. W. Riffle, F. Sogandares, I. C. Williams, M. Young; **382nd**—S. A. Akhtar, C. G. Goodchild, C. M. Heald, H. W. Huizinga, S. A. Khan, P. D. Leiby, H. A. Nathan, A. C. Todd; 383rd—R. D. Lumsden, M. Goldman; **384th**—J. F. Bergner, Jr., E. U. Eni, G. C. Hill, R. E. Millemann, R. C. Watkins, R. D. Winslow; **385th**—L. Al Arif, I. K. de Boehringer, A. Holbrook, G. I. Issa, H. Lembright, L. Mazotti, W. C. McGuire, D. B. Segal, D. E. Worley; **386th**—T. E. Amerault, D. W. Gates, T. O. Roby, P. A. Madden, D. W. Anthony, W. T. Shalkop, A. J. Hasselwander; **387th**—K. R. Barker, D. R. Harlow, G. W. Bird, Y. Tigin.

VICTOR H. DROPKIN
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