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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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On a New Nematode Genus *Nordia* (Dorylaimoidea: Nordianae n. subfam.) with Remarks on the Genus *Longidorella* Thorne, 1939

M. SHAMIM JAIRAJPURI and ATHER H. SIDDIQI*

The taxonomic status of long-speared dorylaimoids, viz., *Dorylaimus microdorus* de Man, 1880 and *Dorylaimus penetrans* Thorne and Swanger, 1936 has long been in doubt and Andrássy (1959) placed them in *Eudorylaimus* Andrássy, 1959. These nematodes possess long, attenuated spears with equal to subequal extensions and do not seem to belong to either *Dorylaimus* Dujardin, 1885 or *Eudorylaimus*. They are most closely related to *Longidorella parva* Thorne, 1939 because of the shape and size of their bodies and the character of the spear and its extension. The only important character in which *L. parva* differs from the above two species is the "basal portion of esophagus set off from the slender anterior portion by a constriction." The apparent similarity between *L. parva*, *D. microdorus* and *D. penetrans* has been confusing. Tarjan (1953, 56) and Altherr (1954) have recorded *D. microdorus* but attributed it to *L. parva*, while Meyl (1954) placed it in *Longidorus* Micoletzkty, 1922. Goodey (1963) has transferred *D. microdorus* and *D. pentrans* under the genus *Longidorella* Thorne, 1939 because he does not consider the esophageal constriction of diagnostic importance. He has emended the generic diagnosis to include these two species of *Dorylaimus* and does not mention the constriction although in defining *L. parva* this character has been included. Thorne and Tarjan (personal communications) as well as the present authors accept the validity of this esophageal character because there are also other genera in Dorylaimoidea which have been erected on this basis alone and which are accepted as valid. Therefore, the inclusion of *D. microdorus* and *D. penetrans* under the genus *Longidorella* is no longer possible. Altherr (1950) described *L. macramphis* and *L. murithi* which do not possess a constriction in the esophagus and therefore do not conform with the generic diagnosis of *Longidorella*.

Three species related to *Dorylaimus microdorus* were recently found in collections from soil about the roots of plants in North India. The size and form of their bodies, greatly attenuated spears and spear extensions, and the gradual expansion of the esophagus to a broad, elongate, basal enlargement indicate that these and similar described species merit generic rank for which the name *Nordia* n. g., is established. This name is composed of the first and last portions of the words North India.

Thorne (1939) doubtfully placed *Longidorella* in Longidorinae Thorne, 1935. Meyl (1961) raised Longidoriane to Longidoridae, included in it the genera *Longidorus*, *Xiphinema* Cobb, 1913 and *Longidorella*. The possession of a long attenuated spear is the only character which relates *Longidorella* and

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Nordia n. g., with other members of Longidoridae. Therefore *Longidorella* and *Nordia* n. g., do not belong to Longidoridae and should be placed in a new subfamily of Dorylaimidae. The great differences in the a and b measurements, the body texture (Longidoridae have a silvery appearance which definitely separates them from the yellow or the brownish color of *Longidorella, Nordia* and other Dorylaimidae). Differences in the cellular structure of the intestine of the two forms are considered to be diagnostic. Therefore Nordianae new subfamily is proposed for the reception of *Nordia* and *Longidorella*. *Nordia* is designated as type genus because both males and females are known.

NORDIANAE n. subfam.

DIAGNOSIS: Dorylaimidae. Body short and robust, with yellowish or brownish appearance. Spear axial, greatly attenuated with long extensions; junction of spear and extensions surrounded by an elongate swelling; guiding ring single, located near the middle of spear. Esophagus about 1/3 body length, comprising an anterior slender and a broad, elongate posterior glandular portions. Vulva slightly posterior to middle of body in all known forms. Ovaries amphidelphic, reflexed. Supplements consisting of an adanal pair and a ventromedian series beginning anterior to range of spicules. Tails of both sexes similar.

TYPE GENUS: *Nordia* n. gen.

OTHER GENUS: *Longidorella* Thorne, 1939

THE GENUS *Nordia* n. gen.

DIAGNOSIS: Nordianae. Body 1 mm. long or less, robust (a = less than 30). Lips prominent or amalgamated, continuous or set off from the body contour. Amphids with broad slit-like apertures. Spear greatly attenuated with equal or subequal extension and without basal knobs or flanges; junction of spear extension and esophagus surrounded by a conspicuous swelling; guiding ring located near the middle of spear. Anterior portion of esophagus narrow, muscular, expanding to a wide elongate basal portion. Vulva transverse, slightly behind middle of body in all known forms. Ovaries amphidelphic, reflexed. Males rare. Supplements consisting of an adanal pair and a ventromedian series beginning anterior to range of spicules. Tails of both sexes similar.

TYPE SPECIES: *Nordia microdorus* (de Man, 1880) n. comb.

Syn. *Dorylaimus microdorus* de Man, 1880
Longidorus microdorus (de Man, 1880) Meyl, 1954
Eudorylaimus microdorus (de Man, 1880) Andrássy, 1959
Longidorella microdorus (de Man, 1880) Goodey, 1963

OTHER SPECIES: *N. penetrans* (Thorne and Swanger, 1936) n. comb.

Syn. *Dorylaimus penetrans* Thorne and Swanger, 1936
Eudorylaimus penetrans (Thorne and Swanger, 1936) Andrássey, 1959
Longidorella penetrans (Thorne and Swanger, 1936) Goodey, 1963

N. macramphis (Altherr, 1950) n. comb.

Syn. *Longidorus macramphis* Altherr, 1950
Longidorella macramphis (Altherr, 1950) Altherr, 1950

N. murithi (Altherr, 1950) n. comb.

Syn. *Longidorella murithi* Altherr, 1950

N. thornei n. sp.

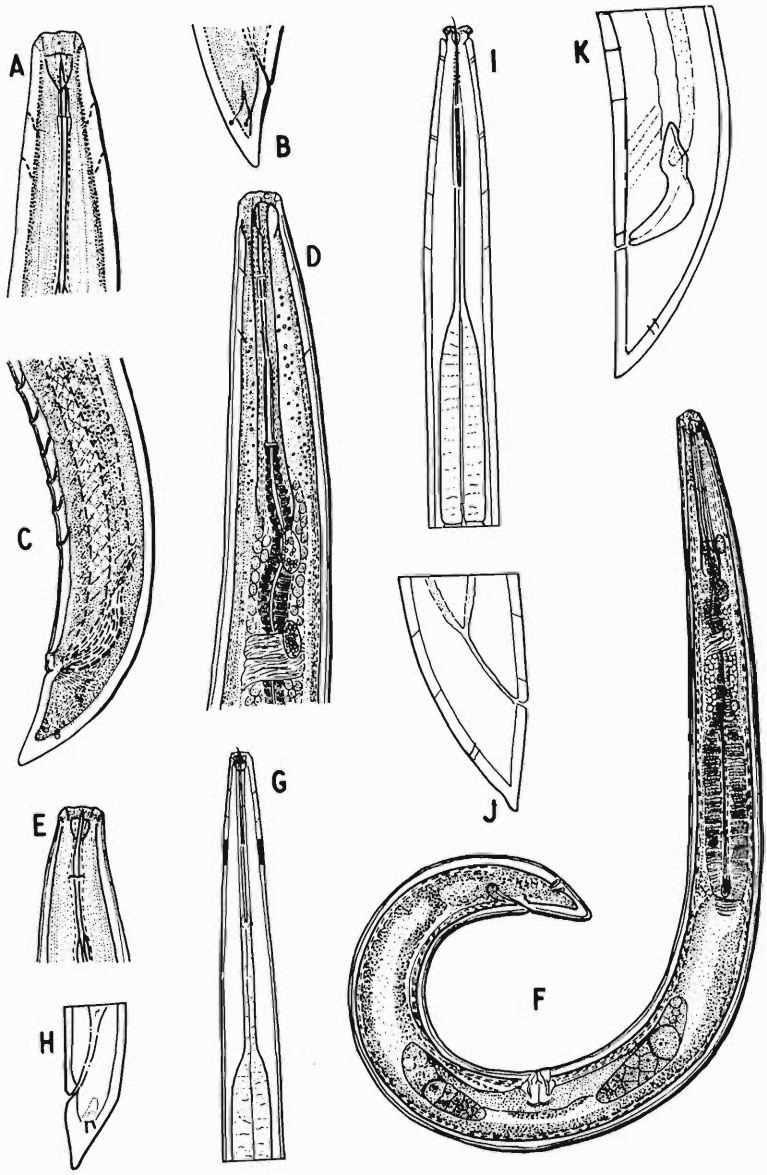

Plate I. Figs. A-C. *Nordia microdorus*. A. Anterior end of female. B. Tail of female. C. Posterior end of male. D-F. *Nordia penetrans*. D. Anterior end. E. Head end. F. Female. G-H. *Nordia macramphis*. G. Esophageal region. H. Tail. I-K. *Nordia murithi*. I. Esophageal region of female. J. Tail of female. K. Posterior end of male. (Figs. A-F. After Thorne and Swanger, 1936; G-K. after Altherr, 1950).

N. okhlaensis n. sp.
N. acutis n. sp.

KEY TO GENERA OF NORDIANAE N. SUBFAM.

Anterior portion of esophagus slender, non muscular, set off by a distinct constriction ..... *Longidorella*
Anterior portion of esophagus narrow but muscular, not set off by constriction ..... *Nordia*

KEY TO SPECIES OF *Nordia n. g.* (Based on females)

1. Tail long (c = less than 20), acute or subacute 2
   Tail short (c = more than 20), bluntly conoid or subdigitate 4
2. Tail length about 3 times anal body diameter (c = avg. 11), spear avg. 43 microns ..... *thornei*
   Tail shorter (c = 15-20) 3
3. Spear 33 microns; tail tapering to an acute terminus ..... *acutis*
   Spear longer avg. 40-45 microns; tail slightly arcuate ventrally, with subacute terminus ..... *microdorus*
4. Tail subdigitate; spear more than 40 microns 5
   Tail convex-conoid; spear less than 40 microns 6
5. Length of basal portion of esophagus 2 times diameter of neck region; spear straight ..... *macramphis*
   Length of basal portion of esophagus 3 times diameter of neck region; spear tip curved dorsally ..... *murithi*
6. Lip region distinct; set off by a depression; spear and extension equal *okhlaensis*
   Lip region continuous; lips low, rounded; spear extension ¾th as long as spear ..... *penetrans*

Nordia microdorus (de Man, 1880) n. comb. (Plate I. Figs. A-C.)

FEMALE: L = 0.6 mm.; a = 19; b = 2.8; c = 18; V = 63

MALE: L = 0.8 mm.; a = 20; b = 3.3; c = 25

DESCRIPTION: Body short and robust. Lip region amalgamated, continuous with body contour. Spear 42 microns long with extension of the same length; guiding ring single. Esophagus beginning as a tube ⅕th as wide as neck, suddenly enlarging slightly anterior to middle until more than half the neck width. Cardia elongate-hemispheroidal; female rectum and prerectum each about as long as anal body diameter. Ovaries reflexed almost to vulva; eggs fill body cavity and are twice as long as wide. Males very rare; supplements 4-6, beginning a tail length in front of anal pair and spaced about ¼th body width apart; prerectum extending to third supplement. Spicula about as long as tail. Tails slightly arcuate ventrally.

Nordia penetrans (Thorne and Swanger, 1936) n. comb. (Plate I. Figs. D-F)

FEMALE: L = 0.6 mm.; a = 80; b = 2.8-3.3; c = 25; V = 60

DESCRIPTION: Short, robust nematodes. Lip region low, rounded, continuous with neck contour. Spear 36 microns long, sometimes curved, its length equal to 3 times width of lip region; spear extension ¾th as long as spear. Esophagus slender anteriorly, enlarged in posterior half until ⅗th as wide as neck. Cardia hemispheroidal; rectum and prerectum each about as long as anal body diameter. Ovaries reflexed ⅔rd distance to vulva; eggs one and one-half times as long as body width; tail somewhat convex-conoid to blunt terminus.

Nordia macramphis (Altherr, 1950) n. comb. (Plate I. Figs. G-H)

FEMALE: L = 0.7 mm.; a = 25; b = 3.1; c = 23; V = 60

DESCRIPTION: Body medially cylindroid, tapering rapidly anteriorly. Cuticle anteriorly thick, finely striated. Contour of head rounded, papillae small;

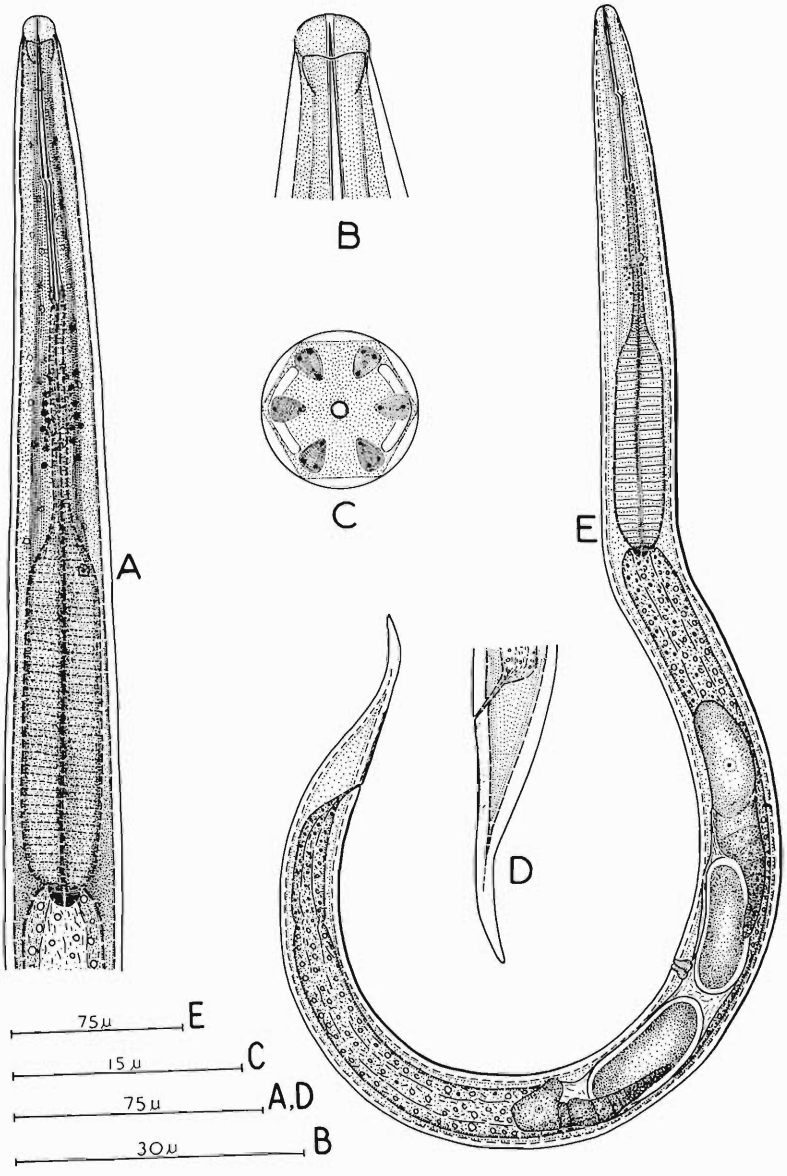

Plate II. Figs. A-E. *Nordia thornei*. A. Esophageal region. B. Head end. C. *En face* view. D. Tail. E. Female.

lips not modifying head contour, continuous with neck. Spear 25% of esophagus length, slightly swollen at its proximal end; extension a little shorter than spear. Esophagus enlarging near middle to a wide elongate posterior portion. Gonads short, reflexed. Rectum length twice anal body diameter. Tail bluntly conical, slightly curved ventrally.

Nordia murithi (Altherr, 1950) n. comb. (Plate I. Figs. I-K)

FEMALE: L = 0.8-0.9 mm.; a = 25; b = 3.1-3.4; c = 21-23; V = 56

MALE: L = 1.2 mm.; a = 21; b = 4.1; c = 25

DESCRIPTION: Body medially cylindroid. Cuticle finely striated. Lip region set off by slight constriction. Spear length 1/6th that of esophagus, curved dorsally; extension equal to spear length. Esophageal swelling about 59% total length of esophagus. Uteri and spermatheca filled with sperms. Ovaries reflexed about half to vulva. Rectum length equal to anal body width; prerectum twice length of rectum? Tail subdigitate, convex-conoid dorsally. Two subdorsal caudal papillae present. Spicula massive, 35 microns long. Supplements consisting of an adanal pair and 6-7 ventromedian ones, beginning opposite proximal end of spicula and spaced 16 microns apart.

Nordia thornei n. sp. (Plate II. Figs. A-E)

10 females: L = 0.77-0.85 mm. (0.82 mm.); a = 21-26 (23); b = 3-3.3 (3.1); c = 10-13 (11); V = 55-59 (57); Spear = 40-47 (43 microns); spear extensions = 37-45 (41 microns).

HOLOTYPE (FEMALE): L = 0.85 mm.; a = 24; b = 3.2; c = 11; V = 55; Spear = 43 microns; spear extension = 40 microns.

DESCRIPTION: Body tapering towards both ends. Cuticle and subcuticle thick, without apparent striations. Head rounded, lip region amalgamated, faintly marked off from body. *En face* view showing 6 distinct lips, 4 submedian and 2 lateral. 16 cephalic papillae distributed as follows: An inner perioral circlet of 6 and an outer circlet of 10, of which one on each lateral, and 2 on each submedian lip. Amphids large, cup-like; their broad slit-like apertures 3/4th as wide as head, situated at base of lips.

Spear long and slender; extension nearly equal to spear. Spear and extension 1/3rd length of neck and 8 times head width. Guiding ring faint, difficult to observe, situated slightly above middle of spear. Nerve ring midway between base of spear etxenison and anterior end of basal portion of esophagus.

Esophagus beginning as slender anterior portion then enlarged to a basal bulb 3 times as long as neck width. Cardia bluntly conoid to hemispheroid. Prerectum 47 microns long. Rectum length about one anal body diameter. Tail length 3 times anal body diameter, the posterior third a little dorsally bent with a subacute terminus. Cuticle of anterior half of tail showing wrinkled appearance ventrally.

Vulva a transverse slit. Vagina 1/4th body diameter. Ovaries amphidelphic and reflexed. Oöcytes arranged in a single row except for a short zone of multiplication. Eggs twice as long as body diameter, 62-64 × 20-30 microns.

MALE: Not found.

HOLOTYPE: Collected August 24, 1962; slide in Zoological Museum, Aligarh Muslim University, Aligarh (U.P.) India.

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

TYPE HABITAT: Soil around the roots of grass, *Cynodon dactylon* Pers.

TYPE LOCALITY: Simla (H.P.) North India; elevation 7,200 feet.
DISTRIBUTION: Dalhousie (H.P.) North India.
DIAGNOSIS: *Nordia* sp., with the above measurements and description; distinguished by the following characters: Lip region amalgamated, faintly

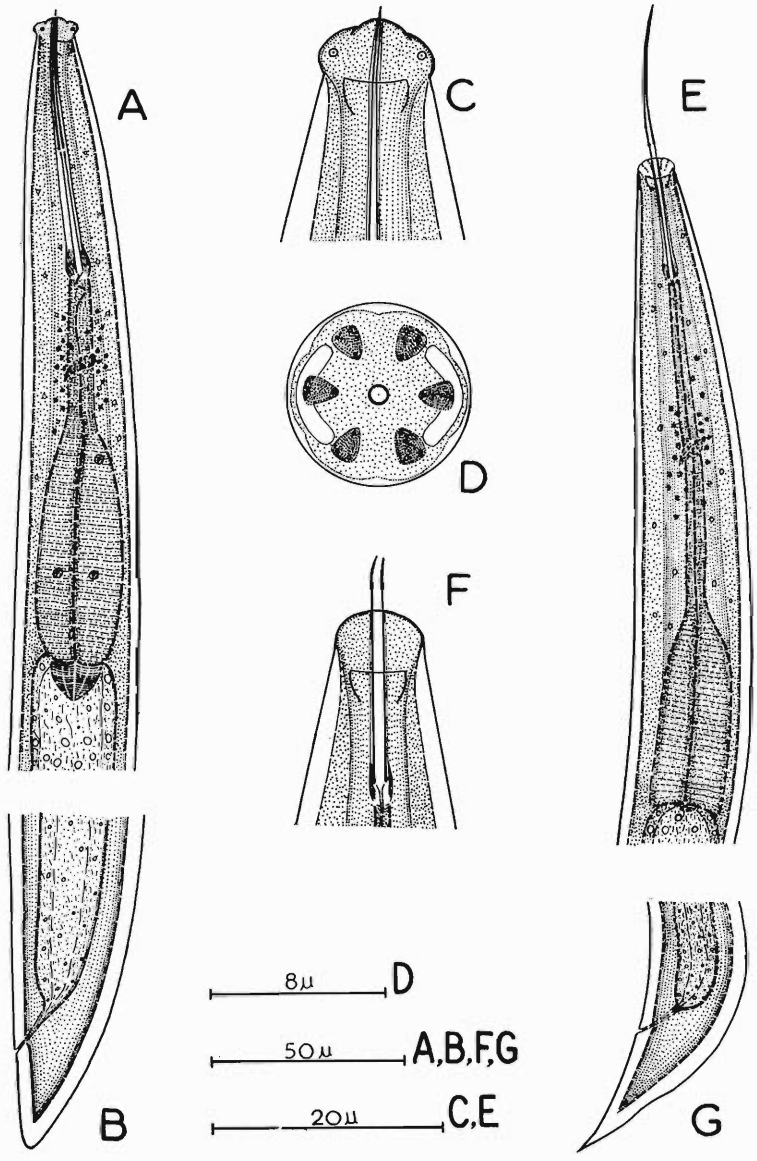

Plate III. Figs. A-D. *Nordia okhlaensis*. A. Esophageal region. B. Tail. C. Head end. D. *En face* view. E-G. *Nordia acutis*. E. Esophageal region. F. Head end. G. Tail.

marked off from body contour; spear and extension equal, each 37-47 microns long; length of basal portion of esophagus about 3 times width of neck; tail length about 3 times anal body diameter, posterior ⅓rd usually a little dorsally bent, with a subacute terminus.

Nordia okhlaensis n. sp. (Plate III. Figs. A-D)

10 females: L = 0.5-0.6 mm. (0.57 mm.); a = 13-16 (14); b = 2.9-3.5 (3.2); c = 22-26 (23); V = 57-61 (59); Spear = 32-36 (34 microns); spear extension = 31-36 (33 microns).

HOLOTYPE (FEMALE): L = 0.6 mm.; a = 16; b = 3.5; c = 24; V = 58; Spear = 33 microns; spear extension = 33 microns.

JUVENILE: L = 0.5 mm.; a = 14; b = 3; c = 20; Spear = 27 microns; spear extension = 28 microns.

DESCRIPTION: Body short and robust, tapering slightly to both extremities. Cuticle and subcuticle thick without apparent striations. Lip region distinctly set off from body contour. *En face* view showing 6 lips and the usual circlet of 6 inner and 10 outer cephalic papillae. Amphids large with broad slit-like apertures situated well below the lip region.

Spear and extension equal, their combined length equal to ⅔th the length of neck region. Spear guiding ring located just anterior to middle of spear, clearly visible in specimens studied in formalin but faint in glycerine preparations. Nerve ring situated midway between base of spear extension and anterior end of basal portion of esophagus.

Base of esophagus twice width of neck. Cardia prominent, conoid. Prerectum and rectum about equal in length. Tail blunt, convex-conoid, its length equal to anal body diameter.

Ovaries amphidelphic and reflexed. Oöcytes arranged in a single row except for a short zone of multiplication. Vulva a transverse slit. Eggs 40-57 x 23-31 microns.

MALE: Not found.

HOLOTYPE: Collected October 30, 1962; slide in Zoological Museum, Aligarh Muslim University.

PARATYPE: 10 females; other data same as for holotype.

TYPE HABITAT: Soil around the roots of banana, *Musa paradisiaca* L.

TYPE LOCALITY: Okhla, New Delhi, North India.

DIAGNOSIS: *Nordia* sp., with the above measurements and description; distinctive in the following characters: Lip region set off by a distinct constriction; lips prominent. Spear and extension equal, 31-36 microns; length of basal portion of esophagus twice width of neck; tail blunt, convex-conoid, about one anal body diameter in length.

Nordia acutis n. sp. (Plate III. Figs. E-G)

HOLOTYPE (FEMALE): L = 0.5 mm.; a = 18; b = 3.2; c = 16; V = 60; Spear = 33 microns; spear extension = 33 microns.

DESCRIPTION: Body tapering to both ends. Cuticle and subcuticle without apparent striations. Head continuous with body contour; lip region amalgamated. Amphids cup-like with slit-like apertures. Spear and extension 8 times head diameter and less than ⅓rd length of neck. Esophagus slender in anterior half; length of basal enlarged portion less than twice width of neck base. Cardia small. Tail length about twice anal body diameter, tapering to an acute terminus. Vulva transverse slit. Ovaries amphidelphic and reflexed. Oöcytes arranged in a single row except for a short zone of multiplication.

MALE: Not found.

HOLOTYPE: A single female collected August 27, 1962; slide in Zoological Museum, Aligarh Muslim University.

TYPE HABITAT: Soil around the roots of *Citrus limon* (L.) Burm.

TYPE LOCALITY: Dalhousie (H. P.) North India

DIAGNOSIS: *Nordia* sp., with the above measurements and description; distinctive in having amalgamated lips continuous with the body contour; length of basal portion of esophagus nearly twice width of neck base; spear and extension equal; tail acute, twice anal body diameter.

THE GENUS *Longidorella* Thorne, 1939

Longidorella is distinctive because of the slender, nonmuscular anterior portion of the esophagus which is set off by constriction. *Longidorella parva* is the only valid species. The inclusion of *L. chappuisi* (Schneider, 1935) Thorne, 1939 and *L. pygmaea* (Steiner, 1914) Thorne, 1939 under the genus *Longidorella* is questionable. Goodey (1963) as well as the present authors consider them *species inquirendae*. *Longidorella multipapillatus* (Schuurmans Stekhoven et Teunissen, 1938) Siddiqi, 1962 must also be regarded as an *inquirendum* since esophageal characters are not known.

SUMMARY

Three new species of a new nematode genus *Nordia* from North India are described and illustrated. *Dorylaimus microdorus* de Man, 1880, *D. penetrans* Thorne and Swanger, 1936, *Longidorella macramphis* (Altherr, 1950) Altherr, 1950 and *L. murithi* Altherr, 1950 are transferred to this new genus; description and illustration of these species is provided. Remarks on the genus *Longidorella* Thorne, 1939 are included. A new subfamily Nordianae is proposed to include *Nordia* and *Longidorella*.

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Precipitinogens in the Excretory Gland Contents and in Extracts of Isolated Tissues of *Stephanurus dentatus*

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Previous studies (Tromba and Baiden, 1960, 1963) showed that whole-worm extracts of *Stephanurus dentatus* contained antigens that formed precipitates with sera from swine infected with this nematode. However, certain antigens in these preparations were also precipitated by sera from helminth-free swine or from those infected with other nematodes. Since chromatographic separation of antigens in whole-worm extracts was only partially successful (Baisden and Tromba, 1963), other methods were sought.

The presence of precipitinogens in nematode metabolic products was suggested by Sarles (1937) to explain the formation of precipitates at the body openings of *Nippostrongylus muris* (*N. brasiliensis*) larvae exposed to immune rat serum. Other investigators have demonstrated the antigenicity of metabolic products present in nematode incubation fluids; however, as pointed out by Weinstein (1960), the specific origin of the antigens and the effect of the imposed conditions on metabolism are unknown. Thorson (1956a, b), seemingly avoided these difficulties by making extracts of organs of *Ancylostoma caninum* suspected of having excretory or secretory functions. Such extracts, however, may contain tissue antigens in addition to excretions or secretions. The extraordinary size of the excretory glands in *S. dentatus* (cephalic glands of Tayler, 1900) gave us an opportunity to collect quantities of gland contents (and hence a metabolic product or products) free of tissue antigens and under essentially normal conditions for antigenic analysis. Occasionally, other worm tissues were isolated in addition to excretory glands because Oliver-Gonzalez (1943) showed that specific antigens in extracts of isolated tissues of *Ascaris lumbricoides* were precipitated by the sera of infected animals.

We have included some protein determinations and a description of the excretory gland cell contents in order to interpret some of the serological reactions. It was also necessary to establish the identity of the organs that we called excretory glands because the term used by Tayler (1900) has been applied to different glands in other nematodes.

MATERIALS AND METHODS

Adult kidney worms removed from ureteral cysts and juveniles collected from the perirenal area of infected swine were washed several times and dissected under magnification in 0.8% NaCl. Dissections were usually completed on the day the worms were collected because storage overnight in saline resulted in some mortality and made dissections of the living worms more difficult. The excretory glands were removed intact by tearing the cuticle at about the level of the base of the esophagus, pulling away the head, and separating the glands from the terminal duct. In most cases, the coelomocytes attached to the glands were not removed. If the glands were ruptured during dissection, the preparation was discarded. After some practice, separation of the excretory glands could be made with the unaided eye.

Excretory gland contents (EGC) were collected by tearing the excised glands and rinsing them rapidly in 2 changes of 0.05M phosphate buffer. The gland tissue with residual contents (EGT) was placed in fresh buffer in a glass tube and homogenized with a motor-driven Teflon pestle. In these and other preparations, the number of worm parts per ml of buffer varied from 5 to 50. For convenience this ratio was expressed as worms/ml. The same day, or after overnight storage, both preparations were centrifuged and the supernatant fluids (su) and sediments (se) separated, preserved, and stored.

In addition to the foregoing, one or more of the following tissue isolations were sometimes made: whole excretory glands (EG); worms with excretory glands removed (R); head (H), including the excretory pore, terminal duct of the excretory glands, mouth parts, and associated tissues; esophagus (E); intestine (I); body fluid (BF); gonads (G); coelomocytes (CO); and cuticle (C), including muscles, lateral canals and their contents. After dissection the tissues were homogenized in buffer as previously indicated and stored. Initially, the tissues were extracted for several weeks; but after some preparations showed evidence of bacterial growth, this period was limited to 1 to 7 days. The preparations were then centrifuged and the supernatant fluids preserved and stored. The sediments were resuspended in buffer, preserved, and stored. Unless otherwise indicated, the following conditions apply to all antigen preparations: storage at 5 C; centrifugation at 20,000 x g at 5 C for 30 min.; and preservation by addition of 0.01 ml of a 3% solution of sodium ethylmercurithiosalicylate per ml of extract.

All preparations were tested against sera from infected swine by an agar diffusion technique (Crowle, 1958), and the resulting patterns evaluated by criteria used previously (Tromba and Baisden, 1963). Briefly these were: Group 1, lines concave toward the serum well and more than half the distance from antigen to serum; Group 2, straight lines about midway between antigen and serum; Group 3, lines concave toward the antigen well and less than half the distance between antigen and serum. Strongly positive ("immune") sera collected from pig 34 during the above studies was used for most of the tests. Serum of approximately equal strength from another experimentally infected animal was substituted after this was exhausted.

Serial twofold dilutions of antigen and serum were made with 0.05M phosphate buffer and 0.8% NaCl, respectively. Titer was expressed as the highest dilution at which lines were visible.

Samples of extract were treated with trichloroacetic acid (final concentration 3%) and the protein content of the resulting precipitates measured by the method of Lowry *et al* (1951) using crystalline bovine albumin as a standard. Values reported as microgm./worm were calculated by dividing the protein content/ml extract by the number of worms/ml.

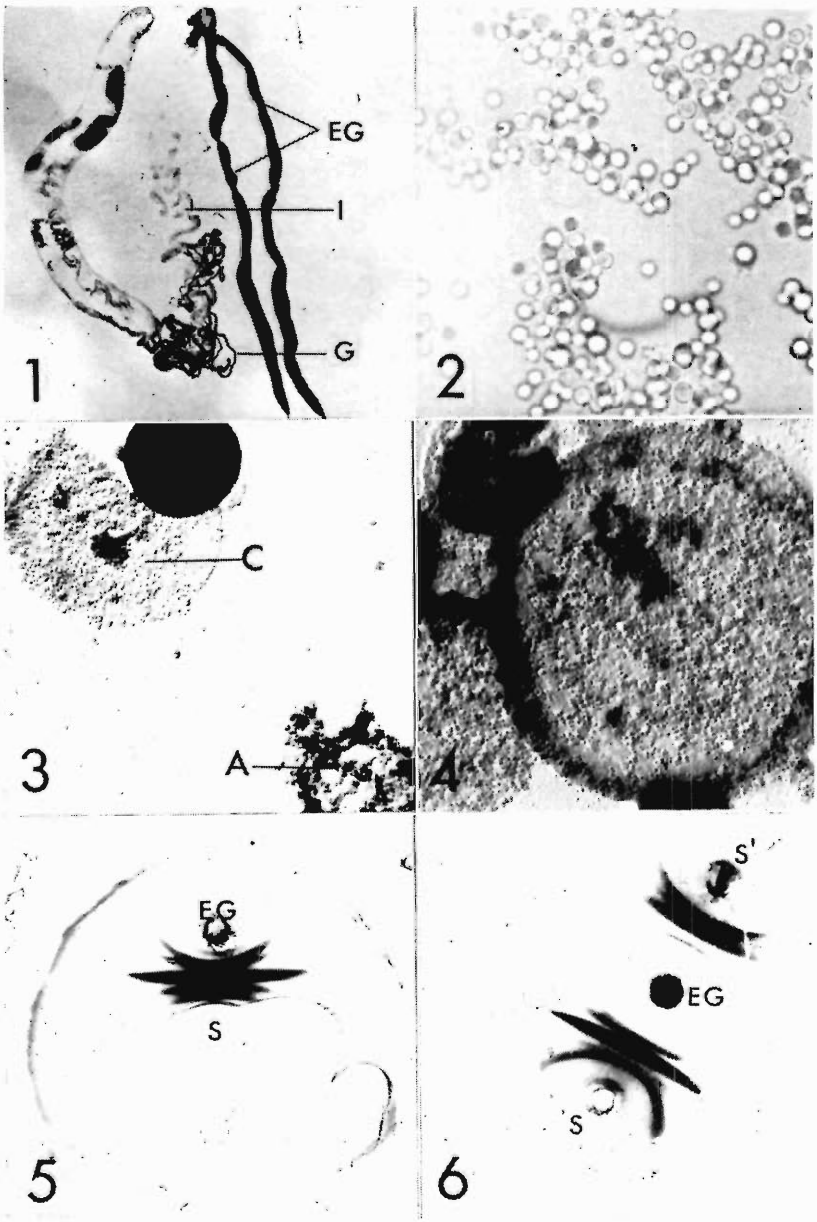
Formed elements in the excretory gland contents were observed unfixed at conventional magnifications. Preparations fixed in dilute osmic acid were placed on grids, shadowed with germanium, and studied in an electron microscope (RCA EMU3-C).

RESULTS

A. MORPHOLOGY OF EXCRETORY GLAND AND CONTENTS. The excretory glands of *Stephanurus dentatus* are elongate, sac-like, subequal structures lying partially enclosed in the convolutions of the intestine and attached to it and to each other by coelomocytes (Plate I, Fig. 1). Tayler (1900) illustrated these

structures, which she called cephalic glands, and stated that they opened on the ventral surface near the anterior end of the esophagus. Our dissections confirmed this and also showed that the glands unite in an excretory sinus, which receives branching ducts from the lateral canals. A very short terminal duct leads from the sinus to the excretory pore.

The gland contents consist of a fluid and formed elements ranging in size from 2.1 to 5.6 microns. These are the bodies referred to as "granules" in



descriptions of other nematodes and as "corpuscles" by Enigk and Grittner (1952). They appeared faintly pink in transmitted light and without discernible structure even at high magnification (Plate I, Fig. 2). However, preparations examined in the electron microscope showed a definite structural organization, which had the appearance of a stroma, (Plate I, Fig. 3 and 4). In addition to the formed elements, irregular aggregations of material were found, which appeared to have no definite structure (Plate I, Fig. 3). After storage in buffer, unfixed preparations showed a gradual decrease in the number of formed elements and increased amounts of the irregular aggregates. The presence of the latter in fresh gland contents indicates that they may be formed in the living worm from disintegrated corpuscles.

B. *Precipitinogens in various isolates:* 1. EXCRETORY GLANDS AND CONTENTS: Extracts of whole excretory glands (EG), isolated from 16 separate worm collections, contained antigens that gave up to 8 bands of precipitate with some sera (Plate I, Fig. 5). Although all extracts did not give the same number of bands, even against the same sera (compare Plate I, Fig. 6 with the previous illustration), Group 1 and Group 2 lines were always present. Usually 2 strong and 2 weaker Group 2 lines were seen; occasionally one Group 3 line was present. Group 1 lines appeared against control as well as "immune" sera. Sediments of these extracts were negative for all precipitinogens immediately after resuspension, but became positive after storage of 2 days or longer.

Gland contents (EGC) were obtained from 4 lots of freshly collected worms. The glands were handled rapidly with a minimum of tearing in order to eliminate or minimize tissue or tissue extracts in the EGC preparations. However, this procedure left a considerable amount of residual gland contents in the EGT preparations. Lots 18, 19, and 20 were each separated into 4 fractions (Table 1) for testing.

With some sera, up to 8 bands of precipitate were formed against both EGC su and EGT su preparations. Patterns produced were identical, or differed only in intensity or slight displacement in position (Plate II, Figs. 1, 2, and 3). They differed only in intensity from patterns produced with whole gland extracts. There is some correlation between reaction intensity and the amount of protein present in the preparations. Whole gland and EGT su extracts, which usually showed the strongest bands, had consistently higher protein concentrations per worm than did EGC su preparations (Table 1). These observations indicate that some or all of the antigens found in gland contents are present, and conceivably have their origin, in gland tissue. There was no evidence in any of the preparations that gland tissue contributed any antigens which were not already present in gland contents. The resuspended sediments of both EGC and EGT formed Group 1 and Group 2 lines; however, they were fewer and weaker than those given by the corresponding su

Plate I

Fig. 1. Dissection of adult female *Stephanurus dentatus*. EG = excretory gland cells. I = intestine, G = gonad.

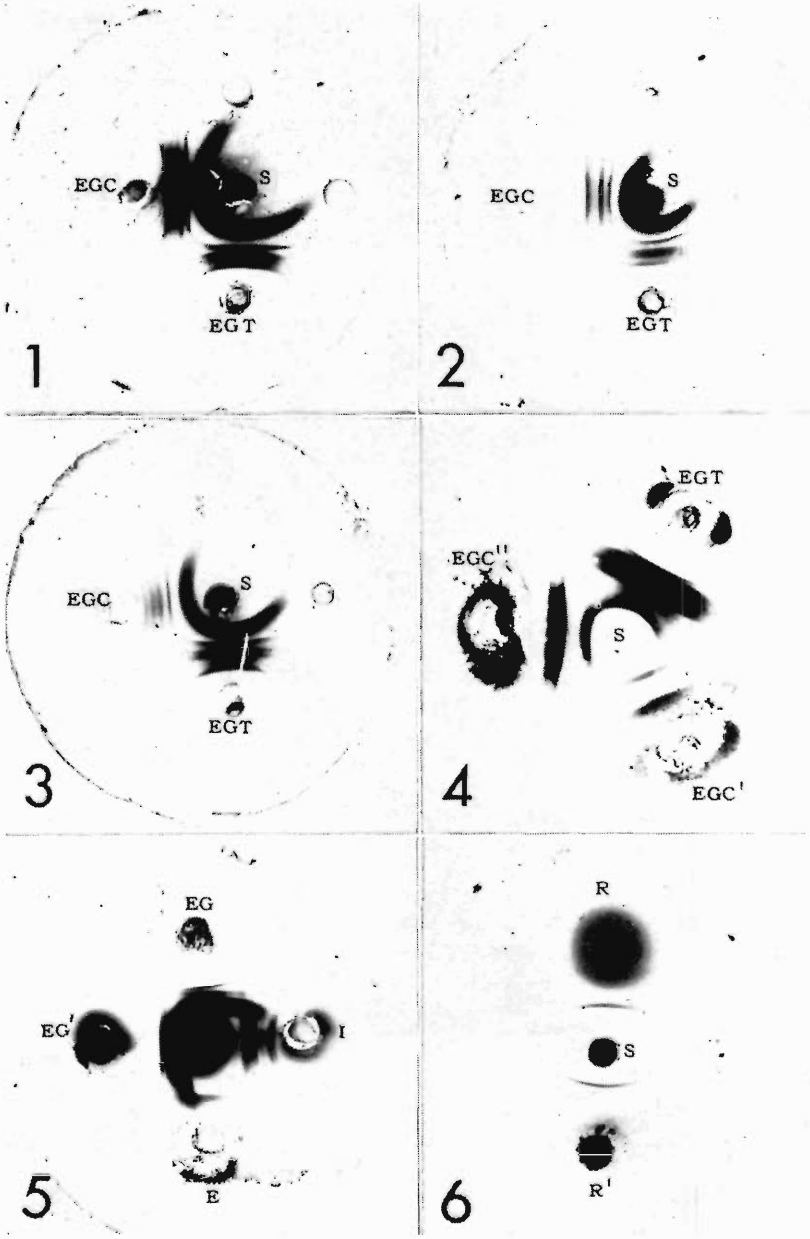
Figs. 2, 3, and 4. Corpuscles (C), and irregular aggregates (A) in the excretory gland fluid of *S. dentatus*. Fig. 2 (×500), Fig. 3 (×5,300), Fig. 4 (×9,200).

Fig. 5. Precipitin reaction of whole excretory gland extract with serum from an infected pig. S, serum 34-20, from 20th bleeding, 19 weeks after infection of pig 34. EG, whole excretory gland extract (EG-4).

Fig. 6. Precipitin reaction of whole excretory gland extract with two sera from an infected pig. S, serum 34-20; S^1, serum 34-24 from 24th bleeding 23 weeks after infection of pig 34. EG, whole excretory gland extract (EG-15).

preparations against the same sera (Plate II, Fig. 4).

2. INTESTINE: Extracts of intestinal tissue selected for study, as well as extracts of other organs and tissues reported here, were all taken from the same lot of worms. The excretory gland preparation from this lot was numbered EG-15. Reactions of intestinal extract (I-2) were compared with EG-15 and also with extracts of the esophagus. Up to 5 Group 2 lines appeared



when I-2 was tested against "immune" sera (Plate II, Fig. 5). In addition, one Group 3 line was usually present. None of the lines appeared to be common to those formed with EG-15. Group 1 lines were either absent or very weak.

3. ESOPHAGUS: Reactions of esophageal tissue extract were much weaker than those of EG-15, and usually weaker than those of I-2 (Plate II, Fig. 5). The single line in Group 2 was apparently not related to any antigen in EG-15. A weak Group 1 line was occasionally present.

Table 1. Protein content and precipitinogen titer of excretory gland contents and extracts of the excretory glands and other tissues of *Stephanurus dentatus*.

Preparation	Source	Extraction time	Protein microgm/worm	Titer*
EGC-16 su	80% juveniles	1 hr	28	+, not titered
EGC-18 su	adults	18 hr	157	1:32
EGC-18 se	adults	2 days	57	1:4
EGT-18 su	adults	18 hr	183	1:64
EGT-18 se	adults	2 days	20	1:2
EGC-19 su	juveniles	18 hr	103	1:8
EGC-19 se	juveniles	2 days	37	1:4
EGT-19 su	juveniles	18 hr	128	1:16
EGT-19 se	juveniles	2 days	10	1:2
EGC-20 su	juveniles	18 hr	94	1:4
EGC-20 se	juveniles	2 days	31	1:4
EGT-20 su	juveniles	18 hr	181	1:16
EGT-20 se	juveniles	2 days	34	1:4
EG-15	90% adults	5 days	345	+, not titered
EG-11	adults	5 days	304	+, not titered
EG-13	adult females	7 days	267	+, not titered
EG-14	adult males	7 days	215	+, not titered
R-6	adult males	7 days	504	+, not titered
BF-1	90% adults	----------	71	neg.
C-2	90% adults	5 days	203	neg.
H-2	90% adults	5 days	22	neg.
I-2	90% adults	5 days	54	+, not titered
E-2	90% adults	5 days	6	1:1, 1:8**
G-2	90% adults	5 days	243	neg.

*Unless specified otherwise, titrations were made against undiluted serum. **Serum diluted 1:8.

Plate II. Precipitin reactions of excretory gland contents, and extracts of the excretory gland and other isolated tissues, of *Stephanurus dentatus* with sera from infected swine.

Fig. 1. S, serum 348-7, from 7th bleeding, 11 weeks after infection of pig 348; EGC, excretory gland contents (EGC-18) supernatant fluids; EGT, extract of excretory gland and residual contents (EGT-18) supernatant fluids.

Fig. 2. S, serum 348-7; EGC, excretory gland contents (EGC-18) supernatant fluids diluted 1:2; EGT, extract of excretory gland and residual contents (EGT-18) supernatant fluids diluted 1:2.

Fig. 3. S, serum 348-7; EGC, excretory gland contents (EGC-20) supernatant fluids diluted 1:2; EGT, extract of excretory gland and residual contents (EGT-20) supernatant fluids diluted 1:2.

Fig. 4. S, serum 34-22, from 22nd bleeding, 21 weeks after infection of pig 34; EGC^1, excretory gland contents (EGC-20) sediments diluted 1:2; EGC^{11}, excretory gland contents (EGC-20) sediments undiluted; EGT, extract of excretory gland and residual contents (EGT-20) supernatant fluids.

Fig. 5. Serum 34-22 in central well; EG, whole excretory gland extract (EG-15) undiluted; EG^1 (EG-15) diluted 1:2; E, extract of esophagus E-2; I, extract of intestine I-2.

Fig. 6. S, serum 34-24; R and R^1, extracts of worm residues.

4. OTHER EXTRACTS: Extracts of the head were usually negative but sometimes showed very weak Group 2 and 3 lines. These reactions were considered to be due to traces of excretory gland fluid or possibly secretions from the esophagus rather than to antigens present in the tissues of the cervical region. A single test of worm residues after removal of the excretory glands showed one fairly strong line in Group 2 (Plate II, Fig. 6). It is not immediately apparent why other lines in the 3 groups, that together are characteristic of I-2 and E-2, were absent. Possibly the extraction procedures were adequate for the relatively small quantities of tissue isolates but not the much bulkier worm residues. The body fluid occasionally showed a Group 2 line which was too weak to compare with the other precipitates. Extracts of the cuticle, gonads, and coelomocytes did not contain any precipitinogens detectable by our methods.

C. PROTEINS IN EXTRACTS AND GLAND CONTENTS: The protein content of a number of whole gland extracts, collections of gland contents, and extracts of various other tissues is given in Table 1. Excretory gland fluid (EGC-16 su), separated from the formed constituents within 1 hour after dissection, contained relatively small amounts of protein. Similar su preparations of lots 18, 19, and 20 that were stored for 18 hours before centrifugation contained 4 to 5 times as much.

EGT su preparations had a higher protein content than EGC su preparations, which may indicate the presence of extractable protein from the gland tissue. It is also probable that homogenization of EGT released soluble materials from the formed elements that were present as residual gland contents. Extraction time, after the first 18 hours, appeared to have little effect on the protein content of the various preparations.

D. TITRATION OF VARIOUS PREPARATIONS: Serial dilutions of some of the preparations were tested against "immune" serum from an experimental case of stephanuriasis. The results of testing the four fractions of 18, 19, and 20 show (Table 1) that there is a correlation between protein content and titer. As expected, the position of a specific line or lines showed a gradual shift toward the wells containing the more dilute antigens. The highest titer for E-2 was obtained at a serum dilution of 1:8.

DISCUSSION

The association of antigenic activity with extracts of the intestine or esophagus and with excretory gland contents of *Stephanurus dentatus* is in accordance with the views first expressed by Sarles (1937) on the relative antigenic importance of metabolic products. Proof of such antigenic activity has been furnished by the serological assay of nematode incubation fluids containing metabolic products (Thorson, 1953; Sadun and Norman, 1957; Soulsby, Sommerville and Stewart, 1959; and Olson, Richards, and Ewert, 1960). However, because of the conditions under which they were collected, the specific origin of these antigens could not be determined. Association of the secretions of a particular organ with antigenic activity was shown by Thorson (1956a, b) for extracts of the esophagus of *Ancylostoma caninum*. Soulsby, Sommerville, and Stewart (1959) and Soulsby and Stewart (1960) demonstrated the antigenicity of "exsheathing fluid" collected from molting infective larvae of *Haemonchus contortus*. According to evidence presented by Rogers and Sommerville (1957) and Sommerville (1957), this fluid is secreted by cells near the base of the esophagus.

In the present study, it has been possible to establish unequivocally that the excretory gland cells of *S. dentatus* are the source of antigens that, free of tissue extracts or other extraneous materials, form specific precipitates with sera from infected animals. Enigk and Grittner (1952) have theorized that the formed elements found in the excretory glands of *Strongylus vulgaris* are the end-products of protein metabolism. Our results with *S. dentatus* show that soluble protein is present in small quantities in the gland fluid. Larger quantities, present in the formed elements, are extractable with buffer. Although our observations on the structure of the corpuscles do not indicate that they themselves are end-products, they do not exclude the possibility that the extractable proteins in the corpuscles and those present in solution in the gland fluid are metabolic end-products. Indeed, the presence of relatively large amounts of dissolved or soluble proteins, among which are potent antigens, suggests that this may be the case.

Antigens in intestinal extracts, which formed 6 precipitate bands with sera from infected animals, were apparently not related to those of the gland contents. However, serum absorption studies are needed before a definitive statement can be made. Although we did not attempt to evaluate the participation of tissue extracts in these reactions, we consider it likely that at least some of the antigenic activity was derived from soluble materials in the lumen.

We were not able to ascertain the source of the antigen in esophageal extracts; but we consider it likely, in view of the reports by Thorson (1956a, b), that it is an esophageal secretion.

Although there is ample contact between the cuticle of *S. dentatus* and the internal milieu of the host, precipitins against cuticular extracts, as reported by Oliver-Gonzalez (1943) from the sera of rabbits infected with *Ascaris lumbricoides,* were never encountered. Precipitinogens in *Ascaris* sperm and egg, also reported by Oliver-Gonzalez, were not found in corresponding tissues of *S. dentatus*. Extracts of coelomocytes, cells considered by Enigk and Grittner (1952) and others to have an excretory function, were also negative. However, since the quantity of coelomocyte extract was limited, we do not consider the failure to find precipitinogens in a few tests as conclusive.

The "prefractionation" by isolation of tissues and organs demonstrates that more antigen-antibody systems are present than those previously determined using whole-worm extract. As such, this only serves to increase the complexity of the relationship. However, all or most of the antigens which may be considered to give nonspecific reactions (Tromba and Baisden, 1963) are apparently restricted to the excretory glands. This indicates that other preparations, particularly intestinal extracts, may be more suitable as diagnostic antigens.

SUMMARY

Extracts of isolated tissues and fluids of *Stephanurus dentatus* were tested for precipitinogens against sera of experimentally infected swine. Tissue-free excretory gland cell contents and extracts of whole excretory gland cells gave 8 bands of precipitate with some sera. A comparison of the reactions indicated that excretory gland cell tissue did not contain any precipitinogens not present in the gland contents.

Analysis of excretory gland contents showed that some protein was present in the fluid portion and a larger amount in the formed elements. Titration of a series of preparations showed a positive correlation between protein content and titer.

Extracts of the intestine formed 6 precipitate bands none of which were apparently related to those of the excretory gland. Extracts of the esophagus formed 1 band which was not related to bands formed against other tissue isolated. The single band formed by the body fluid was too weak for comparison. Extracts of the cuticle, gonads, coelomocytes, and head were negative.

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Isolation and Ecological Observations of *Panagrodontus* sp. (Nematoda: Cephalobidae) in pitcher plants (Sarracenia sledgei)*

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Abstract

Nematodes tentatively assigned to the genus *Panagrodontus* have been repeatedly isolated from the tubes of the pitcher plant, *Sarracenia sledgei* Macfarlane, in southeast Louisiana. The plant infestation varied from 20 to 50% in March and April, but only 3% of plants collected in June contained nematodes. Temperature and possibly nutrients found within the pitcher tube influence the presence of the nematodes. In addition a fungus, *Mucor* sp., a Gram-negative coccus or a Gram-negative bacillus must be present in the plant. In vitro the nematode sex ratio was one male to 10 females on bacterial cultures and one male to 20 females on fungus cultures. Cultures of the nematode have been maintained for over 9 months.

FIELD OBSERVATIONS: Two series of plant collections were made in March and April and another one in June, 1960. In March approximately 50% of the 27 plants used for observation were found to be infested. Individual leaf populations of the nematodes ranged from 4,576 to 44,644, and the male to female sex ratio was 1:10. In the April collections 40% of the plants were infested and individual leaf populations varied from 1,140 to 64,296. The sex ratio remained the same. Of the 71 plants collected in June of 1961 only 2 contained nematodes.

ISOLATION and CULTURING: One inch sections of the pitcher tube were chopped and placed in large test tubes with 25 ml of distilled water and incubated at 25° C for two hours. The nematodes were then poured off, and 5 ml of the suspension was placed in 20 grams of commercial rolled oats (Quaker) in an 100 x 15 mm Petri dish.

Isolations were made to determine the nature and extent of the associated microbial flora by streaking dilutions of the contents of the pitcher plant tube on crystal violet agar, Littman oxgall agar, Wort agar, and nutrient agar. Incubations were at 20° and 37° C for 24 hours. A prevalent Gram-negative bacterium produced large spreading colonies on all media. The *Mucor* fungus was restricted to Littman oxgall agar.

Culturing of the nematodes was unsuccessful until the Gram-negative bacterium was isolated and added to the culture medium. A successful culturing method involved the inoculation of the bacterium and nematodes on the medium simultaneously, followed three days later by the fungus. It has been possible to maintain cultures over 9 months. No other fungus has been found to serve in place of the *Mucor* in the cultures.

The male to female sex ratio in laboratory cultures dropped from 1:10 on bacterial cultures to 1:20 on the fungus cultures.

DISCUSSION AND CONCLUSIONS

It seems from the obvious variation of the microhabitat, temperature, food composition, plant condition, etc., that the nematodes are tolerant of a

*Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, The Louisiana Agricultural Experiment Station; Science Department, State College of Iowa; and Louisiana Division Loyola University.

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wide range of conditions, and that they may persist in the dead and decaying leaves in the soil while awaiting the return of more favorable conditions. As the old leaves decay, new ones are maturing from the same root stock and hence are open to infestation. Nematodes belonging to the Cephalobidae are known to migrate across moist (dew, rain) surfaces of vegetation, Goodey (1951).

Hooker (1875) described 4 zones, the attractive, conducting, glandular and detentive, which composes the pitcher plant leaf. The third zone has numerous gland cells which secrete the digestive enzymes. Microbial action also assists in digesting captured fauna. In *S. sledgei* the glandular zone composes approximately ½ of the total leaf length. Nematodes have been isolated from the third zone under field conditions and from third and fourth zones under greenhouse conditions.

The inhabitants reported by Lloyd (1942) to occur within the tube of the pitcher plant leaf, disregarding "trapped" food material, are extremely diversified. *Nepenthes*, an old world genus of pitcher plants, houses such diverse forms as algae, protozoans, and small tree frogs. *Sarracenia* is known to provide lodging for the larvae of mosquitos and small moths, but no known prior mention has been made of nematodes in the pitcher of either genus.

In Louisiana, invertebrate animals other than nematodes, isolated from the pitcher tubes, include bdelloid rotifers and water mites (Hydroacarina). Protozoans in the form of small ciliates were found, and in one collection from Alton a tree frog was observed in the second zone of the tube.

No evidence could be found of the nematode burrowing up the plant from the soil. Entry into the leaf most probably is affected by an arthropod vector and splashing rain. All other members of the genus have come from the frass of bark beetles.

From the observation made thus far it is indicated that the nematode uses the pitcher plant leaf as a normal microhabitat, since the percent infestation and the population levels are too high to consider the organism as an occasional visitant. Whether or not the nematode has evolved some sort of relationship with or within the plant is not known. The presence of plant enzymes complicates the problem of interdepence between the plant, bacteria, fungus, and nematode in the breaking down and utilization of animal and other remains for food. Therefore, it is not yet known if these organisms are competitive, commensal, or symbiotic.

As mentioned above, the nematode is probably a new species of the genus *Panagrodontus* Thorne, 1935. It is being studied and will be described and named in another paper.

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Odeningotrema apidion n. sp. (Trematoda: Lecithodendriidae) from a Malayan Primitive Primate

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Trematodes appear to be uncommon parasites of tree shrews (Family Tupaiidae) in most localities in Malaya and Borneo. Except for a few records and descriptions of nematodes (Myers, 1960; Dunn, 1963 and in press) the endoparasites of these primitive primates of the Oriental Region are almost unknown, and the parasitological literature contains no descriptions or records for trematodes from these hosts (Yamaguti, 1958). The writer has had the opportunity to examine more than one hundred tree shrews from various parts of Malaysia for helminths and other parasites during 1962 and 1963. Although many cestodes and nematodes have been collected, no trematodes were found until a group of 17 *Tupaia glis* from Kedah Peak (Gunong Jerai) in northwestern Malaya were examined in March 1963. The animals were live-trapped on the mountain at altitudes between 2900′ and 3750′. Thirteen were brought alive to this Institute for dissection under optimal conditions; the other four animals were dissected in the field. In addition to a variety of nematodes and a few cestodes, three species of trematodes were found in the tree shrews. The present paper is concerned with one of these species, a tiny lecithodendriid fluke belonging to the recently-defined Subfamily Odeningotrematinae Rohde, 1962.

The flukes were recovered from the small intestine of one of the 17 animals examined. This animal (R 52,353) was trapped at 3750′ just below the summit of the peak in stunted xerophytic forest on 17 March 1963. The animal, an adult female, was brought alive to Kuala Lumpur, caged temporarily in an animal room, and dissected several days later. Eighteen extremely small flukes were collected, fixed in hot water, and stored in 70% alcohol with glycerine. Fourteen specimens were stained with Semichon's carmine and mounted in Canada balsam. No pressure was applied in fixing or mounting the flukes, aside from the weight of the coverslip itself. The following description is based on observations and measurements of 13 intact specimens.

Odeningotrema apidion n. sp.

DESCRIPTION: Minute trematodes, about half a millimeter in length. Body pyriform, dorso-ventrally flattened; entirely covered with spines, very small at anterior and increasing in size toward posterior end. Maximum breadth of body posterior to mid-body. Oral sucker subterminal, slightly broader than long, and conspicuously smaller than acetabulum. Prepharynx short but well-defined, pharynx broader than long, esophagus relatively long. Bifurcation at level of or slightly anterior to anterior border of acetabulum. Caeca short, ending at mid-acetabular level. Inner margins of caeca often partially overlapping lateral margins of acetabulum. Vitellaria copious, bunches of follicles extending from level of pharynx to near posterior end, and across midline at levels posterior to testes and anterior to acetabulum. Cirrus sac moderately large, curved or hooked, and wholly or partially dorsal to acetabulum. Seminal vesicle divided into two oval or spherical parts. Prostate gland cells conspicuous. Cirrus short, without spines. Genital pore simple, between acetab-

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ulum and side of body, close to antero-lateral margin of acetabulum (on left side in dorsal view). Acetabulum moderately large, slightly anterior to mid-body, at or to the right of midline. Ovary oval or irregularly lobate, greatly variable in size, and usually overlapping right postero-lateral margin of acetabulum. Testes posterior to acetabulum, symmetrical or subsymmetrical, markedly variable in size. Receptaculum seminis round, oval, or oblong, near midline in triangle formed by testes and acetabulum. Laurer's canal present. Uterus, winding irregularly, extends from level of posterior vitellaria to acetabular zone, but not usually beyond lateral margins of testes. Eggs very small, oval, thin-shelled, and operculate. Excretory vesicle Y-shaped.

MEASUREMENTS: Based on 13 specimens; average followed (in parentheses) by range. All measurements in microns.

Length—526 (476-616); maximum breadth—352 (294-406); diameters of oral sucker—66 x 72 (60-74 x 63-84); acetabulum—98 x 102 (84-109 x 88-112); pharynx—26 x 31 (23-32 x 28-35); length of esophagus—50 (35-60); length of caeca—117 (98-140); right testis—107 x 126 (70-133 x 109-144); left testis—116 x 128 (70-151 x 98-154); ovary—87 x 110 (56-123 x 56-147); receptaculum seminis—44 x 60 (35-49 x 43-77).

Egg measurements (average and range for 60 eggs in 6 flukes): 18.5 x 33.5 (17.5-21 x 30-36.5).

Holotype measurements: Length—511; maximum breadth—294; oral sucker—63 x 74; acetabulum—88 x 93; pharynx—26 x 30; esophagus length—60; length of caeca—98; right testis—98 x 109; left testis—105 x 119; ovary—70 x 81; receptaculum seminis—46 x 63.

HOST: *Tupaia glis* (Diard)—the common tupaia or tree shrew.

LOCATION: Small intestine.

TYPE LOCALITY: 3750′ on Kedah Peak (Gunong Jerai), Kedah, Federation of Malaya.

HOLOTYPE: U. S. National Museum Helminthological Collection. Paratypes—helminthological collections of Department of Zoology, University of Malaya and Division of Parasitology, San Francisco Medical Center, University of California.

DISCUSSION

The Subfamily Odeningotrematinae was established to accommodate two new lecithodendriid trematodes recovered from the Malayan slow loris, *Nycticebus coucang,* a primitive primate of the Oriental Region (Rohde, 1962 a). These trematodes, *Novetrema nycticebi* and *Odeningotrema bivesicularis,* considered distinct at the generic level, were shown to differ from other species of the Family Lecithodendriidae in having "a transverse cirrus sac at the level of the anterior margin of the acetabulum, a genital pore between the acetabulum and the side of the body at the level of the anterior margin of the acetabulum, and vitellaria which fill the posterior part and sides of the body, beginning at the level of the pharynx."

The major characters distinguishing *Novetrema* and *Odeningotrema* were shown to be: an acetabulum smaller than the oral sucker in *Novetrema,* and larger than the oral sucker in *Odeningotrema*; eggs of *Novetrema* somewhat larger and less numerous in the uterus than those of *Odeningotrema*; and a cirrus sac larger, longer, and extending well lateral to the acetabulum in *Novetrema* in contrast to a relatively small, compact, cirrus sac hugging the anterior margin of the acetabulum in *Odeningotrema.*

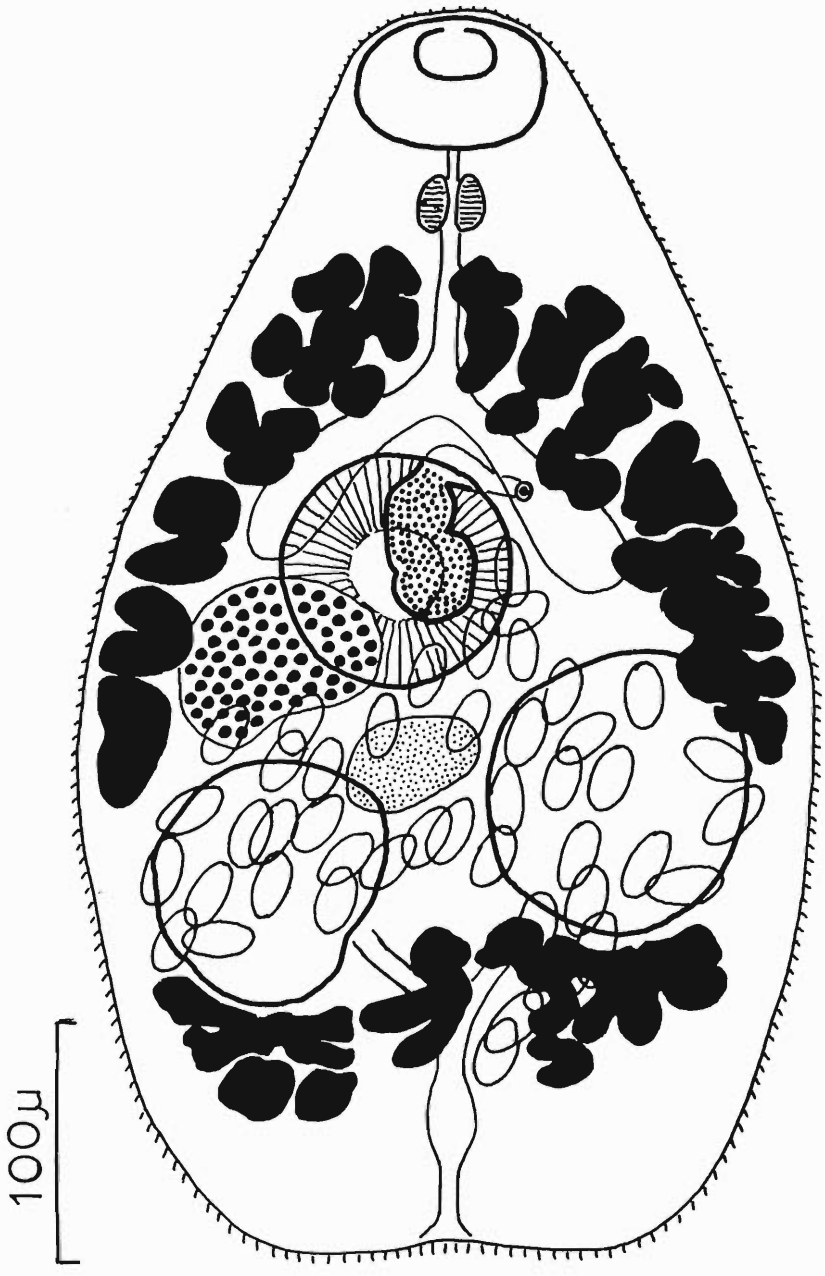

Fig. I. *Odeningotrema apidion* n.sp. Ventral view of holotype, drawn from whole mount. Excretory vesicle partially based on paratype specimens.

Recently Rohde (1962 b) has described a second species of *Odeningotrema* from a Malayan mammal, this time an insectivore, the short-tailed shrew, *Hylomys suillus*. The new species, *O. hypergenitalis*, closely resembles *O. bivesicularis* except in having larger genital organs, shorter eggs, and more copious vitellaria. Rohde also reports (personal communication; in a paper to be submitted) that he has recovered what appears to be *O. bivesicularis* from an insectivorous bat, *Cheiromeles torquatus*, collected at the same locality in Pahang State from which came the infected *Hylomys*. Thus, at this writing, species of *Odeningotrema* have been found parasitizing Malayan primitive primates, insectivores, and bats.

The trematode described herein clearly belongs in the genus *Odeningotrema*, but there has been some difficulty in arriving at a decision concerning the specific status of the parasite because, in many respects, it appears to be an intermediate form between *O. bivesicularis* and *O. hypergenitalis*. In Table I. the average and range measurements for the three forms are compared to emphasize the extent to which *O. apidion* stands between the other two species in measurements. On the basis of these measurements alone (and superficial morphological comparisons) one might be tempted to assign the tree shrew trematode to *O. bivesicularis*, and to designate *O. hypergenitalis* a synonym of *O. bivesicularis*. There are, however, several consistent characters which may be used to distinguish the new form from the other two species. These are: 1. The cirrus sac, which in *O. apidion* is hooked or curved and largely dorsal to the anterior half of the acetabulum, is transverse and almost entirely anterior to the acetabulum in the other species. 2. The caecal bifurcation is just anterior to or at the level of the anterior margin of the acetabulum in *O. apidion* and well anterior to the acetabulum in the other species. 3. The caeca, which are well-separated from the acetabulum in the other two species, run close to the lateral margins of the acetabulum (sometimes with actual overlapping) in *O. apidion*. 4. The eggs of *O. apidion* are definitely smaller than those of *O. bivesicularis* and probably of *O. hypergenitalis* as well.

Although the development of the vitellaria in *O. apidion* is usually much more extensive than that in *O. bivesicularis*, sometimes almost matching that in *O. hypergenitalis*, the writer doubts that the *extent* of this development will prove to be a criterion of much value in distinguishing these and related species. In the members of this Subfamily the extent of vitelline development may prove to be a highly variable character. In a small series of specimens of *Novetrema nycticebi* recently obtained from the intestine of a slow loris, *Nycticebus coucang*, collected at Bukit Lagong Forest Reserve near Kuala Lumpur, the vitelline development was much more extensive than in Rohde's paratype series; indeed nearly as extensive as the development of the vitellaria in *O. hypergenitalis*.

With this description there are now records for trematodes of the genus *Odeningotrema* from four species of Malayan mammals in three Orders. It is clear that these trematodes are not highly host-specific, and their occurrence in tree shrews as well as in lorises probably has no particular phylogenetic significance. It is also unlikely that these trematodes have a high degree of ecological specificity. Species in the genus have now been recorded from animals trapped at nearly 4000′ on a mountaintop with stunted xerophytic vegetation in northwestern Malaya where there is great seasonal variation in rainfall; from animals collected at about 3000′ in Pahang in a humid rainforest area where there is little seasonal change; and from lorises collected near sea-level in a disturbed forest area near Kuala Lumpur where there is

Table I. Three species of *Odeningotrema*: Comparison of important measurements. Average measurement followed by range in parentheses (all measurements in microns). Data for *O. bivesicularis* and *O. hypergenifalis* from Rohde 1962 a and b.

	O. bivesicularis	*O. apidion*	*O. hypergenitalis*
	(10 specimens)	(13 specimens)	(5 specimens)
Length	540 (470-650)	526 (476-616)	630 (570-690)
Breadth	350 (240-410)	352 (294-406)	420 (360-480)
Oral Sucker	76 × 88 (67-86 × 79-94)	66 × 72 (60-74 × 63-84)	67 × 86 (49-76 × 73-92)
Acetabulum	104 × 113 (85-119 × 96-134)	98 × 102 (84-109 × 88-112)	109 × 109 (95-120 × 101-120)
Pharynx	31 × 36 (25-35 × 31-41)	26 × 31 (23-32 × 28-35)	24 × 31 (27-40 × 21-26)
Length Esophagus	51 (22-74)	50 (35-60)	—
Length Caeca	145 (104-166)	117 (98-140)	—
Testes	76 × 106 (79-134 × 43-122)	112 × 127 (70-151 × 98-154)	133 × 149 (122-180 × 110-153)
Ovary	76 × 78 (65-89 × 46-115)	87 × 110 (56-123 × 56-147)	106 × 145 (90-143 × 122-174)
Receptaculum Seminis	43 × 56 (37-68 × 28-57)	44 × 60 (35-49 × 43-77)	65 × 75 (40-98 × 40-92)
Eggs	21 × 40 (18-22 × 36-47)	18.5 × 33.5 (17.5-21 × 30-36.5)	21 × 36 (19-24 × 34-39)

also little seasonal climatic change. It is worth noting, however, that all of the hosts so far recorded for *Odeningotrema* are wholly or partially insectivorous.

One sentence of the diagnosis of the Subfamily Odeningotrematinae (Rohde, 1962 a) which reads "Cirrus sac transverse, at the level of the anterior margin of the acetabulum." (translation) should be amended to read "Cirrus sac transverse, at the level of the anterior margin of the acetabulum, or curved and largely dorsal to the anterior half of the acetabulum."

SUMMARY

A new species of trematode, *Odeningotrema apidion* (Lecithodendriidae: Odeningotrematinae) is described from a Malayan primitive primate, the common tree shrew, *Tupaia glis*. This is the first trematode to be described from a host in the Family Tupaiidae. The presently-known species of *Odeningotrema* have been collected from four species of Malayan bats, insectivores, and primitive primates.

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Rhynconema subsetosa, a New Species of Marine Nematode, with a Note on the Genus *Phylolaimus* Murphy, 1963*

D. G. MURPHY**

A heretofore undescribed species of *Rhynconema* Cobb, 1920 can be found regularly in sand washings from open beaches along the Oregon Coast. Adults are available throughout the year with apparently no major response in life cycle to season. The specimens described below were collected from sand in the mid-tide zone at Governor Patterson Memorial State Park near Waldport, Oregon, on September 8, 1960. Holotype (male), allotype (female) and paratypes (3 males and 3 females) are maintained on slide OSC OM 62, Oregon State University nematode collection, Corvallis, Oregon.

Rhynconema subsetosa, n. sp.

FEMALE (4): L = 0.73 mm. (0.69-0.77), a = 24.2 (22.4-27.9), b = 4.0 (3.8-4.2), c = 8.1 (7.9-8.3), V = 71.5% (70.1-73.0).

MALE (4): L = 0.67 mm. (0.61-0.76), a = 27.5 (24.9-32.8), b = 4.4 (4.2-4.8), c = 8.4 (7.7-8.8).

DESCRIPTION: Cephalic region elongate, stoma long and cylindrical. Cuticle coarsely annulated, annules being directed forward on the anterior portion of the body, and retrorse on the posterior half. Six lips, each bearing one setose papilla. There are six cephalic setae, slightly less than one-half head diameter in length, and three circles of subcephalic setae located between the amphid and the anterior circle of setae. Long, thin, cervical and somatic setae are located at regular intervals. The amphids are circular, 4.5 to 5.0 microns in diameter (slightly greater than 50% of the corresponding head diameter), and positioned immediately above the base of the stoma.

Cephalad the anteriormost region, bearing the papillae and cephalic setae, is readily differentiated from the remainder of the cephalic region by an appearance of a denser, more solid construction. Head diameter at the level of the cephalic setae is five microns, diameter at posterior end of esophagus is approximately 28 microns. Esophagus cylindrical, terminating in a moderate bulb; cardia oval, long axis directed dorso-ventrally. Nerve-ring is at 35% of the esophagus measured from the base of the stoma. Excretory pore not observed. Intestine highly refractive, lumen containing unidentified oval bodies of nine by five microns.

Female monodelphic, ovary reflexed. Male with one genital papilloid supplement one anal diameter anterior to cloacal opening. Spicula are sharply bent about midway in their length. The gubernaculum is plate-like and has a dorsal apophysis.

Tails are conical, curved ventrally: female tail 4.8 anal diameters long, male tail 3.4 anal diameters long.

REMARKS: *R. subsetosa* is most closely related to *R. hirsuta* Hopper, 1961 collected from the Gulf of Mexico, these two being the only described species of the genus with six cephalic setae rather than ten. The Oregon species differs in having a distinct bulb, males with but one preanal supplement (vs. 3 or 5? in *R. hirsuta*), and spicula with a sharp bend centrally (vs. a moderate bend proximally in *R. hirsuta*).

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**On leave to Zoologisches Staatsinstitut, Hamburg, Germany.

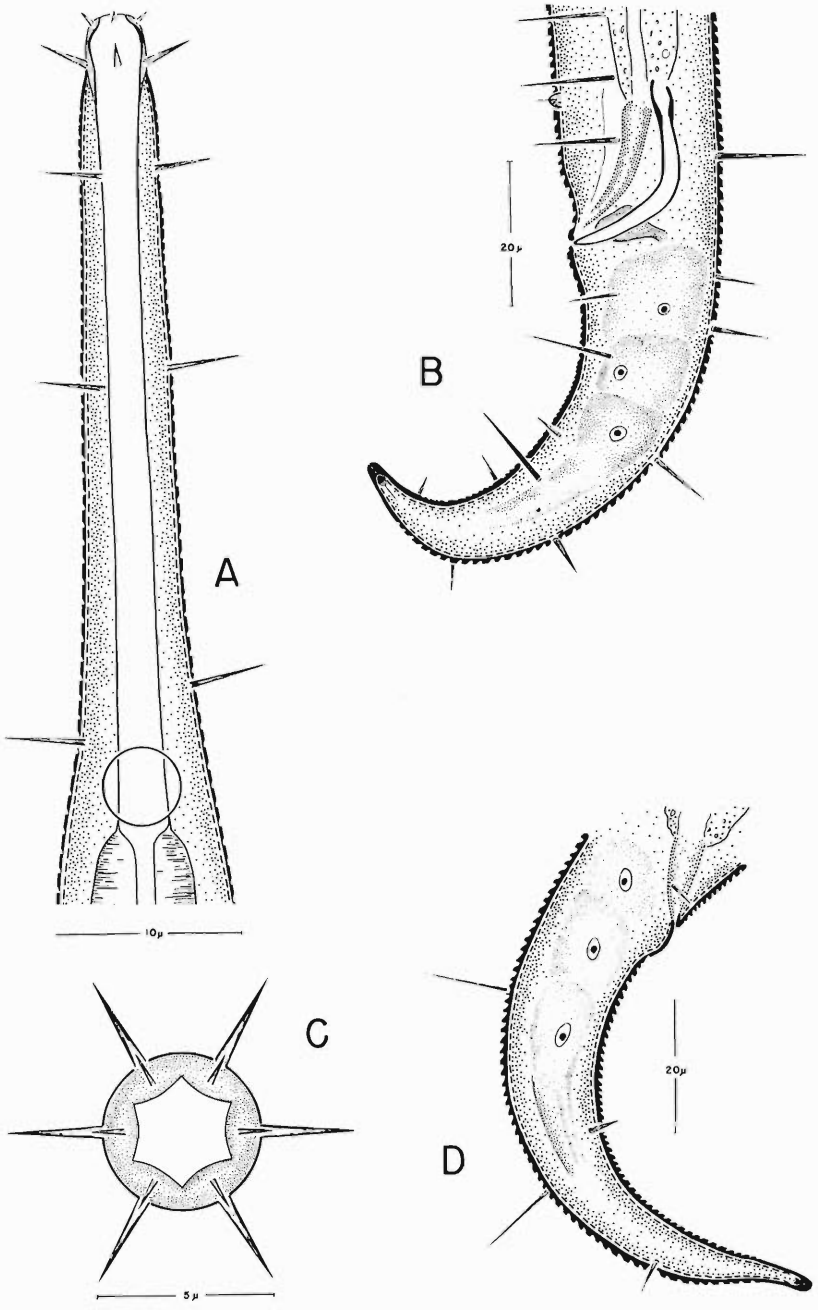

Fig. 1. *Rhynconema subsetosa*, n. sp. A. Anterior region of male. B. Male tail. C. Face view of male. D. Female tail.

Of possible taxonomic significance the new species, in contrast to *R. hirsuta,* possesses three circles rather than one circle of subcephalic setae, and a reflexed rather than outstretched ovary in the females. This latter, especially, may only be a reflection of variation in stages of development.

PHYLLOLAIMUS Murphy, 1963

In the description of this genus I failed to assign a type species, and therefore now designate *P. tridentatus* Murphy, 1963 as type for the genus. Type specimens for the species are deposited in the Oregon State University nematode collection, slide OSC OM 56.

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Studies on Freshwater Larval Trematodes. Part V. A New Polyadenous Xiphidiocercaria, *C. baldai*, from Venezuela.

P. NASIR

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While on a scientific trip to Güiria, coast of the Gulf of Paria, Dr. R. Donoso-Barros, Head of the Biology Departmente, Universidad de Oriente, and Mr. C. Flores, Instituto Oceanográfico, made a collection of snails from Quebrada Seca, a freshwater stream. Six of these snails which later proved to be the specimens of *Australorbis glabratus* (Say) were handed over to the author for the study of larval trematodes, if any. When isolated individually in glass jars containing tap water, only one *A. glabratus* emitted cercariae presented in this paper.

All observations were made on freshly emerged cercariae. Only measurements in mm. are taken on specimens relaxed in neutral red and fixed by addition of an equal volume of ten percent hot Formalin.

Cercaria baldai n. sp. (Fig. 1-2)

DESCRIPTION: Conniae subdivision of polyadenous cercariae. Body 0.264-0.350 × 0.093-0.147. Tail 0.196-0.270 × 0.026-0.031, lodged in a subterminal caudal depression bounded by spined caudal pockets. Body furnished with minute spines and thirteen to fifteen rows of "flagellets." Tail aspinose. Globular bodies irregularly scattered, few, not constituting a remarkable feature of cercaria. Suckers unspined. Oral sucker 0.063- 0.068 in diameter. Ventral sucker 0.041-0.046, located about middle of body. Prepharynx 0.004-0.013 long. Pharynx 0.016-0.018 in diameter. Esophagus 0.013-0.018 long. Ceca extending to anterolateral border of ventral sucker. Stylet without a basal bulb, 0.033-0.039 long, Javelin-shaped. Width of shaft 0.004-0.008; width of shoulder 0.008-0.013; shoulder incomplete ventraly; when viewed from lateral side tip of stylet turned upwards. Stylet glands consisting of eight pairs, coarsely granular, anterolateral to ventral sucker, opening by two pairs of ducts at sides of shoulder. Genital rudiments C-shaped. Ex-

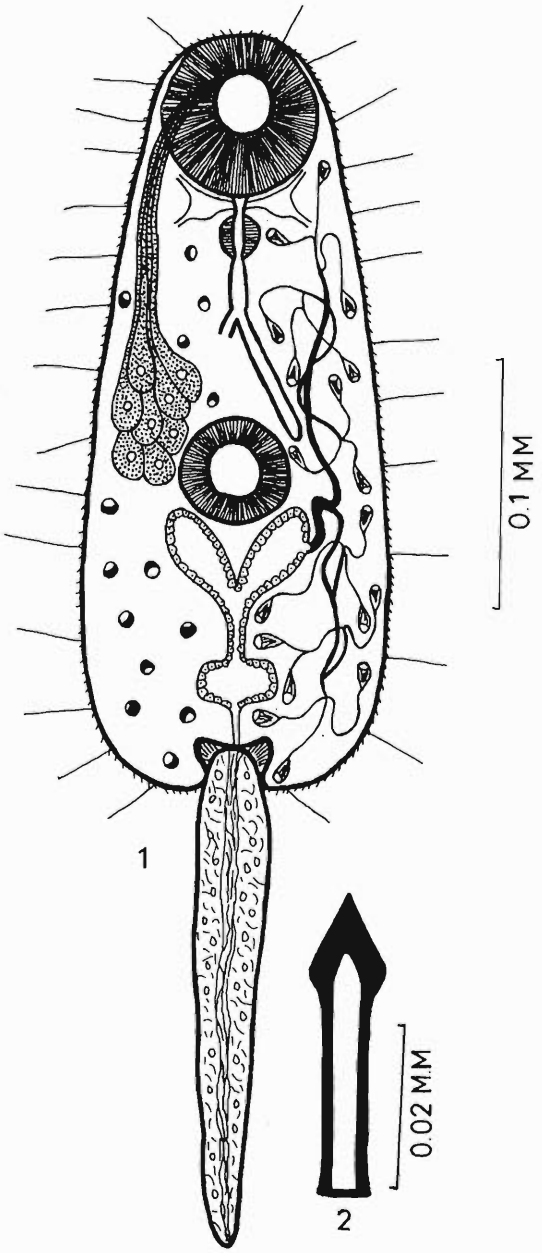

Fig. 1. *Cercaria baldai* showing intestinal cecum, stylet glands, globular bodies, and flame-cells on one side only.
Fig. 2. Stylet of *C. baldai*.

cretory vesicle Y-shaped, lined with cuboidal cells; stem of Y leading into a dilated portion which opens in caudal depression by a narrow duct. Arms of Y extending to posterolateral aspect of ventral sucker. No caudal excretory duct. Main excretory ducts discharging subterminally into corresponding arms of excretory vesicle. Each of main ducts after forming a simple loop receiving anterior and posterior excretory tubules. Flame cell formula: 2[(3+3+3) + (3+3+3)] = 36. Development in sausage shaped sporocysts so characteristic of other cercariae belonging to conniae subdivision. Emergence in early morning and evening hours. Not encysting in open water.

RELATIONSHIP: *Cercaria isocotylea* Cort (1914); *C. polyadena* Cort (1914) = Larva of *Plagiorchis proximus* as described by McMullen (1937); *C. diaphana* Faust (1917); *C. dendritica* Faust (1917); *C. micropharynx* Faust (1917); *C. indicae XVII* Sewell (1922); *C. reptans* Uribe (1925); cercaria of *Dasymetra conferta* as described by McCoy (1928); cercaria of *Pneumatophilus variabilis* as described by McCoy (1928); *C. helvetica XXX* Dubois (1929); *C. sudanensis N° 2* Archibald and Marshall (1931); Cercaria of *Zeugorchis syntomentera* as described by Ingles (1933); *C. acanthocoela* Miller, E. L. (1935); Cercaria of *Plagiorchis muris* as described by McMullen (1937); Cercaria of *P. micracanthos* as described by McMullen (1937); Cercaria of *Tetrapapillatrema concavocorpa* as described by Ralph (1938); *C. holthauseni* Rankin (1939); *C. brevicauda* Byrd and Reiber (1940); *C. diamondi* Brooks (1943); *C. nolfi* Brooks (1943); *C. aalbui* Brooks (1943); *C. eta* Brooks (1948); *C. goodmani* Najarian (1952) = larva of *Plagiorchis goodmani* Najarian (1961); Cercaria of *Plagiorchis (M.) megalorchis* Rees (1952); *C. blukwa* Fain (1953); *C. ramanujami* Peter (1955); cercaria of *Opisthoglyphe locellus* as described by Macy and Moore (1958) and *C. edgbastonensis* Nasir (1960) are other stylet cercariae which like *C. baldai* are characterized with more or less eight pairs of stylet glands and in which the contents of glands are not differentiated into finely or coarsely granular material. Some of these cercariae, *i.e., C. isocotylea, C. polyadena, C. acanthocoela,,* cercaria of *Plagiorchis muris,* cercaria of *Pneumatophilus variabilis, C. nolfi, C. reptans, C. indicae XVII* and cercaria of *Zeugorchis syntomentera* show variations in the number of stylet glands but have to be taken into consideration due to other similarities in their characters, namely, shape and size of stylet and flame cell system.

In order to establish the specific entity of *Cercaria baldai* such characters as shape and size of stylet, extent of esophagus and ceca, definite number of stylet glands, presence or absence of globular refractile bodies, shape of excretory vesicle, origin of main excretory tubes, presence or absence of spines in caudal pockets and a definite number of flame cells have proved to be of diagnostic importance. In the light of these characters and as a result of comparative study, cercaria of *Opisthoglyphe locellus* appears to be the only other larva which resembles *C. baldai* most closely. However, cercaria of *O. locellus* has a smaller stylet, only 25 microns long, main excretory tubes arise terminally, ceca are not discernible and long esophagus extends almost to ventral sucker. All these characters are in direct contradiction with a condition found in *C. baldai,* and, therefore *C baldai* is a distinct species.

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Studies on Freshwater Larval Trematodes. Part III. A New Species of a Strigeid Cercaria, *C. manzanaresensis*, from Venezuela.

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In a previous paper (in press) Nasir described a new species of an echinostome cercaria infecting *Lymnaea* (*Pseudosuccinea*) *columella* Say, collected from Rio Manzanares, near Cumanacoa. The subject of the present investigation was also obtained from *L.* (*P.*) *columella* found in Rio Manzanares about halfway between Cumaná and Cumanacoa. Altogether 59 specimens of *L.* (*P.*) *columella* were collected but only two were discharging *Cercaria manzanaresensis*. Rest of the 57 snails, apparently uninfected, when dissected showed the presence of tetracotyliform larvae in various stages of development and five of them harbored an unknown xiphidiocercaria. Since these xiphidiocercariae did not emerge naturally from their snail hosts, no attempt was made to make a detailed study of these immature larvae. However, some of these cercariae when removed from intercecal spaces of hepato-pancreas were poor swimmers, with poorly developed stylets and in most cases the stylet gland cells were not completely filled with opaque glandular material. Moreover, cuticular spines and those lining the caudal depression were in a poor state of formation. All these underdevelopments, seemingly, allude to the fact that the cercariae, at least those in question in the prevailing circumstances, must spend an extra-sporocystic period in the intercecal spaces of the hepato-pancreas before they are fully mature and eventually make a natural emergence. Stunkard (1930) and Nasir (1960, 1962) observed a similar phenomenon of extra-redial or extra-sporocystic development, as the case may be, in different species of cercariae.

Cercaria manzanaresensis n. sp. (Fig. 1, 2, 3)

DESCRIPTION: Body and tail stem of almost equal length. Furcae slightly larger than either body or tail stem. Body uniformly spinose excepting circumoral spineless area. No oral cap. No forward pointing spines. A pair of "flagellets" about halfway in postacetabular region. A pair of unpigmented eye spots present. Tail stem aspinose, corrugated, bearing 16-18 "flagellets" on either side. 18-20 caudal bodies, last two pairs extending into furcae. Furcae not constricted from tail stem, armed with minute spines. A few scattered bristles on furcae. Ventral sucker not rudimentary, smaller than anterior organ, armed with four concentric rows of spines. Prepharynx and pharynx present. Esophagus, as long as pharynx, not extending to ventral sucker. Ceca septate, extending about halfway in postacetabular region. Penetration glands consisting of three pairs, postacetabular, with finely granular cytoplasm and each enclosing a rounded nucleus. Flame cell formula: $2\ [(2) + (3 + (1))] = 12$. Both complete anterior and posterior excretory commissures present. No ciliations in excretory tubes. Caudal excretory duct dividing posteriorly into two furcal branches which open at outer edges of furcae. Genital rudiments represented by a group of cells anterior to excretory vesicle. Cercariae darting aimlessly in water, occasionally floating with body and tail stem hanging downwards while furcae

stretched apart. Having a tendency to inhabit upper layers of water. Not particularly responding to light. Development in long thread-like knotted sporocysts, characteristic of other strigeid cercariae, with a subterminal birth pore at one end of body. Measurements (in mm.) of ten freshly emerged cercarie, killed by addition of 10% hot formalin. Body 0.147-0.183 × 0.037-0.049; tail stem 0.147-0.178 × 0.035-0.047; furcae 0.169-0.193 long; anterior organ 0.027-0.041 × 0.024-0.027; ventral sucker 0.023-0.038; prepharynx 0.008-0.010 long; pharynx 0.012-0.015 × 0.008-0.012; esophagus 0.012-0.015 long.

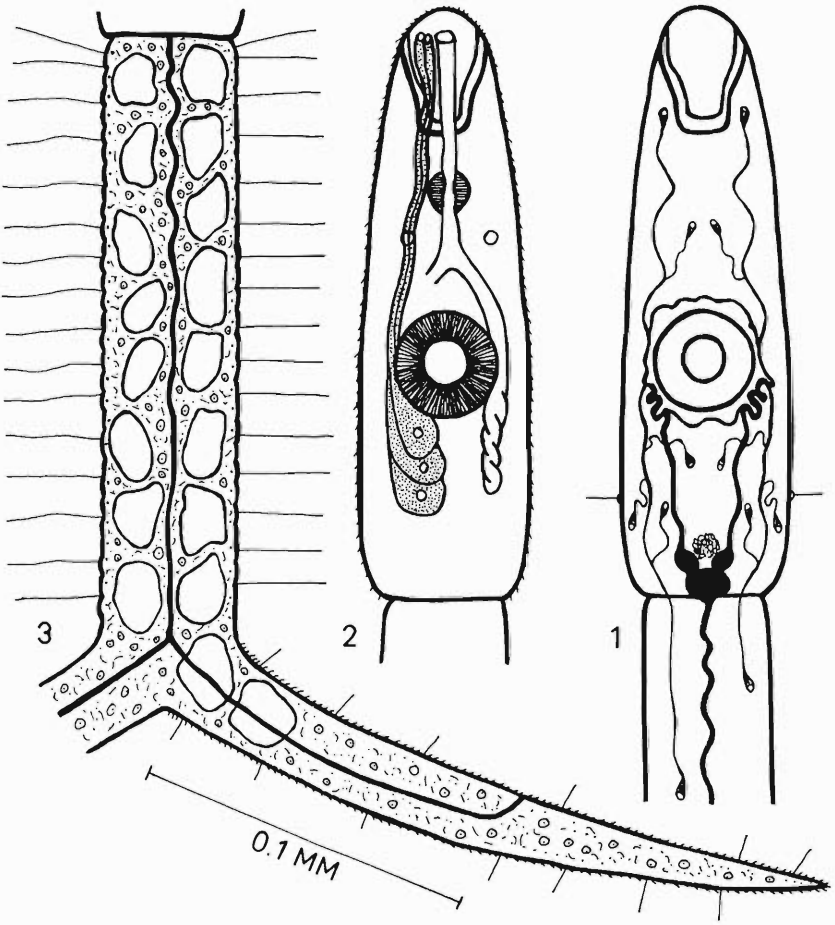

Fig. 1. *Cercaria manzanaresensis* showing flame cell system.
Fig. 2. Intestinal ceca and penetration gland cells drawn on one side only.
Fig. 3. Tail stem and furca of *C. manzanaresensis* showing caudal bodies, flagellets, caudal excretory duct and furcal spination.

DISCUSSION

Cercaria emarginatae Cort (1917), *C. hamata* Miller H. M. (1926), *C. wardlei* Miller H. M. (1926), *C. multicellulata* Miller, E. L. (1936). *C. bessiae* Cort and Brooks (1928), *C. physae* Cort and Brooks (1928), *C. michiganensis* van Haitsma (1930), *Cercaria I* Petersen (1931), *C. frederiksborgensis* Wesenberg-Lund (1934), *C. dohema* Cort and Brackett (1937), *C. scheerpoortia* Porter (1938), *C. riponi* Brackett (1939), *C. wallooni* Olivier (1941), *C. higginsi* Olivier (1941), *C. ancyli* Johnston and Beckwith (1947), *C. Rodhaini* Fain (1953), *C. planorbida* Iles (1959) and *C. roathensis* Erasmus (1960) are other longifurcous pharyngeate distome cercariae which like *C. manzanaresensis* posses six penetration gland cells posterior to ventral sucker. Of all these 18 species of strigeid cercariae *Cercaria roathensis* parasitic in *Planorbis carinatus,* Roath park lake, Cardiff, in Britain, is inseparable from *C. manzanaresensis* in the following points: approximate measurements of body, tail stem, furcae, anterior organ and ventral sucker; lack of apical cap and forward pointing spines; cuticular spination of body and furcae; acetabular spination; extent of intestinal ceca; presence of unpigmented eye spots; flame cell formula and presence of posterior excretory commissure; presence of caudal bodies, flagellets and lack of spination on tail stem. However, in certain other characters the two species are distinctly divergent. The esophagus in *C. manzanaresensis* is short, not extending to ventral sucker, and the ceca are septate in contrast with long esophagus, extending to ventral sucker, and straight ceca of *C. roathensis. C. manzanaresensis* possesses a complete anterior excretory commissure whereas anterior excretory commissure of *C. roathensis* is in the form of two blind ending anterior excretory ducts; the number of caudal bodies in the former ranges from 18-20 in comparison with 13-14 caudal bodies of the latter. The last but not the least difference lies in the phototactic response of the two species. Specimens of *C. roathensis* "concentrate at the side of the tube away from the light and were more numerous in the lower half of the tube" while no such attitude is exhibited by *C. manzanaresensis* which does not show any response to light and the cercariae have a tendency to gather in upper layers of water.

Cercaria bessiae, C. physae, C. rodhaini, C. hamata and *C. multicellulata* have rudimentary ventral suckers and therefore are distinct species from *C. manzanaresensis* where the ventral sucker is not rudimentary. *C. michiganensis, C. wallooni* and *C. higginsi* have 16 flame cells, and 14 flame cells are found in *C. riponi, C, emarginatae, C. wardlei, C. planorbida* as well as in *C. scheerpoortia* in contradistinction with 12 flame cells of *C. manzanaresensis.*

Cercaria dohema and *C. ancyli* have a unique arrangement and appearance of peneration glands, where each penetration gland is tapering posteriorly, unfound in other strigeid cercariae. Besides, these two species are characterized with ten flame cells each.

The flame cell formula of *Cercaria I* and *C. frederiksborgensis* is not known and therefore a fair comparison is not possible.

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Studies on Freshwater Larval Trematodes. Part II. A New Echinostome Cercaria, *C. penalveri*, from Venezuela.*

P. NASIR

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In January 1963, on two different occasions, 73 specimens of *Lymnaea* (*Pseudosuccinea*) *columella Say* were collected from Rio Manzanares near Cumanacoa. Three of the snails, on isolation, were found to discharge an echinostome cercaria without a fin fold on tail while the rest on dissection showed the presence of various precystic stages of unknown tetracotyle as well as echinostome cysts. These echinostome cysts, apparently, belonged to *Cercaria penalveri,* as was evident from their measurements and number of collar spines.

All observations were made on living and naturally emerged cercariae. Only measurements (IN MM.) were taken on fixed material. Fixation was accomplished by relaxing cercariae in a weak solution of neutral red, and then adding 10% hot formalin. This treatment gave the most uniform results.

Cercaria penalveri n. sp. (Fig. 1)

DESCRIPTION: Both surfaces of body armed with transverse rows of spines, right from behind collar to posterior end of body. Posterior to ventral sucker spines decreased in size and rows set further apart. Fine hair-like structures, "flagellets" present on body and tail. Tail unspined, attached subterminally, traversed by two pairs of longitudinal muscle bands, one pair dorsal and other ventral in position. Each muscle band consisting of 5-6 muscle fibres. Posterior extremity of tail invaginable. Collar spines 45 in number, having following arrangement: a group of 6 angle spines and a group of 6 unalternating lateral spines on each side of body while remaining 21 spines set in an alternating dorsal row. Of 21 spines of dorsal row 10 oral, 11 aboral in position. Spines of oral series slightly smaller than those of aboral series. Median spine of dorsal row aboral in position. Ventral sucker protrusible. Esophagus long, dividing almost immediately anterior to ventral sucker, ceca extending to excretory vesicle. Both esophagus and ceca poorly developed, enclosing granular material in contrast to columns of masses found in gut of most other echinostome cercariae. Eight openings at anterior end of body leading into eight ducts, apparently ducts of penetration glands, extending as far back as posterior margin of ventral sucker. Position of penetration glands not determined due to overlying cystogenous gland cells. Cystogenous gland cells enclosing granular and not rod-like cyst material. Excretory vesicle bipartite structure consisting of a smaller anterior and larger more or less rectangular posterior division. Two divisions joined by a narrow duct. Posterior division being a more constant structure. Main lateral excretory tubes, right from ventral sucker to excretory vesicle, contractile. Main excretory tubes, in preacetabular region, enclosing numerous minute excretory granules. Secondary excretory lateral tubules ciliated throughout their extent. Caudal excretory duct dividing into two lateral branches in proximal region of tail. Flame-cell formula: 2 [(3+3+3) +3+ (3+3+3+3)] = 48. Genital rudiments represented by two diffused masses, one in front of and other posterior to ventral sucker. Not negatively photo-

*Cercaria named in the honor of Dr. Luis M. Peñalver who did so much for the establishment of this university.

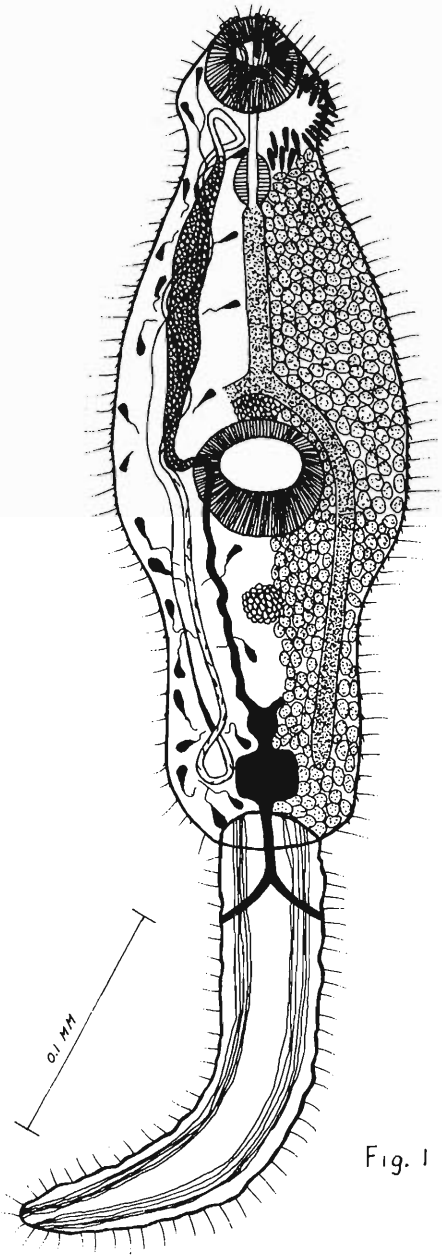

Fig. 1. *Cercaria penalveri* N. SP., collar spines, cystogenous gland cells, intestinal ceca, excretory tubes and flame cells drawn on one side only.

tropic. Cysts (in life) almost spherical, 0.229-0.287 in diameter,

MEASUREMENTS (of ten fixed cercariae): Body 0.312-0.400 × 0.088-0.136. Tail 0.128-0.200 × 0.032-0.056. Oral Sucker 0.037-0.042 × 0.035-0.044. Ventral sucker 0.046-0.054 × 0.048-0.056. Prepharynx 0.008-0.014. Long. Pharynx 0.016-0.025 × 0.010-0.016. Spines (in life) 0.006-0.010 long. Development in typical echinostome rediae with a pharynx, undivided collar, saccate gut and a pair of posterior locomotor appendages.

RELATED SPECIES: cercaria of *Echinoparyphium recurvatum* as described by Mathias (1926) Harper (1929) Azim (1930) Rasin (1933) Wesenberglund (1934), cercaria of *Echinoparyphium flexum* as described by McCoy (1928) Najarian (1954), *Cercaria helvetica XXVI* Dubois (1929), *C. helvetica II* Dubois (1929), cercaria of *Euparyphium murinum* as described by Tubangui (1932), *Cercaria Z* Rees (1932), *Cercaria affinis* Wesenberg-Lund (1934) Ahmed (1959), *C. clelandae* Johnston and Angel (1939), *C. incerta* Komiya (1939) and *C. ellisi* Johnston and Simpson (1944), larva of*Echinoparyphium ellisi* Johnston and Angel (1949) are other echinostome cercariae which like *C. penalveri* lack a fin fold on tail and possess more or less 45 collar spines. Of these ten species of cercariae only *Cercaria Z* and *Cercaria incerta* resemble *C. penadveri* in the following points: A definite number of 45 collar spines, measurements of body and other organ-systems and in having a total number of 48 flame cells. However, they are markedly different species in other fundamental characters. *Cercaria Z* has a group of four angle spines and smaller cysts (0.17 in diam.) in comparison with six angle spines and larger cysts (0.229-0.287) of *C. penalveri*. Moreover *Cercaria Z* is negatively phototropic while *C. penalveri* does not exhibit any such behavior. *C. incerta* also possesses a group of four angle spines in contrast with six angle spines of *C. penalveri*. The most important difference lies in the division of the secondary excretory tubules of the two cercariae. In *C. incerta* each of the secondary excretory tubules bifurcates into two primary excretory tubules at the level of ventral sucker whereas in *C. penalveri* each of the secondary tubules after forming a loop at pharyngeal level continues posteriorly where it forms another loop and then runs anteriorly as a primary excretory tubule ending in three flame cells in the oral region.

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Two New Species of the Nematode Genus *Ektaphelenchus* (Nematoda:Aplelenchoidae) Parasites of Bark Beetles in the Southwestern United States

C. L. Massey*

Nematodes belonging to the genus *Ektaphelenchus* are, for the most part, insect associates or external parasites. The majority of the species are associated with or parasitic on bark beetles. One species, *Ektaphelenchus obtusus* Massey, 1956, was taken from the body cavity of the Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk. This species was also recovered from the egg galleries and from beneath the wing covers of that insect.

The species observed by the writer form small cocoonlike structures beneath the elytra of the host beetle; within the cocoon there may be as many as 50 female nematodes and only 1 male. On numerous occasions the occupants are only females.

Baker, 1962, notes that the genus *Ektaphelenchus* was created by Skrajbin et al., 1954. These writers presented as the genotype *Parasitaphelenchus hylastophilus* form *ateri* Fuchs 1930. Diagnostic characteristics include the flattened caplike head, which is definitely set off. The lips are prominent and distinct. The rectum and anal openings are hardly visible or are absent, the spicules stout, mitten-shaped, with a prominent rostrum. Caudal papillae of the male vary from one to many.

Type specimens of the two new species described are located at Albuquerque, New Mexico, in the collection of the Rocky Mountain Forest and Range Experiment Station.

Ektaphelenchus riograndensis n. sp.

FEMALE (5): 0.75-0.91 mm, a = 0.25-0.34, b = 6.4-8.5, c = ?, V = 80%.

MALE (5): 0.67-0.81 mm, a = 0.24-0.34, b = 6.3-8.0, c = 0.16-0.18.

FEMALE (Fig. 1 A): Lip region caplike, conspicuously flattened. Cuticle moderately thick, marked by moderately coarse transverse striations, the longitudinal striations fine. Spear long, slender, without basal knobs or thickenings. Dorsal esophageal glands at times seven body widths in length. Nerve ring three-fourths body width, posterior to the median bulb of the esophagus. Hemizonid immediately anterior to the excretory pore, which is approximately two body widths posterior to the median bulb. Ovary single, outstretched, in older specimens reaching beyond the distal end of the dorsal esophageal glands; the posterior uterine branch short, usually under a body width in length. Vulva transverse, the lips only slightly protuberant. Anal opening not visible. Terminus subobtuse.

MALE (Fig. 1 C): Testis single, outstretched. Spicules as figured with prominent distal process. Two pairs of ventrosubmedian caudal papillae, one preanal, two postanal. Terminus acute.

DIAGNOSIS: Closely related to *E. tuerkorum* Ruhm 1956. *E. riograndensis* differs in the shape of the ventral rostrum of the spicula, and by the presence of the oddly shaped distal process on that organ.

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*Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, maintained at Fort Collins, Colorado, in cooperation with Colorado State University.

The research reported was completed at the Station's field unit in Albuquerque, New Mexico.

E. riograndensis n. sp. was found associated with *Dendroctonus barberi* Hopk., *Ips integer* Eichh., *Ips ponderosae* Sw., *Ips lecontei* Sw., and *Ips oregonis* Eichh. in *Pinus ponderosa*. It was collected at Bandelier National Monument, New Mexico, and at Prescott, Arizona. Bandelier National Monument is designated as the type locality.

Ektaphelenchus prolobos n. sp.

FEMALE (3): 0.70-0.81 mm, a = 0.35, b = 8.5, c = ?, V = 79%.

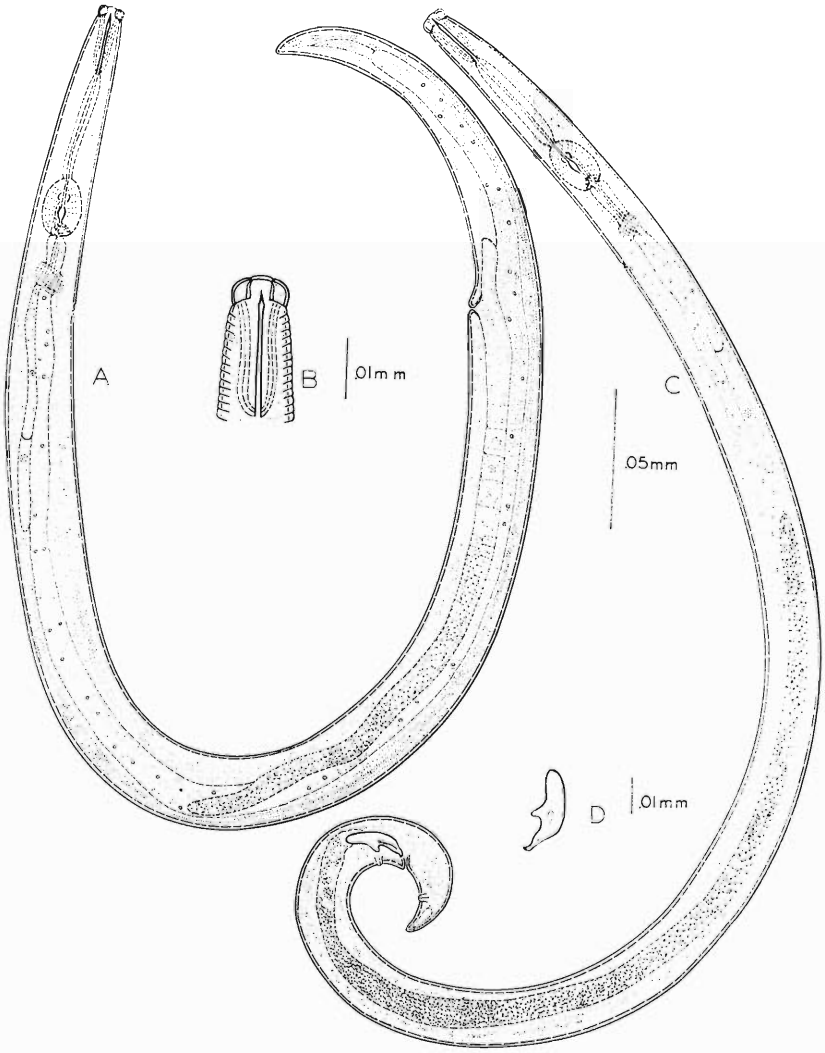

Fig. 1. A-D, *Ektaphelenchus riograndensis* n. sp. A. Female; B. Head; C. Male; D. Spicula.

MALE (3): 0.61-0.66 mm, a = 35.0, b = 7.0, c = 14.0.

FEMALE (Fig. 2 B): Cuticle with moderately fine transverse striations that are more prominent in the region by the head, very fine longitudinal striations; lips offset by a slight constriction, caplike, flattened. Stylet slender, elongate, with basal knobs, twice the length of the body width at the base of the lips. Nerve ring one-half body width, posterior to the median bulb of the esophagus. Excretory pore one body width posterior to the median bulb. Hemizonid apparent immediately anterior to the excretory pore. Ovary single, outstretched, posterior uterine branch not over a body width in length. Vulva transverse, lips not protuberant. Anal opening not visible. Terminus subacute.

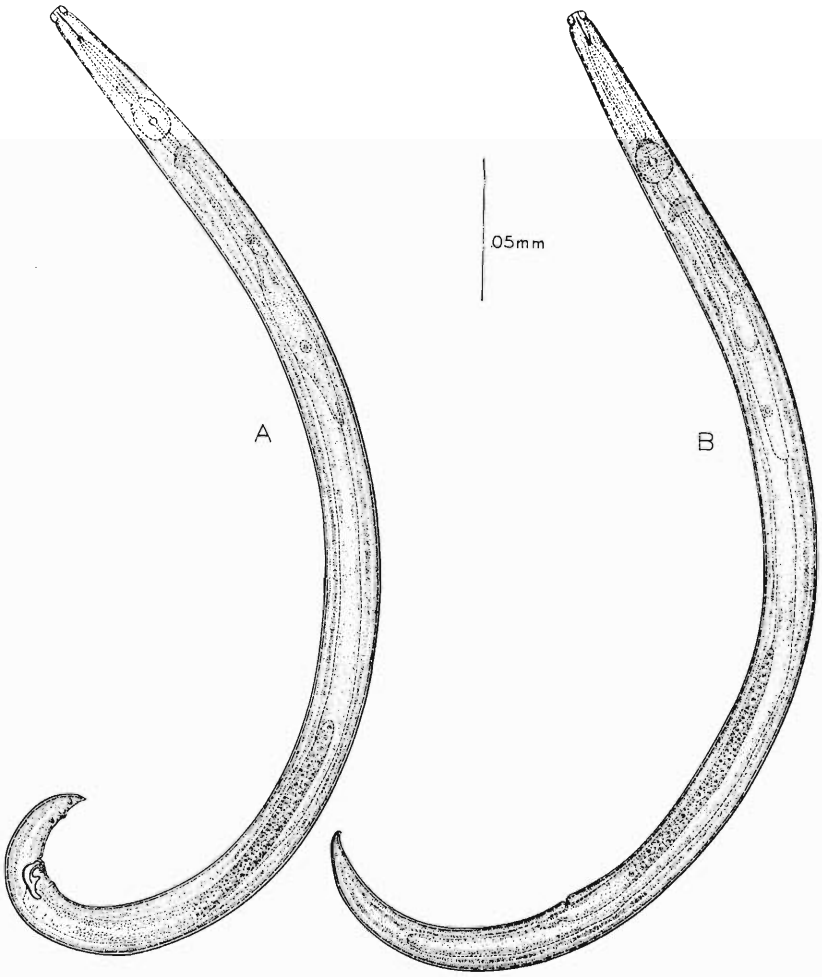

Fig. 2. A-B, *Ektaphelenchus prolobos* n. sp. A. Male; B. Female.

MALE: Testis single, outstretched. Spicula stout, mittenshaped as in Fig. 2 A. There are five pairs of ventro-submedian caudal papillae: one and two preanal, three, four, and five postanal, located as in Fig. 2 A. Terminus acute.

DIAGNOSIS: *E. prolobos* varies from other species in the genus in the number of male caudal papillae and their location, and in the shape of the female tail.

E. prolobos n. sp. was collected from *Abies lasiocarpa* (Hook.) Nutt., where it was associated with *Dryocoetes confusus* Sw. It was collected from the Rio Grande Grant in northern New Mexico near the town of Taos.

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Digenetic Trematodes of Fishes from Palawan Island, Philippines. Part II. Five Opecoelidae, Including Three New Species*

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ**

The digenetic trematodes of this report were part of a collection of parasites of marine fishes made by the junior author while a member of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan, and serving as a guest investigator on the Silliman University-Bishop Museum Expedition to Palawan Island, Republic of the Philippines. Parasites, after washing in saline, were killed in hot water and transferred immediately to FAA fixative. After four to eight hours they were stored in 70 percent alcohol plus two percent glycerine. Staining was variable. All were mounted in balsam. Measurement are in microns.

Opecoelus palawanensis n. sp. (Figs. 1 and 2)

HOSTS: Type, *Parupeneus indicus; P. barberinus* (Mullidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 21 May 1962.

TYPES: USNM Helm. Coll. No. 37890 (one slide of type from *P. indicus*), and No. 37891 (one paratype from *P. barberinus*).

DESCRIPTION (based on five specimens, three mature ones measured and two immature not measured): Body narrow, elongate, unarmed, 2,253 by 255

*Contribution from the Department of Biology, Harpur College State University of New York at Binghamton (J. H. Fischthal).

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(type), depth (dorsoventral extent) in two 189 to 215; forebody short, 242 to 290, narrower than hindbody; posttesticular space (type) 650; posterior extremity round. Oral sucker 70 to 80 by 68 to 82 (depth), subterminal ventral. Acetabulum 104 to 119 by 123 to 143 (depth), on short stalk; bearing four biramous papillae, one on each anterolateral and each posterolateral margin, and four simple papillae, two anteriorly between biramous papillae but more toward acetabular opening and two posteriorly in similar position, simple papillae with short process at base projecting toward acetabular opening; all papillae conical with tip round; biramous papilla, width common base 36 to 37, rami 31 to 72 by 16 to 23 (at base); simple papilla 16 to 26 by 14 to 16 at base and 7 to 9 at tip, basal process length 3 to 4. Sucker length ratio 1:1.30 to 1.56. Prepharynx short, 17 to 24; pharynx round, diameter 53 to 61; esophagus longer than prepharynx, 68 to 106; cecal bifurcation at level of anterior margin of acetabular stalk or slightly anterior. Ceca simple, uniting near posterior extremity, short tube leading to subterminal ventral anus. Excretory bladder tubular, extending forward to ovarian region; pore terminal.

Testes two, tandem, close together, both slightly lobate or anterior testis may be smooth; anterior testis 153 to 224 by 116 to 174, posterior testis (in two) 169 to 230 by 126 to 245; acetabulum to anterior testis 950 to 1,260, to posterior testis (in two) 1,330 to 1,570, distance between testes (in two) 5 to 95. Cirrus sac small, narrow, inconspicuous, 77 to 85 by 22 to 34, at level of cecal bifurcation and anterior portion of acetabular stalk, sinistral; containing muscular cirrus, small pars prostatica, prostate gland cells, and short, tubular seminal vesicle. External seminal vesicle sinuous, narrow, extending 399 to 545 postacetabular (slightly over half way to ovary), not reaching vitellaria. Genital pore left of midline at level of posterior portion of esophagus, 182 to 222 from anterior extremity.

Ovary 135 to 186 by 131 (type), depth (in two) 104 to 116, four lobed, pretesticular, in tandem with testes; acetabulum to ovary 705 to 1,030; ovary to anterior testis 7 to 95. Uterine seminal receptacle; ootype complex overlapping anterior portion of ovary dorsally. Vitelline follicles commencing 190 to 350 preovarian (one fourth to two fifths distance between ovary and acetabulum), also commencing well anterior to ovary in immature specimens, extending without interruption to posterior extremity, invading intergonadal spaces to some extent, confluent posttesticular; vitelline reservoir at anterior portion of ovary. Uterus with few coils between ovary and posterior portion of external seminal vesicle, then ascending either straight or with few slight undulations to metraterm; latter slightly longer than cirrus sac. Eggs large, yellow, operculate, with small knob at anopercular end, 10 measuring 45 to 53 by 28 to 34, younger eggs rounder.

DISCUSSION: In the key to the species of *Opecoelus* prepared by Manter (1954) *O. palawanensis* keyed to *O. tasmanicus* Croweroft, 1947. However, the latter lacked an acetabular stalk, had five or six simple, blunt papillae on each acetabular lip, and the external seminal vesicle did not extend postacetabular. Our species appears closest to *O. adelongatus* described by Nagaty (1954) from *Upenoides vittatus* and *Mulloides auriflamma* from the Red Sea. The number and arrangement of acetabular papillae are as presented for the latter. Nagaty made no mention of a basal process on the simple papillae. Significantly, the vitellaria in all five specimens (mature and immature) of *O. palawanensis* extends well anterior to the ovary, whereas in *O. adelongatus*

it extended only to the ovarian region. Of lesser significance is the round pharynx in the former, whereas it is elongate in the latter.

Pseudopecoeloides carangis (Yamaguti, 1938) Yamaguti, 1940

SYNONYM: *Cymbephallus carangi* Yamaguti, 1938.
HOST: *Sphyraena jello* (Sphyraenidae).
HABITAT: Small intestine.
LOCALITY: Puerto Princesa, Palawan Island, Philippines.
DATE: 20 May 1962.
SPECIMEN DEPOSITED: USNM Helm. Coll. No. 37892.
DESCRIPTION (based on one specimen): Body 1,720 by 330, posterior extremity truncate; forebody 285, hindbody 1,290, posttesticular space 222; oral sucker 99 by 83; acetabulum 145 by 159, completely retracted into body, stalked, no papillae; sucker length ratio 1:1.46; prepharynx length 22, pharynx 68 by 71, esophagus length 121, ceca opening into excretory bladder; anterior testis 203 by 148, posterior testis 222 by 150; acetabulum to anterior testis 560, to posterior testis 770, to posterior extremity of external seminal vesicle 375; ovary 102 by 77, pretesticular, acetabulum to ovary 465; vitellaria uninterrupted opposite gonads, acetabulum to vitelline fields 24 to 46; four eggs 72 to 76 by 41 to 47. Extra ovary present, diameter 73, submedian, dextral, between testes, in contact with posterior testis, slightly overlapping anterior testis ventrally.
DISCUSSION: This species was first described as *Cymbephallus carangi* from *Caranx mertensi* from Japan by Yamaguti (1938). He (1940) added new data from specimens from the same host, and transferred it to his newly created genus *Pseudopecoeloides*. Manter (1940) reported this parasite under its original name from *Selar crumenophthalmus* from Ecuador. Von Wicklen (1946) recorded it from *Polynemus octonemus* from the Gulf of Mexico. Manter (1947) noted its presence in *Caranx bartholomaei* and *C. ruber* from Tortugas, Florida. Yamaguti (1951) recorded it again from *Caranx mertensi* and from *C. equula*.

Plagioporus (*Plagioporus*) *longisaccus* n. sp. (Figs. 3 and 4)

HOST: *Choerodon anchorago* (Labridae).
HABITAT: Small intestine.
LOCALITY: Puerto Princesa, Palawan Island, Philippines.
DATE: 21 May 1962.
TYPE: USNM Helm. Coll. No. 37893.
DESCRIPTION (based on one specimen): Body 990 by 285, elongate, widest at acetabulum, tapering toward both extremities, unarmed; forebody 370,

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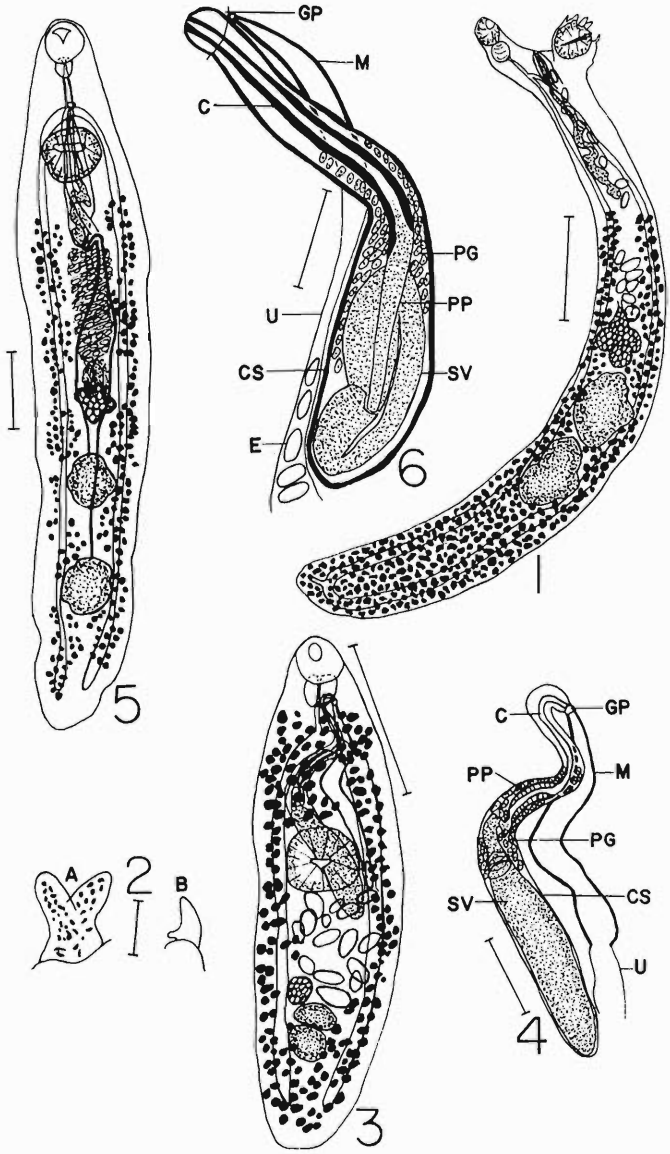
Fig. 1. *Opecoelus palawanensis*, type specimen, anterior fifth of body in dextrolateral view, remainder ventral. Scale 0.3 mm.
Fig. 2. Same. A, Biramous papilla; B, Simple papilla from posterior portion of acetabulum. Scale 0.03 mm.
Fig. 3. *Plagioporus* (*Plagioporus*) *longisaccus*, ventral view of type. Scale 0.3 mm.
Fig. 4. Same. Terminal genitalia, ventral view of type. Scale 0.1 mm.
Fig. 5. *Stenopera rectisaccus*, ventral view of type. Scale 0.3 mm.
Fig. 6. Same. Terminal genitalia, sinistrolateral view of paratype. Scale 0.1 mm.
C, cirrus; CS, cirrus sac; E, egg; GP, genital pore; M, metraterm; PG, prostate gland cells; PP, pars prostatica; SV, seminal vesicle; U, uterus.

hindbody 482, posttesticular space 162. Oral sucker 87 by 70. Acetabulum 138 by 145, just anterior to midbody. Sucker length ratio 1:1.59. Prepharynx short, 10; pharynx round, diameter 55; esophagus 48, longer than prepharynx; cecal bifurcation 191 preacetabular; ceca ending blindly, extending nearly to midlevel of posttesticular space, 85 from posterior extremity. Excretory bladder tubular, extending anteriorly to anterior margin of anterior testis; pore terminal.



Testes two, tandem, in contact, intercecal, transversely elongate; anterior testis 65 by 97, posterior testis 77 by 85; acetabulum to anterior testis 189, to posterior testis 240. Cirrus sac sinuous, very long, extending anteriorly over acetabulum dorsum from 54 postacetabular to posterior region of pharynx, looping to left at genital pore, longitudinal extent 445 by 65 wide, relatively thin walled, containing seminal vesicle, pars prostatica, prostate gland cells, and cirrus. Seminal vesicle long, 305 by 63, commencing postacetabular and terminating preacetabular, with two short loops at distal portion. Pars prostatica thick walled, short, 75 by 17, surrounded by prostate gland cells. Cirrus long, muscular, sinuous within cirrus sac, looping posteriorly to left short distance to genital pore. Latter sinistral to posterior portion of pharynx, 247 preacetabular.

Ovary 75 by 61, 133 postacetabular, submedian to right, slightly overlapping right cecum dorsally, in contact with anterodextral portion of anterior testis. Seminal receptacle transversely elongate, 48 by 26, preovarian, overlapping anterior edge of latter dorsally. Vitelline follicles large, from just postpharyngeal to posterior extremity, confluent dorsally in forebody, filling posttesticular space; vitelline reservoir median. Ootype complex anteromedial to ovary. Uterine coils between anterior testis and acetabulum; metraterm thick walled, long, 295 by 36, commencing sinistral to midacetabular level, sinistral to cirrus sac and following course of latter to genital pore. Eggs large, yellow-brown, 10 measuring 63 to 66 by 31 to 35.

DISCUSSION: This trematode fits readily into the subgenus *Plagioporus* Stafford, 1904. *P. longisaccus* differs from all members of the genus, with two exceptions, in having the cirrus sac extending postacetabular. The exceptions are *P. synagris* described by Yamaguti (1952) from *Synagris* sp. from Japan, and *P. longicirratus* from a triggerfish (Balistidae) from Fiji by Manter (1963). However, both these forms belong in the subgenus *Caudotestis* Isaitschikow, 1928, the latter differing from *Plagioporus* chiefly in not having the ceca extend posttesticular. The species name *longisaccus* (L. *longus*, long; L. *saccus*, sac) refers to the long cirrus sac.

Hamacreadium lethrini Yamaguti, 1934

HOSTS: *Lethrinus hypselopterus* (Lethrinidae), and *Lutjanus gibbus* (Lutjanidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 37894 (one slide with one worm from *L. hypselopterus*), and No. 37895 (two slides with one each from *L. gibbus*).

DESCRIPTION (based on five specimens, four measured): Body 1,870 to 3,080 by 460 to 880; preoral lobe (in three with oral sucker not retracted) 12 to 26; oral sucker 165 to 228 by 179 to 240; acetabulum 245 to 375 by 255 to 385; sucker length ratio 1:1.48 to 1.77; forebody 515 to 1,210, hindbody 885 to 1,500 (includes two with part of hindbody missing); prepharynx length 16 to 63; pharynx 112 to 128 by 114 to 150; esophagus length 97 to 213; testes smooth in younger mature specimen and slightly lobed in fully mature ones; anterior (left) testis 148 to 295 by 133 to 240; posterior testis 179 to 335 by 143 to 265; acetabulum to anterior testis 280 to 390, to posterior testis 365 to 620; posttesticular space, zero to 585; cirrus sac 264 to 550 (longitudinal extent) by 70 to 127; ovary 101 to 310 by 97

to 260; acetabulum to ovary 210 to 255; ovarian lobes two in young mature form and six to eight in fully mature ones; vitellaria commencing postbifurcal, anteriormost margin of vitellaria to acetabulum 225 to 610; genital pore to acetabulum 172 to 460; six older intrauterine eggs 56 to 69 by 39 to 46.

DISCUSSION: Our study was based on one young mature worm from *Lethrinus hypselopterus* (measured) and four fully mature ones from *Lutjanus gibbus* (three measured). Yamaguti (1934) described this species from *Lethrinus haematopterus* from Japan. In one of our specimens the oral sucker was retracted into the body, lying 46 microns posterior to the anteriormost body margin. The acetabular opening is triangular with the apex posteriorly directed, whereas Yamaguti (1934) showed it oval. The posttesticular body was completely missing in one specimen and partly so in another; the lengths in two complete specimens were 555 and 585 microns. The cirrus is protrusible. A short muscular metraterm is present, whereas Yamaguti did not mention one although his figure indicated one. A loop of the uterus extended between the ovary and the anterior testis to the posterior testis in all specimens, whereas Yamaguti did not note this; Manter (1963) indicated a similar variation for *Hamacreadium mutabile* Linton, 1910. The eggs of our specimens were smaller than the 74 to 81 by 45 to 55 listed by Yamaguti.

Stenopera rectisaccus n. sp. (Figs. 5 and 6)

HOST: *Holocentrus violaceus* (Holocentridae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 20 May 1962.

TYPES: USNM Helm. Coll. No. 37896 (one slide of type), and No. 37897 (two slides with one paratype each).

DESCRIPTION (based on three specimens): Body elongate, 2,237 to 2,485 plus (longest worm with part of posttesticular body missing) by 250 to 487, extremities round; preoral lobe (in one) 11, forebody 360 to 420, hindbody 1,806 to 2,100, posttesticular space (in two) 380 to 430. Oral sucker round, 152 to 157 by 148 to 157, subterminal ventral. Acetabulum round to slightly transversely elongate, 184 to 220 by 185 to 230. Sucker length ratio 1:1.21 to 1.40. Prepharynx length (in two) 15 to 41; pharynx slightly longer than wide, 68 to 73 by 61 to 63; esophagus length 109 to 145; cecal bifurcation slightly preacetabular; ceca narrow, extending to within 50 to 150 of posterior extremity. Excretory bladder tubular, extending to ovarian region; pore terminal.

Testes two, tandem, slightly lobed, intercecal, longer than wide, anterior testis at about middle of hindbody; testes 104 to 171 apart; anterior testis 172 to 255 by 121 to 237; posterior testis 186 to 265 by 109 to 255; acetabulum to anterior testis 940 to 1,050, to posterior testis 1,240 to 1,470. Cirrus sac 445 to 570 by 92 to 121, elongate, clavate, straight, median, commencing 177 to 305 postacetabular and terminating 29 to 99 preacetabular at genital pore, may extend posteriorly beyond anterior limit of vitellaria; containing convoluted seminal vesicle, straight, thin walled, short pars prostatica surrounded by prostate gland cells, and straight, long, muscular, thick walled cirrus; cirrus sac tip protrusible through genital pore; latter slightly anterior to cecal bifurcation, very slightly submedian to left. Vasa efferentia very long, uniting to very short vas deferens entering cirrus sac.

Ovary 143 to 172 by 97 to 155, four lobed, median, intercecal, in tandem with testes, 67 to 111 pretesticular, 710 to 805 postacetabular. Seminal receptacle 116 to 140 by 51 to 85, elongate, anterodorsal to ovary; Mehlis gland prominent, anterodorsal to ovary; Laurer's canal opening dorsally anterior to ovary. Vitelline follicles circumcecal, in lateral fields, anterior limit of right field different from left (65-165, 550-275, 95-165 postacetabular in three specimens), terminating near posterior extremity; vitelline reservoir anterodorsal to ovary. Uterus spiraling in diagonal coils intercecally between ovary and posterior portion of cirrus sac, ascending ventral to latter up to anterior portion of acetabulum, then crossing on right; metraterm thick walled, muscular, short, commencing at about level of anterior fifth of acetabulum dorsum. Ten eggs 41 to 51 by 27 to 32, with unipolar filament.

DISCUSSION: *Stenopera* was described by Manter (1933) with *S. equilata* from *Holocentrus ascensionis* from Tortugas, Florida, as type. Sparks (1957) reported this species from the same host from the Bahama Islands. Gupta (1956) described *S. pteroisi* from *Pterois russelli* from India. Comparison of *S. rectisaccus* with the type specimen of *S. equilata* (USNM Helm. Coll. No. 29956) and the description by Manter (1933) indicated several fundamental differences. In the latter the metraterm commences a short distance postacetabular; the cirrus sac is longer and more or less S-shaped, extending around the right border of the acetabulum; the seminal vesicle is straight, and the pars prostatica coiled; the testes are wider than long; and the space between the acetabulum and ovary is relatively shorter. In *S. pteroisi* the cirrus sac is longer and more or less S-shaped; the pars prostatica is coiled; the esophagus is longer and the cecal bifurcation farther preacetabular; the vitellaria extends anteriorly to the level of the acetabulum or slightly preacetabular; and the ovary has seven lobes and is situated an appreciable distance pretesticular. The species name *rectisaccus* (L. *rectus*, straight; L. *saccus*, sac) refers to the straight cirrus sac.

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Some taxonomic studies on the genus *Criconema* (Nematoda:Criconematidae)

A. MORGAN GOLDEN* and WILLIAM FRIEDMAN*

The genus *Criconema* Hofmänner and Menzel, 1914 was thoroughly reviewed and clearly defined for the first time by Taylor (1936) in his monograph of the Criconematinae Taylor, 1936. At that time 8 species, with a key for identification, were included in this genus; two of these were described as new by Taylor, but one of the new species was credited to N. A. Cobb. Another report of the genus was given in 1957, when Chitwood described two new species of *Criconema* and presented a key to 17 species which he included at that time. In 1960 Oostenbrink gave a very detailed account of *Criconema*, made some nomenclatural changes, and presented a key to 23 species which he recognized. The species, *C. limitaneum*, described by Luc as a *Criconema* in 1959 and included in Oostenbrink's key, was transferred to *Criconemoides* by Luc and de Guiran in 1960. For extensive details of the complex history of *Criconema* and its constituent species to about 1959, see the publications by Taylor (1936) and Oostenbrink (1960).

Since 1959 six additional *Criconema* species have been described. Wu in 1960 described *C. celetum* from African violet (*Saintpaulia* sp.) from Quebec, Canada; in 1961 Siddiqi presented descriptions of *C. pruni*, *C. brevicaudatum* and *C. tenuicaudatum* from India; Andrassy in 1962 described *C. hungaricum* from Hungary; and, also in 1962, Siddiqi and Southey described *C. palmatum* from England.

The purposes of the present report are: to describe two new *Criconema* species; to establish and designate type specimens for three previously described species and to give additional data on these and one other species; and to present a key to aid in the identification of the present 30 *Criconema* species.

Criconema eurysoma, n. sp. (Fig. 1 A-C)

MEASUREMENTS: 2 females: Length 0.417 mm. (0.412-0.423); a = 5.0 (5.0-5.1); b = 3.5 (3.5-3.6); c = ?; V = 90%; stylet 85 microns (84-86); total body annules = 44.

HOLOTYPE (female): Length 0.423 mm; a =5.1; b = 3.6; c = ?; V = 90%; stylet 84 microns; total annules = 44. Vulva located on 6th annule from posterior end; excretory pore at 15th annule from anterior end.

MALE: unknown.

DESCRIPTION: Body wide and cylindrical, tapering gradually at each end. Head lightly sclerotized and clearly offset, bearing one very prominent cephalic annule projecting outward and slightly forward which is followed anteriorly by the conspicuous dome-shaped labial region (Fig. 1 B). Cephalic annule bearing a continuous fringe of very small, indistinct bead-like structures or modified spines, producing a somewhat crenate appearance. Second annule (neck or first body annule) reduced in size, projecting forward and without visible spines in specimens examined. Total annules 44, not interrupted by lateral fields. Body annules, especially anterior to region of vulva, projecting forward while annules in vulval region and posterior to vulva

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projecting posteriorly as illustrated (Fig. 1 A). Distinct spines in continuous, comb-like fringe directed posteriorly, numbering about 100 per annule at midbody. Posterior to vulva a few spines become longer and 2 or 3 times the width of the other body spines, appearing somewhat spathulate. Deirids and phasmids not seen. Stylet well developed; basal knobs projecting forward, extending into middle of the criconematoid median bulb. Short isthmus encircled by nerve ring. Esophageal glands forming a small, rather pyriform basal bulb at the base of which is the very indistinct intestine. Excretory pore located at 15th annule from anterior end. Anus not seen clearly and with certainty. Vulva transverse, located on 6th annule which is modified usually as illustrated. Spermatheca absent or not seen. Large muscular uterus followed by single ovary anteriorly outstretched; oocytes in single file except in region of multiplication. Caudal end usually appearing as illustrated (Fig. 1 A).

LARVA (1 specimen) : Length—0.388 mm; a = 5.5; b = 3.7; c = ?. Stylet, 68 microns in length; annules, 44 in number. Body annules and spines similar to females. Excretory pore located at 15th annule from anterior end.

DIAGNOSIS: *Criconema* related to *C. Celetum* Wu, 1960, but differing from this and other described species particularly (1) in having the annules anterior to the vulva projecting forward while the spines extend backwards, (2) in being very wide in relation to length (a = 5.0), and (3) in having excretory pore at 15th annule from anterior end.

HOLOTYPE (female) : Collected by G. Steiner, January 11, 1944 at Beltsville, Maryland. Slide T-41t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARATYPES (female and larva) : same data as holotype. Slides T-116p and T-117p, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

TYPE HABITAT, HOST and LOCALITY: Soil and roots of *Citrus grandis* (L.) Osb. in greenhouse at Beltsville, Maryland.

Criconema sulcatum, n. sp. (Fig. 2 A-G)

MEASUREMENTS: 30 females; length = 0.529 mm. (.437-.635); a = 7.51 (6.51-8.59); b = 3.57 (3.14-4.27); c = ?; V = 91.3% (87.2-94.5); total body annules = 34-46; stylet = 86.9 microns (83.0-96.0).

30 larvae; length = 0.394 mm. (.219-.527); a = 6.52 (5.26-9.0); b = 3.20 (2.41-4.80); c = ?; total body annules = 38-50; stylet = 69.3 microns (49.5-80.0).

HOLOTYPE: (female); length = 0.559 mm., a = 7.69; b= 3.75; c = ?; V = 92.9%; total body annules = 35; stylet = 85.2 microns.

MALE: unknown.

DESCRIPTION: Body relatively stout, tapering gradually at both extremities. Head with one prominent cephalic annule, directed forward and outward, which is followed anteriorly by the sub-lateral lobes around the labial protuberance. First body annule either directed outward or retrorse; succeeding body annules retrorse, averaging about 15 microns apart. In profile, anterior body annules rounded at their edges, becoming increasingly pointed toward the posterior end of the nematode (Fig. 2A). Cuticular scales in 12 longitudinal rows, wider than long; the anterior ones rounded and less prominent than the posterior ones which become longer and irregular in shape. Edge of first annule smooth. Posterior edge of succeeding annules and scales crenate (Fig. 2, B & C). Cuticle of annules with shallow longitudinal grooves

as illustrated. (Fig. 2, B & C). Stylet strong, extending through 6-8 annules, with forward pointing knobs. Dorsal gland opening about 6 microns from base of stylet. Excretory pore 12-15 annules from the anterior end, and 1-4 annules posterior to the base of the esophagus. Ovary single, very long, may extend past the base of the stylet. Vulva 4-8 annules from the terminus. Anus not seen clearly. Terminus with 1 or 2 knobs.

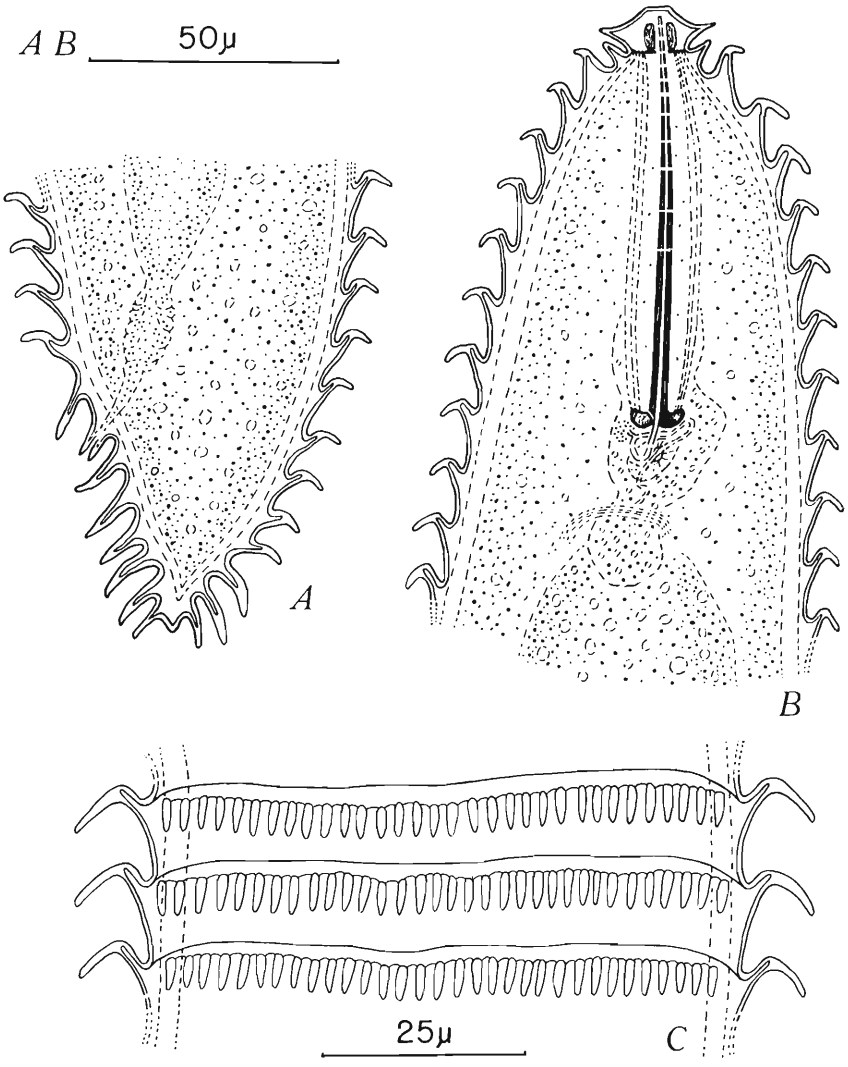


Fig. 1. Female of *Criconema curysoma*, n. sp. A—Posterior portion, B—Anterior portion, C—External view of cutiele at mid-body.

LARVAE: Cuticular scales markedly different from those of the female. First annule crenate. Succeeding annules with scales arranged in 8 longitudinal rows. (Fig. 2, E & F). Each scale with about 6 small rounded protuberances laterally, one anteriorly and one posteriorly.

DIAGNOSIS: *Criconema* differing from other closely related described species principally by single scales or spines arranged in 12 longitudinal rows and by the presence of 34-46 annules with each body annule bearing many longitudinal grooves as illustrated (Fig. 2, B & C).

HOLOTYPE (female): Collected by C. S. Tuthill on November 17, 1958, at Uniondale, Long Island, New York. Slide T-42t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARATYPES (Approximately 200 females and larvae): Specimens deposited in United States Department of Agriculture Nematode Collection, Beltsville, Maryland; University of California Nematode Survey Collection, Davis, California; and Canadian National Collection of Nematodes, Ottawa, Canada.

TYPE HABITAT, HOST and LOCALITY: Soil and roots of *Chrysanthemum* sp. at Uniondale, Long Island, New York.

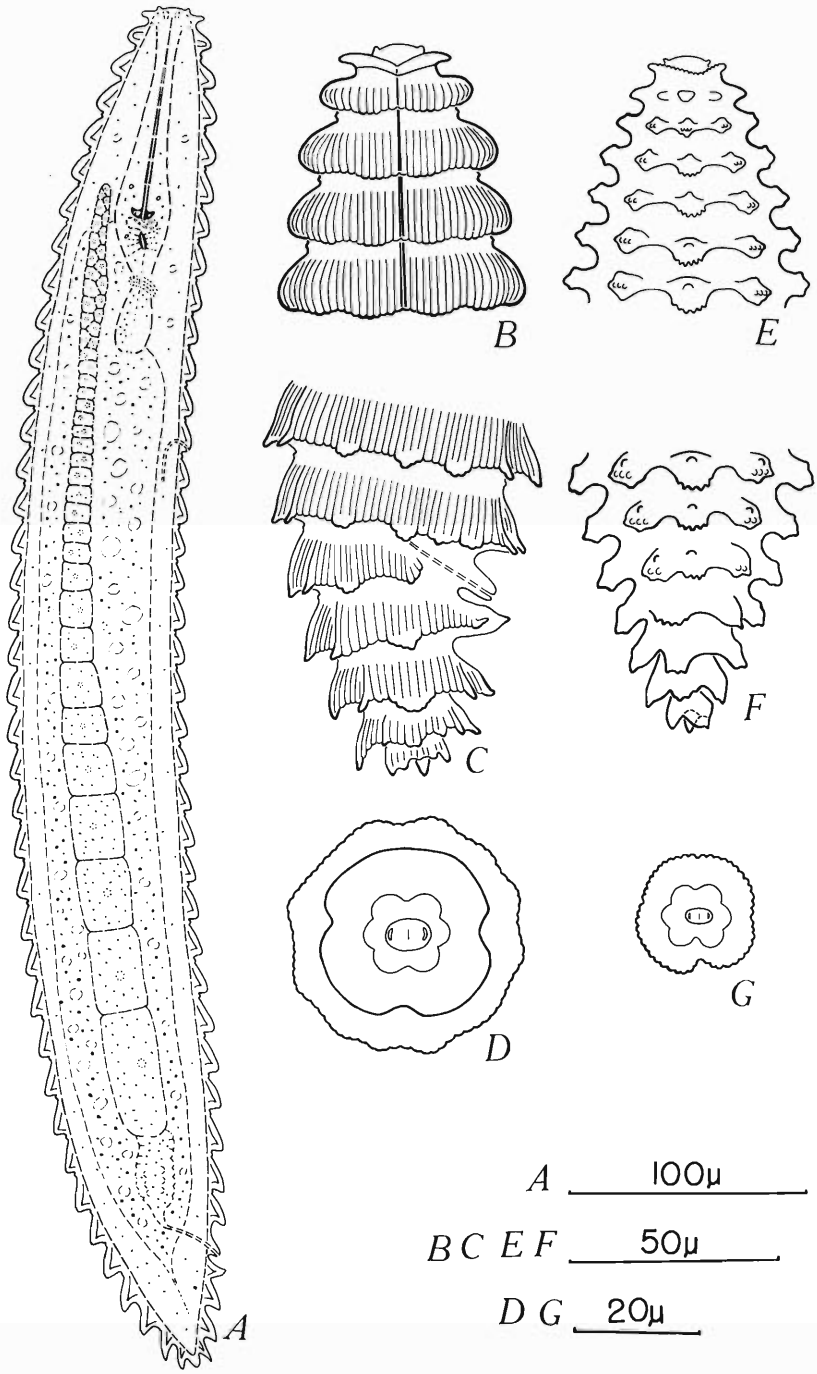
During the study and description of this species, specimens as indicated below were examined from the following areas: 30 females and 51 larvae from the type host and locality, collected by C. S. Tuthill on November 24, 1955; 40 females and 9 larvae, collected from garden soil in Brooklyn, New York by J. Rehn on July 26, 1957; and 30 females and 27 larvae, collected from soil around *Aster trinervius* Roxb. from Japan by A. E. Peters on November 18, 1954.

Criconema palmatum Siddiqi and Southey, 1962 (Fig. 3, A-C)

This interesting species was recently described by Siddiqi and Southey (1962) from several specimens collected from soil around strawberry roots at two locations near Combe Martin, Devonshire, England. An outstanding characteristic of this nematode is the striking hand-like spines which number about 8 per annule and which occur in irregularly alternate positions on adjoining annules.

During the present study on the genus *Criconema*, the writers, unaware of the work by Siddiqi and Southey until its publication late in 1962, prepared a technical description and drawings (Fig. 3, A-C) for a nematode which is evidently *C. palmatum*. Presented in Table 1 are data on key characters of specimens of *C. palmatum* from four new hosts and two new countries of occurrence. It is noted that in these specimens the stylet length is generally a few microns longer than that given by Siddiqi and Southey for their specimens from strawberry, and also, the "a'" value is somewhat less, indicating a greater body width in these specimens from the new hosts and areas. However, since all other characters examined were similar to those given in the original description, these small differences in stylet length and body width are at present best considered as variations of populations within this species rather than as representing a separate taxon.

Fig. 2. *Criconema sulcatum*, n. sp. A—Full-length view of female, B—Anterior part of female, C—Posterior portion of female, D—Outline of 1st and 2nd annules of female head viewed from anterior end, E—Anterior portion of larva, F—Posterior portion of larva, G—Outline of 1st annule of larval head viewed from anterior end.



It is of interest that these specimens were recovered from around woody plants, such occurrence being characteristic of many other *Criconema* species. Also, in all of these samples, relatively many larvae in various stages of development were found in addition to the adults, indicating that the life cycle was being completed.

These specimens have been deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

Criconema inaequale Taylor, 1936. (Fig. 4, A-D)

Through the cooperation of A. L. Taylor, the remaining original material used in the preparation of the description of *C. inaequale* by him in 1936 was made available for study and type designation. Six adult female specimens in balsam were obtained; and when remounted in balsam, these nematodes appeared in very good condition.

Study of the specimens revealed that the measurements and other data were essentially the same as given by Taylor (1936) in the original description. The types as designated herein were selected in consultation with the original author. Some additional measurements are presented.

MEASUREMENT: (Lectotype, female): Length 0.525 mm.; a= 8.6; b = 4.2; c = ?; V = 92%; stylet 68.3 microns; total body annules = 67. Vulva located on 6th annule from posterior end; excretory pore located between 8th and 9th annule from anterior end.

PARALECTOTYPES (5 females): Lenth 0.531 mm. (0.502-0.561); a = 8.5 (7.7-8.9); b = 4.2 (3.4-4.5); c= ?; V = 90.4% (90-91); total body annules 65 (64-66). Vulva located on 5th-6th annule from posterior end; excretory pore located between 8th and 9th annule from anterior end.

LECTOTYPE (female): Collected by A. L. Taylor, August 31, 1933 in woods south of Alexandria, Virginia. Slide T-43t, United States Department of Agricultural Nematode Collection, Beltsville, Maryland.

PARALECTOTYPES (5 females): same data as lectotype. Three specimens in United States Department of Agriculture Nematode Collection, Beltsville, Maryland; 2 specimens in California Nematode Survey Collection, Davis, California.

TYPE HABITAT and LOCALITY: Leaf mould from woods along the Mt. Vernon Memorial Highway about half way between Alexandria and Mt. Vernon, Virginia.

Criconema fimbriatum Cobb in Taylor, 1936 (Fig. 4, E-G)

As with *C. inaequale*, the remaining original material used in the preparation of the published description of *C. fimbriatum* was made available by A. L. Taylor for study and type designation. Only two adult female specimens in balsam were obtained; but when these were remounted in balsam, they were found to be in good condition.

Study of these specimens revealed that with two exceptions, the data and measurements were essentially the same as presented by Taylor (1936) in the original description. The original total length of *C. fimbriatum* was given as 0.57-0.81 mm. with an "a" value of 6. These measurements indicate that this nematode is a very wide one, the body width being as much or more than 100 microns. Examination of the drawings does not agree with this; rather, it suggests that the body width is considerably less, apparently in the range of 50-60 microns. The total length of the present two specimens was found to be

Table 1. Measurements of several key characters of *C. palmatum* from certain new locations and new hosts compared with original data of Siddiqi and Southey.

Host, origin, and date collected	No. of adult specimens examined	Total length (mm.)	Stylet length (microns)	No. of body annules	a (including spines)	b	V%	Vulva from posterior end	Excretory pore (from anterior end
Magnolia grandiflora L. Italy— August 12, 1957	10	0.458 (0.420-0.528)	85.0 (83.0-88.0)	53 (49-57)	6.3 (5.9-7.2)	3.5 (3.1-4.2)	90% (88-91)	5th-6th annule	17th-20th annule
Magnolia sp. Italy— unknown	7	0.450 (0.372-0.550)	85.9 (81.2-88.0)	53 (48-56)	6.6 (5.4-8.2)	3.6 (3.1-4.0)	90.6% (90-91)	5th-7th annule	17th-18th annule
Lobelia sp. Ireland— Oct. 11, 1960	5	0.452 (0.380-0.520)	84.1 (80.6-87.3)	54 (53-56)	6.3 (6.0-6.8)	3.8 (3.7-4.0)	91% (90-92)	6th-7th annule	17th-18th annule
Grape Italy—1961	1	0.454	86.8	56	6.5	3.8	91%	7th annule	obscured by debris
Strawberry England— April 21, 1961	12	0.44 (0.38-0.50)	80 (78-84)	53 (50-57)	10.0 (9.4-11.0) (excluding spines)	3.4 (3.0-3.9)	92.7% (91.4-94.0)	7th annule	17th-19th annule
From Siddiqi & Southey:									

over 100 microns less than the minimum length originally given for *C. fimbriatum*. After consultations with the original author on these two discrepancies in measurements, it was felt and agreed that the total length and "a" value as given below are a more accurate representation of these two characters for this species than was given in the original description. Also, it was established that the description and drawings as presented by Taylor (1936) were based on the specimens collected by him from the area specified herein rather than on the specimens of Cobb mentioned in the description as having been collected near Luray, Virginia.

LECTOTYPE (female): Length 0.430 mm; a = 8.5; b = 3.7; c = ?; V = 87%; stylet 95.2 microns; total body annules = 54. Vulva located on 10th annule from posterior end; excretory pore not been clearly because of position of specimen and presence of debris; approximately 40 spines on each annule at middle of body, not arranged in definite longitudinal rows.

PARALECTOTYPE (1 female); Length 0.405 mm; a = 7.1; b =3.5; c = ?; V = 85%, stylet 96.3 microns; total body annules = 53. Vulva located on 10th annule from posterior end; excretory pore not seen clearly because of position of specimen and presence of debris; spines as on the lectotype.

LECTOTYPE (female); collected by A. L. Taylor, August 31, 1933 in woods south of Alexandria, Virginia. Slide T-44t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARALECTOTYPE (1 female): same data as lectotype. Specimen on slide T-149p, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

TYPE HABITAT *and* LOCALITY: Leaf mould from woods near the Mt. Vernon Memorial Highway about half way between Alexandria and Mount Vernon, Virginia.

Criconema civellae Steiner, 1949 (Fig. 4 H)

This very distinctive species was described by Steiner (1949) from specimens obtained from pummelo (*Citrus grandis* (L.) Osb. growing in a greenhouse at Beltsville, Maryland. Although no type specimens were designated at the time of description, at least some of the specimens used in originally describing this species were retained in the collection at Nematology Investigations, in Beltsville, Maryland. During the course of the present study, these specimens, consisting of one juvenile female and seven larvae in glycerine, were found and remounted glycrine. Examination of this juvenile female showed that it conformed to the description as given by Steiner although somewhat less in total length as would be expected. Data on this specimen, including some measurements not given in the original description, are presented.

MEASUREMENTS (Lectotype, juvenile female): Lenth 0.322 mm; a = 6.0; b = 3.6; c = ?; V = 90%; stylet 64 microns; total body annules = 58. Vulva located at 7tr annule from posterior end; excretory pore not clearly seen because of debris.

PARALECTOTYPES (7 larvae): specimens in various stages of development, giving inconsistent measurements. Cultiicular structure similar to adults, that is, scales in 8 longitudinal rows, with each scale having several (about 4 to 6) stiff, outward- and backward-pointing setae on its posterior edge.

LECTOTYPE (Juvenile female): collected by G. Steiner January 11, 1944 at Beltsville, Maryland. Slide T-45t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

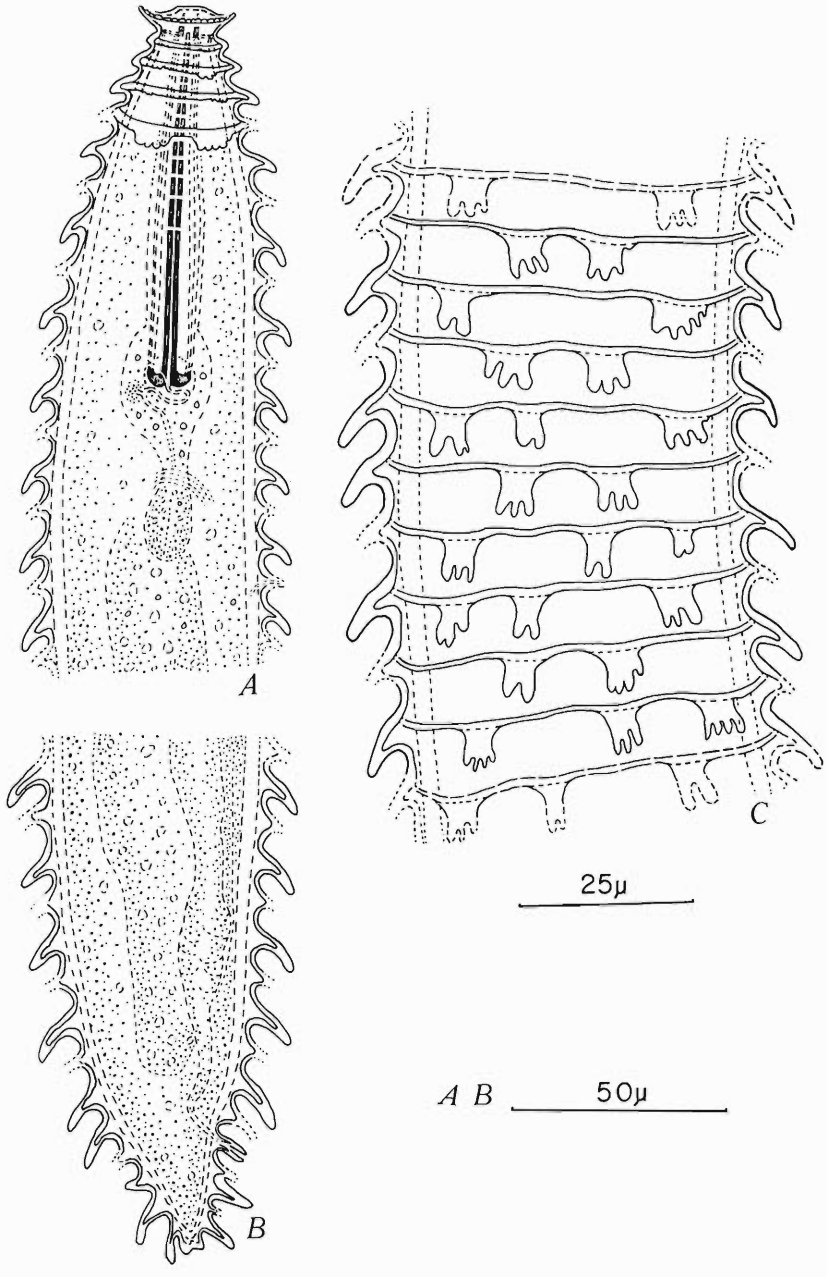


Fig. 3. Female of *Criconema palmatum* Siddiqi and Southey, 1962. A—Anterior portion, B—Posterior portion, C—External view of cuticle at mid-body.

PARALECTOTYPES (7 larvae): same data as lectotype. Five specimens deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland; and 2 specimens in California Nematode Survey Collection, Davis, California.

TYPE HABITAT, HOST and LOCALITY: Soil and roots of *Citrus grandis* (L.) Osb. in the Conservatory greenhouse at Beltsville, Maryland.

On May 13, 1963, the senior author collected a small sample of soil and roots from the type host and locality. Among the nematodes recovered from this sample were a number of adult females and larvae of *C. civellae*. Measurements of 8 of these topotype female specimens were as follows:

Length 0.363 mm. (330-387); a = 5.9 (5.0-6.5); c = ?; V = 89.5% (89-90); stylet 68.4 microns (67.0-70.0); total body annules = 57 (55-60). Vulva located at 7th-8th annule from posterior end; excretory pore not seen clearly because of presence of debris.

These specimens possessed the same characters as the designated types and conformed to the species description as Steiner (1949) presented. Specimens are deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

KEY TO THE SPECIES OF CRICONEMA**

1. Annules 80 or more 2
   Annules 75 or less 6
2. Cuticular spines continuous on each annule; annules 94-108; stylet 66-74 microns ..... *pruni* Siddiqi, 1961.
   Cuticular spines or scales arranged in longitudinal rows of 12 or less 3
3. Annules about 150; stylet 120 microns; scales short and wide, in 8 longitudinal rows ..... *squamosum* (Cobb, 1913) Taylor, 1936.
   Annules less than 120; stylet less than 90 microns 4
4. Scales or spines in 10 longitudinal rows; 86 annules; stylet 66-85 microns ..... *decalineatum* Chitwood, 1957.
   Scales or spines in 6-8 longitudinal rows 5
5. Scales or spines in 6 longitudinal rows, triangular; about 100 annules; stylet 75 microns ..... *guernei* (Certes, 1889) Menzel, 1914.
   Scales or spines in 8 longitudinal rows, setose; about 89 annules; stylet 40 microns ..... *spinalineatum* Chitwood, 1957.
6. Cuticular spines in a continuous fringe 7
   Cuticular scales or spines in a discontinuous fringe 13
7. Stylet 58 microns; 42 annules; 120 short, delicate spines at midbody ..... *brevicaudatum* Siddiqi, 1961.
   Stylet more than 80 microns 8
8. Annules less than 50 9
   Annules more than 50 11
9. Body annules directed forward; spines retrorse ..... *eurysoma*, n. sp.
   Body annules and spines retrorse 11
10. Ventral spines posterior to vulva long and narrow, similar to other body spines ..... *multisquamatum* (Kirjanova, 1948) Chitwood, 1957.
    Ventral spines posterior to vulva broad, with 2-6 finger-like projections; other body spines long and narrow ..... *celetum* Wu, 1960.

**This key is based on females. Juveniles are included only where the female is unknown. The species referred to by Kirjanova (1958) as "*Criconema georgiensis* Kirjan., sp.nov." is not included in this key because of insufficient data in the publication. The actual status of this species remains undetermined.

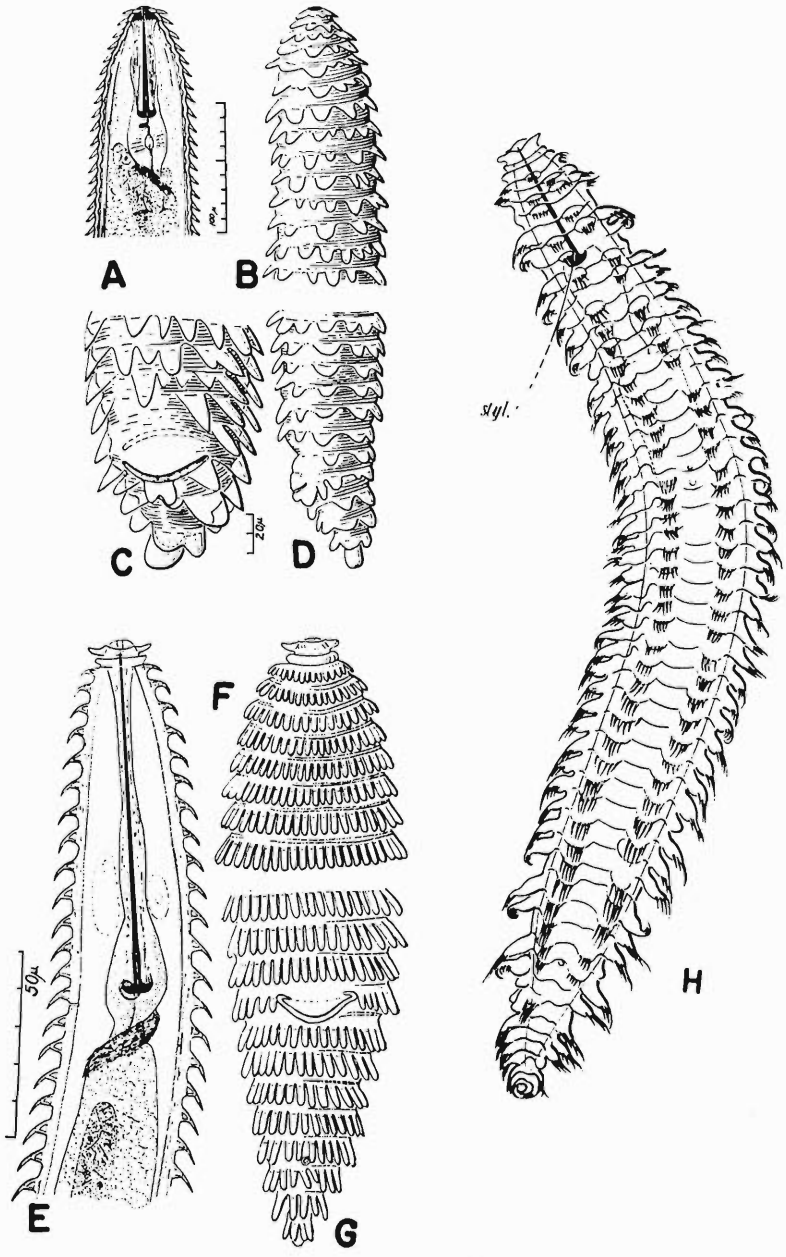


Fig. 4. Reproduction of published drawings (A to G from Taylor, 1936—his figures 1 to 7). A and B anterior portion, C and D portion of female of *C. inaequale;* E and F anterior portion, G posterior portion of *C. fimbriatum.* H (from Steiner, 1949—his figure 22B) full length view of *C. civellae.*

11. Annules about 53 ..... *fimbriatum* Cobb in Taylor, 1936.
Annules 60-70 12
12. About 60 spines at mid-body ..... *menzeli* (Stefanski, 1924) Taylor, 1936.
About 32-50 spines at mid-body ..... *aculeatum* (W. Schneider, 1939) de Coninck, 1943.
13. Scales not in regular longitudinal rows 14
Scales or spines in regular longitudinal rows 15
14. Annules 64-70; stylet about 70 microns; 12-16 scales on each annule ..... *inaequale* Taylor, 1936.
Annules 49-57; stylet 83-88 microns; about 8 hand-like scales on each annule arranged in alternate fashion ..... *palmatum* Siddiqi and Southey, 1962.
15. Spines in 4 longitudinal rows ..... *minutum* (Kirjanova, 1948) Chitwood, 1957.
Scales or spines in 8-20 longitudinal rows 16
16. Scales in 16-20 longiturinal rows, rounded, becoming long and narrow and bifurcate posteriorly ..... *coronatum* (Schuurmans Stekhoven & Teunissen, 1938) de Coninck, 1943.
Scales or spines in longitudinal rows of 16 or less 17
17. Scales or spines in 8-16 longitudinal rows 18
Scales or spines in 8 longitudinal rows 24
18. Spines arranged in groups of 2-3 in each longitudinal rows 19
Scales or spines arranged singly in each longitudinal row 20
19. Spines in 10 longitudinal rows ..... *hungaricum* Andrassy, 1962.
Spines in 14 longitudinal rows ..... *tenuicaudatum* Siddiqi, 1961.
20. Annules 34-46; scales in 12 longitudinal rows ..... *sulcatum*, n. sp.
Annules 57-70 21
21. Scales smooth, in 16 longitudinal rows ..... *minus* (W. Schneider, 1940) de Coninck, 1943.
Scales dentate or with small rounded protuberances 22
22. Scales with small rounded protuberances ..... *triconodon* (Schuurmans Stekhoven & Teunissen, 1938) de Coninck, 1943.
Scales dentate 23
23. Stylet 58-78 microns; scales in 8-11 longitudinal rows ..... *schuurmans-stekhoveni* de Coninck, 1943.
Stylet 84-103 microns; scales in 10-16 longitudinal rows ..... *cobbi* (Micoletzky, 1925) Taylor, 1936
24. Scales bearing 4-6 setose spines posteriad ..... *civellae* Steiner, 1949.
Scales not bearing setose spines 25
25. Annules 53-54 ..... *paxi* (W. Schneider, 1940) de Coninck, 1943.
Annules 60 or more 26
26. Scales mostly longer than wide 27
Scales wider than long or about equal 28
27. Annules 68-71 ..... *murrayi* (Southern, 1914) Taylor, 1936.
Annules about 60 ..... *southerni* (W. Schneider, 1940) de Coninck, 1943.
28. Scales with small rounded protuberances ..... *octangulare* (Cobb, 1914) Taylor, 1936.
Scales smooth 29
29. Stylet about 52 microns ..... *lentiforme* (Schuurmans Stekhoven & Teunissen, 1938) de Coninck, 1943.
Stylet about 90 microns ..... *zernovi* (Kirjanova, 1948) Chitwood, 1957.

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Studies on Campydoridae and Leptonchidae (Nematoda: Dorylaimoidea) with description of *Basirotyleptus basiri* n.gen., n.sp., from India

M. SHAMIM JAIRAJPURI*

Thorne (1935) included Leptonchinae and Campydorinae under Leptonchidae and regarded the genus *Aulolaimoides* Micoletzky, 1914 as having uncertain affinity. However, he was doubtful about the inclusion of Campydorinae in Leptonchidae. Under Leptonchinae the same author (1939) included *Leptonchus* Cobb, 1920; *Tyleptus* Thorne, 1939; *Tylolaimophorus* de Man, 1880; *Dorylaimoides* Thorne and Swanger, 1936; *Doryllium* Cobb, 1920 and *Tylencholaimellus* M. V. Cobb, 1915. To this list Lordello (1955) added *Proleptonchus* and Heyns (1963) *Botalium*. Campydorinae contained *Campydora* Cobb, 1920 as its only representative until Clark (1961) raised it to the family rank and included in it also the genera *Tyleptus* and *Aulolaimoides*. He also shifted *Tylolaimophorus* to Diphtherophoridae. Goodey (1963) agreed with Clark's classification and also included doubtfully *Funaria* van der Linde, 1938 under Leptonchidae. The present author agrees fully with Thorne as far as the inclusion of genera under Leptonchinae is concerned. Clark's action in proposing Campydoridae seems valid since *Campydora* is altogether different from the rest of Leptonchidae, but the inclusion of *Tyleptus* and *Aulolaimoides* is questionable. *Tyleptus* belongs in Leptonchinae and *Aulolaimoides*, like *Campydora*, represents an independent family since it is distinctive from all the Leptonchidae in the characters of

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spear, esophagus, spicula, presence of gubernaculum, and pharyngeal wall supported by minute ribs. The following scheme is proposed to classify the above mentioned genera.

FAMILY AULOLAIMOIDIDAE n. fam.

DIAGNOSIS: Pharyngeal wall supported by minute ribs. Spear compound, flanged. Esophagus a long slender tube ending in a short triquetrous basal sucking bulb. Spicula long, lineate, crooked, with apophyses extending half way to anus. Gubernaculum trough-shaped. Testes apparently paired. Both sexes with filiform tail.

TYPE AND ONLY GENUS: *Aulolaimoides* Micoletzky, 1914.

FAMILY CAMPYDORIDAE (Thorne, 1939) Clark, 1961

DIAGNOSIS EMENDED: Esophagus a slender tube terminating in a definitely set off basal bulb with an elongate triquetrous valvular chamber. Tooth dorsally mural. Prerectum absent, excretory pore present.

TYPE AND ONLY GENUS: *Campydora* Cobb, 1920.

DISCUSSION: A large number of specimens of *Campydora* were found in soil collections from around the roots of apple tree, *Pyrus Malus* L. and pomegranate, *Punica granatum* L. from Srinager (Kashmir) India. Upon study they were found to represent the type species *Campydora demonstrans* Cobb, 1920 with the following differences: the caudal pores in the present material are more towards tail tip and the basal esophageal bulb is smaller in relation to the length of neck.

FAMILY LEPTONCHIDAE Thorne, 1935

DIAGNOSIS EMENDED: Meromyarian. Body cylindroid. Cuticle and subcuticle smooth or finely striated; subcuticle usually provided with dot-like radial elements, especially abundant near tail. Lateral pores in two lines. Spear axial; extension strongly developed, simple or with basal knobs or flanges. Esophagus a slender tube with a short basal bulb which may be set off by a distinct constriction. Esophageal lumen usually not forming a broad triquetrous chamber in the basal bulb. Prerectum present. Testes paired, dorylaimoid. Adanal pair of supplements and lateral guiding pieces present; gubernaculum absent.

TYPE SUBFAMILY: Leptonchinae Thorne, 1935.

OTHER SUBFAMILIES: Tylencholaimellinae n. subfam.; Tyleptinae n. subfam.

KEY TO THE SUBFAMILIES OF LEPTONCHIDAE

1. Head provided with six small perioral liplets; lumen of basal bulb forming a small triquetrous chamber ..... Tyleptinae
   No such structures present 2
2. Spear extension flanged or knobbed ..... Tylencholaimellinae
   Spear extension simple ..... Leptonchinae

SUBFAMILY LEPTONCHINAE Thorne, 1935

DIAGNOSIS EMENDED: Spear extension simple, weakly or strongly sclerotized; arcuate or angular. Esophagus a slender tube with a short basal expansion (rarely reaching one third the neck length in some *Dorylaimoides*) which is set off by a constriction in *Proleptonchus*.

TYPE GENUS: *Leptonchus* Cobb, 1920.

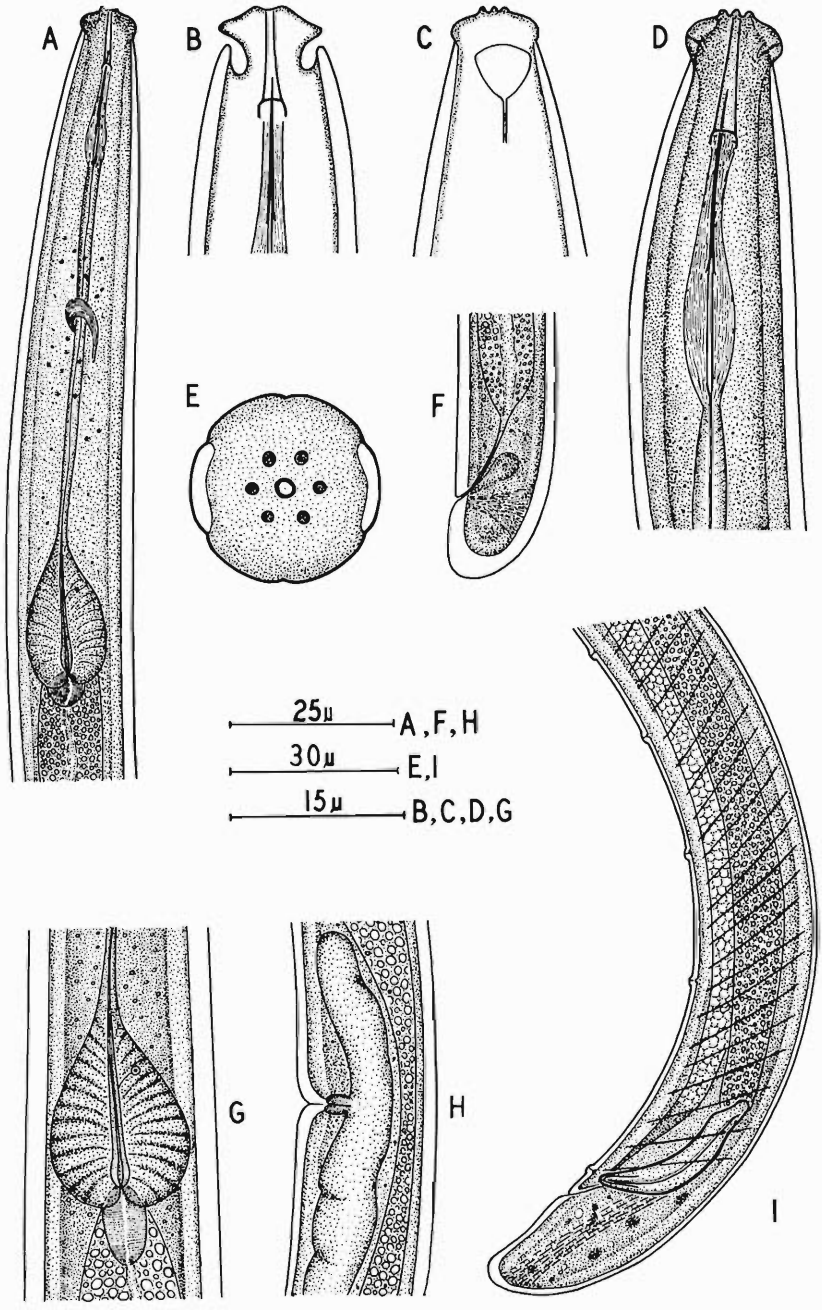

Fig. 1. A-H, *Basirotyleptus basiri* n.gen, n.sp. A. Esophageal region; B. Head, dorsoventral view; C. Head, showing amphid; D. Head end; E. *En face* view; F. Tail end; G. Basal esophageal bulb; H. Vulvar region.
I. male tail of *Tyleptus striatus* Heyns, 1963

OTHER GENERA: *Dorylaimoides* Thorne and Swanger, 1936; *Proleptonchus* Lordello, 1955.

DISCUSSION: *Funaria* van der Linde, 1938 may also belong here, but since the character of spear extension is not known it seems best to regard it as *genus inquirenda*.

KEY TO THE GENERA OF LEPTONCHINAE

1. Stoma flask-shaped; basal bulb set off ..... *Proleptonchus*
   Stoma not flask-shaped; basal bulb not set off 2
2. Spear extension arcuate; basal bulb length less than twice width and about one sixth of neck length ..... *Leptonchus*
   Spear extension angular; basal bulb length twice neck width or more and usually one fourth to one third of neck length ..... *Dorylaimoides*

SUBFAMILY TYLENCHOLAIMELLINAE n. subfam.

DIAGNOSIS: Spear extension provided with well developed basal flanges or knobs. Esophagus a slender tube with a short basal bulb set off by a distinct constriction.

TYPE GENUS: *Tylencholaimellus* M. V. Cobb, 1915.

OTHER GENERA: *Doryllium* Cobb, 1920; *Botalium* Heyns, 1963.

KEY TO THE GENERA OF TYLENCHOLAIMELLINAE

1. Ovaries paired; ventromedian supplements several ..... *Botalium*
   Ovary and ventromedian supplement single 2
2. Spear with dorsal stiffening piece ..... *Tylencholaimellus*
   Spear without dorsal stiffening piece ..... *Doryllium*

SUBFAMILY TYLEPTINAE n. subfam.

DIAGNOSIS: Head provided with six perioral liplets. Spear extension simple. Esophagus a slender tube till it expands to the pyriform basal bulb. Bulbar lumen in two sections, posterior one forming a narrow, triquetrous, valvular chamber.

TYPE GENUS: *Tyleptus* Thorne, 1939.

OTHER GENUS: *Basirotyleptus* n. gen.

DISCUSSION: Presence of six perioral liplets in *Tylolaimophorus* de Man, 1880 indicates its affinities with this subfamily, but the character of the flanged extension shows relationship with Tylencholaimellinae. The small triquetrous valvular chamber in the basal bulb which is a diagnostic feature of Tyleptinae is also not described. Further information on the morphology is needed to determine its exact taxonomic status, but for the present it seems appropriate to consider it as *genus inquirenda* under Tyleptinae.

Goodey's (1963) action in considering *Triplonchium* Cobb, 1920 a synonym of *Tylolaimorphorus* is not justified, since *Triplonchium* does not have the perioral circlet of minutely mammiform papillae typical of *Tylolaimophorus*, and the spear as illustrated by de Man (1880) has little resemblance to that of *Triplonchium*.

KEY TO THE GENERA OF THE SUBFAMILY TYLEPTINAE

1. Spear dorylaimoid; stoma and guiding ring simple ..... *Tyleptus*
   Spear slender, needle-like with narrow lumen and obscure aperture; stoma conical, strongly sclerotized; guiding ring strongly sclerotized with two backwardly directed extensions ..... *Basirotyleptus*

GENUS *Tyleptus* Thorne, 1939

A large number of male and female specimens of *Tyleptus* were collected at various occasions from Aligarh (U.P.), Jorhat (Assam) and Trivandrum (Kerala) India. Measurements and morphology of these worms closely conform with *Tyleptus striatus* Heyns, 1963 reported from South Africa. The diagnosis of *Tyleptus*, the descriptions of *T. striatus*, and the type species *T. projectus* Thorne, 1939 are based on females only. Now that males are available, their description is provided and the diagnosis of the genus is emended to include their characters.

DIAGNOSIS EMENDED: Body cylindroid. Cuticle and subcuticle with fine transverses striations. Lateral chords with two lines of coarse ducts reaching to the lateral pores. Six conspicuous, projecting liplets around the oral aperture. Spear dorylaimoid with strongly sclerotized extension surrounded by conspicuous muscles. Guiding ring simple. Esophague a slender tube till it expands to a pyriform basal bulb. Bulbar lumen in two sections, the posterior one forming a narrow, triquetrous, valvular chamber. Vulva transverse; ovary single, opisthodelphic and reflexed. Testes paired; one reflexed, the other outstretched. Spicula well developed, ventrally arcuate. Lateral guiding pieces present. Supplements an adanal pair and a series of 2 to 4 ventromedians in *T. striatus*. Tail of both sexes somewhat similar .

Tyleptus striatus Heyns, 1963 (Fig. 1, I)

FEMALES (5): L = 0.75-0.10 mm.; a = 27-42; b = 3.6-4.5; c = 63-71; V = 33-36.

MALES (5): L = 0.76-0.85 mm.; a = 33-35; b = 4.0-4.7; c = 40-53.

DESCRIPTION (male): Similar to female in general morphology. Testes paired; posterior one reflexed, anterior outstratched. Supplements an adanal pair and a series of 2 to 4 ventromedians beginning well above spicula and spaced at regular intervals. Spicula 25-28 microns long, well developed. Lateral guiding pieces present. Tail bluntly rounded, somewhat similar to female.

GENUS *Basirotyleptus* n. gen.

A large number of nematodes resembling *Tyleptus* in general shape and appearance, type of esophagus, reproductive organs and tail, were recently collected from soil around the roots of tea plants, *Thea sinensis* L., from Tocklai Experimental Station, Jorhat (Assam) India. Having a different type of spear, extension, stoma and guiding ring, these worms represent a new genus and species. The name *Basirotyleptus basiri* in honour of Professor M. A. Basir is proposed.

DIAGNOSIS: Body short, cylindroid. Cuticle and subcuticle transversely striated, the latter provided with dot-like radial elements, especially abundant near tail end. Lateral chords broad. Mouth surrounded by six small liplets. Spear slender, needle-like, its lumen narrow and aperture obscure; extension simple, sclerotized, surrounded by conspicuous sheath of muscles. Stoma conical, strongly sclerotized, with two posteriorly directed extensions. Esophagus a slender tube till it expands to a pyriform basal bulb. Lumen of basal bulb in two sections, the posterior one forming a narrow, triquetrous, vavular chamber. Vulva transverse; ovary opisthodelphic and reflexed. Tail short, hemispheroid. Males unknown.

TYPE AND ONLY SPECIES: *Basirotyleptus basiri* n. sp.

Basirotyleptus basiri n. sp. (Fig. 1, A-H)

FEMALES (20): L = 0.50-0.58 mm.; a = 21-28; b = 4.8-6.0; c = 45-58; V = 34-40.

HOLOTYPE (Female): L = 0.58 mm.; a = 28; b = 5.6; c = 58; V = 38.

DESCRIPTION: Body cylindroid, ventrally arcuate when relaxed, blunt at both extremities. Cuticle and subcuticle distinctly striated. Lips somewhat conoid, the region marked off from the body contour by a distinct depression. Amphids cup-like, their slit-like apertures occupying about half the head width. *En face* view showing six small liplets around the cuticularized oral opening; amphidial slits situated laterally; papillae not seen. Spear slender, needle-like; extension simple and sclerotized. Extension nearly half the spear length; combined length of spear and extension about three times the width of lip region. Stoma conical strongly sclerotized. Guiding ring single, heavily sclerotized, with two extensions directed backwards. Esophagus a slender tube till it expands to a pyriform basal bulb. Lumen of basal bulb in two sections, the posterior one forming a narrow triquetrous, valvular chamber. Only the dorsal esophageal gland nuclei visible. Nerve ring midway of esophagus. Cardia prominent, hemispheroid, about a quarter of neck width. Vulva transverse; Vagina thick walled, about half the corresponding body width. Ovary opisthodelphic and reflexed half way back to vulva. Anterior uterine sac more than one body width long. Sperms not present in the uteri. Rectum about one anal body width long; prerectum twice as long as rectum (two to four times as much in paratypes). Tail hemispheroid, about one anal body width long, with thick cuticle at the terminus. Caudal pores obscure due to abundance of radial elements.

MALE: Not found.

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

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

SUMMARY

A revised classification of Campydoridae and Leptonchidae (Nematoda: Dorylaimoidea) is proposed and a new family Aulolaimoididae and two new subfamilies Tylencholaimellinae and Tyleptinae are erected. *Basirotyleptus basiri* n. gen., n. sp., is described and illustrated from India. Remarks on the occurance of *Campydora demonstrans* Cobb, 1920 and *Tyleptus striatus* Heyns, 1963 are also included.

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Two New American Dagger Nematodes (*Xiphinema:* Dorylaimidae) Associated with Citrus, with Comments on the Variability of *X. bakeri* Williams, 1961

A. C. TARJAN*

Among the nominal species of *Xiphinema,* only *X. americanum* Cobb, 1913; *X. chambersi* Thorne, 1939; *X. index* Thorne and Allen, 1950; *X. obtusum* Cobb in Thorne, 1939; and *X. truncatum* Thorne, 1939, have been reported from the United States. *Xiphinema* species reported from citrus hosts are *X. americanum* Cobb, 1913 (which is a common associate of citrus in Florida); *X. attorodorum* Luc, 1961 from Guinea; *X. basiri* Siddiqi, 1959 from India (which is also associated with citrus in Puerto Rico); *X. campinense* Lordello, 1951 (= *X. elongatum* Schuurmans Stekhoven and Teunissen, 1938) from India (Timm and Ameen, 1960); *X. chambersi* Thorne, 1939 (which has been found associated with citrus in central Florida); *X. ifacolum* Luc, 1961 from Guinea; and *X. obtusum* from California. This paper deals with three additional species from the United States, *X. coxi* n. sp., *X. vulgare* n.sp., and *X. bakeri,* of which the new species also are associates of citrus roots in central Florida, U.S.A.

Xiphinema coxi n. sp .(Fig. 1)**

MEASUREMENTS: Type Population: Females (15): Length = 3.58 (3.06-4.00) mm; a = 74.7 (66.0-82.3); b = 8.4 (7.5-9.2); c = 65.8 (58.8-82.3); V = $^{12\ (9\text{-}15)}$ 44 (40-46) $^{11\ (6\text{-}16)}$; stylet = 194 (185-210) microns.

Holotype (female); Length = 3.69 mm; a = 81.9; b = 8.0; c = 65.6; V = ^{13} 44 ^{10}; stylet = 199 microns.

POPULATION DESCRIPTION: Body position somewhat arcuate, with post-vulvar portion more curved than anterior portion (Fig. 1A). Anterior and posterior extremities tapering. Labial region about 15 (15-16) microns wide, slightly offset; labia completely amalgamated. Amphid apertures located at base of labia, about ⅔rds to ¾ths as wide as labial region. Cuticle faintly striated transversely; striations more pronounced in caudal region. Cuticle mainly appearing to be of two layers; cuticle at mid-body 2.9 (2.2-3.2) microns thick. Cuticle thickest on dorsal side of tail, where it appears to be composed of three layers; the outermost area 1.4 (1.0-1.8) microns wide, the next layer 0.9 (0.6-1.2) microns wide, and the innermost layer 4.0 (3.5-4.4) microns wide. Greatest width of caudal cuticle 6.3 (5.4-6.8) microns.

Body bearing somatic pores which originate as a simple line of lateral pores directly posterior to the labial region. Dorsal and ventral (or subdorsal and subventral) pores also appearing on some specimens in cervical region occupied by the anterior portion of the stylet. Lateral pores, sometimes becoming sublateral, usually continuing to area of body occupied by vulva where several pores, in apparent random arrangement, are situated. Pores continuing posteriorly from vulva as a single dorso-sublateral line,

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*Florida Citrus Experiment Station, Lake Alfred. Florida Agricultural Experiment Stations Journal Series No. 1702.

Grateful appreciation is extended to Dr. M. Luc for the loan of the holotype and paratype specimens of *X. setariae;* to Dr. J. B. Goodey for specimens of *X. vulgare* from Indonesia; to Dr. I. Andrássy for specimens of *X. coxi* from Germany; to Dr. T. D. Williams for paratypes of *X. bakeri;* and to Dr. H. J. Jensen, Mr. B. E. Hopper and Mr. R. P. Esser for specimens of *X. bakeri* from Oregon, Kentucky, and Florida, respectively.

**Named in honor of the late Dr. Carroll E. Cox, Professor of Plant Pathology, University of Maryland, College Park.

but sometimes accompanied by a single ventro-sublateral line as well, to tail which usually bears two, but sometimes three, pores. When the tail shows only two pairs of pores (Fig. 1B, C), there is always a third preanal pair. When there are three pairs on the tail (Fig. 1J), a fourth pair of ventro-sublateral pores may be found about one anal body width preanally.

Stylet composed of odontostyl 122 (113-127) microns long, and basal portion, less sclerotized, 72 (68-82) microns long (Fig. 1E). Basal portion of stylet with knobbed area 14 (13-15) microns wide. Guiding ring double in adult specimens. In juveniles the anterior ring appears to be molted with the odontostyl. In the holotype and five other adult females, the double ringed guiding collar averaged 14 (6-24) microns long, enveloping the odontostyl about 23 (8-34) microns anterior to the junction of the two stylet parts. In the remainder of the specimens the lightly sclerotized anterior ring was 19 (14-24) microns anterior to the stylet junction while the heavier sclerotized posterior ring was 19 (12-24) microns posterior to the junction. In these specimens the total length to the guiding collar was 38 (35-40) microns.

Esophagus bearing a sagittate, sclerotized structure close to the lumen in most specimens (Fig. 1I). This structure appears to be a vestigial odontostyl primordium found only in adult specimens and never in juveniles where a "reserve" stylet reposes in the esophagus (Coomans and de Coninck, 1963). Although this barb usually points anteriorly and is located 32 (15-50) microns behind the stylet, four specimens show the barb pointing posteriorly and situated 61 (55-76) microns behind the stylet.

Esophagus usually convoluted anterior to basal esophageal bulb, which measures 97 (87-103) microns long and 20 (19-20) microns wide (Fig. 1F) and contains three glands. The single gland located anteriorly in the esophageal bulb may be located either in a dorsal, ventral, or lateral area of the bulb. In 14 individuals studied, it was located ventrally in seven, dorsally in five, and laterally in two. This anterior esophageal gland is the most conspicuous of the three glands. It measures 7.7 (7.5-8.0) microns wide, and 9.2 (8.7-9.6) microns long, the latter being the portion connected to the esophageal lumen. Its prominent nucleus is situated 11 (9-14) microns behind the anterior part of the esophageal bulb. The two posterior glands are usually in close proximity although their nuclei may be as much as 16 microns apart. They may be located together in the dorsal, ventral, or lateral sectors of the esophagus but often are separated, occupying different esophageal sections. These glands are located 49 (42-61) microns from the anterior part of the esophageal bulb. In 14 individuals studied, 15 gland nuclei were located in the ventral sector, 12 nuclei in the dorsal sector, and one located laterally. In six individuals the lower glands were separated, occupying different esophageal sectors.

A saccate to bluntly conical esophago-intestinal valve measuring 9 (8-11) microns long and 12 (10-15) microns wide is positioned between the esophagus and the intestine (Fig. 1F).

Vulva usually transverse. Sexual system amphidelphic, reflexed (Fig. 1D). Female gonad complex, beginning as an ovary with two- or even three-rank oocytes, which in the growth zone becomes single-ranked. Shortly before the point of flexture, the ovary differentiates into oviduct which soon forms a sphincter valve (Fig. 1H). Adjacent to this valve, the gonad expands into a uterine chamber, the other end of which contains a pronounced lumen which may act as spermatheca, and in which sperm-like bodies have been located. This chamber may be constricted or may continue non-constricted

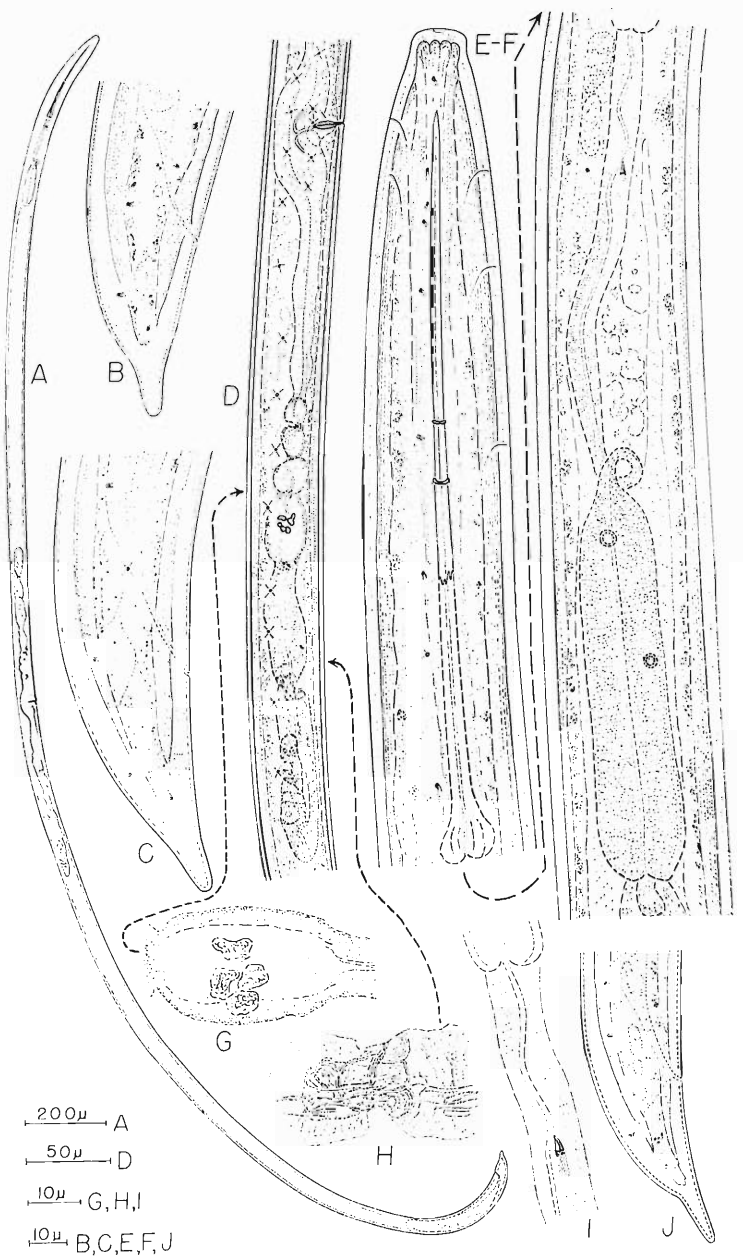

Fig. 1. *Xiphinema coxi* n. sp. A, Holotype female. B, Paratype tail. C. Holotype tail. D, Vulva and posterior gonad of holotype. E-F, Esophagus of holotype (in two parts). G, "Z" organ with arrow showing position in posterior gonad. H, Sphincter valve with arrow showing position in posterior gonad. I, Sagittate barb positioned in the esophagus. J, Tail of female from *Cocos nucifera*.

to an unusual oval-shaped organ (Fig. 1G). Structural details of this organ are as already presented by Luc, 1958 and 1961, who named it the "Z" organ. The sclerotized pieces or apophyses contained within the organ number four or five and may be of varying shape and position. A band of muscle tissue seems to underlie the epithelial covering of the uterus which exhibits minute longitudinal and transverse folds suggesting expansive capabilities so as to accommodate passage through of the egg. The remainder of the uterus is usually convoluted up to the reniform uterine chamber into which the vagina leads. One paratype female contained a large egg positioned at the proximal end of the uterus. The egg shell measured 414 microns long and 78 microns wide while the densely granulated egg was 357 microns long and 68 microns wide.

A pre-rectum, discernible by its lighter texture and about 625 microns long, leads to the conical, ventrally convex, digitate tail (Fig. 1B, C, J) of which the terminal non-protoplasmic portion is 20 (18-21) microns long. Tail 1.8 (1.5-2.0) times as long as width of body at anus.

Male: unknown.

DIAGNOSIS: Among those *Xiphinema* species with amphidelphic gonads and with vulva situated near 44% of the body length, *X. coxi* is closest to *X. attorodorum* Luc, 1958 and *X. ebriense* Luc, 1958 from which it differs by its larger size, measurements and ratios, and tail shape. It is also close to *X. hallei* Luc, 1958 from which it differs by the more forward position of the vulva on the body (44% as contrasted to 47%) and by the shorter tail (about 1.8 anal body widths long as contrasted to 4-5 anal body widths long) which is of different shape. It can be separated primarily by tail shape from *X. flagellicaudatum* Luc, 1961 which has a filiform tail; from *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939 and *X. index*, which have more obtuse tails with distinctly set-off terminal "peg-like" projections; from *X. rotundatum* Schuurmans Stekhoven and Teunissen, 1938, and *X. yapoense* Luc, 1958 which have hemispherical tails; and from *X. vanderlindei* Heyns, 1962, which has an elongate-conical tail. Two other species with some general characteristics resembling *X. coxi* are *X. italiae* Meyl, 1963 and *X. obtusum;* these, however, have different measurements and tail shapes and have been assigned to *species inquirendae.*

HOLOTYPE: Female collected by S. Malo, August 25, 1959. Slide 6, Tray 2, Cabinet C-2724, Nematode Collection, University of Florida Citrus Experiment Station, Lake Alfred.

PARATYPES: One female, Slide 7, Tray 2, Cabinet C-2724, Nematode Collection, University of Florida Citrus Experiment Station, Lake Alfred; two females and one juvenile, United States Department of Agriculture Nematode Collection, Beltsville, Maryland; one female, Nematology Department Collection, Rothamsted Experimental Station, England; one female and seven juveniles, Nematology Laboratory Collection, I.D.E.R.T., Abidjan, Ivory Coast; one female, University of California Collection, Davis. Eight females in author's possession.

TYPE HABITAT*: Soil around roots of Temple orange scion on sour orange (*Citrus aurantium* L.) rootstock.

TYPE LOCALITY: Citrue grove owned by M. S. Whaley, two miles south of Orsino ,Merritt Island, Florida. Florida State Road Department General

*This species reproduced and maintained itself on potted Duncan grapefruit seedlings in the greenhouse for one and a half years.

Highway and Transportation Map: Range 36E, Township 23S, Section 12, Brevard County.

OTHER LOCALITIES: One female from soil around *Cocos nucifera* L., Key West, Florida, collected by L. G. van Weerdt, January 23, 1958; and 18 females from soil around grass roots, Aschersleben, German Democratic Republic, collected by R. Fritzche, July 16 ,1962.

DISCUSSION: A mixed population of nematodes from Aschersleben, East Garmany was found to contain *X. diversicaudatum* and numerous specimens of *X. coxi*. Tail shapes of the German population were identical with those of the type Florida population. Measurements of the German population were: Length = 4.09 (3.69-4.48) mm; a = 68.9 (63.8-74.3); b = 10.1 (9.1-11.9); c = 74.5 (65.8-85.6); V = ^{16} 44 (42-45) ^{15}; anterior stylet portion = 127 (119-133) microns; posterior stylet portion = 73 (57-83) microns; total stylet length = 200 (186-209) microns; tail/anal body width = 1.5 (1.3-1.7). The female sexual system of the German population was similar to that of the type population except that the "Z" organ was smaller and not as sharply defined, although the sclerotized pieces contained within were distinct in most specimens.

Xiphinema vulgare n. sp. (Fig. 2A-C, E-F)

MEASUREMENTS: Type Population: Females (13): Length = 2.65 (2.36-2.84) mm; a = 56.3 (52.3-61.4); b = 7.2 (6.7-8.0); c = 53.2 (48.1-57.7) V = $^{9\ (7\text{-}12)}$ 39 (37-40) $^{10\ (7\text{-}12)}$; stylet = 181 (175-193) microns.

Holotype (female): Length = 2.66 mm; a = 55.5; b = 7.7; c = 50.7; V = ^{7} 39 ^{10}; stylet = 177 microns.

ALLOTYPE (male): Length = 2.52 mm; a = 64.5; b = 6.9; c= 45.8; stylet = 188 microns; spicule = 64 microns.

OTHER POPULATIONS—Female (13): Length = 2.63 (2.47-2.75) mm; V = 39 (36-42); stylet = 179 (169-185) microns.

DIFFERENTIAL DESCRIPTION: Holotype: Labia completely amalgamated, separated from rest of body by slight incisure (Fig. 2A). Integument bearing a single line of dorso-sublateral pores extending from the tail to the labial region; two ventro-sublateral pores situated at approximately 1 and 3 anal body widths anterior to anus. Two adjacent caudal pores situated post-equatorially on tail (Fig. 2C). Stylet consisting of odontostyl 104 microns long and basal portion 73 microns long; total stylet length 177 microns. Female gonads amphidelphic, reflexed. Vulva transverse; uterine chamber spacious, elliptical; uterus narrows to form a proximal, globose swelling and distal elongated pouch-like swelling with transverse cells. Adjoining this pouch is a sphincter valve which connects to a part of the oviduct with thin-walled, transversely elongated, punctate cells and which reflexes and connects to the ovary (Fig. 2B). Tail conical with dorsal side distinctly convex to a level with the end of the protoplasmic region of the body where the tail becomes digitate, having relatively straight but tapering sides and a finely rounded terminus. Tail 1.8 times as long as width of body at anus.

ALLOTYPE: A single line of dorso-sublateral pores extending from tail to an area between the posterior end of the stylet and the anterior end of the esophageal bulb where the pores become lateral and extend up to the labial region. A ventro-sublateral line of pores extending posteriorly from a position 79% of the body length to the end of the tail. Tail with 5-6 pairs of pores (Fig. 2F). Supplements 4, one adanal, and three spaced about one

body width apart, beginning two and a half body widths anterior to adanal supplement. Spicules 64 microns long. Tail 1.6 times as long as width of body at anus.

DIAGNOSIS: *Xiphinema vulgare* n. sp. is distinctive because it is an amphidelphic species with vulva situated at approximately 39% of the body length, and with a conical digitate tail. It differs from *X. index*, *X. diversicaudatum* and *X. mammillatum* Schuurmans Stekhoven and Teunissen, 1938, which have hemispherical tails with set-off, digitate termini. *Xiphinema attorodorum* has a longer, thinner, and more uniformly tapering tail. The tail length/anal body width ratio is 2.5 for *X. elongatum* Schuurmans Stekhoven and Teunissen, 1938 (syn: *X. campinense* and *X. pratense* Loos, 1949) whereas it is only 1.8 for *X. vulgare;* then, too ,the tail shapes differ. *Xiphinema coxi* has a different vulva position, body dimensions, and gonad structure than *X. vulgare*. Three other species now in *species inquirendae*. *X. grande* Steiner, 1914, *X. obtusum*, and *X. parasetariae* Luc, 1958 have different tail shapes.

Xiphinema vulgare n. sp. is closest in appearance and measurements to *X. setariae* Luc, 1958, but is shorter, thinner, with shorter odontostyl in relation to the basal portion of the stylet, and with a shorter and less pronounced digitate portion of the tail (Fig. 2C, D).

HOLOTYPE AND ALLOTYPE: Collected May 4, 1961 by S. Buck. Slides 11 and 12 respectively, Tray 2, Cabinet C-2724, Nematode Collection, Florida Citrus Experiment Station, Lake Alfred.

PARATYPE: Four females and two juveniles, Slide 13, Tray 2, Cabinet C-2724, Nematode Collection, Florida Citrus Experiment Station, Lake Alfred; five females deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland, and in the Nematology Laboratory Collection, I.D.E.R.T., Abidjan, Ivory Coast; four females deposited in Nematology Department Collection, Rothamsted Experimental Station, England, six females and 11 juveniles in author's personal collection.

TYPE HABITAT*: Soil from around the roots of tangerine budded to Cleopatra rootstock (*Citrus reticulata* var. Cleopatra Blanco).

TYPE LOCALITY: Fifty yards west of Building No. 25, Florida Citrus Experiment Station, Lake Alfred.

OTHER LOCALITIES: Seventeen females from soil around Zoysia grass roots, Winter Haven, Florida; two females from citrus root soil, Lake Alfred, Florida; two females from cactus root soil, Lake Alfred, Florida; two females from centipede grass root soil, Fort Pierce, Florida; nine females from citrus root soil, Tampa, Florida; one female and 23 juveniles from soil around roots of West Indian cherry, Isabella, Puerto Rico; three females from citrus root soil, Gurabo, Puerto Rico; and six females from citrus root soil, Djakarta, Indonesia.

DISCUSSION: The similarity of *X. vulgare* n. sp. to *X. setariae* instigated a close study of the holotype and 36 paratype specimens of the latter species collected from soil around roots of *Setaria megaphylla* from Adiopodoume, Ivory Coast, and six specimens collected from banana and corn growing in two locations in western Nigeria. Comparison of type females of both species (Table 1) shows *X. setariae* to be larger than *X. vulgare*. In an

*Picked specimens, isolated from soil by a sugar flotation technique and inoculated to potted Duncan grapefruit seedlings have shown population increases of as much as 300% during an eleven-month period in the greenhouse.

effort to express numerically the difference shown between tails of these two species (Fig. 3C, D), the width of the tail at the terminus at the protoplasmic portion of the body was measured. This measurement indicated difference between the two species, as did the ratio of the tail length divided by the width of the body at the level of the anus.

A study of 26 females of each species showed that the total stylet length for *X. setariae*, the longer species, was correspondingly greater. The odontostyl was longer for *X. setariae* as compared to *X. vulgare* while the lengths of the basal portion of the stylet were about the same for both species.

Likewise indicating marked differences between species were the tail length, length of the terminal non-protoplasmic portion of the tail, and the ratio obtained in dividing the former by latter (Table 1). All populations of *Xiphinema vulgare* obtained consistently yielded these same differences as compared to the Ivory Coast and Nigerian populations of *X. setariae*.

Measurements of six females of *X. vulgare* collected from citrus root soil in Djakarta, Indonesia were: length = 2.53 (2.33-2.66) mm.; a = 59.8 (54.3-67.5); b = 6.3 (5.9-6.7); c = 49.3 (44.0-53.4); V = ♀ 41 (39-42) ♀; odontostyl = 113 (107-119) microns; basal stylet portion = 70 (66-74) microns; total stylet length = 182 (176-189) microns; WTP (see Table 1) = 10 (8-12) microns; tail length = 52 (47-53) microns; LNT = 18 (15-19) microns; tail length/LNT = 2.9 (2.6-3.4). Hence ,these females conformed well to the differential criteria characterizing the type population of *X. vulgare*, despite the variability in number of caudal pores which ranged from one to four pairs (Fig. 2E).

Table 1. Comparative measurements of *X. setariae* and *X. vulgare*.

	X. setariae	*X. vulgare*
	(n = 13)	(n = 12)
Body Length in mm	2.98 (2.81-3.16)	2.65 (2.36-2.84)
a	63.7 (60.2-67.4)	56.3 (52.3-61.4)
b	7.2 (6.6-8.1)	7.2 (6.7-8.0)
c	50.7 (46.3-54.3)	53.2 (48.1-57.7)
Vulva %*	37 (35-39)	39 (37-40)
Anterior Gonad %**	8 (6-12)	9 (7-12)
Posterior Gonad %**	9 (6-13)	10 (7-12)
ABW***	28 (26-31)	27 (25-29)
Tail Length/ABW	2.1 (1.9-2.3)	1.8 (1.7-2.0)
WTP† in microns	13.7 (12.1-15.5)	10.6 (8.5-12.5)
	(n = 26)	(n = 26)
Odontostyl in microns	120 (110-130)	109 (104-120)
Basal Portion of Stylet in microns	70 (64-82)	72 (67-78)
Total Stylet Length in microns	190 (183-200)	181 (175-193)
Odontostyl/Total Stylet Length	63 (58-67)	60 (59-63)
LNT‡ in microns	25 (23-29)	17 (13-20)
Tail Length/LNT	2.3 (2.1-2.7)	3.1 (2.7-4.0)
Tail Length in microns	59 (55-63)	49 (46-53)

*Distance of vulva from anterior end expressed as percentage of total length.
**Length expressed as percentage of total body length.
***Width of body at level of the anus.
†Width of the tail at level of the terminus of the protoplasmic portion of the body.
‡Length of terminal non-protoplasmic portion of tail.

Table 2. Measurements on populations of *Xiphinema bakeri*.

	Raspberry Soil Hatzic British Columbia	Dahlia Soil Canby, Oregon	Maple Soil Helmick Park Corvallis, Oregon	Hardwood Forest Soil Mammoth Cave Kentucky	Carya Soil Monticello Florida
No. of Specimens	(3)	(5)	(12)	(3)	(3)
Length in mm	4.31	3.73	4.00	3.32	2.57
	(3.87-4.54)	(3.59-4.02)	(3.16-4.59)	(3.19-3.48)	(2.44-2.67)
a	68.1	67.8	71.0	58.2	51.6
	(62.4-79.0)	(59.8-75.6)	(61.1-81.9)	(56.0-61.0)	(45.2-56.2)
b	8.1	8.7	9.1	7.3	6.4
	(7.9-8.3)	(7.9-9.2)	(7.0-10.9)	(7.0-7.5)	(6.1-6.6)
c	69.8	76.3	69.7	68.4	56.3
	(66.1-76.0)	(69.0-91.3)	(58.4-83.6)	(61.4-72.5)	(53.0-60.1)
V%*	32	32	29	28	28
	(29-33)	(32-34)	(26-30)	(27-29)	(28-29)
Anterior Gonad %**	8	8	11	10	10
	(5-10)	(7-9)	(6-14)	(9-10)	(9-12)
Posterior Gonad %**	10	8	12	19	16
	(8-13)	(6-11)	(5-17)	(13-24)	(15-16)
Odontostyl in microns	137	130	126	117	121
	(131-143)	(125-133)	(118-135)	(111-129)	(118-124)
Basal portion of Stylet in microns	79	69	76	66	70
	(75-85)	(59-75)	(65-82)	(63-73)	(67-73)
Total Stylet Length in microns	216	199	202	183	191
	(206-223)	(190-208)	(195-217)	(173-202)	(184-196)
Tail/Anal Body Width	1.4	1.4	1.5	1.3	1.3
	(1.3-1.6)	(1.1-1.6)	(1.1-1.7)	(1.2-1.4)	(1.2-1.3)

*, **See footnotes, Table 1.

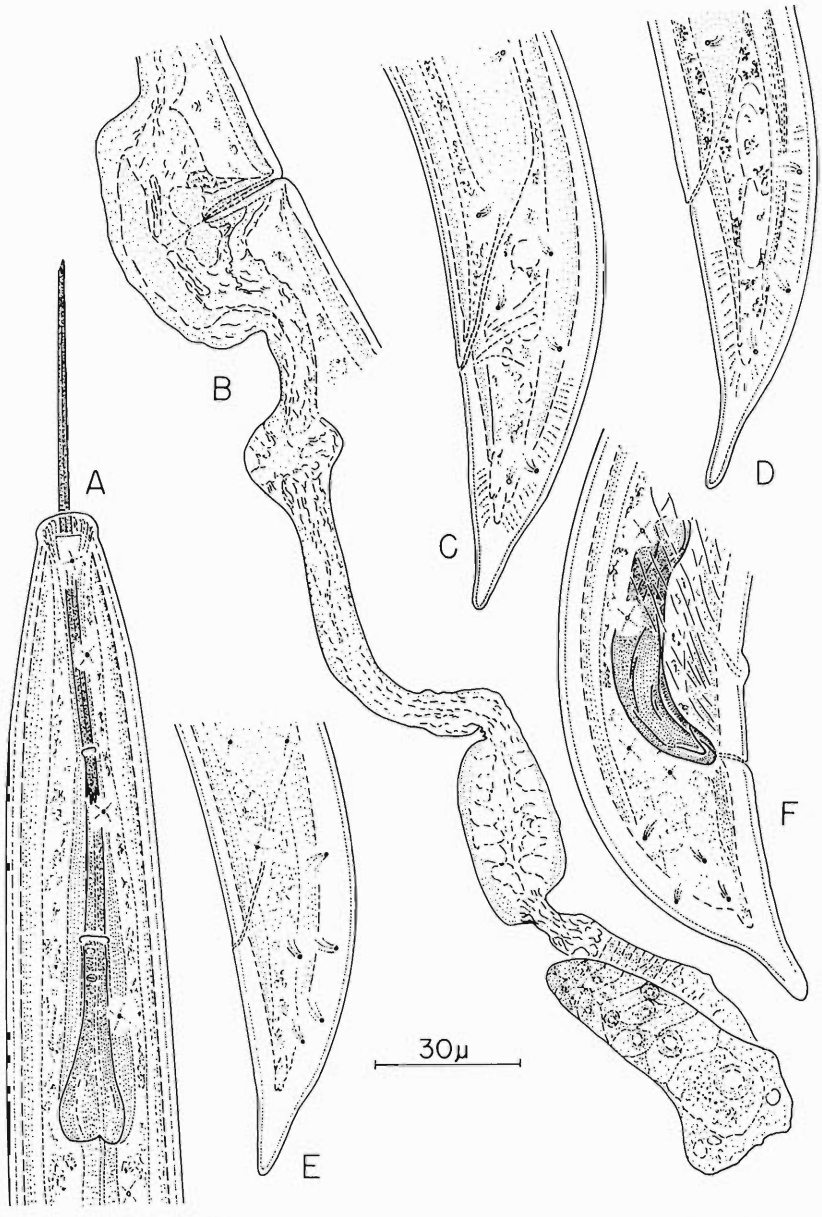

Fig. 2. A-C, E-F. *Xiphinema vulgare* n. sp. A, Anterior end of holotype female. B, Anterior gonad of paratype female. C, Tail of holotype female. E, Tail of female from Indonesia. F, Tail of allotype male. D, *Xiphinema setariae* Luc, 1958, Tail of paratype female from the Ivory Coast.

VARIABILITY OF *Xiphinema bakeri* WILLIAMS, 1961 FROM THE UNITED STATES

Four collections of *X. bakeri* from the United States—two from Oregon, one from Kentucky, and one from north Florida—exhibited sufficient similarity to the type populations from soil around raspberry roots in Hatzic, British Columbia, to be specifically identified. Yet, rather gross dissimilarities in body measurements and ratios exist, particularly between populations at least 2,000 miles separated. Table 2 lists these data.

The population from Canby, Oregon, although slightly smaller in length, otherwise conformed closely to the type specimens from British Columbia. The 12 specimens from Corvallis, Oregon, exhibited their greatest variability in the more anterior position of the vulva (29%), which is claimed to be one of the more important diagnostic features for this genus (Luc and Tarjan, 1963). Yet this measurement does not differ appreciably from that given by Williams, 1961, for his "second" collection. Both populations collected from the southeastern United States differed in almost all body measurements from the northwestern nematodes. Whereas the type population was described as having a "lip region set off by a slight but well-marked incisure," some paratype specimens personally inspected showed no incisure. The population from Oregon invariably showed a definite incisure, while those from Kentucky and Florida had labial regions only slightly set-off or apparently continuous with the rest of the body (Fig. 3A). Gonad structure did not differ between populations, consisting of the usual spacious uterine chamber and very long oviduct, with sphincter valve, which reflexed before the gonads and terminated in the ovary (Fig. 3B). Tails of the specimens from Mammoth Cave, Kentucky showed marked differences in the length and width of the terminal "peg-like' 'portions (Fig. 3C, E) as well as in the tail/anal body width ratios. Tails of the specimens from Monticello, Florida, were shorter and exhibited a shorter "peg-like' 'terminus (Fig. 3D) than for the Kentucky specimens or for the type populations (Fig. 3F). Tails of all populations contained either two or three pairs of pores (Fig. 3C-F).

The apparent differences between the northwestern and southeastern populations of this species in body length, a, b, posterior gonad length, stylet length, and tail/anal body width ratio suggest consideration of a subspecific category for the southeastern populations. However, the small number of specimens collected from the southeast coupled to the uncertainty that would be created by basing a nominal category on measurements alone suggests that the most prudent approach would be to ascribe the aforementioned variation as only geographical and not specific.

SUMMARY

Xiphinema coxi n. sp. associated with citrus roots in Florida and with grass roots in East Germany has amphidelphic gonads which exhibit a "Z" organ, a vulva located at 44% of the body length, and a conical, ventrally-convex, digitate tail. *Xiphinema vulgare* n. sp. is associated principally with citrus in central Florida, but is also found in Puerto Rico and Indonesia. It is an amphidelphic species with vulva at approximately 39% of the body length and with conical digitate tail. It differs from the most closely related species, *X. setariae* Luc, 1958 mainly by the length of the terminal "peg-like" portion in relation to tail length. Four collections of *X. bakeri* Williams, 1961 are discussed and compared.

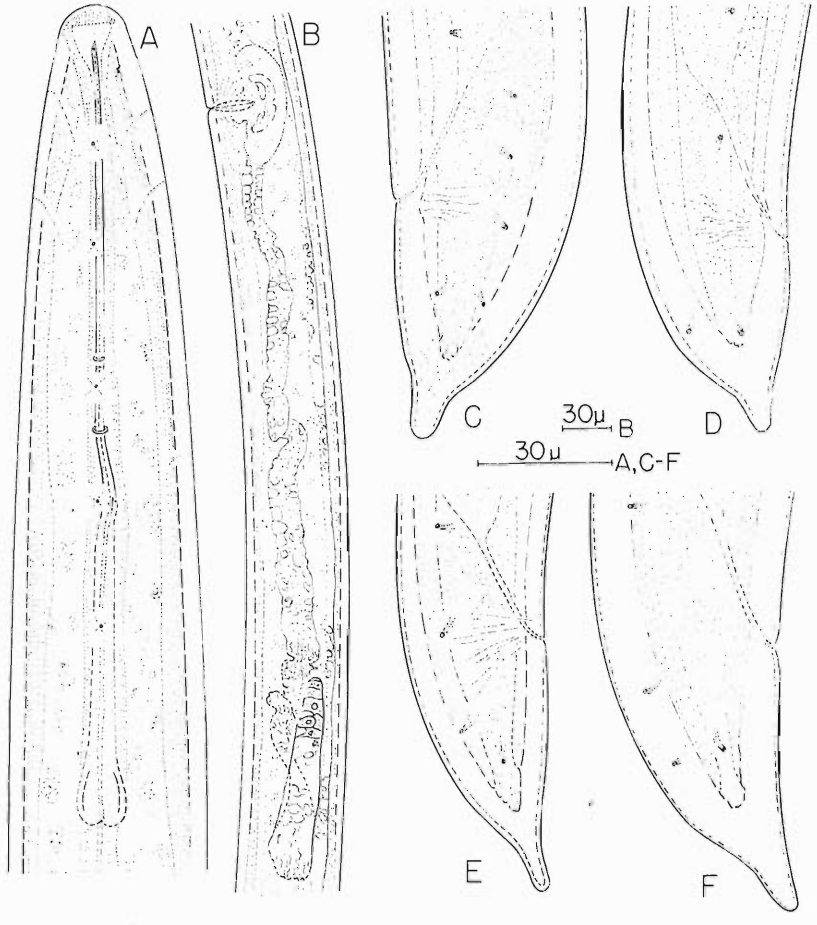

Fig. 3. *Xiphinema bakeri* Williams, 1961. Female from Mammoth Cave, Kentucky: A, Anterior end; B, Posterior gonad; C, Tail. D, Tail of female from Monticello, Florida. E, Tail of another female from Mammoth Cave, Kentucky. F, Tail of paratype female from Hatzic, British Columbia.

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Studies on *Gyrodactylus macrochiri* n. sp. (Trematoda:Monogenea) from *Lepomis macrochirus**

GLENN L. HOFFMAN AND ROBERT E. PUTZ

In September, 1960 we noted a large population of *Gyrodactylus* sp. on young bluegills (*Lepomis macrochirus*) held in our steel research troughs at 12°C. Each time thereafter when we transferred small bluegills from the hatchery ponds to our 12°C water a large population of *Gyrodactylus* soon became evident. The infection has persisted in our facilities as well as in the hatchery and adjacent waters (1961-1963). Because of its prevalance on the bluegill we propose to name it *Gyrodactylus macrochiri* of the bluegill.

MATERIALS AND METHODS

All fish were obtained from the Leetown National Fish Hatchery except one lot from the National Fish Hatchery, Lamar, Pennsylvania, one lot from farm ponds in the vicinity of Leetown, and the green sunfish, creek chub, shiner, and *Cottus* from the Leetown Run.

Gyrodactylus was removed from the fish with 1:4000 formalin in 15-45 min. and fixed and preserved in 10% formalin. Some were so removed but flattened slightly under cover glass pressure and fixed with Bouins' fixative. Whole mounts were stained with Harris' hematoxylin. Sections were made of heavily infected fish fixed with Bouin's and stained with Harris' hematoxylin and eosin.

All measurements are in microns.

Gyrodactylus macrochiri n. sp. (Fig. 1)

DESCRIPTION (based on 10 specimens removed with 1:4000 formalin and fixed in 10% formalin): Body small, cylindrical and fusiform, 413 (350-470) by 78 (60-110). Cuticle thin and smooth. Opisthaptor 97 (80-110) by 90 (85-95), a concavo-convex disc opening ventro-posteriorly; armed with 2 anchors, 2 bars, haptoral shield, and 16 marginal hooklets. Anchors moderately stout, 10-11 in greatest diameter and 69 (65-74) from base to point of greatest curvature with root 24 (23-26) and strongly recurved points 29 (27-32). Ventral bar 27 (25-30) by 7 (6-8), gently curved with antero-

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*We wish to thank Dr. Goran Malmberg, University of Stockholm, Sweden for preliminary examination of our material; Mr. Howard Jackson who designed and performed the Experimental Infection and Mssrs. Arnold Golding and Erwin W. Steucke Jr. for valuable assistance while trainees at Leetown. Mr. C. E. Dunbar kindly prepared the histological sections.

lateral projections 7 (6-8). Attached to posterior of ventral bar is the ventral shield 18 (16-20) by 17 (15-18). Dorsal bar delicate 25 (23-29) by 2 and 3 with a C-shaped notch at midpoint of the posterior edge; terminal bullae 7-9 in greatest diameter. Marginal hooklets 8 (7-9) by 5-6 with lamellae 8-11 and long thin shafts 26 (24-27) by *ca.* 1, terminated proximally by a small bulb. The head organ consists of a pair of antero-lateral, papillate organs, the ducts of which lead into a set of glands anterior to the pharynx and another posterio-lateral to it. Also seen in this region is a pair of vesicles which were seen to fill and empty as do contractile vacuoles of protozoa. The bilobed pharynx, 43 (40-48) by 36 (33-38) leads directly into the intestine which immediately bifurcates into 2 large crura. Ovarian complex consists of an ovary of several cells and usually a large developing egg (12-28) all enclosed in a thin-walled sac which appears to be connected to the posterior end of the uterus. Uterus *ca.* midway in body and usually contains an embryo. Vitellaria consist of several elongated bodies postero-lateral to the ceca. Testis is ovoid, 22 by 32 (14-26 by 28-36) and immediately posterior to ovary. The cirrus pouch, 14 (13-15) has 5-6 stylets 3-4 long and one large spine *ca.* 6 long.

HOSTS: NATURAL HOSTS—*Lepomis macrochirus* Rafinesque (bluegill), *L. cyanellus Rafinesque* (green sunfish).

EXPERIMENTAL HOSTS—*Micropterus salmoides* (black bass), *Salmo gairdneri* (rainbow trout), *Salvelinus fontinalis* (brook trout) and *Cottus bairdi* (sculpin).

LOCATION: Skin.

LOCALITY: Leetown (Kearneysville), West Virginia; Lamar, Pennsylvania.

TYPE: U. S. National Museum Helm. Coll. No. 60174.

PARATYPE: U. S. National Museum Helm. Coll. No. 59885.

COMPARISON: Of the species of *Gyrodactylus* found in North America, *G. macrochiri* most nearly resembles *G. eucaliae* Ikezaki and Hoffman, 1957 in size and armature. However, the marginal hooklet has a shorter root and the notch in the dorsal bar is C-shaped, whereas it is simple in *G. eucaliae*. It resembles *G. atratuli* Putz and Hoffman, 1963 but the ventral bar projection is much shorter and the anchor and hooklets are of different shape. It somewhat resembles *G. bairdi*, (Wood and Mizelle, 1957), *G. limi*, (Wood and Mizelle, 1957), and *G. richardsonius*, (Wood and Mizelle, 1957), but none of these has a notch in the dorsal bar and the projections of their ventral bars are not as great. *G. macrochiri* was apparently misidentified as *G. elegans* by Hargis (1953).

REPRODUCTIVE SYSTEM AND EARLY DEVELOPMENT

The precise identification of the reproductive organs of *Gyrodactylus* is very difficult because of their small size. In his revision of the superfamily Gyrodactyloidea Price (1937) defines *Gyrodactylus* as "with the characters of the subfamily (Gyrodactylinae)" and refers back to the family Gyrodactylidae for internal anatomy where: "ovary V-shaped, or lobed, post-testicular. Vitellaria absent or united with ovary. Vagina absent." Either these structures in *Gyrodactylus* vary greatly among different species or various authors have erroneously identified them. At least 5 types of female reproductive structures have been described. In *G. funduli*, *G. stephanus* and the family description the ovary is considered post-testicular (Hargis, 1955; Price, 1937). In the generalized description of *Gyrodactylus* by Malmberg

(1956) the ovary consists of completely separated lobes. In *G. elegans sinicus* the ovary is pre-testicular but very large (Yin and Sporston, 1948).

In *G. bullatardus* Turnbull, 1956 and *G. macrochiri* n. sp., the pre-testicular ovary consists of several cells enclosed in a thin-walled sac apparently attached to the posterior of the uterus. Ikezaki and Hoffman (1957) (*G. eucaliae*) apparently mistook part of the vitellaria for testis and the testis for ovary. The structure they call ootype is probably the ovarian sac complex. In *G. prolongis* the ovary is partially dorsal to the testis and both are some distance posterior to the "ootype" (Hargis, 1955).

In *G. macrochiri* n. sp., which we studied from cross sections and whole mounts, the ripe egg, presumably fertilized, passes into the uterus at or shortly after birth of the previous daughter. Division must take place almost instantly because no un-divided eggs in-utero were found in the many specimens examined. The first embryonic stage seen (Fig. 2) consists of 3 large daughter cells (14-24 microns) and several smaller cells (7 microns). This is very similar to that described by Kathariner (1904) for *G. elegans* v. Nordm. Several small cells can be seen attached to the anterior and posterior inner wall of the uterus. We presume that the anterior ones function, at least in part, as a birth pore sphincter and the posterior ones as a pore sphincter for the passage of the egg into the uterus. The next embryonic stage (Fig. 3) consists of many small cells (5 microns). The egg in the ovarian sac is now about 12 microns in diameter. The next embryonic stage (Fig. 4) consists of cells 3 microns in diameter and marginal hooklets. The egg in the ovarian sac is 20 × 28 microns in diameter. A later stage (Fig. 5) shows the anchors partly formed and the egg is about the same size, 26 × 19 microns. The anchors are completely formed in Fig. 6 and the egg is 24 × 28 microns in diameter. Further development proceeds with the phenomenon of a second embryo forming inside the first, a third forming inside the second, and sometimes a fourth forming inside the third (Kathariner, 1904).

INTENSITY AND DURATION OF INFECTION

During the first 6 months of age of the fish the population of *Gyrodactylus* on the small bluegills at 12°C was usually very high. This temperature is probably much below optimum for the bluegill but more nearly optimum for *Gyrodactylus macrochiri,* and the massive infection persisted a long time. On December 21, 1960, the following counts were obtained from 19 fish which were 2.5-7 cm. long and approximately 5 months of age: anal fin—11(3-40), dorsal fin—17(6-25), Caudal fin—8(0-30), right pectoral fin—4(0-20), left pectoral fin—7(0-28), left ventral fin—6(0-20), right ventral fin (omitted). Total on fins 60(16-165).

During examinations of bluegills for other parasites we had observed that in contrast to the above high population of *Gyrodactylus*, the bluegills taken directly from the outside rearing ponds and the Leetown stream were carrying none or only several per fish. It also appeared that the older the fish, the fewer the *Gyrodactylus*. Several bluegills were kept under observation for 1½ years under the same conditions that encouraged heavy parasite populations on the very young fish, *i.e.*, small stainless steel trough with running spring water at 12°C. At 11 months in the lab one 9 cm. fish had 4 *Gyrodactylus* on its fins, at 12 months two 7-9 cm. fish had 1 and 20 respectively. At 2 years 10 fish 7-12 cm. long, had 0-65 (ave. 18). The 5-month-old

fish had 3 times as many as the oldest ones, but although the older fish were only *ca.* twice as long, their surface area was *ca.* 5 times as great. Therefore, the smaller fish had *ca.* 15 times as many *Gyrodactylus* per unit area as the larger ones.

ENVIRONMENT

Little is known of the factors influencing *Gyrodactylus* populations other than that they must not be lethal for the host and that crowding of the fish host often results in larger populations of *Gyrodactylus*. Malmberg (1956) discusses the influence of eutrophic and oligotrophic water, pH, accumulation of metabolic by-products, temperature and the host's secretions on Swedish species.

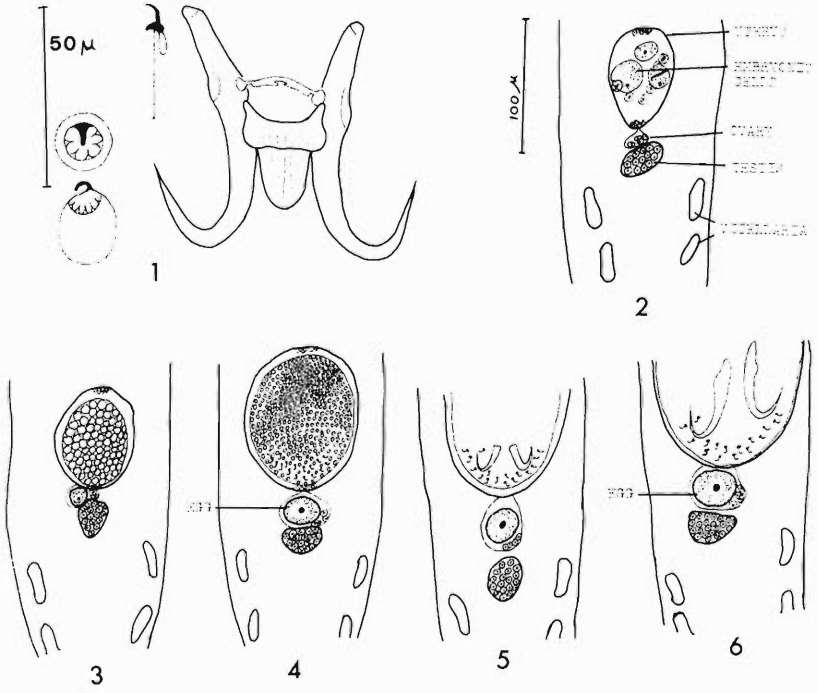

Fig. 1. Chitinoid armature of *Gyrodactylus macrochiri* n. sp. drawn by aid of microprojection. Both top and side views of the cirrus pouch are shown. The opisthaptor was flattened.

Figs. 2-6. Early development of *Gyrodactylus macrochiri* n. sp. Composite drawings to scale from cross sections and whole mounts, some of which were slightly flattened.

Fig. 2. Earliest in-utero stage seen.

Fig. 3. Embryo in many-celled stage. Oldest egg now visible.

Fig. 4. Older many cell stage. Marginal hooklets present. Egg in ovarian-sac has grown considerably.

Fig. 5. Anchor hooks are about half-formed.

Fig. 6. Development of embryo nearly completed. Egg now at maximum size.

We noted that the *Gyrodactylus* disappeared from bluegills that were transferred from the 12°C running water troughs to glass aquaria at room temperature (17-24°C). The Leetown spring water is fairly hard (262 ppm total hardness) with pH *ca.* 7.3. In an attempt to determine whether the increaser temperature, increased light, or accumulation of metabolic by-products caused the disappearance, the following experiments were performed with 6 mo. old infected bluegills:

EXPER. 1. Twenty-one bluegills were moved to aquaria at room temperature. At 1 day, 4 fish examined, many *Gyrodactylus;* at 2 days, 2 fish examined, moderate *Gyrodactylus;* at 9 days, 1 fish examined, 1 *Gyrodactylus;* at 20 days, 1 fish examined, 0 *Gyrodactylus;* at 29 days, 1 fish examined, 0 *Gyrodactylus.*

EXPER. 2. Ten bluegills aged 6 mo. which were heavily infected were moved to glass aquaria and 10 were moved to darkened aquaria, both at room temperature. At the end of 6 days none of 14 survivors had any *Gyrodactylus.* Controls at 12°C in light were still infected.

EXPER. 3. Same as Exper. 2 but water changed daily, results same.

EXPER. 4. Six fish were placed in glass aquaria at room temperature and supplied with enough 12°C running spring water to keep the water clear. At 5 days 3 fish had 0, 1, and 6 *Gyrodactylus* respectively and at 7 days 3 fish had none.

EXPER. 5. Six fish were placed in a glass aquarium and immersed in the 12°C water. At 15 days 5 fish averaged 5(1-14) *Gyrodactylus* on the fins. Controls in the trough averaged 3 (0-8). At 44 days none of those in the aquarium had any *Gyrodactylus* although the controls were still infected.

EXPER. 6. Twenty-five bluegills were placed in a glass aquarium immersed in the 12°C water. These fish were heavily infected. Between 2 and 8 days 18 dead fish were still heavily infected. At 9 days, 3 were moderately infected, and at 14 days 2 had 15 and 16 respectively on the fins.

Therefore we conclude that (1) 12°C is nearer optimum than 20°C for *G. macrochiri,* (2) subdued light at 20°C does not enhance *G. macrochiri* populations (3) the accumulation of metabolic by-products of small fish at 20°C does not enhance the population of *G. macrochiri.* Malmberg (1956) was not able to maintain *Gyrodactylus* in aquaria, but Turnbull (1956) maintained *G. bullatrudis* on guppies.

THE EFFECT OF CONCOMITANT INFECTION

One lot of *ca.* 100 bluegills 3-7 cm. long with a very sparse population of *G. macrochiri, Trichodina* sp., and *Ichthyophthirius multifilis* were moved from a pond to a stainless steel trough with 12°C running water. At 17 days the "Ich" population had become very great but caused no mortalities. At 20 days the "Ich" population was still high. At 26 days the "Ich" had greatly declined but the fish had many large white patches. During this "Ich" epizootic there were very few *G. macrochiri* present. At 27 days no ectoparasites were found on 3 fish examined. At 28 days a few *Gyrodactylus* were found on one dead fish. It is quite probable that the presence of *I. multifilis* or the stressed epithelium of the fish during an "Ich" attack inhibits *Gyrodactylus.* Dogiel, Petrushevski and Polyanski (1958, p. 46) have reviewed the "interdependence of members of a parasite fauna" and mention several cases of apparent concomitant antagonism. At 48 days no

"Ich" or *Trichodina* were seen but the *G. macrochiri* population had become very great and typical symptoms, including death from *Gyrodactylus* disease, occurred.

EXPERIMENTAL INFECTION

It is well-known that *Gyrodactylus* is easily transferred from fish to fish of the same species (Bychowsky, 1957, Malmberg, 1956) but for fish-cultural practices we wished to know how they could be transferred and how long it would take for the *G. macrochiri* population to develop at 12°C. In the following, 4 bluegills which had been disinfected with 1:4000 formalin were used for each experiment. Compressed air was supplied to each aquarium. Total counts during the experiments were made on fish anesthetized with MS-222.

EXPER. 1. Skin scrapings containing *G. macrochiri* were placed with fish in a 2 gal. aquarium; 14 days 5.8 (4-9) per fish present; 27 days 1.3 (0-4) per fish present.

EXPER. 2. Fish parasitized with *G. macrochiri* were placed with disinfected fish in a 2 gal. aquarium; 14 days 5 (4-6) per fish present; 27 days 0 (donors neg. also) per fish present.

EXPER. 3. Disinfected fish were placed in a wire basket set on bottom of trough containing many infected fish; 14 days—7.3 (6-9) per fish present; 27 days—11 (4-18)—2 died before final count.

EXPER. 4. As Exper. 3 but basket was suspended; 14 days—2.3 (0-4) per fish; 27 days—0 (3 died before final count).

EXPER. 5. Control—6 disinfected fish only in 2 gal. aquarium at 12°C; 14 days—0 per fish present; 27 days—0 per fish present.

From these experiments it is obvious that *G. macrochiri* is easily transferred from fish to fish or contaminated material to fish. Those fish having access to the bottom of the trough became more heavily parasitized than the others. Counts were also made of the parasites on each fin and the body. The most heavily infected in decreasing order were caudal fin, dorsal fin and anal fin.

HOST SPECIFICITY

Gyrodactylus macrochiri was found on *Lepomis macrochirus* and *Lepomis cyanellus* from the ponds and water supply of the Leetown hatchery but was not found on *Micropterus salmoides, Semotilus margarita, Carassius auratus, Rhinichthys atratulus, Salmo gairdneri, Salmo trutta* or *Salvelinus fontinalis.* Because it is commonly mentioned that *Gyrodactylus* will transfer to new hosts in the crowded conditions of a fish hatchery, the following experiments were set up. Heavily infected bluegills were kept in stainless steel troughs containing about 100 liters of running spring water at 12°C. Test fish were kept in wire baskets in the same trough.

EXPER. 1. One *Semotilus atromaculatus;* 5 days, neg.; 3 mo, neg. One *Cottus bairdi;* 5 days, 3 on fins.

EXPER. 2. 20 *Salmo gairdneri* fry; 2 days, one with one *Gyrodactylus;* 7 days, 2 with one *Gyrodactylus* each; 9 days, 7 with no *Gyrodactylus,* one with 5 *Gyrodactylus;* 14 days, one with no *Gyrodactylus,* one with 5 *Gyrodactylus.*

EXPER. 3. 20 *Salvelinus fontinalis* fry; 4 days, one fry had one *Gyrodactylus* (remainder died).

EXPER. 4. One *Notropis* sp.; 8 days, no *Gyrodactylus.*

EXPER. 5. 11 *M. salmoides,* 2-3" 2 days, 9 with no *Gyrodactylus;* 37 days, 1 with no *Gyrodactylus;* 60 days, 1 with moderate number of *Gyrodactylus* (not counted). This fish had not been eating and was greatly emaciated.

Although *G. macrochiri* was transferred to hosts other than the normal one it is doubtful that these infected abnormal hosts would be found in nature. There was no indication that any significant distress was caused to the abnormal hosts by the presence of *G. macrochiri.* Mr. Elmo Barney, Coleman National Fish Hatchery, Anderson, California, has told us that all of their ponds of *Salmo gairdneri* were infected with *Gyrodactylus,* but he has not found any *Gyrodactylus* on any of the *Oncorhynchus tshawytscha* in 31 adjacent ponds.

SUMMARY

Gyrodactylus macrochiri n. sp. is described from the bluegill, *Lepomis macrochirus,* and green sunfish, *L. cyanellus. Micropterus salmoides* was infected experimentally but is considered an accidental host. The early development and reproductive system are discussed. Experimentally, 12°C was nearer optimum than 20°C, subdued light and removal of waste did not enhance the *G. macrochiri* population. A severe *Ichthyophthirius* epizootic reduced the *G. macrochiri* population temporarily. *Gyrodactylus*-free bluegills became infected when placed in wire baskets among infected fish, but those which could contact the bottom became more heavily infected. The bluegill appears to be the primary host but a very few *G. macrochiri* were transferred to *Micropterus salmoides, Salmo gairdneri, Salvelinus fontinalis,* and *Cottus bairdii* under crowded conditions with heavily infected bluegills. There was no evidence of distress produced by the few worms on the abnormal hosts.

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Hypsoperine graminis (Nematoda: Heteroderidae), a New Genus and Species of Plant-Parasitic Nematode.

E. B. SLEDGE AND A. MORGAN GOLDEN*

An undescribed nematode of the family Heteroderidae was found as a parasite of St. Augustine grass, *Stenotaphrum secundatum* (Walt.) Kuntze, by the senior author in Florida in 1959. Under natural conditions infected grass became chlorotic and died. The infected roots at most had only slight swellings at the sites of nematode infections. Most mature female nematodes were completely embedded in the roots, but some protruded conspicuously with only the head and neck embedded in the host tissue. The occurrence of this nematode was reported by van Weerdt, et al (1960). Sledge (1962) found in host tests in the greenhouse that it completed its life cycle on six out of nine grass varieties tested, but did not develop through a life cycle on plants other than grass, such as, tomato, squash, corn, carrot, grapefruit, and peach. He noted small galls and considerable root necrosis on infected grasses, but observed no symptoms of parasitism on other inoculated plants. Histological studies of infected St. Augustine grass roots revealed the presence of giant cells.

Preliminary examination of specimens indicated that this nematode was a new species of the genus *Meloidogyne*. However, the results of closer study led the authors to believe that this form represents a new genus which occupies a position between *Heterodera* and *Meloidogyne*, being closer to the latter. A description of this new genus and new species is presented herein.

Hypsoperine n. gen.

DIAGNOSIS: Heteroderidae with pronounced sexual dimorphism. Female body white, oval, with very thick cuticle and well-defined, protruding neck usually situated to one side. Body cuticle composed of fine transverse striae which form a perineal pattern. Vulva and anus situated posteriorly on a slight protrusion. Median esophageal bulb prominent. The two convoluted ovaries become obscured as eggs develop and the uterus expands. Eggs are deposited outside of the body in a gelatinous matrix. Stylet distinct, with rounded knobs.

Male undergoes complete metamorphosis. Adult body cylindroid, vermiform with strong cephalic framework. Stylet stout with prominent knobs. Esophageal glands well developed. Tail devoid of caudal alae. Testis one. Hemizonid located posterior to the excretory pore in the larvae of some species.

TYPE SPECIES: *H. graminis*, n. sp.

DISCUSSION: The name *"Hypsoperine"* suggests "elevated perineum." It is of the neuter gender and is the latinized combination formed from the Greek "hypsos," meaning high, and "perineos" referring to the area between the anus and the genital opening.

Hypsoperine graminis, n. gen., n. sp.

MEASUREMENTS: 20 females (Fig. 1 & 3)—Length 0.726 mm (0.586-0.841); width 0.472 mm (0.280-0.680); stylet 12.46 microns (11.70-13.44).

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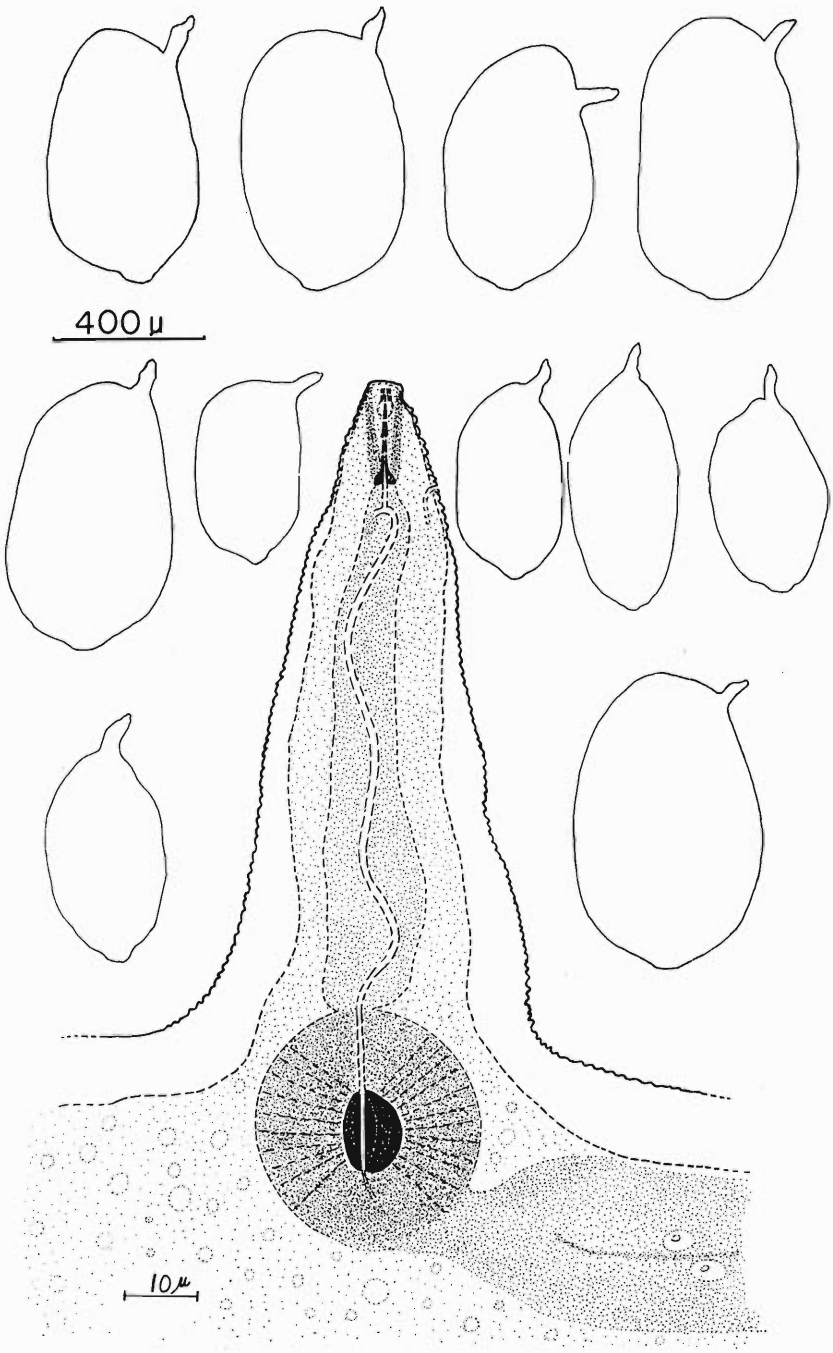

Figure 1. Drawings of females of *H. graminis*, n. gen. n. sp. (Outlines of 11 females and details of anterior portion of 1 specimen).

HOLOTYPE (female): length 0.816 mm; width 0.490 mm; stylet 12.3 microns.

Body white and oval with protruding neck usually situated well to one side of median plane through vulva. Cuticle finely annulated and quick thick, measuring 21.50 microns (16.80-32.48) at the thickest point on body. Head bearing no annules and not distinctly set off from neck. Lip region variable in exact shape but apparently with circumoral elevation; cephalic framework indistinct. Cephalids not observed. Stylet knobs rounded posteriorly. Esophagus well developed with elongate, cylindrical procorpus and large spherical metacorpus provided with heavily sclerotized valve. Esophageal glands with three prominent nuclei. Junction of esophagus and intestine obscure. Orifice of the dorsal gland 3.73 microns (3.36-4.48) posterior to base of stylet. Excretory pore distinct; generally located about on a level with knobs of unprotruded stylet. Ovaries two, becoming indistinguishable as the uterus becomes packed with eggs and enlarges, eventually filling the body cavity. Vulva and anus situated posteriorly on a slight but distinct button-like protrusion of the body. Perineal pattern coarse, with rather high arch, and lateral lines not completely interrupting transverse striations. Eggs deposited in a gelatinous matrix.

MEASUREMENTS: 20 males (Figs. 2, A & B)—Length 1.512 mm (1.275-1.734); a = 43.50 (37.38-50.39); b = 7.25 (6.42-8.2); c = 187.31 (131.70-273.93); stylet 18.31 microns (17.92-19.00).

ALLOTYPE (male): Length 1.420 mm; a = 41.20; b = 7.1; c = 220.0; stylet 18.40 microns.

Body cylindroid, vermiform, tapering gradually at the ends. Body width 34.89 microns (30.80-42.00). Head slightly offset from body. Cuticular annulation distinct. Annules approximately 2.5 microns wide in the middle region of the body, becoming smaller toward both ends of the body. Lateral field 7.98 microns (7.28-8.40) wide, with 4 lines; not aerolated except in extreme anterior portion. Cephalic framework prominent. Stylet stout, with knobs rounded posteriorly but not so rounded anteriorly. Orifice of the dorsal gland 2.48 microns (1.68-2.80) posterior to base of stylet. Median bulb elongate with well developed sclerotized valve. Length of esophagus (from anterior end to base of esophagus) 212.15 microns (202.0-224.0) and from center of median bulb to base of stylet 74.0 microns (68.0-81.0). Hemizonid prominent, located in the first two annules anterior to the excretory pore. Hemizonion small but distinct, located about 8 annules posterior to the excretory pore. Spicules arcuate 28.26 microns (28.0-29.12) long. Gubernaculum 8.12 microns (7.84-8.40) in length. Tail length 8.46 microns (5.60-11.20). Phasmids about 4 microns from tail tip.

MEASUREMENTS: 20 second-stage larvae (Figs. 2, CDE)—Length 0.475 mm (0.420-0.510); a = 31.74 (28.77-33.99); b = 2.33 (2.10-2.95); c = 6.07 (5.71-6.78); stylet 12.61 microns (11.70-13.44).

Body cylindrical, vermiform, tapering considerably toward posterior end. Width 15.00 microns (14.56-15.68). Head not offset from body and bearing no visible annules. Cuticular annulation of the body well marked. Lateral field 4.52 microns wide (3.92-5.04), with 4 lines. Cephalic framework indistinct. Stylet weak with rounded knobs. Orifice of the dorsal gland 2.49 microns (2.24-2.80) posterior to base of the stylet. Median bulb elongate with prominent sclerotized valve. Length of esophagus (from anterior end to base of esophagus) 200.4 microns (179.0-224.0), and from center of median bulb to base of stylet 43.33 microns (39.0-47.0). Hemizonid located

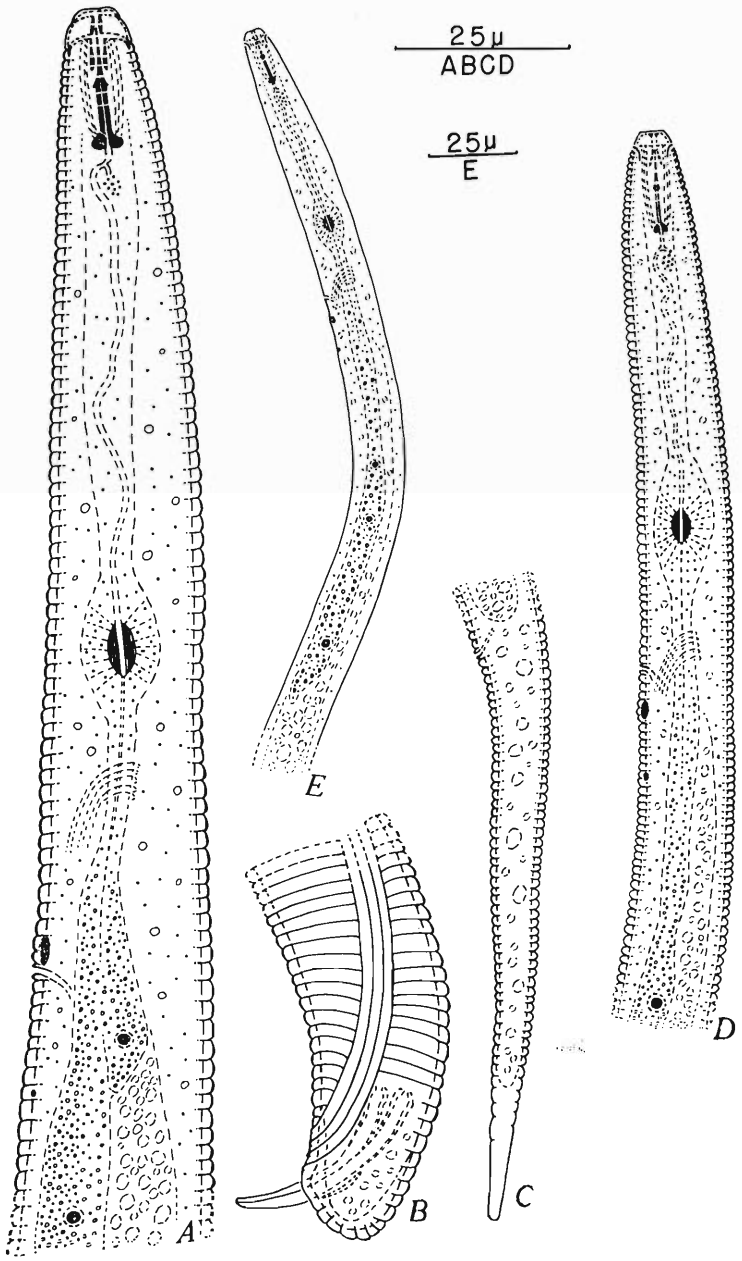

Figure 2. Drawings of male and larva of *H. graminis*, n. gen., n. sp. A & B—Anterior and posterior portions of a male. C, D & E—Anterior and posterior portions of a larva.

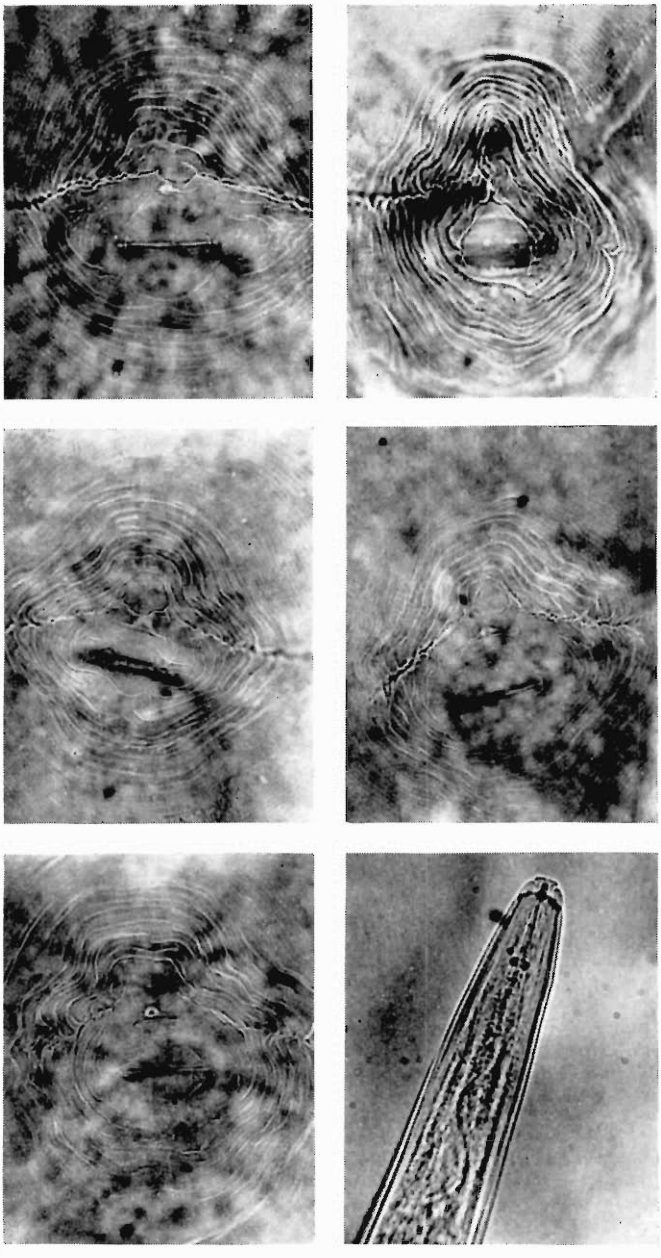

Figure 3. Photomicrographs of *H. graminis*, n. gen., n. sp. (five perineal patterns of females and, lower right, ventral view of anterior portion of a male).

approximately 4-5 microns posterior to the excretory pore. Tail 78.28 microns (68.0-88.0) long. Hyaline portion of tail 18.54 microns (14.0-22.4) long. Caudal ratio A 4.32 (3.90-4.92).* Terminus rounded.

DIAGNOSIS: *Hypsoperine* with above measurements and description. Separated from the one other species especially by having a coarse, very distinct perineal pattern.

HOLOTYPE: Female: Collected by E. B. Sledge, July 27, 1962. Winter Haven, Florida. Slide T-47t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

ALLOTYPE: Male: same data as holotype. Slide T-48t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

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

TYPE HABITAT, HOST, and LOCALITY: Roots of *Stenotaphrum secundatum* (St. Augustine grass) in lawn around Division of Plant Industry Laboratory, Winter Haven, Florida.

Other species: *Hypsoperine acronea* (Coetzee, 1956) n. comb.; Syn. *Meloidogyne acronea* Coetzee, 1956, n. syn.

DISCUSSION: In describing this species, Coetzee (1956) pointed out that the posterior portion of the female body "is drawn out into a distinct rounded protuberance on which are situated vulva and anus." Also, among other things, she noted that the female "cuticle is tougher." Examination by the authors of specimens of *H. acronea* from the type host and locality confirmed the observation of Coetzee, including the presence of a thick cuticle; and furthermore, it was found that the basic shape of the female is oval as is the species described herein rather than pear- or flask-shaped as is characteristic of *Meloidogyne* species. These and other differences given in the description indicate that *H. acronea* and *H. graminis* n. sp., form a distinct and separate group of nematodes although closely related in many respects to *Meloidogyne* species.

H. acronea has a faint, very indistinct perineal pattern easily distinguishing it from the one other described *Hypsoperine* species.

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*Calculated by dividing the length of the hyaline portion of the tail by its width at its beginning anteriorly. (Golden and Cobb, 1963).

Remarks on the Genus *Oxyspirura* (Nematoda: Thelaziidae) with description of *Oxyspirura (O.) basiri* n. sp.

ATHER H. SIDDIQI AND M. SHAMIM JAIRAJPURI*

The genus *Oxyspirura* Drasche in Stossich, 1897 is very host-specific and has a large number of species reported from different types of birds from various parts of the world. Usually each host species has its own species of nematode as pointed out by Ali (1960). There are now 49 species in all divided into three subgenera: *Oxyspirura* (Drasche in Stossich, 1897) Skrjabin, 1931; *Cramispirura* Skrjabin, 1931 and *Yorkeispirura* Skrjabin, 1931. The diagnosis of these three subgenera is based on the divided (*Yorkeispirura*) or undivided buccal capsule (*Oxyspirura* and *Cramispirura*) and equal (*Cramispirura*) or unequal spicules (*Oxyspirura*). Yeh Liang-Sheng (1957) considered this division unsuitable, whereas recently Barus (1963) further subdivided the subgenera *Oxyspirura* and *Yorkeispirura* and added two more subgenera *Skrjabinispirura* and *Caballeroispirura*. Under *Skrjabinispirura* he included those species of *Oxyspirura* which do not possess cephalic alae and may or may not have a gubernaculum. To *Caballeroispirura* he transferred those species of *Yorkeispirura* which have cephalic alae and a gubernaculum. The presence or the absence of cephalic alae and gubernaculum are not characters of subgeneric status, and therefore erection of subgenera cannot be justified only on these two bases. The subgenera *Skrjabinispirura* and *Caballeroispirura* are therefore considered here as synonyms of *Oxyspirura* and *Yorkeispirura* respectively. The new species described by Barus (1963) as *Oxyspirura* (*Skrjabinispirura*) *rysaryi* now becomes *O.* (*O.*) *rysaryi* (Barus, 1963) n. comb.

As regards the importance of divided or undivided character of the buccal capsule, it has been used quite frequently as a character of generic rank for various groups of nematodes and it is here proposed that *Yorkeispirura* should be raised to the status of an independent genus *Yorkeispirura* (Skrjabin, 1931) n. grad., thus making the following combinations necessary: *Y. tanasijtchuki* (Skrjabin, 1916) n. comb., *Y. hispanica* (Yeh Liang-Sheng, 1957) n. comb., *Y. mansoni* (Cobbold, 1879) n. comb., *Y. tsingchengensis* (Hsü, 1933) n. comb., *Y. octopapillata* (Caballero, 1942) n. comb., and *Y. navali* (Caballero, 1936) n. comb.

Two species of the subgenus *Oxyspirura* were collected from the orbital cavities of two birds in Aligarh, India. The one from the pied crested cuckoo, *Clamator jacobinus* (Boddaert) represents a new species, for which the name *Oxyspirura* (*O.*) *basiri* is proposed after Professor M. A. Basir; whereas the other species *O.* (*O.*) *buccosulcata* Singh, 1948 has been recorded from a new host, the pied myna, *Sturnus contra* L.

Oxyspirura (*O.*) *basiri* n. sp. (Figs. A-D)

DESCRIPTION: Based on two males and a single female: Worms slender, cylindrical body, bluntly rounded anteriorly and sharply tapering posteriorly. Anterior end surrounded by broad cephalic alae. Cuticle thin and transparent, marked by fine transverse striations. Head simple, 60-83 microns in diameter. Four pairs of prominent submedian papillae and a pair of amphids

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situated laterally. Buccal capsule heavily cuticularized, 28-30 × 22-28 microns. Nerve ring situated near junction of muscular and glandular portions of esophagus. Excretory pore located at 0.30-0.34 mm. from anterior end.

MALE: Males measure 10.6-12.2 × 0.29-0.33 mm. Esophagus 0.76-0.81 mm. long with an anterior muscular portion 0.14-0.16 mm. and a posterior glandular portion 0.62-0.65 mm. Ratio between muscular and glandular portions of esophagus 1:4. Tail ventrally hooked, and sharply pointed at tip, 0.22-0.34 mm. long.

Testis extends up to 80% of body length and then reflexes to about half of its length. Three pairs of precloacal and four pairs of postcloacal papillae; last pair of postcloacals somewhat bigger in size and located at about one third of tail length from cloaca. Spicules very unequal in size and different in form; smaller one stout with blunt ends and measures 206-220 × 36-43 microns; longer comparatively delicate, pointed at tip, and measures 480-564 × 14-20 microns; spicule ratio 1:2.5.

FEMALE: Slightly larger than males; it measures 13.6 × 0.30 mm. Muscular portion of esophagus 0.15 mm. and glandular portion 0.67 mm; ratio between muscular and glandular portions of esophagus approximately 1:4. Tail conical and sharply pointed, 0.55 mm. long.

Vulva situated near posterior end, less than a tail length above anus. Vagina a short, prominent cuticularized tube extending inword and forward. Gonads prodelphic and reflexed. Uteri packed with embryonated eggs measuring 40-43 × 28-29 microns.

RELATIONSHIP: In the possession of cephalic alae and three pairs of precloacal and four pairs of postcloacal papillae the present species *O.* (*O.*) *basiri* n. sp., comes closest to *O.* (*O.*) *kaitingensis* Hsü, 1933 (Hsü, 1933) and *O.* (*O.*) *otocompsa* Rasheed, 1960 (Rasheed, 1960) but differs from them markedly in the larger size of the body, esophagus, tail, spicules and eggs. The disposition of the pre- and postcloacal papillae is also different.

Oxyspirura (*O.*) *buccosulcata* Singh, 1948

Only a single male specimen of this species was found. In the presence of wing-like cephalic alae, the number of pre- and postcloacal papillae, and in various body measurements the present material is in complete agreement with that of Singh (1948). However, there are some differences in the nature of the cuticle, structure of the spicules and in the disposition of the pre- and postcloacal papillae. In the present material the cuticle is distinctly transversely striated; the posterior ends of both the spicules are blunt and only the larger spicule is anteriorly cephalated; the last pair of precloacal papillae is almost cloacal, whereas the last pair of postcloacal papillae is not located near the caudal end as shown by Singh (1948).

SUMMARY

Barus (1963) proposed two more subgenera, *Skrjabinispirura* and *Caballeroispirura* under the genus *Oxyspirura* Drasche in Stossich, 1897 in addition to already existing *Oxyspirura*, *Yorkeispirura* and *Cramispirura*. *Skrjabinispirura* and *Caballeroispirura* are here considered as synonyms of *Oxyspirura* and *Yorkeispirura* respectively, since their diagnoses are based merely on the presence or absence of cephalic alae and gubernaculum which are characters of not more than specific rank. The species *O.* (*S.*) *rysavyi* Barus, 1963 now becomes *O.* (*O.*) *rysavyi* (Barus, 1963) n. comb. The subgenus *Yorkeispirura* in having a divided buccal capsule is here raised to an independent genus *Yorkeispirura* (Skrjabin, 1931) n. grad. A new

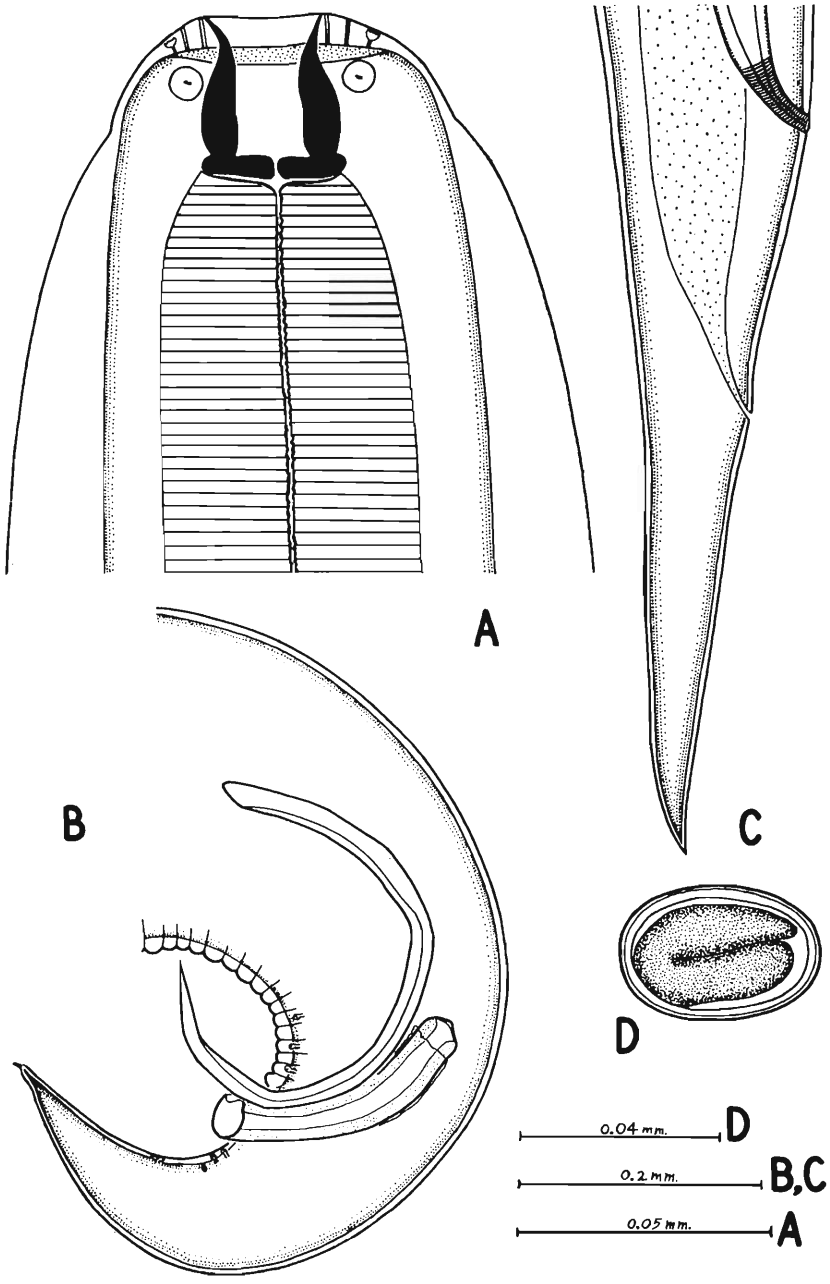

Fig. A-D. *Oxyspirura* (*O*). *basiri* n. sp. A. Anterior end of male. B. Posterior end of male. C. Posterior end of female. D. Egg.

species *O.* (*O.*) *basiri* from *Clamator jacobinus* and a known species *O.* (*O.*) *buccosulcata* Singh, 1948 from a new host, *Sturnus contra* are reported from Aligarh, India.

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Immunization Against the Cattle Lungworm: Experiments on Oral Vaccination of Calves with X-irradiated *Dictyocaulus viviparus* Larvae*

JOHN T. LUCKER AND H. H. VEGORS

The purpose of this paper is to report on two experiments carried out to test the reported efficacy of oral vaccination of calves with X-irradiated *Dictyocaulus viriparus* larvae for protection against patent infection with this lungworm.

Experiment 1 was patterned after the pioneering one of Jarrett *et al.* (1957) in which vaccination with 4,000 irradiated larvae was found to induce a high level of protection. However, both the level of the challenge exposure and the interval from vaccination to challenge were intentionally greater than in the Scottish workers' comparable test. The results have been reported heretofore in abstract only (Lucker and Vegors, 1960).

Experiment 2 was intended to ascertain whether the rate of exposure to the X-irradiation might be an important determinant of the induced level of resistance. Otherwise, it was patterned after the test of Jarrett *et al.* (1959), in which double vaccination with 1,000 larvae was found to give complete protection against challenge with 10,000 larvae, where the intervals between vaccinations and from second vaccination to challenge were 42 and 51 days, respectively.

MATERIALS AND METHODS

CULTURE OF LARVAE: Infective *D. viriparus* larvae were reared by us from first-stage larvae isolated by baermannization from feces of infected calves.

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The writers are grateful to Dr. Howard L. Andrews and Mr. R. W. Swain, National Institutes of Health, Bethesda, Maryland, who placed X-ray machines at our disposal, calibrated the outputs, and operated or supervised operation of the sources during irradiation of larvae used in both experiments; to Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, who supplied the Dictol and some of the normal larvae used in Experiment 2; and to Dr. D. O. Everson, formerly of ARS Biometrical Services, Beltsville, Maryland, who analysed the data on worm loads in Experiment 2 for statistical significance.

Those used in Experiment 1 were obtained from moist, animal-charcoal cultures kept seven days at room temperature (68° to 75° F.). Those used in Experiment 2 were cultured in water about ½ cm deep in Syracuse watchglasses kept seven days at 68-70° F. Larvae were washed from the cultures, passed through a fine screen, sedimented, and stored in water at about 42° F. until used. Also used in Experiment 2 were infective larvae obtained from Fort Dodge Laboratories, Fort Dodge, Iowa. They were reared directly in and flushed from fecal cultures, essentially as described by Jarrett *et al.* (1957), and washed on screens prior to sedimentation and storage. The designations BPL and FDL are hereinafter applied to larvae reared at the Beltsville Parasitological Laboratory and at the Fort Dodge Laboratories, respectively.

IRRADIATION OF LARVAE: Both FDL and BPL larvae were exposed to X-rays while in water 1 cm deep until, according to calibrations in air, the source had delivered 40,000 roentgens at the surface of the water. BPL larvae were irradiated in distilled water at the National Institutes of Health, Bethesda, Maryland, under supervision of radiation physicists, who also calibrated the sources used.

EXPERIMENT 1. The X-ray source was a 3 Mev Van De Graaff generator, operated at about 2.5 Mv and 0.75 ma without external filtration (9.6 mm lead HVL). The distance from the target to the surface of the water in the petri dish, which contained the larvae with some very finely divided charcoal, was 40 cm. The dose-rate was about 2,650 roentgens per minute (r/m).

EXPERIMENT 2 FDL larvae, according to the information given to us, were irradiated at about 100 r/m by a source operated at 150 kv and 12 ma with filtration by 0.25 mm Cu and 1 mm Al. The maximum temperature of the medium was about 60° F. At intervals of about 1 hour irradiation was interrupted for about five minutes and the medium stirred.

With BPL larvae, the procedure was as follows. Three aliquots were taken from an agitated suspension. Each was placed in a 2-inch stender dish in a larger glass vessel containing cold water. For irradiation of two of the aliquots, a 250 kv unit, operated at 140 kv with filtration by 0.25 mm Cu and 1 mm Al (0.65 mm copper HVL), was used. One was exposed with the unit operated at 10 ma and a dose-rate of 200 r/m, the other at 20 ma and a dose-rate of 400 r/m. The distance from the target to the surface of the medium was about 15 cm. The maximum period of continuous irradiation was 55 minutes. The medium was stirred and recooled at the end of each such period; its temperature during irradiation ranged from 62° to 70° F. The third aliquot was irradiated with the aforementioned Van De Graaff generator; the target to surface distance was 40 cm. On the first occasion of its use, the generator was operated at about 2.5 Mv and 0.8 ma; the dose-rate was about 2,900 r/m. On the second occasion, unexpected difficulties forced operation mostly at about 2.2 Mv and 0.6 ma and, though peaks of operation at 2.6 Mv and 0.8 ma were attained, the dose-rate averaged only about 1,200 r/m.

ANIMALS: All calves were Holsteins, reared and kept at all times in masonry pens in barns under conditions that precluded extraneous exposure to *D. viviparus* and other nematodes. Except as otherwise noted, test and control calves were penned in pairs. Pairs were matched on the basis of age, weight, and prior average daily gain. Eight calves about 4.5 months old on day 0, the day larvae were first administered to any calf, were used in Experiment 1, six as test animals and two as controls. Twenty calves,

which were about three months old on day 0, were used in Experiment 2. Eight were used as the four vaccinated pairs and four as controls; these twelve were necropsied at the end of the experiment. The other eight were used as follows: Four were vaccinated, each with a unit of a different one of the four vaccines under test, when the principal pairs were first vaccinated, and the remaining four were so vaccinated when the principal pairs were again vaccinated. Each was kept in a separate pen and was necropsied about 11 days post-vaccination.

Mice and guinea pigs also were used in Experiment 2 to compare the ability of the larvae from the different vaccines and of normal larvae to reach the lungs of these hosts.

ORIGIN, COUNTING AND ADMINISTRATION OF LARVAE: Doses of BPL larvae simultaneously administered to different individuals, whether calves or rodents, consisted of aliquots of a suspension of the desired kind of larvae, *viz.*, normal, or irradiated at a particular rate. The number of larvae/ml of suspensions was determined from the number counted in samples, only those that were active or tightly coiled and of normal appearance being enumerated. FDL vaccine was received in small bottles each containing a vaccination unit, which, so far as known, was an aliquot of a single suspension of simultaneously irradiated larvae. Dosage for vaccinations I and II in Experiment 2 was based on the number of larvae counted in an FDL vaccination unit.

The irradiated and normal larvae administered for immunization in Experiment 1 were initially part of a single suspension of normal larvae. As previously noted, the same is true of the differently irradiated BPL larvae used for each vaccination in Experiment 2. Challenge doses in this experiment were aliquots of a suspension of half and half FDL and BPL normal larvae. BPL normal larvae given to rodents were from the suspension that was the source of the aliquots irradiated.

A metal dosing pipette fitted with a rubber bulb was used for administration of larvae to calves; administration to rodents was by means of a plastic stomach tube or metal dosing pipette attached to a small glass syringe.

DETERMINATION OF OUTPUTS OF FIRST-STAGE LARVAE: In Experiment 1, from days 20 to 92 after vaccination, a rectal sample of at least 200 gm of feces was collected every other day from each test calf; thereafter, until shortly before challenge, it was collected as least biweekly. In Experiment 2, a sample was collected daily from each vaccinated calf from days 20 to 32 after each vaccination; in the rest of the period prior to challenge, collections were weekly. In both experiments, the feces of the controls were sampled weekly prior to challenge. Beginning 17 to 20 days postchallenge and until shortly before necropsy, the feces of all calves were sampled daily. All samples were baermannized and any larvae recovered enumerated directly or by a dilution-counting method. Outputs of larvae were computed from the average numbers of larvae recovered per gram of feces and the weights of the appropriate 24-hour fecal deposits of the calf-pairs.

CLINICAL AND POSTMORTEM OBSERVATIONS: With exceptions noted below, the calves were weighed weekly and were otherwise clinically examined as follows: Body temperature was determined daily. Respiratory rate, the occurrence of coughing, and abnormal lung sounds on auscultation, were noted daily Monday through Friday throughout Experiment 1. In Experiment 2, they were noted at an average interval of three days for six weeks after vaccination I and eight weeks after vaccination II; from days 8 to 26

postchallenge, they were noted more frequently, the occurrence and intensity of coughing being noted daily. The calves killed about 11 days postvaccination in Experiment 2 were not examined clinically after vaccination.

At necropsy, the lungs of all calves were observed grossly for extent of consolidation, edema, emphysema, and inflammation and mucopurulent material in the air passages. Numerical scores were used to express the observed degrees of damage. They were based more on relative severity within the necropsy-series than on absolute criteria. However, in general, very slight damage in calf lungs was denoted by 0.25 and progressively greater damage by larger numbers up to 3.0 for very extensive and severe pathologic change. Lungs of rodents were examined for numbers of petechiae and extent of pneumonic areas.

RECOVERY AND ENUMERATION OF WORMS FROM CALVES: The lungs and trachea were removed intact at necropsy to a tray containing 0.8 per cent saline solution. All observed worms and fragments thereof were collected with forceps as the following processing steps were carried out: (1) opening of the trachea, which was thereafter scrubbed and rinsed in saline in a bucket; (2) opening of the respiratory tree as completely as possible with the lungs dorsal-side upwards in the tray; (3) cutting the lobes into fist-sized chunks and completion of opening of the bronchioles. The worms so collected were promptly preserved, usually in hot alcohol.

The chunks were soaked, squeezed, and rinsed in the saline in the bucket, then removed to another tray. The sediment from the bucket and first tray was preserved and stored in a container marked "washings."

Next the chunks were diced with serrated-blade postmortem shears into about ½-inch pieces which were suspended in saline in battery jars overnight. The diced tissue was removed in the morning and the sediment that settled in the jars after several hours was preserved and stored in a container marked "Baermann."

The number of worms recovered from a calf was determined as follows: The aforementioned sediments, plus any worms initially collected with forceps and later cut up and added to the sediments as mentioned below, were placed in suspension separately or after consolidation. From at least one 1/20 aliquot of the suspension, all intact worms and head-end and tail-end pieces were collected and counted under a dissecting microscope. Specimens somewhat less than 1 mm long were detected by this method. In Experiment 1, if the number of worms collected initially with forceps was estimated to exceed 300, about 50 to 75 intact ones of each sex were taken at random for subsequent measurement and were individually counted. Those not so counted were cut up finely for good dispersion in liquid and added to the sediments prior to suspension of the latter. If the number so collected was estimated to be less than 300, all intact worms and terminal pieces were counted individually. In Experiment 2, all of the intact worms and terminal pieces collected initially, plus others removed from the sediments prior to suspension, were individually counted.

RECOVERY AND ENUMERATION OF WORMS FROM RODENTS: The lungs were finely cut up and baermannized overnight in saline solution in small funnels. To determine the numbers of larvae recovered, 15 ml of fluid was withdrawn per funnel and the larvae in aliquot samples were counted.

MEASUREMENT OF WORMS: Where the number of worms recovered postchallenge was small, the length of all available intact specimens was deter-

mined. Where the number was large, 50 of each sex were taken at random and measured.

DATA OF EXPERIMENT 1

On day 0, each calf of three pairs received 4,000 larvae for immunization; pair N-larvae (tables 1 and 2) received normal larvae, whereas pairs X-larvae 1 and X-larvae 2 (tables 1 and 2) received irradiated larvae. On day 129, each of these calves and each calf of the control pair received 14,800 normal larvae for challenge. One calf of each pair was necropsied on day 34 and the other on day 36 postchallenge.

FINDINGS PRIOR TO CHALLENGE: Vaccination with the irradiated larvae evidently did not result in patent infection since the feces of all four calves so vaccinated were negative for first-stage larvae on all examinations. Both calves vaccinated with normal larvae became substantially infected with mature worms. The patent period was about two months in one of them and about four months in the other. On the average they eliminated 5,225,000 first-stage larvae from days 20 to 128 postvaccination.

This pair (N-larvae) also evidenced typical signs of severe lungworm disease. Elevation in body temperature was last observed about eight weeks postvaccination, but respiratory distress continued for a longer period. One calf of pair X-larvae 2 coughed rather persistently during the second month postvaccination and showed signs of minor pulmonary involvement for a longer period. That these symptoms were due to vaccination seemed doubtful because the other three identically vaccinated calves were asymptomatic. The average daily weight gain of pair X-larvae 2 was inferior to that of the control pair. However, this likewise was not clearly due to vaccination because pair X-larvae 1 gained as well as the controls.

FINDINGS POSTCHALLENGE: Postchallenge each pair previously vaccinated with irradiated larvae eliminated at least as many first-stage larvae in its feces as the control pair, whereas output by pair N-larvae was negligible (table 1). These findings indicated that pair N-larvae was barely susceptible to patent infection on challenge, whereas all other pairs were at least moderately susceptible.

The clinical signs were fairly consistent with these parasitological indications. Three of the four calves vaccinated with irradiated larvae showed significant elevation in body temperature on a few more days than either control calf. No appreciable temperature response was observed in the

Table 1. Total outputs of first-stage lungworm larvae in feces of calves from time of challenge exposure (14,800 larvae) to necropsy (Experiment 1).

Pair	No. larvae/calf
	Vaccinated with normal larvae
N-larvae	1,165
	Vaccinated with irradiated larvae
X-larvae 1	1,528,000
X-larvae 2	778,500
Average	1,153,250
	Unvaccinated
Control	698,000

remaining one or in pair N-larvae. Respiratory rate was markedly accelerated in all calves roughly one-half of the time beginning about seven days post-challenge. The incidence and severity of other signs of respiratory distress in three calves of the X-larvae pairs were about as in the controls; the other was affected a little less, and the N-larvae pair much less, than the controls. The rate of gain of all pairs was affected adversely; that of the control pair was affected most and that of pair N-larvae least.

The degree of lung damage observed grossly at necropsy was least in pair N-larvae (table 2). In only one calf of the X-larvae pairs was the damage ranked as less severe than in either control calf.

The numbers of worms recovered at necropsy (table 2) indicated that the N-larvae pair was resistant to infection on challenge. However, the four calves that had been vaccinated with irradiated larvae were not demonstrably resistant, since the average number of worms recovered from them approximated that recovered from the controls. One calf of pair X-larvae 2 yielded fewer worms than either control.

Only the worms from the positive N-larvae calf were smaller on the average than the measured worms from the controls (females, 18 mm *vs.* 33 mm; males, 14 mm *vs.* 25 mm).

DATA OF EXPERIMENT 2

Each calf of four pairs was vaccinated with 1,260 larvae on day 0 and 1,520 on day 49. The pairs were designated by the rate of irradiation of the vaccines received as follows: 100 r/m, 200 r/m, 400 r/m, and 2,050 r/m (Av., used as a designation of convenience, as the larvae received by this pair on days 0 and 49 were irradiated at 2,900 r/m and 1,200 r/m, respectively). For challenge, each calf of these pairs and each of four controls received 10,000 normal larvae on day 104; necropsy was at about one month postchallenge. As previously noted, eight other calves were vaccinated once and were necropsied about 11 days postvaccination.

Table 2. Lungworms recovered and lung damage at necropsy of calves about 1 month after challenge exposure of 14,800 larvae (Experiment 1).

Pair		Worms No.	Lung damage Score*
		Vaccinated with normal larvae	
N-larvae		0	0.75
		74	1.25
	Average	37	1.00
		Vaccinated with irradiated larvae	
X-larvae 1		911	2.50
		1,215	2.25
X-larvae 2		134	1.00
		693	2.25
	Average	738	2.00
		Unvaccinated	
Control		281	2.25
		1,319	2.25
	Average	800	2.25

*Scores ranging from 0.25 for very slight to 3.0 for very extensive and severe damage.

The infectivity of the vaccines and of normal larvae to rodents was compared as follows: The vaccines given calves on day 0 and normal larvae were tested with pairs of mice given about 2,500 larvae/mouse and single guinea pigs given about 5,800 larvae each (test 1, table 3); necropsy was at 42 hours postexposure. The vaccines given calves on day 49 and normal larvae were tested with pairs of guinea pigs given about 4,500 larvae/animal (test 2, table 3); necropsy was at 74 hours postexposure.

FINDINGS IN RODENTS: The results of the tests with rodents, which are summarized in table 3, indicated that infectivity, as determined by percentage recovery of larvae administered, was not directly related to irradiation or to the rate of irradiation. The two highest percentages of recovery were from guinea pigs and were of normal FDL larvae and BPL larvae irradiated at 400 r/m (12.6 and 11.6%). Larvae irradiated at 1,200 r/m and 2,900 r/m were at least as invasive on the average as those irradiated at 100 r/m. Degree of lung damage was not well correlated with numbers of larvae recovered.

FINDINGS IN CALVES AT 11 DAYS POSTVACCINATION: No larval or small adult worms were found in 1/10 of the sediment obtained by the procedure previously described (Materials and Methods) from the lungs of 7 of the 8 calves in this group. The total indicated recovery from the eighth calf, which was vaccinated on day 0 with larvae irradiated at 100 r/m, was 30 worms. Histologic examinations for worms immobilized in tissues were not made.

Minor damage to the lungs was observed in all four of the calves vaccinated on day 0; it was scored highest (1.0) in the one given larvae irradiated at 100 r/m and lowest (0.25) in the one given larvae irradiated at 200 r/m. Scarcely any damage, and no difference associated with rate of irradiation, occurred in the four vaccinated on day 49.

FINDINGS IN TEST PAIRS PRECHALLENGE: No evidence was obtained that either vaccination resulted in development of mature worms in any calf of the test pairs; all examinations of their feces and those of the controls were negative for first-stage larvae.

No significant elevation in body temperature and no coughing due to vaccination I or II were observed. Vaccination I was not observed to cause

Table 3. Results of administration of irradiated and normal lungworm larvae to mice and guinea pigs (Experiment 2).

Kind of larvae	Percentage recovered			Lung Damage Score*		
	Test 1**		Test 2***	Test 1**		Test 2***
	Mice (Av.)	G. pigs	G. pigs (Av.)	Mice (Av.)	G. pigs	G. pigs (Av.)
Normal (BPL)	1.0	6.9	3.5	2.0	2.0	1.0
Normal (FDL)	—	—	12.6	—	—	3.5
Irradiated						
100 r/m (FDL)	1.0	1.0	3.8	4.0	1.0	3.5
200 r/m (BPL)	0.4	—	1.5	1.0	—	2.5
400 r/m (BPL)	0.6	11.6	1.4	3.0	4.0	2.5
1,200 r/m (BPL)	—	—	5.5	—	—	3.0
2,900 r/m (BPL)	0.6	2.6	—	5.0	3.0	—

*Scores ranging from 1.0 for a few petechiae to 5.0 for extensive pneumonic damage.
**Test of vaccines given to calves on day 0.
***Test of vaccines given to calves on day 49.

significant increase in respiratory rate. However, about one month after vaccination II, a slight increase in rate occurred for about three consecutive days in most of the calves, notably those given larvae irradiated at the higher rates. No definite evidence was obtained that vaccination had any effect on average daily weight gain.

FINDINGS POSTCHALLENGE: The outputs of first-stage larvae (table 4) indicated that among the vaccinated pairs the challenge exposure resulted in establishment of fewest mature worms in pair 100 r/m, next fewest in pair 2,050 r/m and most in pair 200 r/m.

All calves showed definite signs of reaction to the challenge exposure. Pair 100 r/m coughed on nearly as many days as the controls, but not as frequently or as hard. Average respiratory rate increased from 33 before challenge to 67 postchallenge; in the controls, it increased from 33 to 75.

In all other vaccinated pairs, coughing was nearly as frequent and hard as among the controls. Average respiratory rate was higher than in the controls (86 *vs.* 75), but the percentage increase from the rate before challenge was not any higher than in the controls.

Significant elevation in body temperature was not observed in pair 100 r/m. It occurred in all other groups. The average observed duration was as follows: Controls, six days; pair 200 r/m, five days; pair 400 r/m, three days; pair 2,050 r/m, two days.

The average daily weight gain of pair 100 r/m was not affected. The rate of gain of the other vaccinated pairs and of the controls was about equally reduced.

Thus, pair 100 r/m reacted in a manner consistent with the antemortem parasitological evidence of its resistance. However, pair 2,050 r/m was markedly affected clinically despite its apparent resistance, as sometimes occurs where strong resistance has been induced by initial infection with normal larvae.

At necropsy, least gross damage to the lungs was observed in pair 100 r/m and next least in pair 2,050 r/m, whereas the average score of each of the other vaccinated pairs was but little lower than that of the controls (table 5).

The numbers of worms recovered at necropsy from all calves and the averages for the several groups are given in table 5. On statistical analysis

Table 4. Total outputs of first-stage lungworm larvae in feces of calves from time of challenge exposure (10,000 larvae) to necropsy (Experiment 2).

Pair*		No. larvae/calf
		Vaccinated
100 r/m		200
200 r/m		1,225,570
400 r/m		44,800
2,050 r/m		2,075
	Average	318,180
		Unvaccinated
Control 1		805,610
Control 2		6,039,630
	Average	3,422,620

*Vaccinated pairs designated by rate of irradiation of vaccines received; see text.

of these data by Duncan's multiple range test*, ARS Biometrical Services reported as follows: The mean number of worms from the controls is: (1) significantly greater ($P < 0.05$) than the mean numbers from pairs 100 r/m and 2,050 r/m, but (2) not significantly greater than the mean numbers from pairs 200 r/m and 400 r/m. The mean numbers from pairs 100 r/m, 400 r/m, and 2,050 r/m do not differ significantly from one another.

The average length of the measured worms from pair 100 r/m was very much less than that of those from the controls (females, 14.3 mm *vs.* 37.8 mm; males, 10.9 mm *vs.* 28.0 mm). The females and males from pairs 400 r/m and 2,050 r/m also were inferior in length to those from the controls, but in a much lesser degree.

DISCUSSION

Jarrett *et al.* (1957) initially designated the levels of irradiation of the vaccines which they found to induce effective resistance as levels "A" and "B" and stated that vaccination with larvae irradiated at level "B" was preferable because it caused less lung damage. Data in their next report (1958) indicated than level "B" was 40,000 r. No other information on irradiation of the larvae used by them was available to us when Experiment 1 was started (Jan., 1959). Hence, several reports (Alicata, 1951; Gould *et al.*, 1953; Kim, 1957; Levin and Evans, 1942; Sadun *et al.*, 1957; Villella *et al.*, 1958) on the effects of X-irradiation on the ability of larvae of other nematodes to develop

*See: "Mean Separation by the Functional Analysis of Variance and Multiple Comparisons" by E. L. LeClerg (ARS-20-3, ARS, USDA, May, 1957).

Table 5. Lungworms recovered and lung damage at necropsy of calves about 1 month after challenge exposure of 10,000 larvae (Experiment 2).

Pair*		Worms No.	Lung damage Score**
		Vaccinated	
100 r/m		25	0.50
		75	1.00
	Average	50	0.75
200 r/m		1,235	2.00
		1,410	2.00
	Average	1,322	2.00
400 r/m		232	1.50
		409	2.00
	Average	320	1.75
2,050 r/m		27	1.00
		167	1.50
	Average	97	1.25
		Unvaccinated	
Control 1		323	2.25
		2,908	1.75
Control 2		1,344	2.75
		3,024	2.25
	Average	1,900	2.25

*Vaccinated pairs designated by rate of irradiation of vaccines received; see text.
**Scores ranging from 0.25 for very slight to 3.0 for very extensive and severe damage.

in and immunize a host were examined for clues to a rate of irradiation suitable for use in the experiment. No evidence was noted that dose-rate was, or had been deemed, critical. Moreover, radiobiologists we consulted expressed the opinion that the biologic effect of rates ranging from about 200 r/m to 3,000 r/m would be substantially alike. As previously mentioned, the irradiated larvae used in Experiment 1 were exposed to 40,000 r at 2,650 r/m and vaccination with these larvae did not induce effective resistance under the conditions of the experiment.

This finding soon came to the attention of investigators associated with the experiments carried out by Jarrett *et al.*, or with the development and production of Dictol**, who promptly suggested that vaccination in Experiment 1 was ineffective because our vaccine was irradiated at too fast a rate. They informed us that the dose rates used in the experiments of Jarrett *et al.* and for the production of Dictol were only 200 r/m (see: Jarrett *et al.*, 1960) and 100 r/m, respectively.

However, in Experiment 2, which tested the rate of irradiation as a determinant of antigenicity of *D. viviparus* larvae exposed to 40,000 r, the means of the numbers of worms recovered postchallenge from the pairs of calves vaccinated with larvae irradiated at the extremes of rate tested did not differ significantly. Hence, the conclusion that rate of irradiation was not the critical determinant of efficacy of vaccination in this experiment is considered warranted.

On this premise, some other factor or factors must have been responsible for the lesser effectiveness and ineffectiveness of vaccination in pairs 400 r/m and 200 r/m, respectively, and the wide range in average worm load that occurred among the vaccinated pairs.

Unequal vigor of larvae prior to irradiation could not have been an appreciable factor in the dissimilar results of vaccination with the BPL vaccines because each vaccine was an irradiated aliquot of the same agitated suspension of normal larvae. However, as previously noted, BPL and FDL larvae were not reared by the same method. The larvae from which the FDL vaccine (Dictol) was produced were cultured by a technique basically like that described by Jarrett *et al.* (1957), who reported that their method yields larvae of uniform high infectivity, whereas methods involving the use of the Baermann apparatus give rise to larvae highly variable in infectivity. Nevertheless, in Experiment 2, the pair vaccinated with FDL vaccine and pair 2,050 r/m, which received BPL vaccine, yielded substantially similar numbers of worms at necropsy. Moreover, in the tests with guinea pigs, irradiated BPL larvae were at least as invasive as irradiated FDL larvae.

So far as we have been able to learn by consultation with radiobiologists it is very unlikely that such variation as occurred in the postchallenge worm loads of the test pairs was caused by differences in attenuation of the vaccines due to qualitative differences in the rays emitted by the several X-ray sources used for irradiation. The information and opinions expressed to us were as follows: (1) The biologic effect of a given total dosage of high-voltage rays is somewhat, but not substantially, less than that of an equal dosage of low-voltage rays. (2) The rays emitted by the source operated at 140 kv for preparation of two of the BPL vaccines and by the one operated at 150 kv

**Trade name of the vaccine produced by Allen and Hanburys, Ltd. under a patent based on the procedures developed by Jarrett *et al.* The vaccine also has been produced for experimental use only by Fort Dodge Laboratories, Fort Dodge, Ia., under arrangements with the British firm.

for the preparation of Dictol would have very similar characteristics and would not differ appreciably from those emitted by the source used by Jarrett *et al.*, which was operated at 140 kv and 5 ma, with external filtration of 0.25 mm Cu and 1 mm Al (Jarrett *et al.* 1960).

Seemingly noteworthy in this connection is that Jarrett *et al.* (1957; 1960) reported vaccination effective not only where the larvae were irradiated at 40,000 r, but also where the level was "slightly above" 40,000 r. Moreover, their data on worms recovered at 35 days postvaccination indicate that exposure to 20,000 r attenuated the larvae nearly as much as exposure to 40,000 r. Thus, whether even total X-ray dosage is highly critical within such limits is open to question. Poynter *et al.* (1960) concluded that larvae irradiated at 50,000 r were able to invade the lungs.

In view of the foregoing considerations, we deem the variation in the postchallenge worm loads of the test pairs of Experiment 2 attributable mostly to variability among calves in capacity to acquire resistance in response to similar levels of antigenic stimulation.

That wide variation in initial susceptibility to infection with normal larvae is characteristic of calves is virtually beyond contravention. This variation is evident in the worm loads of the control calves of the experiments reported here. Jarrett *et al.* (1957) reported that recovery from ten calves simultaneously given a lose of 4,000 larvae ranged from 180 to 1,788 worms. Additional evidence could be cited.

Considerable evidence of variability in capacity to develop resistance also exists. Weber and Lucker (1959) found one of three calves that had eliminated an initial infection to be highly susceptible to reinfection on challenge and slightly susceptible to a third infection after elimination of the second one. Cornwell (1960) gave 10,000 larvae to a calf that had been doubly vaccinated, but was passing a few larvae from an infection acquired on pasture, and found that the larvae/gm count increased materially and the feces remained positive for eight weeks after the artificial challenge. Ten weeks after this challenge, the calf was returned to pasture and again acquired a patent infection. Jarrett *et al.* (1957) reported data showing that of ten calves initially infected with 2,500 larvae each, six were apparently completely immune to reinfection six months later, whereas four were not; three of the four were also somewhat susceptible to a third infection.

The results of Experiment 2 contraindicate irradiation of the vaccines at different rates as an adequate explanation of the fact that single vaccination with 4,000 larvae was not effective in Experiment 1, whereas it was effective in the comparable experiment of Jarrett *et al.* (1957; 1958; 1960). The interval from vaccination to challenge was 129 days in our experiment and less than half that interval in theirs and the challenge doses were 14,800 and 4,000 larvae, respectively. These differences may have been responsible for the unlike results of the experiments. Michel and Shand (1955) concluded that naturally acquired resistance can be broken down by heavy exposure and that its potency diminishes with time. Michel (1962) concluded that artificially induced resistance began to decline about 110 days after initial infection. That the larvae used for preparation of the vaccine used in Experiment 1 were reasonably vigorous is evidenced by the fact that the calves given 4,000 normal larvae of the same suspension acquired substantial and highly pathogenic infections.

Vaccination with larvae rapidly irradiated by means of the Van De Graaff generator was effective in Experiment 2 and ineffective in Experiment 1.

However, double exposure to antigen (Exp. 2) evidently stimulates resistance much more effectively than single exposure (Exp. 1). Jarrett *et al.* (1957) originally reported than single vaccination with 1,000 larvae gave a good level of protection, though less than single vaccination with 4,000. Subsequently, however, they reported (1959) an experiment in which the following mean numbers of adult worms were recovered from groups of calves challenged with 10,000 larvae: Control group, 897; group singly vaccinated with 1,000 larvae, 820; group doubly vaccinated with 1,000 larvae, 0. In relation to the probable importance of the period from vaccination to challenge mentioned above, the fact is noteworthy that in the experiment in question, 93 days elapsed from single vaccination to challenge and only 51 days from second vaccination to challenge. Another point of interest is that in the same experiment the use of 4,000, instead of 1,000, larvae for the second vaccination was of no advantage.

Our results in Experiment 2 are not the only existing evidence that even double vaccination does not invariably afford perfect protection against natural or artificial challenge: Englebrecht (1961) found the procedure very effective against challenge with 4,000 larvae, but did recover a few worms postchallenge. Edds *et al.* (1963) reported rather similar results at the same level of challenge, but in one test they recovered from the vaccinated calves about one-third the number of worms recovered from the controls. They also reported evidence of good protection against natural challenge in some tests. However, in one of two trials on pasture in Florida, their vaccinated calves yielded about one-third, and in the other about three times, the number of worms found in the controls. Cornwell (1960) observed symptoms and detected low levels of infection where the level of challenge exposure on pasture apparently was not high. In an instance where the interval between vaccinations was longer than recommended, he noted symptoms in all of 4 calves and recovered 850 worms from one that was killed *in extremis*. He recovered about 15,000 worms from a calf that died of husk after it was doubly vaccinated as recommended and placed on a heavily contaminated pasture. He also has reported (1962) that all of ten doubly vaccinated calves became infected on artificial challenge with 5,000 to 10,000 larvae. The patent period ranged from 1 to 81 days and maximum counts of larvae ranged from 0.7 to 71.8/gram of feces.

SUMMARY

Two experiments were carried out to test the efficacy of oral vaccination with X-irradiated *Dictyocaulus viviparus* larvae for protection against patent infection with this lungworm of cattle. The larvae were irradiated in water until 40,000 roentgens were delivered at its surface.

In the first experiment, each of four calves was singly vaccinated with 4,000 larvae irradiated at a rate of about 2,650 r/m. At about four months postvaccination, the calves were not resistant to a challenge dose of about 15,000 normal larvae. The average number of worms recovered from them about one month postchallenge was about equal to the average number recovered from two control calves.

To test rate of vaccine-irradiation as a factor in efficacy of vaccination, a second experiment was performed in which pairs of calves were doubly vaccinated with larvae irradiated rapidly and at three much slower rates. The interval between vaccinations with roughly 1,000 larvae was seven weeks. The interval from second vaccination to challenge with 10,000 larvae was

about two months. The irradiation rates tested were 100 r/m, 200 r/m, 400 r/m, and the combination of 2,900 r/m for initial, and 1,200 r/m for second, vaccination. The average numbers of worms recovered about one month post-challenge from the pairs vaccinated with larvae irradiated at these rates were 50, 1,322, 320 and 97, respectively. The average number recovered from four control calves was 1,900. Rate of irradiation is rejected as the prime determinant of efficacy of the vaccinations because the postchallenge worm loads did not vary directly or inversely with the rate. Variation in capacity to develop resistance is deemed largely responsible for the observed differences in efficacy of the vaccinations.

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Neosteganoderma glandulosa gen. n., sp. n. (Trematoda: Steganodermatidae) from an Atlantic Fish*

MITCHELL A. BYRD**

ABSTRACT

Neosteganoderma glandulosa gen. n., sp. n. (Trematoda: Steganodermatidae) from the hindgut of a fish (*Polymixia lowei* (Günther)) from the Straits of Florida is described.

The new trematode genus described herein was among a collection of parasites made in 1960 aboard the United States Fish and Wildlife Service Vessell Silver Bay. All specimens were relaxed in sea water saturated with Chloretone and subsequently were fixed in F.A.A. Specimens were stained in hematein and mounted in permount. All measurements are in millimeters except egg-size which is in microns.

FAMILY STEGANODERMATIDAE

Neosteganoderma glandulosa gen. n., sp. n. (Figs. 1 to 3)

HOST: *Polymixia lowei* (Günther).

HABITAT: Large Intestine.

LOCALITY: Hosts were collected at a depth of 290 fathoms in a 40 foot balloon trawl. Collecting station was located at 23° 40′ North Latitude and 79° 18′ West Longitude (Straits of Florida).

DATE: 5 November 1960.

SPECIMENS DEPOSITED: Holotype, U.S.N.M. Helminthological Collection No. 60166.

PARATYPE: U.S.N.M. Helminthological Collection No. 60166.

DESCRIPTION (based on 52 specimens, measurements 30): Body covered with minute spines, larger spines on hindbody (spines more noticeable on very young specimens); fusiform with long slender forebody, blunt, more rounded hindbody; 1.80 to 3.42 long; greatest width at level of acetabulum, .74 to 1.26: Oral sucker ovoid, .19 to .23 wide; acetabulum very wide, often extending transversely beyond level of lateral body margin, .24 to 1.22 wide. Sucker ratio 1:3.9 to 1:1.6, forebody .95 to 1.75. Mouth terminal; oral sucker muscular. Short, narrow prepharynx, .01 to .03; pharynx, .06 to .10 long by .06 to .09 wide; esophagus long, .50 to .98 long, bifurcating immediately in front of cirrus pouch; ceca thin-walled usually terminating just anterior to posterior margin of acetabulum.

Genital pore on left side of body, slightly anterior to acetabulum, pore surrounded at distal end by collar-like mass of gland cells which lie outside cirrus sac. Testes two, lateral, symmetrical, tending to be round, immediately post-acetabular, rarely reaching acetabulum; right testis, 0.29 to 47 long by 0.31 to 0.43 wide, left testis, 0.24 to 0.42 long by 0.25 to 0.42 wide. Cirrus sac elongate, slightly overlapping anterior margin of acetabulum, extending anterior from acetabulum toward right side of body, looping transversely across body toward genital pore; transverse portion extending nearly full width of body; basal portion of cirrus sac contains coiled seminal vesicle which opens into short prostatic vesicle, terminating in cirrus. Cirrus

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**College of William and Mary, Williamsburg, Virginia.

Appreciation is extended Mr. James Sterling and Dr. E. B. Joseph respectively for collection and identification of hosts.

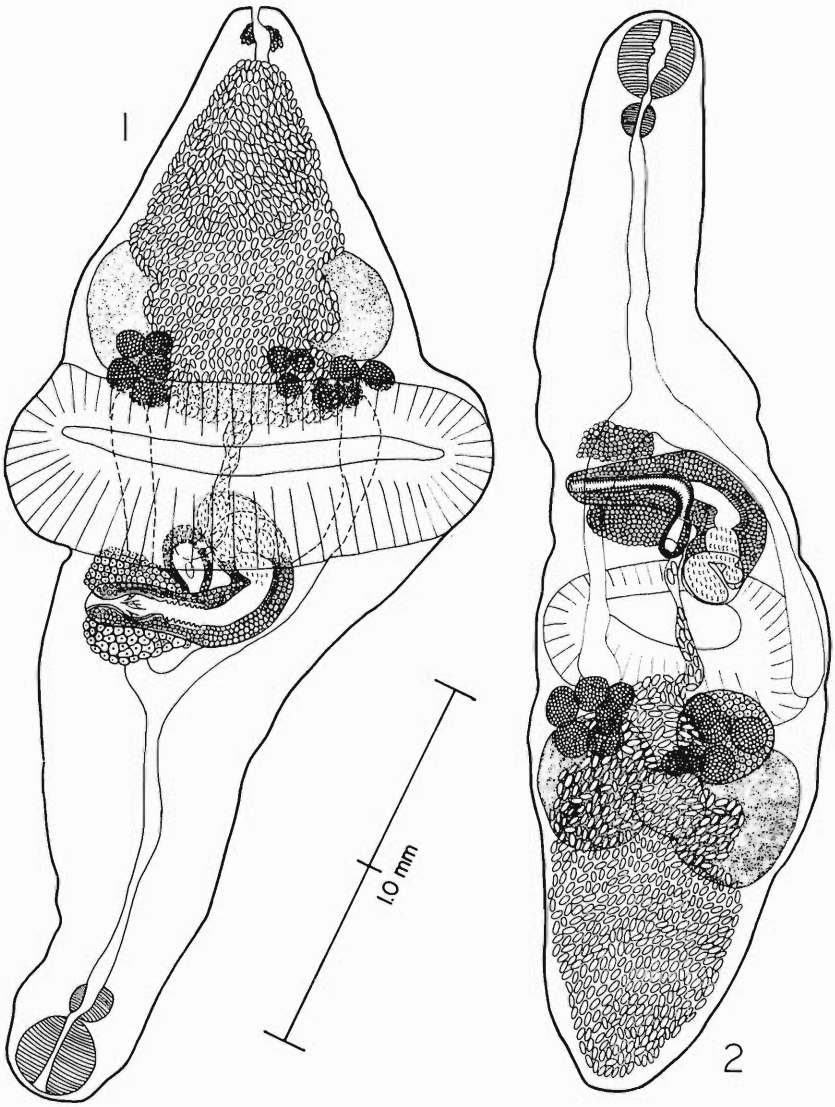

Figure 1. *Neosteganoderma glandulosa* from *Polymixia lowei*. Ventral view of holotype.

Figure 2. *Neosteganoderma glandulosa* from *Polymixia lowei*. Dorsal view of paratype.

eversible, proximal portion with serrated margins, spined, spines in groups of two or three.

Ovary large, ovoid, usually indistinct, median, between testes, immediately postacetabular, rarely reaching acetabulum, 0.21 to 0.27 long by 0.19 to 0.25 wide. Seminal receptacle, postovarian, spherical 0.23 long by 0.22 wide, dorsal to testes. Vitelline follicles in two distinct clusters of follicles, 5 to 9 follicles in each cluster, post-testicular, clusters slightly overlapping acetabulum, follicles 0.07 to 0.11 long by 0.08 to 0.12 wide, clusters overlapping anterior margins of testes, not extending beyond lateral margins of testes. Uterus filling hind body, entering metraterm; metraterm tubular, thick-walled at base, entering genital pore from ventral side; unspined. Eggs yellow, embryonated, 26 to 31 long by 12 to 15 wide.

Excretory vesicle tubular, concealed by eggs in most specimens, terminal end surrounded by a few scattered cells. Generic Diagnosis of *Neosteganoderma*.

Body covered with minute spines, very conspicuous in young specimens,

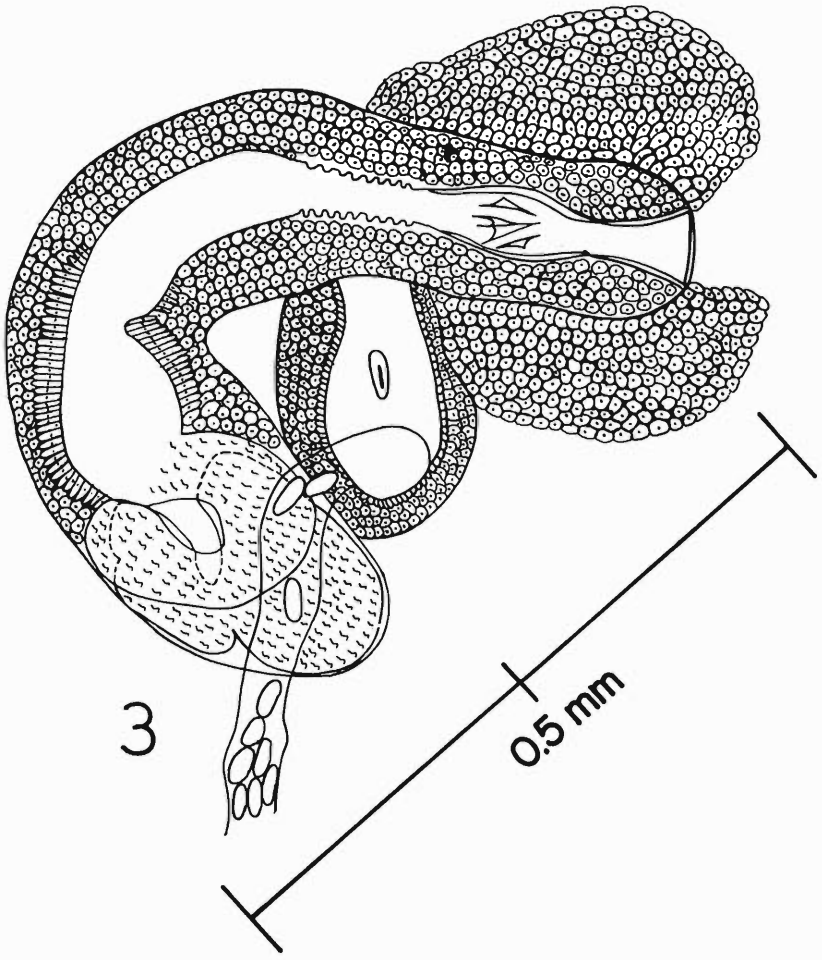

Figure 3. *Neosteganoderma glandulosa* terminal genital ducts of holotype.

apparently absent in older specimens, fusiform, long slender forebody, shorter more rounded hindbody. Acetabulum very large, extending across entire body, usually forming lateral extension beyond body margin; ceca reaching mid or postacetabular region; genital pore lateral, opening to left. Testes ovoid, symmetrical, cirrus sac overlapping anterior margin of acetabulum, distal portion horizontal, cirrus armed with spines and with serrated edge, distal end of cirrus sac surrounded by mass of glandular cells which sometimes appear to form lobes around genital pore, seminal vesicle looped, tubular. Ovary ovoid, antero-dorsal to right testis, slightly overlapping acetabulum; seminal receptacle present, ovoid; uterus filling entire hind-body, metraterm present; vitelline follicles few, in two post-acetabular clusters, sometimes overlapping acetabulum slightly, not extending beyond testis.

Esophagus long, prepharynx short. Excretory vesicle tubular. Eggs embryonated.

DISCUSSION: The genus *Neosteganoderma* is closely related to *Lepidophyllum* Odhner, 1902, *Steganoderma* Stafford, 1904, *Urinatrema* Yamaguti, 1934, *Paralepidophyllum* Yamaguti, 1934, and *Botulisaccus* Caballero, Bravo, and Grocott, 1955.

Dollfus (1952) in a review of the Zoogonidae proposed that the genera *Deretrema* Linton, 1910, *Brachyenteron* Manter, 1934, *Pseudochetosoma* (Dollfus, 1951) Dollfus, 1952, and *Steganoderma* Stafford, 1904 (synonyms: *Lecithostaphylus* Odhner, 1911; Nordosttrema Issaitschikow, 1928; *Proctophantastes* Odhner, 1911) comprising the subfamily Steganodermatinae be raised to the rank of family, Steganodermatidae, to include those genera which possess follicular vitellaria. He further proposed that the Zoogoninae characterized by compact non-follicular vitellaria be the only subfamily of the Zoogonidae. He placed *Lepidophyllum* in a second subfamily Lepidophyllinae. This view is accepted here.

Botulisaccus Caballero, Bravo and Grocott, 1955 also belongs in the Steganodermatidae. *Neosteganoderma,* on the basis of its follicular vitellaria also should be placed in the family.

Manter (1954) pointed out the difficulties of separating *Brachyenteron, Steganoderma,* and *Deretrema.* Although *Steganoderma macrophallus* Szidat and Hani, 1951, in contrast to other members of the genus, possesses vitellaria at the acetabular level, the distribution of vitellaria appears to be a convenient character for separating the genera of Steganodermatidae into two groups. One group with vitellaria lateral and/or preacetabular would include *Deretrema* and *Brachyenteron;* the second group with vitellaria lateral or largely postacetabular would include *Steganoderma, Urinatrema, Lepidophyllum, Paralepidophyllum,* and *Botulisaccus.* Manter (1954) questioned the validity of *Pseudochetosoma* as a genus separate from *Deretrema.*

Neosteganoderma clearly belongs with the second group. It differs from all other genera of this group on the basis of the very large acetabulum, posterior position of acetabulum, the large glandular mass surrounding the genital pore, and the much elongated esophagus and forebody. It differs further from *Botulisaccus* on the basis of its preacetabular cirrus sac.

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Digenetic trematodes of fishes from Palawan Island, Philippines. Part III. Families Hemiuridae and Lepocreadiidae*

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ**

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

FAMILY HEMIURIDAE

Hemiuris sigani n. sp. (Fig. 1)

HOST: *Siganus striolatus* (Siganidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 21 May 1962.

TYPE: USNM Helm. Coll. No. 60401.

DESCRIPTION (based on single specimen): Body elongate, widest at vitellaria, with ecsoma; total length 1,809; body proper 1,254 by 595, ecsoma 555 by 425. Preoral lobe 11, forebody 184, acetabulum to posterior extremity of body proper 892. Cuticular plications visible laterally to level of anterior portion of posterior testis on right and anterior portion of ovary on left; dorsal and ventral surfaces with plications over entire worm, less distinct on body proper posterior to disappearance of lateral plications and on ecsoma. Oral sucker 73 by 77, subterminal, ventral. Acetabulum 178 by 206, at about level of anterior fifth of body proper. Sucker length ratio 1:2.45. No visible prepharynx; pharynx 53 by 56, partially overlapping oral sucker dorsally; esophagus 41 long; bifurcation at anterior margin of acetabulum; ceca extending into ecsoma slightly more than half way, postcecal space 255 (right) and 290 (left). Excretory bladder tubular, sinuous, extending to ovarian region; arms uniting dorsal to oral sucker-pharynx junction; pore terminal.

Testes two, smooth, transversely elongate, slightly oblique, postacetabular, anterior testis slightly overlapping posterior one dorsally; anterior testis 150 by 230, posterior 152 to 213; acetabulum to anterior testis 172, to

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posterior testis 295; latter to ovary 22. Seminal vesicle postacetabular, bipartite, both chambers thick-walled and muscular, transversely oriented left (proximal) to right (distal); left chamber 73 by 96, slightly overlapping anterior testis dorsally, right chamber 77 by 61; acetabulum to seminal vesicle 116. Pars prostatica dextral, slightly sinuous, long, extending from ventral surface of right chamber of seminal vesicle to short distance preacetabular, thick-walled, muscular; surrounded by prostate gland cells throughout length but more prominently postacetabular. Sinus sac 196 by 31, extending anteriorly from just preacetabular to genital pore on oral sucker, relatively thin-walled. Union of pars prostatica with metraterm at proximal end of sinus sac, entering latter as hermaphroditic duct; duct 196 by 15, relatively thick-walled, muscular. Genital pore a transverse slit, ventral, submedian to left, between posterior lip and posterior margin of oral sucker, 16 anterior to posterior margin of latter.

Ovary 133 by 172, transversely elongate, smooth, in tandem with testes, close to posterior testis, 467 postacetabular. Ootype complex posterodorsal to ovary. Seminal receptacle 92 by 66, posterosinistral to ovary, overlapping latter and left vitellarium ventrally. Vitelline lobes two, symmetrical, compact, smooth, in contact; right vitellarium 165 by 167, in contact with ovary, left vitellarium 145 by 165; acetabulum to vitellaria 587, latter to ecsoma 138 (right), 153 (left). Uterus much coiled, dorsal to gonads and vitellaria but may overlap some of margins ventrally, separating posterior testis from ovary; uterus extending into ecsoma 111, one fifth length of latter; metraterm long, extending over most of acetabulum. Eggs numerous, 10 measuring 20 to 23 by 10 to 12.

DISCUSSION: *Hemiuris sigani* differs from all known species of the genus in having both chambers of the seminal vesicle thick-walled and muscular. It appears closest to *H. appendiculatus* (Rudolphi, 1802) Looss, 1899.

Aphanurus stossichi (Monticelli, 1891) Looss, 1907

SYNONYMS: *Distomum ocreatus* Monticelli, 1887, Stossich, 1888, 1898, nec Rudolphi, 1819, nec Olsson, 1867; *Apoblema stossichi* Monticelli, 1891; *Hemiurus stossichi* (Monticelli, 1891) Lühe, 1901; *Aphanurus virgula* Looss, 1907; *Aphanurus harengulae* Yamaguti, 1938.

HOST: *Harengula dispilonotus* (Clupeidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 19 May 1962.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 60402.

DESCRIPTION (based on single specimen): Body 589 by 111, entirely annulated, no ecsoma; preoral lobe 5, forebody 109, hindbody 311, postovarian space 191, postuterine space 65, postcecal space 35. Oral sucker diameter 40; acetabulum 69 by 83, at level of anterior body fourth; sucker length ratio 1:1.73; pharynx 26 by 24; esophagus 17 long; anterior (right) testis 46 by 44, posterior testis 38 by 45; acetabulum to anterior testis 54, to posterior 73; sinus sac median, 72 by 14; hermaphroditic duct 72 by 6; sac and duct overlapping anterior fifth of acetabulum and opening anteriorly into median genital pore just 5 posterior to oral sucker and ventral to midlevel of pharynx; pars prostatica long, surrounded by large prostate gland cells overlapping ceca, posterior portion of acetabulum and both testes; seminal vesicle 43 by 33, acetabulum to latter 31; ovary 35 by 66, overlapping both testes ventrally, acetabulum to ovary 85; vitellarium 65 by 85, overlapping

ovary and posterior testis ventrally, acetabulum to vitellarium 103; 10 eggs measuring 19 to 25 by 9 to 13.

DISCUSSION: Slusarski (1957) reviewed the various forms designated as *Aphanurus stossichi* sensu latu, noting that some were supposed to have a rudimentary ecsoma, some not. He concluded that the entire group needed restudy since the various authors ascribing an ecsoma to their forms did not document their conclusions. Our specimen resembles Yamaguti's (1938) de-

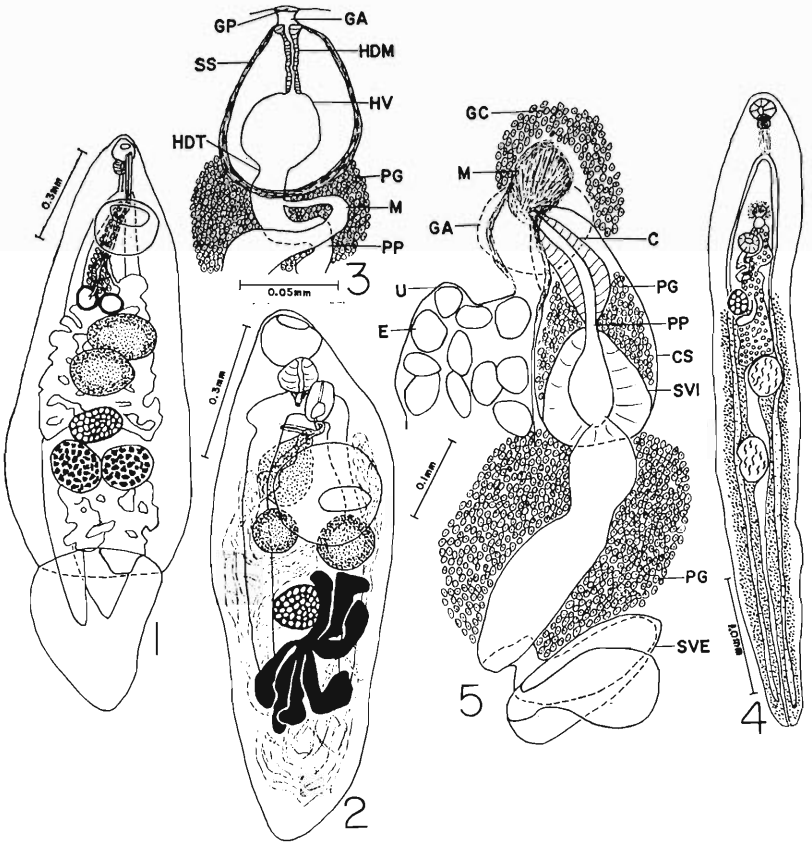

Figure 1. *Hemiuris sigani*, ventral view of type specimen.
Figure 2. *Brachadena cheilionis*, ventral view of type specimen.
Figure 3. Same. Terminal genitalia, ventral view.
Figure 4. *Aephnidiogenes barbarus*, ventral view.
Figure 5. Same. Terminal genitalia, dorsal view. C, cirrus; CS, cirrus sac; E, egg; GA, genital atrium; GC, gland cells; GP, genital pore; HDM, muscular portion of hermaphroditic duct; HDT, thin-walled portion of hermaphroditic duct; HV, hermaphroditic vesicle; M, metraterm; PG, prostate gland cells; PP, pars prostatica; SS, Sinus sac; SVE, external seminal vesicle; SVI, internal seminal vesicle; U, uterus.

scriptions of *A. stossichi* from *Dorosoma thrissa* and *A. harengulae* (syn. of *A. stossichi*) from *Harengula zunasi* from Japan; it also fits Yamaguti's (1953) description of *A. harengulae* from *Clupea clupeoides* from Celebes.

Lecithocladium augustiovum Yamaguti, 1953

HOST: *Caranx affinis* (Carangidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 19 May 1962.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 60403 (three slides with one specimen each).

DESCRIPTION (based on six specimens, five measured): Total length 2,713 to 3,177; body proper 1,716 to 1,906 by 269 to 350, ecsoma 997 to 1,292 by 213 to 299; forebody 491 to 598, acetabulum to posterior extremity of body proper 951 to 1,097; preoral lobe 7 to 16; plications on entire worm, much less distinct on ecsoma; oral sucker (in 6) 243 to 258 by 187 to 232; acetabulum (in 6) 229 to 244 by 232 to 258; sucker length ratio 1:0.90 to 0.95; pharynx 121 to 188 by 88 to 101; anterior testis (in 4) 140 to 198 by 96 to 195, posterior testis (in 4) 133 to 202 by 114 to 191; acetabulum to anterior testis 399 to 568, to posterior 560 to 759; seminal vesicle 309 to 449 by 107 to 166, maximum thickness of muscular wall 30 to 59; acetabulum to seminal vesicle 23 to 177; hermaphroditic duct 368 to 589 long; genital pore at anteroventral portion of oral sucker; ovary 77 to 147 by 96 to 203; acetabulum to ovary 851 to 1,074; ootype complex 52 to 118 by 74 to 128, distinct, compact, posterodorsal to ovary; 7 vitelline lobes, 4 right and 3 left (in 3) or 3 right and 4 left (in 2); uterus extending into ecsoma 667 to 905; 25 older intrauterine eggs measuring 16 to 24 by 6 to 12. One specimen with only single testis 269 by 225, much larger than in those with 2, distance postacetabular 476.

DISCUSSION: This species was described by Yamaguti (1953) from *Rastrelliger* (=*Scomber*) *kanagurta* from Celebes, and redescribed by Velasquez (1962) from *R. chrysozonus* from Visayan Islands and Luzon Island, Philippines. The latter stated that her specimens were smaller than Yamaguti's, but actually they were at the upper limit or larger; Yamaguti's figures for body length were for the entire worm and not for the body proper alone.

Lecithochirium magnaporum Manter, 1940

HOST: *Euthynnus yaito* (Scombridae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 20 May 1962.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 60404 (four slides with one specimen each).

DESCRIPTION (based on 19 specimens; four measured in ventral or dorsal view and four in lateral view): Ecsoma retracted in all specimens. Total length 1,246 to 1,715; width at genital pore 152 to 185, at ovary 280 to 445; depth at genital pore 145 to 195, at ovary 250 to 425; preoral lobe 12 to 31, forebody 275 to 450, hindbody 705 to 1,085, preacetabular pit opening to acetabulum 26 to 122; oral sucker, length 77 to 99, width 87 to 90, depth 76 to 97; acetabulum, length 208 to 320, width 232 to 290, depth 182 to 235;

sucker length ratio 1:2.45 to 3.48; pharynx, length 46 to 58, width 50 to 53, depth 41 to 56; esophagus 25 to 60 long; right testis, length 97 to 182, width 85 to 162, depth 126 to 167, lying anterior to left testis in 3 of 8 specimens; left testis, length 104 to 155, width 95 to 174, depth 115 to 157, lying anterior to right testis in 5 of 8 specimens; seminal vesicle tripartite, posterior margin from 16 preacetabular (in 1) to maximum of 101 posterior to anterior margin of acetabulum (in 7); posterior chamber of seminal vesicle, length 97 to 140, width 59 to 68, depth 65 to 90; middle chamber of seminal vesicle, length 48 to 75, width 47 to 51, depth 46 to 68; anterior chamber of seminal vesicle looping posteriorly, then anteriorly, width 25 to 33, depth 27 to 39; pars prostatica short, relatively straight, length 26 to 85, width 13 to 19, depth 19 to 22, surrounded by prostate gland cells; sinus sac longer than wide, passing from posterodorsal to anteroventral, actual length (lateral view in 4) 88 to 138, longitudinal extent (ventral or dorsal view in 4) 69 to 98, width 49 to 93, depth 57 to 94, containing large vesicular cells surrounding hermaphroditic duct; prostatic vesicle cell lined, length 15 to 31, width 24 to 28, depth 26 to 37; hermaphroditic duct short, actual length (lateral view in 4) 62 to 90, longitudinal extent (ventral or dorsal view in 4) 10 to 41, width 29 to 38, depth 36 to 43, with circular muscles internally giving ringed appearance and prominent plaited longitudinal muscles externally giving longitudinally striated appearance, striations continuing to genital pore and giving its border a conspicuous radially striated appearance; genital pore large, transverse slit, ventral to esophagus-cecal bifurcation, 111 to 264 preacetabular; ovary, length 90 to 138, width 116 to 180, depth 119 to 172, 185 to 440 postacetabular; vitelline lobes 6 to 8, variations in 8 specimens (right-left) 3-3, 3-4, 3-4, 3-4, 3-5, 4-3, 4-3, 4-4; metraterm uniting with male duct just anterior to prostatic vesicle; 34 eggs measuring 20 to 24 by 11 to 15.

DISCUSSION: This species was described by Manter (1940) from *Paralabrax humeralis*, *Euthynnus alletterata*, *Seriola dorsalis*, and (?) *Epinephelus* sp., from the Galapagos Islands. Montgomery (1957) reported it from *Pneumatophorus japonicus diego* and *Trachurus symmetricus* from California. Manter gave the sucker length ratio as 1:2 or slightly less (oral sucker 135 to 150 in diameter, acetabulum 262 to 292). Montgomery noted a ratio of 1:2.3 to 2.4. Study of the type specimen of *L. magnaporum* (USNM Helm. Coll. No. 9366) from *Paralabrax humeralis* indicated an oral sucker 121 by 148 and an acetabulum 281 by 286; sucker length ratio 1:2.32. In a paratype specimen on the same slide the oral sucker was 109 by 131 and the acetabulum 281 by 283; sucker length ratio 1:2.58. Manter noted the sinus sac as wider than long (60 to 102 by 94 to 153). The sinus sac in the type specimen was 121 by 155, and in the paratype 62 by 59. Manter recorded the egg size as 15 to 19 by 8 to 9. Eight eggs in the type were 15 to 21 by 8 to 10, and 8 in the paratype 20 to 26 by 11 to 12.

Paraplerurus sauridae Fischthal and Kuntz, 1963

HOST: *Trichiurus haumela* (Trichiuridae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 19 May 1962.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 60405 (three slides with one specimen each).

DESCRIPTION (based on six specimens, four measured): Body proper 3,083 to 5,155 by 1,442 to 2,040 (at ovary); cesoma 3,181 to 3,950 by 1,044 to

1,649 (at uterus end); body proper plus ecsoma 7,033 to 9,693; ecsoma retracted into body proper 483 to 1,488; actual overall length 5,545 to 8,389; preoral lobe 55 to 84; forebody 941 to 1,496; acetabulum to posterior extremity of body proper 1,137 to 4,165; oral sucker 360 to 460 by 406 to 417; acetabulum 851 to 1,005 by 920 to 1,074; sucker length ratio 1:2.02 to 2.53; pharynx 154 to 199 by 146 to 180; ceca into ecsoma 2,616 to 3,912; testes symmetrical or slightly oblique; right testis 372 to 468 by 506 to 782, left testis 368 to 606 by 537 to 675; acetabulum to right testis, overlapping to 15; acetabulum to left testis, overlapping to 253; seminal vesicle tripartite, posterior chamber 239 to 404 by 184 to 283, middle chamber 166 to 250 by 110 to 299, anterior chamber 162 to 221 by 92 to 176; posterior end of seminal vesicle to posterior margin of acetabulum 439 to 939; prostatic vesicle 81 to 162 by 140 to 188; sinus sac (in 2) 239 to 302 wide; genital pore to acetabulum (in 2) 243 to 445; ovary 514 to 548 by 629 to 828; acetabulum to ovary 591 to 1,012; ootype complex 208 to 414 by 483 to 682; ovary-ootype complex 614 to 706 by 629 to 828; vitelline lobes 3 right, 4 left; uterus into ecsoma 905 to 2,768; 15 eggs measuring 16 to 23 by 8 to 13.

DISCUSSION: Fischthal and Kuntz (1963) described this genus and species from *Saurida undosquamis* from Egypt . The testes, sinus sac, prostatic vesicle, ovary, and ootype complex in the Palawan specimens are wider than in those from Egypt, but we do not believe these differences are sufficient for creating a new species.

Brachadena cheilionis n. sp. (Figs. 2 and 3)

HOST: *Cheilio inermis* (Labridae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 20 May 1962.

TYPE: USNM Helm. Coll. No. 60406.

DESCRIPTION (based on single specimen): Body 1,130 by 400, somewhat flattened, widest at acetabulum, tapering toward both extremities, no ecsoma, cuticle smooth; preoral lobe 10, forebody 290, hindbody 630, postovarian space 450. Oral sucker 99 by 128; acetabulum 210 by 235; sucker length ratio 1:2.12. No visible prepharynx; pharynx 68 by 87; esophagus short; cecal bifurcation midway between suckers; ceca extending into postovarian space one fourth to two fifths length of latter, ceca to posterior extremity 345 (right), 259 (left). Excretory bladder tubular, pore terminal.

Testes 2, symmetrical, separated, partly overlapping acetabulum and ceca; right testis 102 by 110, left testis 114 by 125. Seminal vesicle large, 148 by 123, thick-walled, over right side of anterior two thirds of acetabulum as well as partly lateral to latter, overlapping right cecum dorsally, extending slightly preacetabular; posterior end of seminal vesicle to posterior margin of acetabulum 75. Pars prostatica long, mostly dextral, winding in narrow space between seminal vesicle and sinus sac, overlapping right cecum dorsally, entering posterior end of sinus sac. Prostate gland cells surrounding pars prostatica and base of sinus sac. Sinus sac 87 by 68, elongate oval, thick-walled, muscular, ventral to cecal bifurcation, sinistral to midline; distance to acetabulum 47. Union of pars prostatica and metraterm at posterior border of sinus sac, entering latter as hermaphroditic duct; latter differentiated into three portions: relatively thin-walled, straight tube 10 by 10 (posterior portion); round vesicle 37 by 36 (middle portion); thick-walled, muscular tube (anterior portion) 33 by 12 (proximal) to 21 (distal). Herma-

phroditic duct opening into very small, spool-shaped genital atrium 5 by 6 (middle) to 9 (proximal and distal ends). Genital pore transverse slit, sinistral to midline at level of posterior portion of pharynx; distance to acetabulum 137.

Ovary round, diameter 97, median, slightly overlapping both ceca ventrally; acetabulum to ovary 90. Ootype complex postovarian. Vitellaria ventral, of 7 elongate lobes united centrally, posteroventral to ovary; 4 lobes directed posteriorly beyond cecal ends, 3 anterosinistrally with one slightly overlapping left testis ventrally; acetabulum to vitellaria 35, vitellaria to posterior extremity 230. Uterus much coiled; dorsal to vitellaria, dorsal and ventral to parts of gonads, circumcecal, overlapping posterior and lateral portions of acetabulum, one coil on left extending slightly preacetabular; descending posteriorly beyond cecal ends and vitellaria, ascending on right between seminal vesicle and acetabulum dorsum; uterus to posterior extremity 85. Metraterm short, preacetabular, ventral to portion of pars prostatica. Eggs numerous, very small, 10 measuring 15 to 16 by 9 to 11.

DISCUSSION: The only species in the genus is *B. pyriformis* Linton, 1910. As noted by Manter (1947) it has been observed in a variety of fish hosts from the Atlantic coast of the United States and the Gulf of Mexico; Manter and Van Cleave (1951) and Bravo (1956) reported it from the Pacific coast of California and Mexico respectively. Yamaguti (1954) considered *Brachadena* a synonym of *Lecithophyllum* Odhner, 1905. Bravo (1956) and Margolis (1958) reviewed *Brachadena* and considered it valid, the latter author doing so on the basis of the centrally united vitelline lobes. According to Margolis the presence or absence of a genital atrium had not been determined for this genus. We can now report one as noted in *B. cheilionis*. Study of one of Linton's (1910) specimens of *B. pyriformis* from *Haemulon macrostomum* (USNM Helm. Coll. No. 8490) also indicated the presence of a very small genital atrium. We recognize the validity of *Brachadena* on the same basis as cited by Margolis. *B. cheilionis* can be differentiated from *B. pyriformis* in having much smaller eggs, much longer vitelline lobes, and a large seminal vesicle mainly dorsal to and overlapping two thirds of the acetabulum rather than one mainly preacetabular and barely overlapping the acetabulum.

FAMILY LEPOCREADIIDAE

Aephnidiogenes barbarus Nicoll, 1915 (Figs. 4 and 5)

HOST: *Pomadasys hasta* (Pomadasyidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 21 May 1962.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 60407 (four slides with one specimen each).

DESCRIPTION (based on 11 specimens, 10 measured): Body elongate, 3,404 to 5,395 long in 5 with normal posttesticular space, 2,981 to 3,280 long in 5 with part of posttesticular space missing but healed over; 591 to 767 wide, more or less of uniform width from anterior extremity to just posttesticular, then tapering to posterior extremity; extremities rounded. Forebody 836 to 1,208; hindbody 2,394 to 3,981 in 5 normal specimens, 1,657 to 2,034 in 5 with abbreviated posttesticular body; posttesticular space 1,235 to 2,063 in 5 normal specimens, 84 to 383 in 5 abbreviated ones. Cuticle thick where

spined, thin otherwise; spines larger and distribution denser more anteriorly, terminating at varying levels in different specimens, from level of anterior margin of acetabulum to past middle of posttesticular space in complete individuals. No eyespot pigment. Preoral lobe 18 to 79. Oral sucker 147 to 236 by 136 to 239, subterminal, ventral. Acetabulum 121 to 220 by 121 to 217, round to slightly transversely elongate. Sucker length ratio 1:0.71 to 0.95. Prepharynx short, length where visible in four 14 to 47; pharynx 96 to 132 by 85 to 132, round to slightly longitudinally elongate and less frequently transversely elongate, overlapping oral sucker dorsally; esophagus 74 to 228 long, thick-walled, muscular; cecal bifurcation closer to oral sucker than acetabulum; ceca lined with prominent cells internally, tend to follow lateral contours of gonads but latter may slightly overlap them dorsally, terminating near posterior extremity. Excretory bladder elongate, tubular, passing left of posterior testis and right of anterior testis to ovarian region; pore terminal.

Testes 2, oblique, entire to slightly lobed, usually longer than wide, usually separated from each other, postovarian and separated from latter; anterior testis 298 to 399 by 224 to 360, sinistral; posterior testis 287 to 422 by 217 to 360, dextral; acetabulum to anterior testis 491 to 860, to posterior 813 to 1,512; ovary to anterior testis 101 to 368; testes in one specimen overlap 18, in contact in another, up to 314 apart in remainder. Vasa efferentia long, entering next to one another proximal end of external seminal vesicle. Latter long, much convoluted, tubular, commencing at level of anterior margin of ovary or preovarian, entering posterodorsal portion of cirrus sac; distal portion surrounded by prostate gland cells. Cirrus sac 173 to 294 by 110 to 202, elongate, oval to pyriform, muscular, relatively thin-walled; over right portion of acetabulum, extending 12 and 48 beyond posterior margin of latter in two, to posterior margin in one, and 7 to 88 anterior to posterior margin in remainder; containing internal seminal vesicle, pars prostatica, prostate gland cells, and cirrus. Internal seminal vesicle 74 to 158 by 85 to 173, usually slightly wider than long but may be round, extremely thick-walled, muscular, with relatively small lumen. Pars prostatica short, cell lined, thin-walled. Cirrus 63 to 132 by 48 to 70, variable in size depending on state of contraction, thick-walled, muscular, protrusible, distal end in some attenuated into papilla-like portion, opening independently into genital atrium. Prostate gland cells surrounding distal portion of internal seminal vesicle, pars prostatica, and proximal portion of cirrus. Genital atrium 66 to 107 by 62 to 121, large, usually slightly wider than long but may be round, walls prominent but relatively thin, preacetabular; atrium may project from body surface as large papilla. Genital pore median to slightly submedian to left, 33 to 128 preacetabular.

Ovary 176 to 246 by 162 to 237, round to longitudinally or transversely elongate, dextral, entire, 103 to 399 postacetabular, pretesticular. Oviduct leading from median side of ovary, proximal portion an ovicapt. Ootype complex median to ovary. Laurer's canal from seminal receptacle. *Latter* large, posterior and medial to ovary, dorsal to uterus. Uterus pretesticular, intercecal, left of ovary, ventral to ootype complex. Metraterm sinistral to cirrus sac, commencing at level of anterior portion of acetabulum, extending slightly anterior to cirrus sac, genital atrium, and genital pore, turning ventrally, then posteroventrally, to open independently into genital atrium, distal portion forming very muscular, thick-walled vesicle or dilation. Vitelline follicles small, numerous, circumcecal, commencing beside ovary on

right but somewhat variable on left, confluent to some degree posttesticular, may overlap gonads slightly; vitelline reservoir dorsal to uterus. Eggs operculate, yellow, 16 measuring 58 to 71 by 38 to 49.

DISCUSSION: We are designating our specimens as *A. barbarus* solely on the basis of its presence in the same host species and general geographical area as recorded by Nicoll (1915). The latter inadequately described this species from Australia, the account of the genitalia being especially poor. Yamaguti (1934, 1939) reported this parasite from *Parapristipoma trilineatum* (Pomadasyidae) from Japan. Yamaguti (1934), without naming it, briefly described what he (1939) further described and called *A. isagi* from the same host and country as the latter. Dollfus and Capron (1958) stated that it seemed very probable that *A. barbarus, A. isagi,* and *A. major*, the latter described by Yamaguti (1934) from *Plectorhynchus pictus* (Pomadasyidae) from Japan, represent a single species. Thomas (1960) declared *A. isagi* a synonym of *A. barbarus*. We concur in both conclusions. Nicoll (1915) for *A. barbarus* and Yamaguti (1939) for *A. isagi* specifically noted that the cirrus sac was entirely preacetabular and globular to oval in shape. We believe these observations to be in error, and that what was called the cirrus sac actually was the genital atrium (see discussion below of *A. senegalensis* Dollfus and Capron, 1958).

Dollfus and Capron (1958) described *A. senegalensis* from *Labrax punctatus* (Serranidae) from Senegal, separating it from the above species in possessing an uterine seminal receptacle rather than a true one. The cirrus sac was described as entirely preacetabular, short, thick-walled and dorsoventrally oriented, appearing as a regular circle in ventral view; it contained a very small, very short cirrus, but lacked an internal seminal vesicle. Through the courtesy of Dr. R. P. Dollfus we were able to study 6 whole mount syntypes of this species. Our study indicated that their description and illustration of the male and female reproductive systems were incorrect. Instead they should be, with minor variations, as described by Thomas (1960) for the form he called *A. barbarus* from *Pomadasys* (=*Pristipoma*) *jubelini* (Pomadasyidae) from Ghana, a whole mount specimen of which we were able to study through the courtesy of Dr. J. D. Thomas. A true seminal receptacle and Laurer's canal were present; in Dollfus and Capron's figure 2 the structure labeled "R" is the ovicapt containing sperm released from the true seminal receptacle rather than being an uterine seminal receptacle; vasa efferentia entered the proximal end of the external seminal vesicle which commenced at the level of the anterior margin of the ovary or preovarian; the cirrus sac (in 4) measured 148 to 208 (longitudinal extent) by 73 to 87, overlapping one to two thirds of the left side and margin of the acetabulum; preacetabularly the cirrus sac turned ventrally at almost a right angle, then proceeded to the genital atrium; an internal seminal vesicle was present. Dollfus and Capron's cirrus sac actually is the genital atrium. In their specimens and in Thomas' the cell lined pars prostatica appeared well developed, being about 1.5 times as long as the cirrus. In one specimen the testes were only 22μ apart. Three of the 6 specimens of *A. senegalensis* showed abnormalities: one with the anterior end from the level of the distal portion of the esophagus missing; a second with only one testis and most of the posttesticular body missing; a third also with most of the posttesticular body missing, the posttesticular space measuring only 150μ. We declare the form described by Thomas (1960) as *A. barbarus* a synonym of *A. senegalensis;* Thomas did not mention the latter species in his paper. We

consider *A. senegalensis* a valid species, differing from *A. barbarus* as described by us in the morphology of the terminal genitalia.

Razarihelisoa (1960) described *A. dollfusi* from a single specimen from *Abudefduf sexfasciatus* (Pomacentridae) from Madagascar, and stated it closely resembled *A. senegalensis* in possessing an uterine seminal receptacle. We believe this observation in error and that a true seminal receptacle is present. The characters used for separating *A. dollfusi* from *A. senegalensis* were variable ones; the terminal genitalia were inadequately described. Therefore, it is difficult to ascertain whether *A. dollfusi* is a valid species, synonym of *A. senegalensis*, or should be declared a species inquirienda. We favor synonymy with *A. senegalensis*.

Manter (1947) transferred *A. levenseni* (Linton, 1907) Nicoll, 1915, to *Lepidapedon* Stafford, 1904. Dollfus and Capron (1958) indicated that *A. ptochus* Nicoll, 1915, did not belong in *Aephnidiogenes* Nicoll, 1915, while Thomas (1960) declared it a species inquirienda.

The genus *Holorchis* Stossich, 1901, has been variously related to *Aephnidiogenes* by Dollfus (1946), Manter (1954), Dollfus and Capron (1958), Yamaguti (1958), Thomas (1960), and Skrjabin and Koval (1960). All but Yamaguti placed these two genera in Lepocreadiidae Nicoll, 1935.

Holorchis legendrei was described by Dollfus (1946) from *Mullus surmuletus* (Mullidae) from the Atlantic; from a specimen from *Mullus barbatus* from the Mediterranean, he (1948) added to and corrected in part his earlier description. He (1946) indicated that the cirrus sac contained a large, sinuous, thick-walled, muscular ejaculatory duct (but no cirrus) surrounded by large prostate gland cells; the metraterm was short and surrounded by longitudinal muscles; and the testes were smooth. Through the courtesy of Dr. R. P. Dollfus we were able to study 12 syntypes in whole mount and three in serial frontal and sagittal sections. Our study indicated that the cirrus sac contained a short, tubular, relatively thin-walled, slightly muscular internal seminal vesicle continuous with the external seminal vesicle; this was followed by a longer, thicker-walled, very muscular, tubular internal seminal vesicle; a short, muscular, tubular, cell lined pars prostatica was next; a very short, muscular cirrus opened into the short, tubular genital atrium. The distal thicker-walled portion of the internal seminal vesicle in sections showed a relatively thin inner circular muscle layer and a much thicker outer longitudinal muscle layer. The pars prostatica in sections showed an inner layer of cells, a thin middle layer of circular muscles, and a very much thicker outer layer of longitudinal muscles; it was much thicker-walled and muscular proximally than distally; the cells were not readily detectable in whole mounts. The cirrus was composed of a thick inner circular and equally thick outer longitudinal muscle layers. The metraterm also consisted of an inner cell layer, and inner circular and outer longitudinal muscle layers. The testes in all whole mounts and sectioned specimens showed deep crypts, the walls of each crypt in contact so that a lumen was not visible. It is our opinion that *H. legendrei* is a valid species.

Holorchis rhabdosargi was described from 3 specimens as *Aephnidiogenes rhabdosargi* by Prudhoe (1956) from *Rhabdosargus sarba* (Sparidae) from South Africa. Thomas (1960) placed it in *Holorchis*. Prudhoe noted the male and female complexes opening to the exterior by separate pores, and a cirrus sac containing an ejaculatory duct which was exceedingly muscular distally, a large mass of well developed prostate gland cells, and a small

portion of the seminal vesicle. Through the courtesy of Dr. S. Prudhoe we were able to study one cotype in whole mount and one in serial sagittal section. Our study revealed that the terminal genitalia were almost exactly like that described by us for *H. legendrei*. In the whole mount specimen of *H. rhabdosargi* a very small, shallow, thin-walled genital atrium was present, the male and female pores opening into it; the sections did not show this portion of the worm. Dollfus (1946) originally noted separate male and female pores for *H. legendrei*, but (1948) corrected this observation in reporting a superficial genital atrium. It is our opinion that *H. rhabdosargi* is a valid species and that it belongs in the genus *Holorchis* as reassigned by Thomas (1960).

Holorchis pulcher was described by Manter (1954) from *Latridopsis ciliaris* from New Zealand. Yamaguti (1958) transferred this species to his newly created genus *Pseudoholorchis*. Manter noted that the internal seminal vesicle was small, ovoid, followed by a prostatic vesicle of about the same size; the cirrus was long, coiled when retracted, often protruded as a muscular tube. Examination of whole mounts of the type specimen and a paratype (USNM Helm. Coll. No. 49123) indicated that the internal seminal vesicle started at the proximal end of the cirrus sac as a thin-walled tube continuous with the external seminal vesicle and then enlarged into a relatively thicker-walled, muscular, ovoid chamber; an ovoid, cell lined prostatic vesicle followed the latter, slightly overlapping it ventrally; the cells lining the prostatic vesicle continued into the so-called cirrus a short distance (actually a continuation of the pars prostatica) before becoming the cell free cirrus. On the basis of the morphology of the terminal genitalia we agree with Yamaguti (1958) in his erecting the genus *Pseudoholorchis* for this species and consider it valid.

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Notes on Four Helminths from the Clawed Toad, *Xenopus laevis* (Daudin), in South Africa*

MARY HANSON PRITCHARD

This paper is based on specimens collected at the Jonkershoek Fish Hatchery near Stellenbosch, South Africa. I wish to express thanks to the Department of Nature Conservation (Cape Province) for the privilege of visiting Jonkershoek, and to the staff at the Hatchery for their assistance. I am also grateful to the South African Museum for furnishing transportation between Cape Town and Jonkershoek; to Dr. Mary Beverley-Burton, Dr. Allen McIntosh, and Dr. P. L. G. Benoit for lending specimens for comparisons; and to Dr. Harold W. Manter for his interest and suggestions.

Three specimens of *Xenopus laevis* (Daudin), the clawed toad or "platanna," were examined and all contained parasites. Trematodes were killed under slight coverglass pressure with cold F.A.A.; cestodes were relaxed in cold water and fixed with cold F.A.A. Representative specimens of the helminths are deposited in the South African Museum (S.A.M.), Cape Town, and in the United States National Museum Helminthological Collection (U.S.N.M.).

Egg measurements are in microns; other measurements are in millimeters.

TREMATODA

Monogenea

Polystomatidae Gamble, 1896

Protopolystoma xenopi (Price, 1943) Bychowsky, 1957 (Figs. 1-6)

SYN. *Polystoma xenopi* Price, 1943
Polystoma x. victoriani Vercammen-Grandjean, 1960

INCIDENCE: 4 specimens from 3 of 3 hosts.
LOCATION: Urinary bladder.
SPECIMENS DEPOSITED: S.A.M. No. A29342; U.S.N.M. No. 60146.

DISCUSSION: This species has been reported from *Xenopus laevis* by Price (1943, specimen from South Africa), Williams (1960, specimens from University College, London, presumably imported from Africa), Vercammen-Grandjean (1960, specimens from The Congo), and Beverley-Burton (1962, specimens from Southern Rhodesia). No two of the descriptions agree in details nor does any one of them fully and accurately describe the species.

Through the kind cooperation of Dr. Allen McIntosh (Beltsville Parasitological Laboratory), Dr. Mary Beverley-Burton (her personal collection), and Dr. P. L. G. Benoit (Musée Royal de l'Afrique Centrale, Tervuren, Belgium) I have borrowed, respectively, the holotype, three of Beverley-Burton's specimens, and one of Vercammon-Grandjean's specimens. I can confirm that all have parallel cecal anastomoses between the right and left medial diverticula posterior to the ovary (Fig. 1); all have 16 atrial spines of alternating size (Fig. 2); all have one pair of large haptoral hooks with rather wide roots about as long as the shank (Fig. 3); all have a total of 16 bifid hooklets (one on the "plug tissue" of each of the six haptoral suckers, three anterior to each anterior sucker near the edge of the haptor, and four in a transverse line on the lobe-like fold between the large hooks (Fig. 4); all

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have two simple, slightly curved, longer hooklets between the large hooks (Fig. 5). The body may be rounded or elongate-oval depending upon fixation, the anastomoses may vary from three to six, and the ceca may or may not appear to join posteriorly.

Vercammen-Grandjean (1960) proposed a new subspecies, *Polystoma xenopi victoriani,* which now appears unjustified. He based it on the presence of cecal anastomoses, but three such structures are present in the holotype of *P. xenopi.* He reported only eight atrial spines and less bifid hooklets, but the holotype of *P. x. victoriani* clearly shows the 16 atrial spines of alternating size characteristic of the genus, and the hooklets are the same as in specimens from other localities.

Beverley-Burton (1962) did not report the hooklets of her specimens, but they agree with the holotype in number, arrangement, size, and shape. The large haptoral hook was drawn foreshortened, making it appear considerably more slender than comparable hooks on other specimens. One of the three specimens has two small, apparently longitudinal anastomoses between successive diverticula.

Williams's specimens are not available. Her (1960) Figure 9 shows several anastomoses not all of which are parallel, and, more importantly, she reports that the hooklets between the large hooks are all bifid, four rather than six in number, and that the central two lie slightly anterior to the others "as in *Polystoma integerrimum*" (Fig. 6). Another species may be represented.

Digenea

Plagiorchiidae Lühe, 1901

Oligolecithus jonkershoekensis n. sp. (Figs. 7-9)

INCIDENCE: 61 specimens from 1 of 3 hosts.

LOCATION: Intestine.

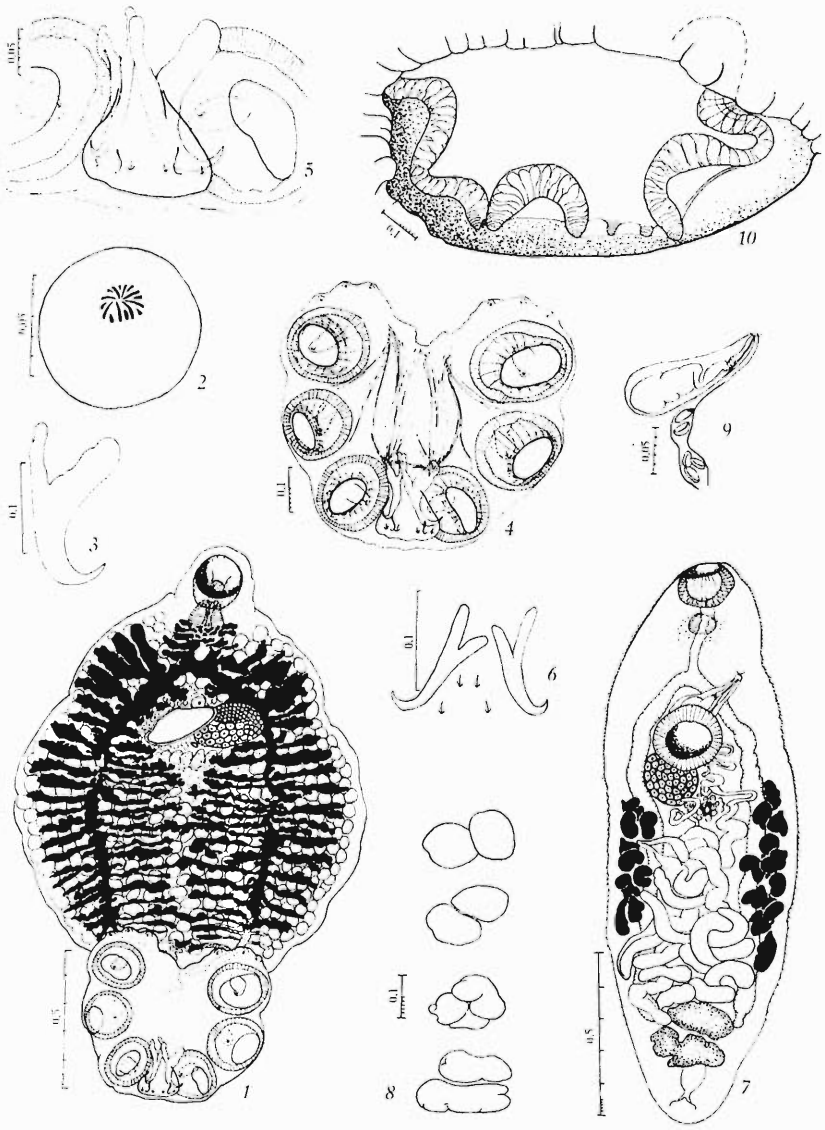
HOLOTYPE: S.A.M. No. A29343.

SPECIMEN DEPOSITED: U.S.N.M. No. 60147, paratype.

DESCRIPTION (measurements on 17 specimens): Body 0.771 to 1.635 long by 0.268 to 0.503 wide, anterior end often bent ventrally; body spines extending posteriorly in decreasing numbers, ventrally to level of testes and dorsally to level of ovary. Parenchyma of forebody vesicular. Oral sucker rounded, 0.106 to 0.154 wide; acetabulum rounded, 0.121 to 0.194 wide, about one-third body length from anterior end; sucker ratio 1: 1.0 to 1.3. Prepharynx often contracted, up to 0.035 long; pharynx 0.048 to 0.058 long by 0.053 to 0.075 wide, with accompanying cells; esophagus 0.048 to 0.128 long; cecal bifurcation nearer acetabulum than oral sucker, ceca extending to level of anterior testis.

Testes (Fig. 8) 0.056 to 0.134 long by 0.104 to 0.201 wide, near posterior end of body, usually oblique but varying from almost tandem to almost symmetrical, usually transversely elongate but sometimes subglobular, smooth to slightly lobed; cirrus sac (Fig. 9) 0.096 to 0.232 long by 0.040 to 0.064 wide, overlapping acetabulum slightly or not at all, posterior end between median line and right cecum, containing coiled seminal vesicle, short pars prostatica, and short, straight ejaculatory duct. Genital pore midway between cecal bifurcation and left side of body.

Ovary subglobular, 0.101 to 0.161 long by 0.112 to 0.181 wide, between right cecum and median line, overlapping acetabulum or immediately post-



All figures were drawn with the aid of a camera lucida. Each scale has its value indicated in millimeters.

Figs. 1-5. *Protopolystoma xenopi* from *Xenopus laevis*, Jonkershoek. 1. Ventral view. 2. Detail of atrial spines. 3. Large haptoral hook. 4. Opisthaptor. 5. Detail of posterior hooklets and large hooks.

Fig. 6. *P. xenopi*, enlargement of posterior hooks and hooklets as shown by Williams (1960, Fig. 9).

Figs. 7-9. *Oligolecithus jonkershoeki* from *Xenopus laevis*, Jonkershoek. 7. Holotype, ventral view. 8. Testes of four paratypes. 9. Cirrus sac of paratype.

Fig. 10. *Progonimodiscus doyeri* from *Xenopus laevis*, Jonkershoek; aperture of acetabulum showing edge of pedunele.

acetabular; Mehlis' gland posteromedian from ovary; seminal receptacle immediately postovarian, oval or rounded, 0.027 to 0.056 long by 0.027 to 0.030 wide; Laurer's canal present; uterine coils descending to level of anterior testis, surrounding lateral and anterior margins of anterior testis, and then ascending to genital pore, occasionally overlapping anterior edge of posterior testis laterally but never invading posttesticular space; metraterm about half as long as cirrus sac; eggs 19 to 26 long by 10 to 16 wide, majority 21 to 24 by 11 to 14. Vitelline follicles relatively large, subglobular to bilobed, 8 to 10 on right side and 10 to 13 on left side but always more on left side, extending from level of ovary about two-thirds distance to anterior testis, usually extracecal but occasional follicles may lie in intercecal space dorsal to uterus; main vitelline ducts converge at level of Mehlis' gland; vitelline reservoir dorsal to center of Mehlis' gland.

Excretory pore subterminal, ventral; excretory vesicle Y-shaped, stem sinuous and extending to level of Mehlis' gland, arms continuing anteriorly lateral to ovary and acetabulum.

DISCUSSION: *Oligolecithus elianae* Vercammen-Grandjean, 1960, the type and only previously described species, is from the small intestine of *Xenopus laevis victorianus* Ahl. taken near Lake Kivu in The Congo. *O. jonkershoekensis* differs from *O. elianae* in a more anterior acetabulum, ceca ending at the level of the anterior testis rather than extending into posttesticular space, vitellaria that rarely extend posteriorly as far as the anterior edge of the anterior testis and never more posteriorly, and uterine coils surrounding the anterior and lateral margins of the anterior testis. Only one egg size, 32 by 14, is given for *O. elianae*, and if this is typical for the species, the eggs of *O. jonkershoekensis* are shorter and rounder.

Sarumitrema Beverley-Burton, 1963, is closely related to *Oligolecithus*, but differs in the following respects: the testes are "always postcaecal and any oblique appearance is due to asymmetrical body contractions" (Beverley-Burton, personal communication); the vitelline follicles are much smaller and more numerous, the anterior follicles extending to the anterior edge of the acetabulum; and the acetabulum is smaller than the oral sucker. These are not strong generic characters, but they are supported by the fact that *Sarumitrema* was found only in *Rana* and *Bufo* even although 27 *Xenopus laevis* were examined.

Paramphistomidae Fischoeder, 1911

Progonimodiscus doyeri (Ortlepp, 1926) Vercammen-Grandjean, 1960

(Fig. 10)

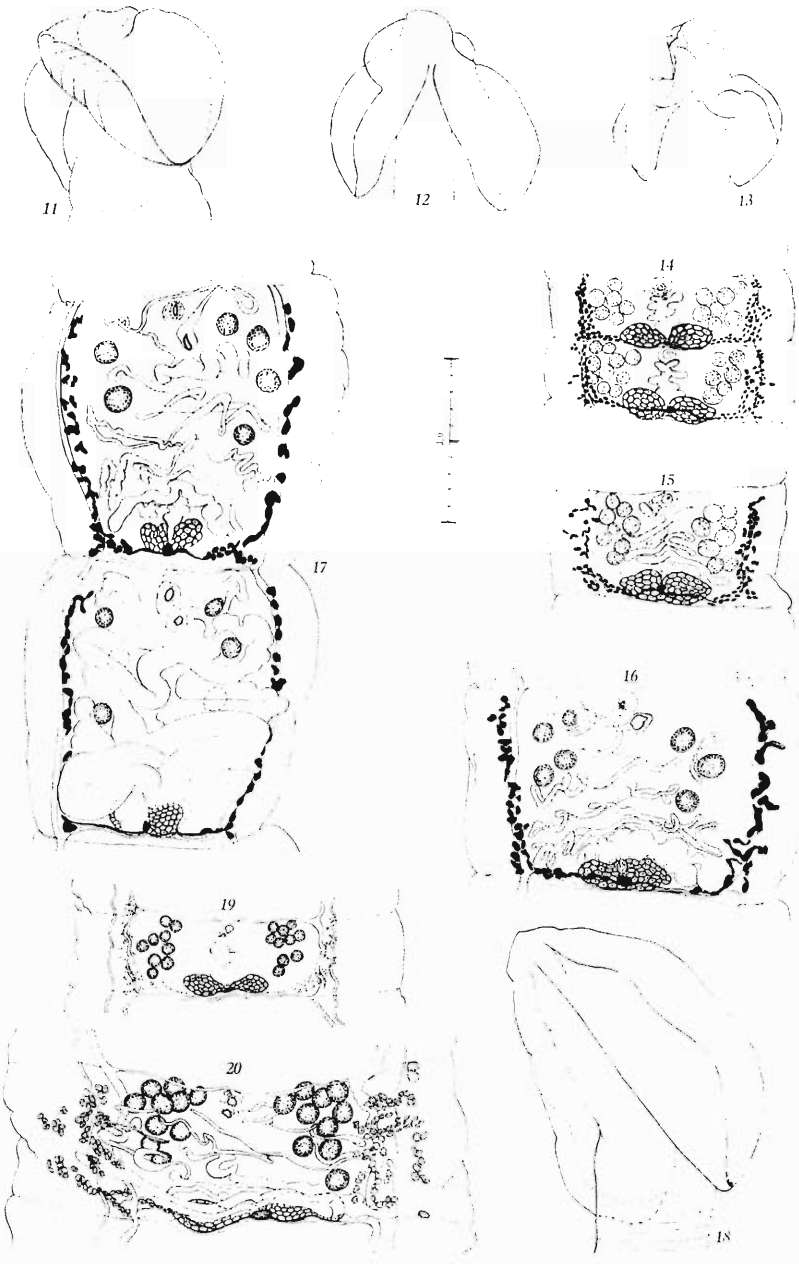
SYN. *Diplodiscus doyeri* Ortlepp, 1926
Progonimodiscus d. victoriani Vercammen-Grandjean, 1960

INCIDENCE: 3 specimens from 3 of 3 hosts.

LOCATION: Rectum.

SPECIMENS DEPOSITED: S.A.M. No. A29344; U.S.N.M. No. 60148.

BRIEF DESCRIPTION (specimens more or less contracted): Body 1.475 to 2.178 long by 1.039 to 1.541 wide; acetabulum 0.643 to 1.039 long by 0.938 to 1.374 wide, central peduncle with numerous folds; pharynx foreshortened, 0.235 to 0.382 wide, pouches 0.154 to 0.214 long by 0.134 to 0.181 wide; esophagus foreshortened; esophageal bulb 0.154 to 0.235 long by 0.121 to 0.154 wide; ceca short, divergent; testis subtriangular, 0.255 to 0.302 long



Figs. 11-17. *Cephalochlamys namaquensis* from *Xenopus laevis*, Jonkershoek; specimens from host with 20 strobilae. 11. Scolex with rounded bothridia. 12. Scolex with elongate bothridia. 13. Scolex with ruffled bothridia. 14. Proglottids 35 and 36. 15. Proglottid 43. 16. Proglottid 74. 17. Proglottids 102-103.

Figs. 18-20. *C. namaquensis* from *Xenopus laevis*, Jonkershoek; specimens from host with four strobilae. 18. Scolex. 19. Proglottid 52. 20. Proglottid 79.

by 0.302 to 0.415 wide, submedian, postcecal; seminal vesicle tubular, 0.181 to 0.214 long by 0.054 to 0.067 wide; genital pore at level of pharyngeal pouches; ovary 0.168 long by 0.201 wide and lateral to testis in one specimen, not seen in others; yellow eggs in posterior part of body 80 to 93 long by 45 to 56 wide; embryonated eggs closer to genital pore 112 to 136 by 72 to 96; vitellaria in two, more or less subdivided, lateral, postcecal groups, totaling 17, 22, and 25 respectively in three specimens.

DISCUSSION: This species has been reported from the same host in South Africa (Ortlepp, 1926), Uganda (Baylis, 1934), The Congo (Vercammen-Grandjean, 1960), and Southern Rhodesia (Beverley-Burton, 1963). Without doubt the *Diplodiscus subclavatus* (Pallas, 1760) Diesing, 1836 of Grobbelaar (1922) from South Africa is also this species, as suggested by Vercammen-Grandjean (1960).

Vercammen-Grandjean (1960) transferred *Diplodiscus doyeri* to a new genus, *Progonimodiscus*. In lieu of a generic diagnosis, a table separated *Progonimodiscus* from *Diplodiscus* by the more anterior genital pore, postcecal vitellaria, a single testis, short ceca, and egg masses both anterior and posterior to the ceca (i.e., cecal tips). Another important character is the ruffled peduncle centering the acetabulum. *Basidiodiscus* Fischtal and Kuntz, 1959, from freshwater fishes of the Nile is a related genus.

Vercammen-Grandjean also named two subspecies, *P. d. doyeri* (Ortlepp, 1926) and *P. d. victoriani*, which in his opinion differed in the relative size and position of the ovary and testis, number** of vitelline follicles, number of folds in the acetabular peduncle, size of the eggs, and size of the body. Through the courtesy of Dr. P. L. G. Benoit (Musée Royal de l'Afrique Centrale) I have borrowed the holotype of *P. d. victoriani* and this specimen shows that the folds of the acetabular peduncle are irregular and not necessarily six in number, that eggs vary from 80 by 48 in the posterior part of the body to 128 by 72 near the genital pore, that the ovary lies posterolateral to the testis and is larger than the testis, that the vitellaria are in two groups (14 right, 12 left) totaling 26. Further, from his table (1960, p. 64) body length varies from 1.98 to 3.71 and overlaps body length of *P. d. doyeri* (1.76 to 3.0). Finally, Bravo Hollis (1941), who redescribed Ortlepp's specimens, confirms (personal communication) that eggs of a paratype retained in her collection vary from 78 by 49 in the posterior part of the body to 143 by 87 near the genital pore. Any remaining differences are well within the limits of species variation and the subspecies are unjustified.

CESTDOA
Pseudophyllidea Carus, 1863
Cephalochlamydidae Yamaguti, 1959
Cephalochlamys namaquensis (Cohn, 1906) Blanchard, 1908

SYN. *Chlamydocephalus namaquensis* Cohn, 1906
Dibothriocephalus xenopi Ortlepp, 1926
Cephalochlamys xenopi (Ortlepp, 1926) Baylis, 1934
Pseudocephalochlamys xenopi (Ortlepp, 1926) Yamaguti, 1959
(Figs. 11-20)

**He actually reported vitelline follicles "15 to 18 microns" for *P. d. doyeri* and "18 to 22 microns" for *P. d. victoriani*, but it is evident that he meant numbers of follicles rather than size.

INCIDENCE: 4 specimens from one host; 20 from another.

LOCATION: Duodenum.

SPECIMENS DEPOSITED: S.A.M. No. A29345, A29346; U.S.N.M. No. 60149.

DESCRIPTIVE NOTES: Strobilae with scolex vary from 11 to 88 mm. long, most exceeding maximum of 19 mm. recorded for the species. Bothridia rounded (Fig. 11), elongate (Fig. 12), or somewhat ruffled (Fig. 13). Neck region short; proglottids mature quickly. Testes vary from four to 20 per segment, usually one to three more on one side than other, and usually more numerous in smaller, more anterior proglottids. Eggs 27 to 43 by 21 to 32 microns. Lateral bands of vitellaria converge medianly at posterior edge of proglottid but do not pass posterior to ovary. Vitelline ducts, however, pass either ventrally or posteriorly to ovary, and vitelline reservoir ventral to median part of ovary. Narrow, dorsal excretory ducts either median or lateral to ventral ducts and have no transverse connections.

Typical of the 20 strobilae found together is one about 88 mm. long with 111 more or less distinguishable proglottids. In 30th proglottid (0.235 long by 1.139 wide) testes and ovary well developed; uterus begins to coil in 35th (Fig. 14), and well developed in 43rd (Fig. 15). Segment 74 (Fig. 16) represents a mature proglottid; while 103rd (1.642 long by 1.541 wide) is one of few gravid proglottids (Fig. 17).

Proglottids of specimens living in less crowded conditions distinctly shorter and wider. One such strobila about 165 mm. long has about 140 proglottids. Scolex (Fig. 18) 1.943 long; 52nd proglottid (Fig. 19) 0.402 long by 1.776 wide and compares in maturity to proglottid 30 of example above, though both genital pores are already present. Proglottid 79 (Fig. 20) 0.570 long by 2.680 wide and mature. Fully gravid proglottids not present.

DISCUSSION: Mettrick (1960, 1963) described specimens from *Xenopus laevis* in Southern Rhodesia, reviewed the history of the species and the synonymies involved. Although Cohn (1906) reported eggs 75 by 40, later authors found considerably smaller eggs—37 by 26 (Ortlepp, 1926) 24 by 16 (Mettrick, 1960), and 27 to 43 by 21 to 32 (above).

SUMMARY

One new trematode, *Oligolecithus jonkershoekensis,* is described from *Xenopus laevis* (Daud.) in South Africa. Descriptive notes are given for two trematodes, *Protopolystoma xenopi* (Price, 1943) Bychowsky, 1957, and *Progonimodiscus doyeri* (Ortlepp, 1926) Vercammen-Grandjean, 1960; and for one cestode, *Cephalochlamys namaquensis* (Cohn, 1906) Blanchard, 1908.

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ERRATA

In my paper "A New Genus and Species of Monogenetic Trematode from a Shark, with a Review of the Family Microbothriidae Price, 1936" which appeared in vol. 30(2), pp. 213-218 of these Proceedings, several unfortunate errors occur which are not the responsibility of either the editor or printer. These are:

On page 213 Neodermophtheriinae should have been Dermophthiriinae and on the same page, and elsewhere, *Neodermophtherius* and *Dermophtherius* should have been *Neodermophthirius* and *Dermophthirius*, respectively. EMMETT W. PRICE

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