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# Proceedings of the Helminthological Society of Washington

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## A Revised Definition of the Monogenetic Trematode Genus *Dactylogyrus*, with Descriptions of Four New Species<sup>1</sup>

CHARLES E. PRICE<sup>2</sup>

The first mention of the genus *Dactylogyrus* in North America was by Hess (1928) who reported the presence of undetermined species on the gills of large- and smallmouth basses, carp, goldfishes, and "other fishes." The validity of this observation is questionable in part, because species of *Dactylogyrus* generally do not occur on the gills of basses and sunfishes. Hess was more correct (1930) when he referred to dactylogyrids specifically from the gills of carp and goldfishes, as shown subsequently by Mueller and Van Cleave (1932).

Descriptive research within this genus in North America began when Mueller and Van Cleave (1932) described *D. extensus* from the gills of the carp, *Cyprinus carpio* Linnaeus.

Prior to the present publication, 56 species of *Dactylogyrus* had been recorded for this continent. Of these, Mizelle and his students described 34 species (Mizelle, 1937, 1938, 1962; Mizelle and Donahue, 1944; Mizelle and Klucka, 1953; Mizelle and Price, 1964; Mizelle and Regensberger, 1945; Monaco and Mizelle, 1955; Price and Mizelle, 1964; Wood and Mizelle, 1957); Mueller, 17 species (1936, 1938); Putz and Hoffman, 1 species (1965); Seamster, 3 species (1948, 1960), and Mueller and Van Cleave, 1 species (1932). This study adds four new species to the list of North American *Dactylogyrus*.

### REVISION OF THE GENERIC DIAGNOSIS

An improved generic diagnosis is needed for *Dactylogyrus*. As a base from which to launch the task of synthesizing a new diagnosis, var-

ious quotes from Yamaguti (1963) are given. First, his definition of the genus:

"Dactylogyridae, Dactylogyrinae. Anchors supported by a single rod-shaped bar. Vas deferens usually (always?) looped around intestinal limb. Vesicula seminalis formed by mere dilation of vas deferens. Two prostatic reservoirs present. Cirrus mostly tubular, with accessory piece. Vagina single, exceptionally double, may or may not have sclerotized supporting structures, with submarginal aperture. Parasites of freshwater teleosts."

In his key to the Dactylogyrinae, Yamaguti (1963) gives as his points of differentiation of *Dactylogyrus* "Anchor blades without marked change in curvature; bar rod-shaped; marginal hooklets usually of uniform size."

Other aspects of diagnosis can be taken from Yamaguti's description of the subfamily Dactylogyrinae: "Dactylogyridae: Opisthohaptor with one pair of anchors, supported by one or two connecting bars, and with 14 marginal hooklets; sclerotized accessory structures of haptor absent. Eyes present. Testis and ovary rounded or elongate, former posterior to latter. Vagina present."

Certain aspects of these diagnostic points warrant examination.

Current diagnoses indicate presence of 14 hooks in *Dactylogyrus*. Mizelle and Price (1963), employing phase-contrast microscopy, showed that in a study of 21 *Dactylogyrus* species, all but four of them actually possessed 16 hooks. These "extra" hooks (designated "4A" so as not to interfere with the present system of hook designation of North American forms) are quite small, but are nevertheless readily observable with the phase-contrast microscope. Both Mizelle and Price feel that

<sup>1</sup> This study was supported by the Faculty Research Fund of the Department of Biology, North Texas State University.

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the presence of these hooks is a constant feature of *Dactylogyrus*, and future descriptions of species from this genus should include reference to them. This additional pair of hooks is not restricted to North American *Dactylogyrus* members, as was shown by the fact that two Old World *Dactylogyrus* species possess them, namely, *D. anchoratus* (Dujardin, 1845) Wagne-ner, 1857, and *D. vastator* Nybelin, 1924.

The question concerning the number of haptor bars present in various species has been a troubled area of *Dactylogyrus* classification since the establishment of the problematical genus *Neodactylogyrus* by E. W. Price (1938). Yamaguti (1963) accepts this genus as a valid one; he takes many North American parasites originally described as *Dactylogyrus* species and transfers them to *Neodactylogyrus*. Yamaguti (1963) gives as the main feature of *Neodactylogyrus* "(forms) in which the anchors are supported by two (dorsal and ventral) bars, which may be similar or dissimilar." This genus has been rejected by Mizelle and his students on several occasions (Mizelle and Donahue, 1944, etc.) on grounds that the ventral bar is subject to pronounced morphological diversity, and is in some cases missing entirely from some of the members of a given species. Yamaguti himself states in a footnote that this ventral bar may be so much reduced or so poorly sclerotized that it "may be overlooked even by careful workers."

Stenzel (1963) has proposed the introduction of two recently coined terms into the taxonomic literature. One of these, *archekastic* (ARCHI-, Greek for "ruling" or "governing," and -HEKASTOS, Greek, for "every one") refers to a feature that is invariably present in every one of the individual units of a given taxon. The other word, *archestinic* (-ESTINOI, Greek, for "some"), is used in reference to a feature that is present or developed in a special form, number, or size in some individual units of a given taxon. This worker feels that unless a character is archekastic in nature, it should not be employed as a character for generic diagnosis. As the main feature for establishment of *Neodactylogyrus* is the presence of a ventral bar which aids in support of the anchors, this supposedly qualifying feature is archestinic in nature, as shown by the highly variable nature

(and sometimes apparent complete absence) of the ventral bar.

Reference should perhaps be made to *Paradactylogyrus* Thapar (1948), which genus was relegated to subgeneric status (of *Dactylogyrus*) by Tripathi (1959). Study of both generic diagnosis and illustrations indicates that this genus differs from *Dactylogyrus* only by the presence of an unpaired sclerotized structure located centrally in the haptor; Thapar (1948) termed this structure an "onchium."

The inclusion of members of *Neodactylogyrus* and *Paradactylogyrus* as species of *Dactylogyrus* would seemingly constitute a contribution to monogenean taxonomy. By establishing new taxa to include forms which differ from *Dactylogyrus* by presence of unpaired structures in the haptor not only creates unnecessary taxonomic confusion, but also goes far toward lessening the prestige and value which should be accorded the rank of genus.

A vagina is not always described in descriptions of *Dactylogyrus*, as this structure is not invariably sclerotized. A vagina is without doubt present in all or nearly all specimens, but is difficult or impossible to observe in some cases. It would seem that presence and location of the vagina cannot be considered an archekastic trait.

Concerning the statement in Yamaguti's key to the effect that the marginal hooklets (haptor hooks) are of a uniform size, the literature indicates that investigators who make painstaking measurements report almost invariably that members of hook pairs 1 and 5 are considerably shorter than members of the remaining pairs. It often happens that additional discrepancies in size occur among the hooks of various species. To quote a rather extreme case, I am presently describing several *Dactylogyrus* species from Africa. One of these forms possesses a complement of hooks exhibiting a great range in hook lengths. The shortest hook is ca. 40% as long as the corresponding anchor, whereas the longest is slightly longer than the associated anchor.

The arrangement of hooks on the haptor should be referred to in descriptions, as the hooks are arranged (with minor deviations) in a generalized spatial pattern. This sequence is discussed by Mizelle and Crane (1964) and illustrated for *Dactylogyrus* by Mizelle (1962).



In agreement with Hargis (1955), it is possible and indeed quite probable that some of the nonsclerotized features of monogeneid parasites mentioned by Yamaguti are of generic magnitude. The "soft" parts should be described in as much detail as possible in all cases, along with complete descriptions of the "hard" parts, *i.e.*, the sclerotized ones. Although the individual researcher must decide upon the characters which he feels are most important from a taxonomic view, an original or comparative description should be complete in as many aspects as possible. Only complete descriptions are likely to pass the test of time.

In view of the foregoing observations and opinions, a modified diagnosis of *Dactylogyrus* is hereby proposed:

"Dactylogyridae, Dactylogyrinae: Haptor with one pair of anchors (dorsal), their bases supported by a (usually) simple haptor bar. A ventral bar may be present or absent. Hooks 16 (8 pairs), one pair relatively very small. Hooks normally similar in shape and usually subequal in size, except for members of pair 4A. In some, additional small sclerotized structures may be present in haptor. Four eyespots. Copulatory complex composed of a tubular cirrus and an accessory piece, the latter generally basally articulated to the former. Vagina present or absent, variable in position; a seminal receptacle usually present. Prostatic reservoir single or double. Seminal vesicle formed by simple dilatation of vas deferens. Vas deferens usually (always?) looped around intestinal limb. Testis postovarian. Intestinal crura confluent posteriorly. Parasites of freshwater fishes."

#### MATERIALS AND METHODS

The host specimens utilized in this study were obtained by seining or trapping in the localities indicated. The live hosts were frozen as suggested by Mizelle (1938a). The parasites were recovered and otherwise treated as prescribed by Price and Mizelle (1964), and measurements made as outlined by Mizelle and Klucka (1953). All measurements are given in microns. Average measurements are given first, followed by minimum and maximum values enclosed within parentheses. Appropriate measurements and illustrations were made with the aid of a calibrated filar

micrometer ocular and a camera lucida, respectively.

#### DESCRIPTIONS OF NEW SPECIES

All new species here described exhibit a distribution of hooks very similar to the generalized arrangement described by Mizelle and Crane (1964). Additionally, the "extra" haptor hooks (4A) of *Dactylogyrus* (Mizelle and Price, 1963) were observed in all species. References to these features are omitted in individual descriptions.

#### *Dactylogyrus centrarchidi* n. sp. (Figs. 1-5)

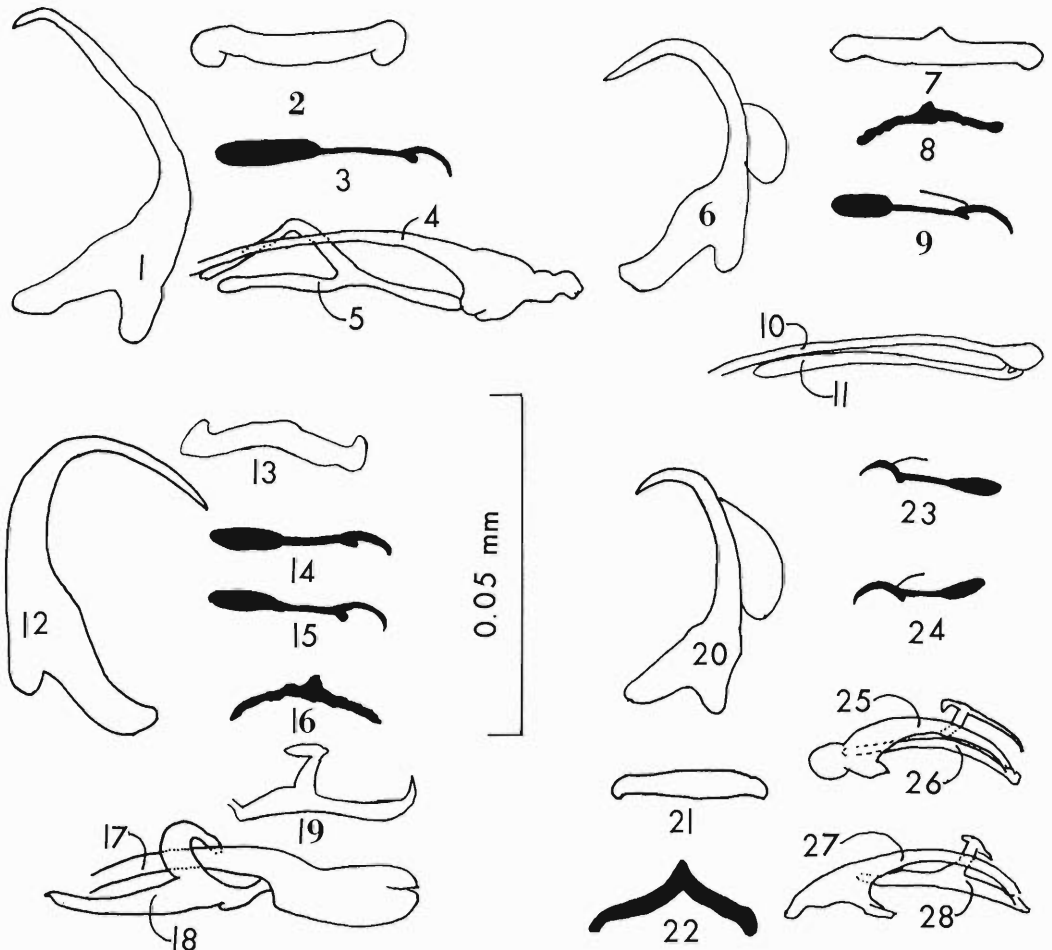
HOST AND LOCALITY: *Lepomis auritus* (L.), the red-breasted sunfish; Milner's Branch, 3 mi. SE of Hollanville, Georgia.

NUMBER OF SPECIMENS STUDIED: Three.

TYPES: Holotype deposited in USNM Helm. Coll., No. 61350, Washington, D.C. Paratypes in author's collection.

DESCRIPTION: A dactylogyrid of moderate size, provided with a thin cuticle; length 461 (433 to 485), greatest width of body 86 (75 to 95), near midlength. A single cephalic lobe anteriorly; lateral cephalic lobes moderately developed. Two pairs of eyespots, members of posterior pair larger and closer together than other members. Several eyespot comprising granules observed in cephalic region. Pharynx subcircular, muscular (ventral view); transverse diameter 32 (29 to 36). Peduncle short and stout, with result that haptor is poorly set off from body proper. Haptor subovate in outline; length 70 (62 to 78), width 76 (66 to 83).

One pair of anchors, located dorsally (Fig. 1). Anchor composed of: (1) a solid base provided with well-developed roots, the superficial much longer than deep root, (2) a solid shaft, and (3) a modified point; length 42 (38 to 45), width of base 14 (13 to 16). A stout, simply constructed dorsal bar connects the anchor bases (Fig. 2); length 31 (28 to 34). Hooks 16 in number (8 pairs), similar in shape (except for 4A), and with a large range of lengths. Each hook composed of: (1) a solid elongate base, (2) a solid, narrow shaft, and (3) a sickle-shaped termination provided with an opposable piece (Fig. 3).



Figures 1-28. Camera lucida illustrations of sclerotized structures of new *Dactylogyrus* species. Figs. 1-5: *D. centrarchidi*. 1, anchor; 2, dorsal bar; 3, hook; 4, cirrus; 5, accessory piece. Figs. 6-11: *D. georgiensis*. 6, anchor; 7, dorsal bar; 8, ventral bar; 9, hook; 10, cirrus; 11, accessory piece. Figs. 12-18. *D. katherineae*. 12, anchor; 13, dorsal bar; 14, 15, hooks; 16, ventral bar; 17, cirrus; 18, 19, accessory pieces. Figs. 20-28. *D. seamsteri*. 20, anchor; 21, dorsal bar; 22, ventral bar; 23, 24, hooks; 25, 27, cirri; 26, 28, accessory pieces.

Hook lengths: no. 1, 27 (25 to 28); no. 2, 31 (29 to 34); no. 3, 30 (28 to 32); no. 4, 20 (18 to 22); no. 5, 26 (25 to 28); no. 6, 21 (19 to 22), and no. 7, 22 (20 to 23). Hook 4A not suitable for measurement.

Cirrus tubular, provided with accessory piece (Figs. 4, 5). Cirrus arising from expanded base, becoming a tube of decreased diameter distally; length 45 (41 to 48). Accessory piece basally articulated to cirrus,

bifurcated near its midlength into two rami. Main ramus parallel to cirrus tube; accessory ramus attached to main ramus in a 65 degree angle, curving partially around cirrus tube, then becoming recurved with its distal aspect approximately parallel to cirrus tube; length 29 (26 to 33). One prostatic reservoir. Vagina not sclerotized, opening ventrally near right body margin just posterior to copulatory complex. Gonads ovate, the ovary pretesticular.

Vitellaria moderately developed, forming lateral bands. Intestinal crura confluent posteriorly.

DISCUSSION: The anchors of this new form resemble those of *D. aureus* Seamster, 1948; the anchors of each parasite possess rather unusual recurved points. The accessory pieces of the two species are quite different. The accessory pieces of *D. centrarchidi* and *D. eucalius* Mizelle and Regensberger, 1945 show similarity, but comparison of other sclerotized structures separates the two without difficulty.

With an increasing number of new species being added to the genus, it is becoming increasingly more difficult to establish morphological affinities among *Dactylogyrus* species. This is especially true in regard to the haptor armament. It is true that a small minority of species can be readily recognized because of striking features of the anchors, bars, and/or hooks, but study of the genus as a whole indicates that a generalized architecture exists. Such a situation does not facilitate establishment of distinct species. Most diversity exists in the copulatory complex, but even here, some generalizations are noticed, especially in regard to the cirrus. The accessory piece, however, is quite variable in morphology, thereby furnishing (generally speaking) a reliable guide in comparative studies.

*D. centrarchidi* is the first species of *Dactylogyrus* to be initially recovered from the gills of a sunfish (family Centrarchidae). The only previous record of a *Dactylogyrus* being reported from a centrarchid was by Hargis (1953), when he recovered *D. aureus* Seamster, 1948 from the gills of both *Chaenobryttus coronarius* (Bartram) and *Lepomis macrochirus* Rafinesque. Hargis considered both occurrences as cases of accidental parasitism.

The possibility of accidental parasitism must be considered in cases such as the present one. There is little doubt that valid cases of accidental parasitism do occur among all groups from time to time. In the light of the close degree of host-specificity existing between monogeneans and their hosts, I feel that true cases of accidental association within the Monogenea are exceedingly rare. The very intimate physiological and immunological relationships that must exist between a parasite and its host seemingly negate the idea that

accidental parasitism is likely to be a very common occurrence.

While the possibility of accidental parasitism should be considered in all questionable cases, then, it should not be used indiscriminately as an argument against a given host harboring a given parasite. This is another aspect of parasitology that can be resolved definitely only by recourse to controlled experimentation.

*Dactylogyrus georgiensis* n. sp.

(Figs. 6-11)

HOST AND LOCALITY: *Campostoma anomalum* (Rafinesque), the stoneroller; ditch within city limits of Milledgeville, Georgia.

NUMBER OF SPECIMENS STUDIED: Three.

TYPES: Holotype deposited in USNM Helm. Coll., No. 61351, Washington, D.C. Paratypes in author's collection.

DESCRIPTION: A dactylogyrid of moderate size, provided with a thin cuticle; length 351 (326 to 370), greatest width of body 70 (61 to 84), near midlength. Two pairs of eyespots, all about equal in size. Eyespots tend to dissociate. Peduncle wide and thick, the pentagonal haptor fairly well set off from body proper; length 56 (48 to 62), width 84 (76 to 89).

The dorsally located pair of anchors are each composed of: (1) a solid base provided with a short deep and an elongate superficial root, (2) a solid shaft, and (3) a solid point, the shaft and point meeting at the suggestion of an angle (Fig. 6); length 39 (36 to 42), width of base 17 (15 to 18). Anchor wings present. Hooks 16 in number (8 pairs), all similar in shape and size except members of pair 4A. Each hook consists of: (1) a solid, elliptical base, (2) a solid, narrow shaft, and (3) a sickle-shaped termination provided with an opposable piece (Fig. 9). Hook lengths: nos. 1 and 2, 24 (22 to 27); no. 3, 26 (24 to 27); no. 4, 25 (24 to 27); no. 4A, 9 (8 to 11); no. 5, 22 (20 to 23); nos. 6 and 7, 23 (21 to 24). Dorsal bar simple, a projection pointing anteriorly, and with inflated ends (Fig. 7); length 24 (21 to 26). Ventral bar vestigial, of irregular outline (Fig. 8); length 17 (15 to 20).

Copulatory complex consisting of a cirrus and basally articulated accessory piece (Figs. 10, 11). Cirrus a simple tube, arising from

an expanded base; length 39 (36 to 41). Accessory piece simple, a sclerotized bar, slightly expanded distally, lying approximately parallel to cirrus tube; length 33 (30 to 35). One prostatic reservoir. Gonads ovate-elliptical in outline, ovary pretesticular. Intestinal crura confluent posteriorly.

DISCUSSION: Anchors, bars, and hooks of *D. georgiensis* resemble those of *D. simplex* Mizelle, 1937 quite closely, but copulatory complexes differ significantly. Absence of an accessory ramus on accessory piece is a rare feature for *Dactylogyrus*. The only other North American species possessing this simplified type of accessory piece are: *D. claviformis* Mizelle and Klucka, 1953; *D. extensus* Mueller and Van Cleave, 1932, and *D. scutatus* Mueller, 1938. The present new form is readily separated from these species by comparison of other sclerotized parts.

*Dactylogyrus katherineae* n. sp.  
(Figs. 12–18)

HOST AND LOCALITY: *Campostoma anomalum* (Rafinesque), the stoneroller; ditch within city limits of Milledgeville, Georgia.

NUMBER OF SPECIMENS STUDIED: Five.

TYPES: Holotype deposited in USNM Helm. Coll., No. 61353, Washington, D.C. Paratypes in author's collection.

DESCRIPTION: A dactylogyrid of moderate size, provided with a smooth cuticle; length 360 (334 to 392), greatest body width 73 (68 to 81). Well-defined anterior and lateral cephalic lobes. Two pairs of eyespots, members of posterior pair larger. Pharynx sub-circular in dorsal view; transverse diameter 23 (20 to 24). Peduncle short and stout, with result that haptor is well set off from body proper. Haptor subtriangular in outline, much wider than long; length 59 (55 to 65), width 94 (88 to 104).

One pair of anchors, dorsally located. Anchor comprised of: (1) a solid base equipped with roots, superficial root much longer, (2) a solid shaft, and (3) a solid point (Fig. 12); length 33 (31 to 36), width of base 15 (14 to 17). Anchor bases connected by a dorsal bar, bent in central portion and with expanded ends (Fig. 13); length 23 (21 to 24). Ventral bar variable in shape, with a built-up central region (Fig. 16); length 17 (15 to

20). Hooks 16 in number (8 pairs), similar in shape and subequal in size (except for 4A). Each hook comprised of: (1) a solid, elongate base, (2) a solid shaft, and (3) a sickle-shaped termination provided with an opposable piece; a posteriorly projecting appendage arises opposite opposable piece and extends for ca. 50% of shaft length (Figs. 14, 15). Hook lengths: no. 1, 18 (17 to 19); nos. 2 and 5, 20 (19 to 22); no. 3, 29 (28 to 31); no. 4, 21 (20 to 23); no. 6, 22 (20 to 23); and no. 7, 23 (21 to 24). Hook 4A not suitable for measurement.

Copulatory complex a cirrus with basally articulated accessory piece (Figs. 17, 18, and 19). Cirrus arising from an expanded base; length 39 (36 to 43). Accessory piece bifurcated, primary ramus terminating in a sharp, recurved point; secondary ramus arising near midpoint of primary ramus, curving partially around cirrus tube; length 23 (21 to 25). Gonads elliptical-ovate, testis postovarian. A single prostatic reservoir emptying into cirrus base via a short duct. Vagina not observed with certainty; a nonsclerotized atrium apparently opening ventrally near right body margin.

Vitellaria moderately developed, the vitelline granules consistent in size and color, and exhibiting a tendency toward forming broad lateral bands, approximately coextensive with intestinal crura, the crura confluent posteriorly.

DISCUSSION: The anchors and bars closely resemble those of *D. atromaculatus* Mizelle, 1938. The accessory piece resembles that of *D. bychowski* Mizelle, 1937, except that the digitiform process arising from the cirrus base in *D. bychowski* is lacking in this form. There exists little doubt that *D. atromaculatus* is the nearest morphological relative of *D. katherineae*.

*Dactylogyrus seamsteri* n. sp.  
(Figs. 20–28)

HOST AND LOCALITY: *Phenacobius mirabilis* (Girard), the suckermouth minnow; Milner's Branch, 3 mi. SE of Hollanville, Georgia.

NUMBER OF SPECIMENS STUDIED: Five.

TYPES: Holotype deposited in USNM Helm. Coll., No. 61354, Washington, D.C. Paratypes in author's collection.

DESCRIPTION: A dactylogyrid of moderate size, provided with a thin cuticle. A moderate

constriction just anterior to level of copulatory complex; length 595 (578 to 652), greatest body width 88 (79 to 101). Anterior cephalic lobes prominent, and lateral cephalic lobes vestigial. Two pairs of eyespots, members of posterior pair larger. One specimen possessing five eyespots, all of equal size. Pharynx sub-circular, somewhat elongate longitudinally in ventral view; transverse diameter 24 (21 to 27). Peduncle unusually elongate, the haptor well set off from body proper. Haptor much wider than long; length 64 (59 to 69), width 90 (85 to 99).

The pair of dorsally located anchors are each comprised of: (1) a solid base equipped with prominent roots, (2) a solid shaft, and (3) a solid point (Fig. 20); length 32 (30 to 35), width of base 12 (11 to 14). Anchor wings present. Dorsal bar simple, ends smaller than shaft (Fig. 21); length 19 (18 to 21). Ventral bar widely "V"-shaped, larger than dorsal (Fig. 22); length 25 (23 to 27). Hooks 16 in number (8 pairs), similar in shape and with a narrow range of lengths (Figs. 23, 24). Each hook comprised of (1) a solid, elongate base, (2) a solid, narrow shaft, and (3) a sickle-shaped termination provided with an opposable piece. A posteriorly projecting appendage arises opposite opposable piece, extending for a short distance. Hook lengths: nos. 1 and 6, 21 (20 to 22); no. 2, 21 (19 to 22); no. 3, 24 (22 to 25); nos. 4, 5, and 7, 22 (21 to 23), and no. 4A, 9 (8 to 11).

Copulatory complex of a cirrus and an accessory piece (Figs. 25, 26, 27, and 28). Cirrus arising from an inflated base of variable morphology, the tube traveling through a continuous arc of ca. 45 degrees; length 24 (21 to 27). Accessory piece complex, its variation illustrated in Figs. 26 and 28; length 21 (19 to 24). Vagina is nonsclerotized, an atrium opening ventrally near right body margin. A single prostatic reservoir, folded back upon itself. Gonads ovate, the ovary pretesticular.

Vitellaria well developed, comprised of large granules, the granules forming two lateral bands. Intestinal crura confluent posteriorly.

Discussion: A ventral bar larger than the dorsal is an unusual feature of North American *Dactylogyrus*. The haptoral armament of *D. seamsteri* is of the generalized pattern referred to earlier (except for the large ventral bar).

The cirrus looks very much like that of *D. californiensis* Mizelle, 1962, but the accessory pieces of the two are quite different. The accessory piece of this new form does, however, resemble that of *D. mizellei* (Price, in press). The bars and anchors of *D. mizellei* are easily distinguishable from those of *D. seamsteri*.

#### SUMMARY

A short history of research in North American *Dactylogyrus* is recounted. Various taxonomic aspects of the genus are discussed, and a revised generic diagnosis is proposed. Four new species of *Dactylogyrus* are described: *D. centrarchidi* from *Lepomis auritus* (L.); *D. georgiensis* and *D. katherineae* from *Camptostoma anomalum* (Rafinesque); and *D. seamsteri* from *Phenacobius mirabilis* (Girard). The possibility of *D. centrarchidi* from the gills of *L. auritus* being a case of accidental parasitism is briefly discussed.

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***Monobothrioides woodlandi* sp. nov. (Cestoidea: Caryophyllidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa**

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According to Wardle and McLeod (1952) two species of *Monobothrioides* Fuhrmann and Baer, 1925 (Caryophyllidea: Lytocestidae) have been described from African siluroid fish: *M. cunningtoni* Fuhrmann and Baer, 1925 from *Auchenoglanis occidentalis* Cuv. and Val. in Mtondwe Bay, Lake Tanganyika (Fuhrmann and Baer, 1925), and *M. chalmersius* (Woodland, 1924) Hunter, 1930 from *Clarias anguillaris* (Linn.) in the Nile River at Khartoum (Woodland, 1924). This paper is the report of a third species collected in 1961 by the junior author from the intestine of two *Clarias mellandi* Boulenger taken from Lake Chali in the Bangweulu Swamp complex in north Zambia (Northern Rhodesia), Africa. Lake Chali is formed by the Chambezi River and is approximately 8 km from the junction of the Chambezi and Luapula rivers. It lies approximately 50 km SE of Samfya, a small village on the east shore of Lake Bangweulu. Lake Bangweulu, the source of the Congo River, is located at latitude 11°S and longitude 30°E and is drained by the Luapula River.

The description is based on whole mounts of 3 gravid, 3 mature, and 38 immature worms and a posterior half of a gravid worm. Mid-sagittal and cross sections were made from different parts of a mature worm. Comparative material included the following from Woodland's collection in the British Museum: *M. cunningtoni*, slide numbers 1927.8.6.1 to 11 (whole mounts) and 1927.8.6.12 to 25 (sections), all of the slides were marked "Type"; and *M. chalmersius*, slide numbers 1961.3.14.42 to 46 (two with sections) and *Lytocestus filiformis* (Woodland, 1924) Hunter, 1930 slide numbers 1923.12.4.2, 1961.3.14.1 to 9 (32 whole mounts), and 1961.3.14.10 to 41 (sections), all of the slides of both species

were marked "cotype." This material was examined by the senior author during tenure of an NIH postdoctoral research grant No. EF 11,462.

Measurements are in millimeters unless otherwise stated.

DESCRIPTION

*Monobothrioides woodlandi* sp. nov.  
(Figs. 1-6)

DIAGNOSIS: (N = the sample observed or measured.) With characters of the genus. Immature worms 1.6 to 4.3 long (N = 14). Gravid worms 4.5 to 4.6 long by 1.1 to 1.3 wide (N = 3). Scolex well defined, compressed, with longitudinal ridges. Outer longitudinal muscles poorly developed and consisting of small fascicles. Inner longitudinal muscles a prominent dorsal and ventral band of closely arranged fascicles and of two separate fascicles at ends of each band. Testes number from 177 to 203 (N = 6; mean 189), 0.056 to 0.096 in diameter (N = 30, 10 each from 3 gravid worms; mean 0.075). Testicular field extending from cirrus sac to approximately one-half way to tip of scolex. Cirrus sac pyriform. External seminal vesicle absent. Vitellaria in preovarian region, only, annularly arranged around testes and extending from ovary to base of scolex. Vitellaria 0.013 to 0.053 in diameter (N = 30, ten each from three gravid worms; mean 0.032). Ovary distinctly follicular and extending to posterior tip of worm. Osmoregulatory canals diffuse with no definite number in sections from middle of body. Seminal receptacle absent. Ova 0.062 by 0.041, not embryonated when laid, shell smooth (N = 3, measured in utero from distal part of uterus).

HOST: *Clarias mellandi* Boulenger (Cypriniformes: Clariidae).

SITE OF INFECTION: Intestine.

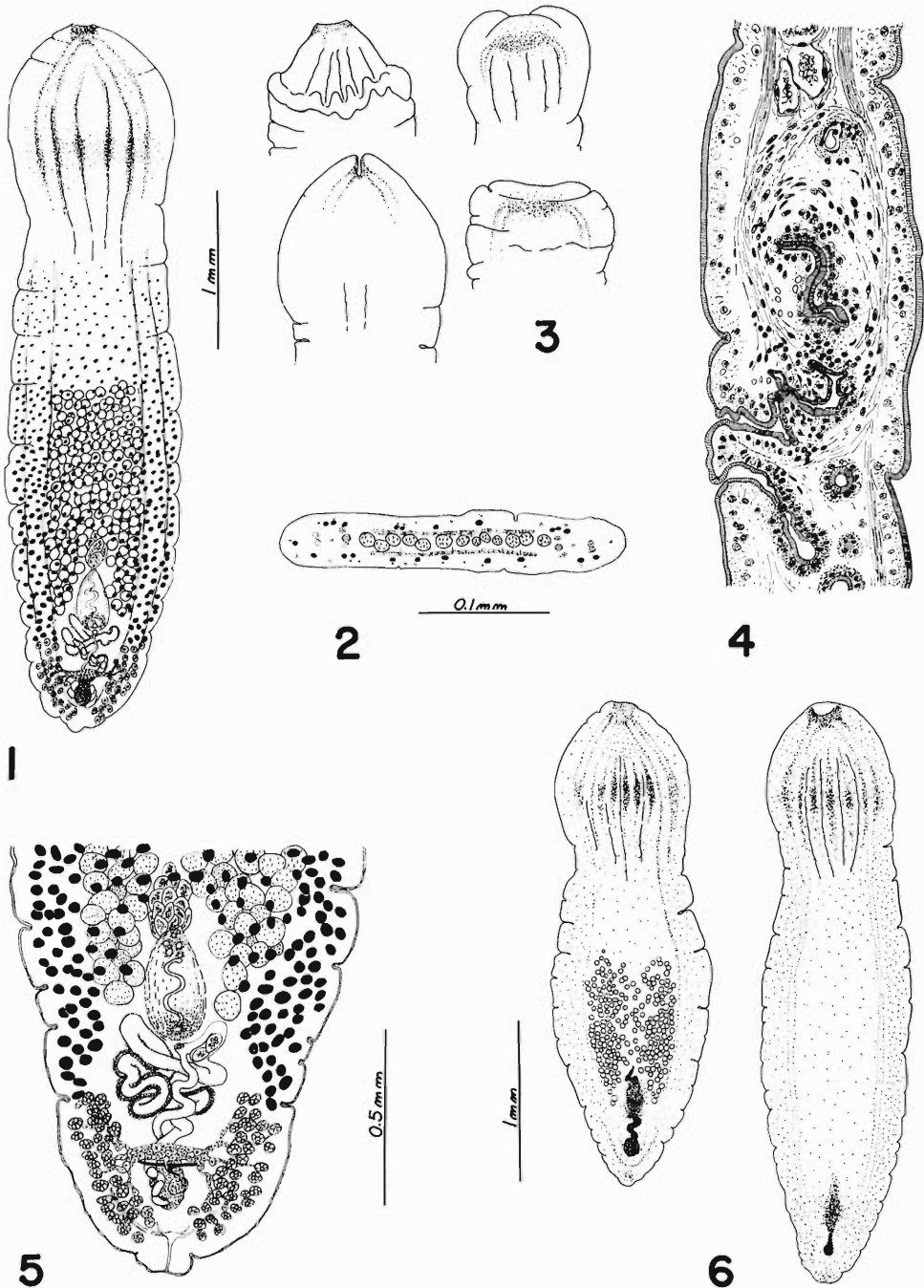
HOLOTYPE: USNM Helm. Coll. No. 61727.

PARATYPES: USNM Helm. Coll. No. 61728 (one whole mount); British Museum, Depart-

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Figures 1-6. *Monobothrioides woodlandi* sp. nov. 1, Whole mount. 2, Cross section through testes showing cortical vitellaria. 3, Variations in scolex shape. 4, Midsagittal section through cirrus sac and gonopores. 5, Posterior part of body. 6, Immature stages.

ment of Zoology, Helm. Coll. No. 1967.1.16.1 (one whole mount).

Supplementary material consists of ten slides (USNM Helm. Coll. No. 61729) with sections and 14 immature worms; and of three slides (British Museum, Department of Zoology, Helm. Coll. No. 1967.1.16.2 to 4) with eight immature worms. The remaining 16 worms are in the senior author's collection.

#### DISCUSSION

Despite differences in states of contraction (Fig. 6) all of the worms exhibited the same general body shape; none had any trace of a cercomer. The compressed scolex bearing longitudinal ridges can assume a variety of shapes but in each case the terminal glandular (?) portion remains as a darkly stained region (Fig. 3). Unlike other species in the genus and most other species in the order Caryophyllidea, the anterior vitellaria progressively become very small, up to one-fifth the size of those in the mid-body region (Fig. 1). The follicular ovary (Fig. 5) is similar to that of *Crescentovitus biloculus* Murhar, 1963. Anterior to the ovarian commissure the relatively short uterus is clothed with small uterine glands. The female gonopore opens a short distance posterior to that of the male (Fig. 4).

The arrangement of two bands of inner longitudinal muscles with two small fascicles at each end of each band appears to be a unique feature of this species. Two lateral nerve trunks can be seen between the medial two fascicles at the end of each band (Fig. 2). The outer longitudinal muscle development is more like that of *M. cunningtoni* than *M. chalmersius*. Neither the presence nor absence of an operculum could be positively established by study of the ova in utero.

Woodland (1937) is alone in including two additional species in the genus *Monobothrioides*: *M. filiformis* (Woodland, 1923) Woodland, 1937 from Africa, and *M. indicus* (Moghe, 1925) Woodland, 1937 from India. Fuhrmann and Baer (1925) removed *Caryophyllaeus filiformis* Woodland, 1923 to the genus *Lytocestus* Cohn, 1908 where it subsequently has been recognized by Hunter (1930), Wardle and McLeod (1952), Yamaguti (1959), and Mackiewicz (1962). The unspecialized scolex of this species neither has

furrows or grooves nor a terminal introvert and therefore cannot be placed in the genus *Monobothrioides*.

Following the suggestion of Woodland (1926) Hunter (1930) removed *Caryophyllaeus indicus* Moghe, 1925 to the genus *Lytocestus* where it subsequently has been recognized by Moghe (1931), Wardle and McLeod (1952), Anthony (1952), Lynsdale (1956), Yamaguti (1959), Gupta (1961), Furtado (1963), and Murhar (1963). According to Moghe's redescription (1931) and illustrations furrows were sometimes absent on the scolex while on others they (p. 84), ". . . continue for some distance on the body"; a terminal introvert is not mentioned. In the absence of comparative material and of a more precise description of the scolex of *Lytocestus indicus*, it seems unwise to make the generic changes proposed by Woodland (1937).

The small size and distinct shape of *M. woodlandi* serves to distinguish this species from *M. cunningtoni* which may be up to 40 mm long and is distinctly filiform in shape. Unlike *M. cunningtoni* the longitudinal extent of the uterus of *M. woodlandi* is less than twice the length of the ovary and the vitellaria extend to the ovary. *M. woodlandi* and *M. chalmersius* are generally similar to each other except that the latter species is a larger, more robust worm lacking the distinct shape of *M. woodlandi*. Furthermore, the scolex of *M. woodlandi* is from one-third to one-fourth the length of the worm while in *M. chalmersius* it is from one-seventh to one-eighth. The most pronounced difference concerns the development of the outer longitudinal muscles. In *M. woodlandi* these muscles consist of small, widely spaced fascicles, quite different from the prominent bandlike layer of the inner longitudinal muscles. In *M. chalmersius*, the inner and outer longitudinal muscles are similar to each other. These differences, plus those of body size, distribution of the organs, and the different hosts serve to distinguish *M. woodlandi* from the other species. On a morphological basis, *M. woodlandi* is more closely related to *M. chalmersius* than to *M. cunningtoni*. The new species is named in honor of Dr. W. N. F. Woodland, British parasitologist.

Several hundred *Clarias mossambicus* Peters

examined by the junior author at the same time contained no caryophyllaeids. According to Corbet (1961: 74) *C. mossambicus* feed, "... mainly on ostracoda and aquatic insects until they reach the size of about 3 cm after which they feed progressively more on fishes." However, Graham (1929) has reported oligochaetes, the intermediate host in known caryophyllaeid life cycles, as a food item. Unfortunately, the food habits of *C. mellandi* are too poorly known to fully explain the apparent case of host-specificity between this species and *C. mossambicus*. However, the carnivorous food habits of *C. mossambicus* strongly suggest that it probably has an ecological rather than a physiological basis.

#### ACKNOWLEDGMENTS

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**Studies on Freshwater Larval Trematodes. XV. An Unusual Xiphidiocercaria, *Cercaria pifanoi*, from Venezuela<sup>1</sup>**

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While surveying trematode infestations of freshwater snails from various parts in the eastern region of Venezuela, the authors have encountered a stylet cercaria which due to the number and peculiar position of its penetration glands, characteristic shape of the intestinal ceca and in the possession of an extraordinary excretory system occupies a unique position among the known xiphidiocercariae. There does not appear, in literature, any other species with which the subject of present investigation can be compared and, therefore, the cercaria in question has been considered a new species.

*Cercaria pifanoi* n. sp.  
(Fig. 1 and 1a)

**DESCRIPTION:** Body uniformly spinose, beset with seven rows of setae arising from prominent papillae. Tail aspinose, without a fin membrane, subterminally attached in a caudal depression bordered with caudal pockets; caudal pockets lined with needlelike spines. Stylet with a basal bulb. Suckers without special spines or papillae. Prepharynx present. Pharynx isodiametric. Esophagus straight for most of its part, being segmented just before bifurcation; intestinal ceca typically segmented, extending as far back as anterior end of excretory vesicle. Penetration glands in two grape-like bunches, pre-, para-, and postacetabular, with a transverse union anterior to acetabulum; each grapelike bunch consisting of 21 penetration glands, each gland with a well-marked nucleus and granular contents; three penetration ducts on each side of body, opening anteriorly in oral orifice; excretory vesicle not extending to ventral sucker, elongate with a variable number of lateral outgrowths; posterior one-third of excretory vesicle surrounded by a glandular structure, probably "excretory gland," having coarsely granular contents; main excretory tubes arising laterally from excretory

vesicle and dividing, some distance anterior to limits of penetration glands, into anterior and posterior lateral collecting excretory tubules; flame cell formula:  $2[(3+3+3) + (3+3+3+3)] = 42$ . Development in unbranched

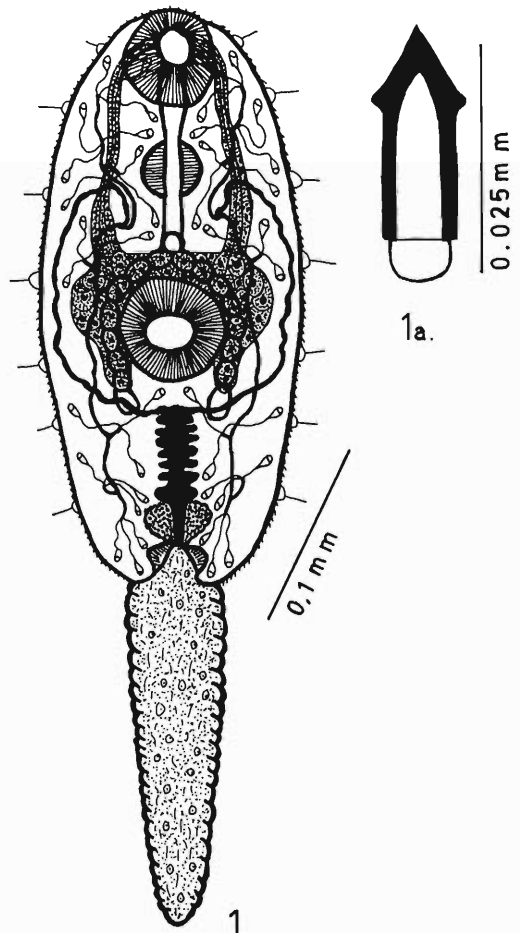


Fig. 1. *Cercaria pifanoi* n. sp., ventral view.

Fig. 1a. Stylet of *C. pifanoi*, ventral view indicating basal bulb.

<sup>1</sup> Named after Dr. Félix Pifano, Instituto de Medicina Tropical, Universidad Central de Venezuela.

anteroposteriorly elongate sporocysts. Measurements (in mm) of freshly emerged cercariae killed by plunging into 10% hot formalin: body  $0.297\text{--}0.330 \times 0.066\text{--}0.143$ ; tail  $0.121\text{--}0.279 \times 0.044\text{--}0.066$ ; oral sucker  $0.044\text{--}0.055$  in diameter; ventral sucker  $0.044\text{--}0.066$  in diameter; prepharynx  $0.012\text{--}0.025$  long;

pharynx  $0.026\text{--}0.033$  in diameter; stylet including basal bulb  $0.025\text{--}0.027$  long,  $0.008\text{--}0.010$  wide at shoulder,  $0.008$  wide at shaft.

Host: *Pomacea glauca*.

LOCALITY: San Juan de Macarapana, Quebrada, 20 km east of Universidad de Oriente, Cumaná, Venezuela.

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**Studies on Monogenetic Trematodes. XXXII.**  
**New Species of Marine Gyrodactylinae from California**  
**with the Proposal of *Archigyrodactylus* gen. n.**

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Hosts were seined from the Pacific Ocean, Bodega Bay (Sonoma Co.), California. Host preparation and methods concerning preparation, observation, measurement, illustration, and description of new gyrodactylid species were employed as given by Mizelle and Kritsky (1967). The pharyngeal width is the greatest transverse diameter for this organ. Measurements are in microns. Paratypes of each species are in the authors' collection.

*Archigyrodactylus* gen. n.

DIAGNOSIS: Gyrodactylidae, Gyrodactylinae: Body elongate with a thin smooth cuticle; divisible into a cephalic region, trunk, peduncle, and haptor. Cephalic lobes poorly developed or absent, each containing portions of head organs and usually a spicule. Head organs opening terminally and infrequently also bilaterally. Cephalic glands bilaterally disposed, composed of large distinctly nucleated cells in anterolateral areas of trunk. Eyes absent. Pharynx composed of one or two parts; gut indefinite. Gonads immediately postuterine. Cirrus ventral, median or submedian, located at pharyngeal or postpharyngeal level, and with one large spine and one to several spinules. Genital pore ventral, submedian, and postpharyngeal. Uterus usually containing an embryo which may contain an embryo of the

next generation. Older embryo often rotated as much as  $180^\circ$  from an inverted U-shape. Vitellaria composed of individual masses at level of and posterior to gonads. Vagina and genitointestinal canal absent. Haptor primitive, poorly or not at all set off from body and consisting of a posteroventral prominence enveloped by a skirtlike structure containing 16 radially arranged hooks of similar shape and size whose points frequently project to produce a scalloped border. One pair of anchors (ventral) in haptoral prominence. Anchor bases comparatively wide, relatively short, and frequently divided into superficial and deep roots. Cavity of anchor points attenuated distally. Anchor folds, knobs, and membranes absent. Bars absent; superficial bar "replaced" by (or modified as) a thin sclerotized plate. Parasitic on external surface and/or gills of marine fishes.

TYPE SPECIES: *Archigyrodactylus archigyrodactylus* sp. n.

TYPE HOST: *Cymatogaster aggregata* Gibbons.

The genus *Archigyrodactylus* gen. n. is related to *Gyrodactylus* but differs from it in several respects. Most conspicuous perhaps is the primitive type of haptor which frequently is not set off from the body and consists of the posterior end of the trunk or peduncle

bearing anchors, plate, and hooks.\* The anchors differ markedly from those of *Gyrodactylus* spp. in that the bases are broad and the deep and superficial roots (when present) or the areas from which they develop, are of approximately the same length, whereas in *Gyrodactylus* spp. the superficial root is elongate and the deep root is represented principally by a knob which may or may not be situated on a prominence. The cavity in the anchor point of *Gyrodactylus* spp. has a uniform hairlike lumen whereas in *Archigyrodactylus* spp. it is relatively large proximally and tapers to a point distally. The older embryos of *Gyrodactylus* spp. described in this paper and those observed by Mizelle and Kritsky (1967) and Crane and Mizelle (1967) are consistently oriented as an inverted U, whereas in *Archigyrodactylus* spp. they may be rotated as much as 180° from this position.

*Archigyrodactylus archigyrodactylus* sp. n.  
(Figs. 1–6, 38)

This species is based on 20 specimens (Holotype, USNM Helm. Coll. No. 61686) from the shiner perch (gills), *Cymatogaster aggregata* Gibbons.

Cuticle infrequently thickened on posterior trunk and peduncle; length 282 (228–352), width 61 (51–75). Cephalic lobes incipient to absent, usually one spicule each. Head organs well developed, weakly striated longitudinally. Cephalic glands conspicuous, secretion granules not observed. Pharynx of two parts of similar width, lumen conspicuous; width 25 (22–28). Gut not observed. Peduncle short to elongate, approximately wide as haptor. Haptor subovate; width 52 (44–64), length 52 (44–65). Anchors with well-developed superficial and deep roots (Figs. 1, 2), length 24 (22–26), base width 11 (9–13). Haptoral plate subovate (Figs. 3, 4). Hook shank without terminal enlargement. Hooklet heel globose, toe shelfless with concave posterior border, shaft comparatively

heavy. Lamella arises acutely near shank mid-length, terminates broadly near hooklet tip (Fig. 6). Hook length 23 (22–24); length of hooklet 4. Gonads ovate; testis sinistro-ventral, slightly larger than ovary. Two embryos in one specimen, others one each. Embryos variously oriented to maximum of 180° from inverted U-shape. Cirrus in all specimens, spinelets 2–4 (Fig. 5).

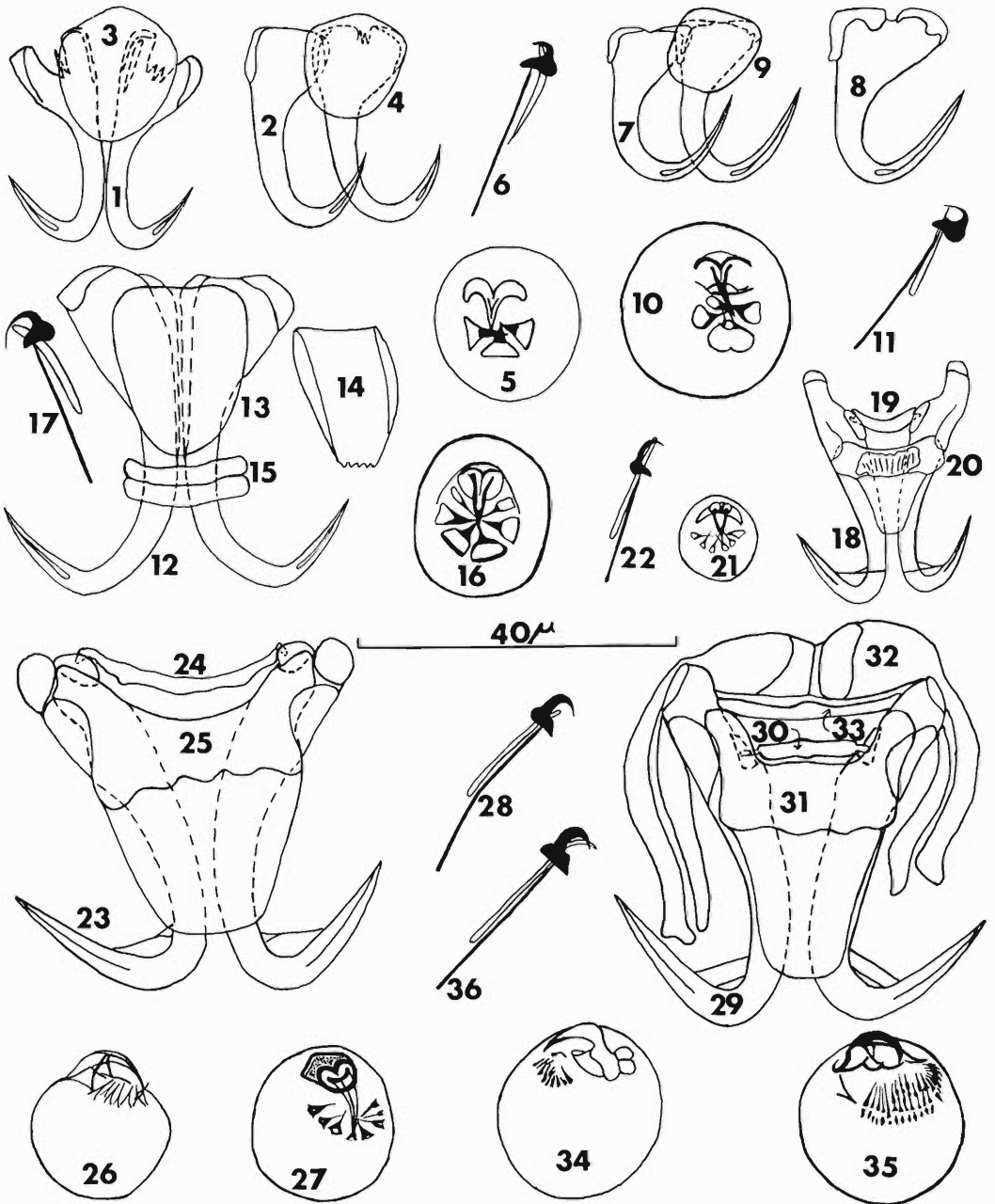
Hooklet points and shafts develop simultaneously and are the first sclerotized structures to appear. Initially they form an irregular or concentric ring, with the points directed inward. Hooklet bases differentiate next and at their completion the hook shanks and anchor points become visible. Anchor points frequently differentiate as wavy structures that later become characteristically curved as in the adult. Succeeding development is the same as that for *Gyrodactylus* spp., and the younger embryo develops late (Mizelle and Kritsky, 1967). The haptoral plate was not observed in embryos. *Archigyrodactylus archigyrodactylus* is the type species of the genus.

*Archigyrodactylus atherinops* sp. n.  
(Figs. 7–11, 40)

This description is based on 11 specimens (Holotype, USNM Helm. Coll. No. 61687) from the topsmelt (skin), *Atherinops affinis* (Ayres).

Cuticle frequently with transverse striations in trunk midregion, infrequently thickened progressively from cirrus to haptor; length 198 (136–253), width 64 (43–93). Cephalic lobes incipient or absent; spicules not observed. Head organs poorly developed, usually longitudinally striated. Secretion granules usually in cephalic glands. Pharynx of one part; width 29 (18–41). Gut not observed. Peduncle broad, often nonexistent. Haptor margin rounded, or truncate; width 48 (30–71), length 39 (30–48). Anchor roots poorly differentiated, margins heavily sclerotized (Figs. 7, 8). Anchor length 23 (21–24), base width 13 (12–16). Haptoral plate subquadrate (Fig. 9). Proximal hook shank enlargement inconspicuous or absent. Hooklet heel globose; toe with concave posterior border, shelf indicated. Lamella ends in point near hooklet tip (Fig. 11). Hook length 24 or 25; hooklet length 4 or 5. Gonads of two or more pairs of irreg-

\* Oncomiracidia possess marginal haptoral hooks on the unmodified posterior end of the trunk (Bychowsky, 1957, Fig. 116). The monopisthocotylean haptor bearing the full complement of sclerotized structures, differentiates later and consists of a distinct organ of attachment set off from the trunk by a peduncle (Figs. 37, 39, 42). The haptor becomes further modified in many Polyopisthocotylea. Any condition between that in the oncomiracidium and the monopisthocotylean adult possessing a typical haptor is considered primitive.





ularly ovate testes, one pair intertesticular sub-ovate ovaries. One embryo each in seven specimens, others none. Four embryos in inverted U-shape; one rotated 90°, two rotated 180°. Cirrus in all specimens; with 3–5 spinelets, terminal largest and usually with a median indentation (Fig. 10).

Sequential development of sclerotized armament in this species is similar to that in *Archigyrodactylus archigyrodactylus* sp. n. except that the anchor points, hooklet bases, and hook shanks apparently differentiate simultaneously. *A. atherinops* most closely resembles *A. archigyrodactylus* sp. n. It differs from this species in the morphology of the cirrus, anchors, and hooks (Figs. 1–11).

*Archigyrodactylus leibyi* sp. n.  
(Figs. 12–17, 41)

This species is described from 12 specimens (Holotype, USNM Helm. Coll. No. 61688) from the skin of spotfin surfperch (type host), *Hypocritichthys analis* (Agassiz), silver surfperch, *Hyperprosopeus ellipticum* (Gibbons), and walleye surfperch, *H. argentium* Gibbons.

Cuticle usually transversely striated from cirrus to haptor, infrequently thickened in posterior trunk half; length 279 (251–294), width 72 (56–82). Cephalic lobes incipient or absent, one or two spicules each. Head organs well developed, emptying terminally and bilaterally. Cephalic glands infrequently with secretion granules. Pharynx of one part, sub-ovate, width 30 (28–32). Gut not observed. Peduncle broad to nonexistent. Haptor usually poorly set off from body, margin rounded, undulate or truncate; width 70 (59–81), length 48 (43–53). Anchor bases infrequently di-

vided into roots (Fig. 12). Anchor length 41 (40–43), base width 15 (14–18). Haptoral plate variable (Figs. 13, 14). Two fleshy bars present across anchor shafts (Fig. 15). Hook shank not proximally enlarged; hooklet base large, toe shelfless; recurved point extends to level of base. Lamella terminates bluntly near hooklet point (Fig. 17). Hook length 22 or 23, hooklet length 5. One pair ovaries, two pairs smaller testes (bilateral). Two embryos in one specimen; others one each. All older embryos oriented as inverted U. Cirrus in each specimen; with 5–8 spinelets (Fig. 16).

Embryos insufficient to determine sequential differentiation of sclerotized structures. The second embryo in *Archigyrodactylus leibyi* develops late (Mizelle and Kritsky, 1967). The closest relative of this species is *A. atherinops* sp. n. from which it differs in the size and morphology of the anchors and haptoral plate, and morphology of the cirrus (Figs. 7–17).

*Gyrodactylus aggregata* sp. n.  
(Figs. 18–22, 39)

The following description was made from 20 specimens (Holotype, USNM Helm. Coll. No. 61689) from the shiner perch (skin), *Cymatogaster aggregata* Gibbons.

With characters of the genus as emended by Mizelle and Kritsky (1967). Cuticle transversely striated in cephalic area; length 220 (189–258), width 55 (39–85). Cephalic lobes frequently exaggerated by contraction; spicules not observed. Head organs usually conspicuous, longitudinally striated. Anterior part of pharynx somewhat conical; posterior larger, width 18 (16–21); gut indefinite. Peduncle variable, short, and moderate to broad; haptor

←

Figures 1–6. *Archigyrodactylus archigyrodactylus* sp. n. 1, 2, Anchors. 3, 4, Plates. 5, Cirrus. 6, Hook.

Figures 7–11. *Archigyrodactylus atherinops* sp. n. 7, 8, Anchors. 9, Plate. 10, Cirrus. 11, Hook.

Figures 12–17. *Archigyrodactylus leibyi* sp. n. 12, Anchor. 13, 14, Plates. 15, Fleshy bars. 16, Cirrus. 17, Hook.

Figures 18–22. *Gyrodactylus aggregata* sp. n. 18, Anchor. 19, Deep bar. 20, Superficial bar. 21, Cirrus. 22, Hook.

Figures 23–28. *Gyrodactylus bodegensis* sp. n. 23, Anchor. 24, Deep bar. 25, Superficial bar. 26, 27, Cirri. 28, Hook.

Figures 29–36. *Gyrodactylus vancleavei* sp. n. 29, Anchor. 30, Deep bar. 31, Superficial bar. 32, Horseshoe-shaped structure. 33, Accessory tissue band. 34, 35, Cirri. 36, Hook.

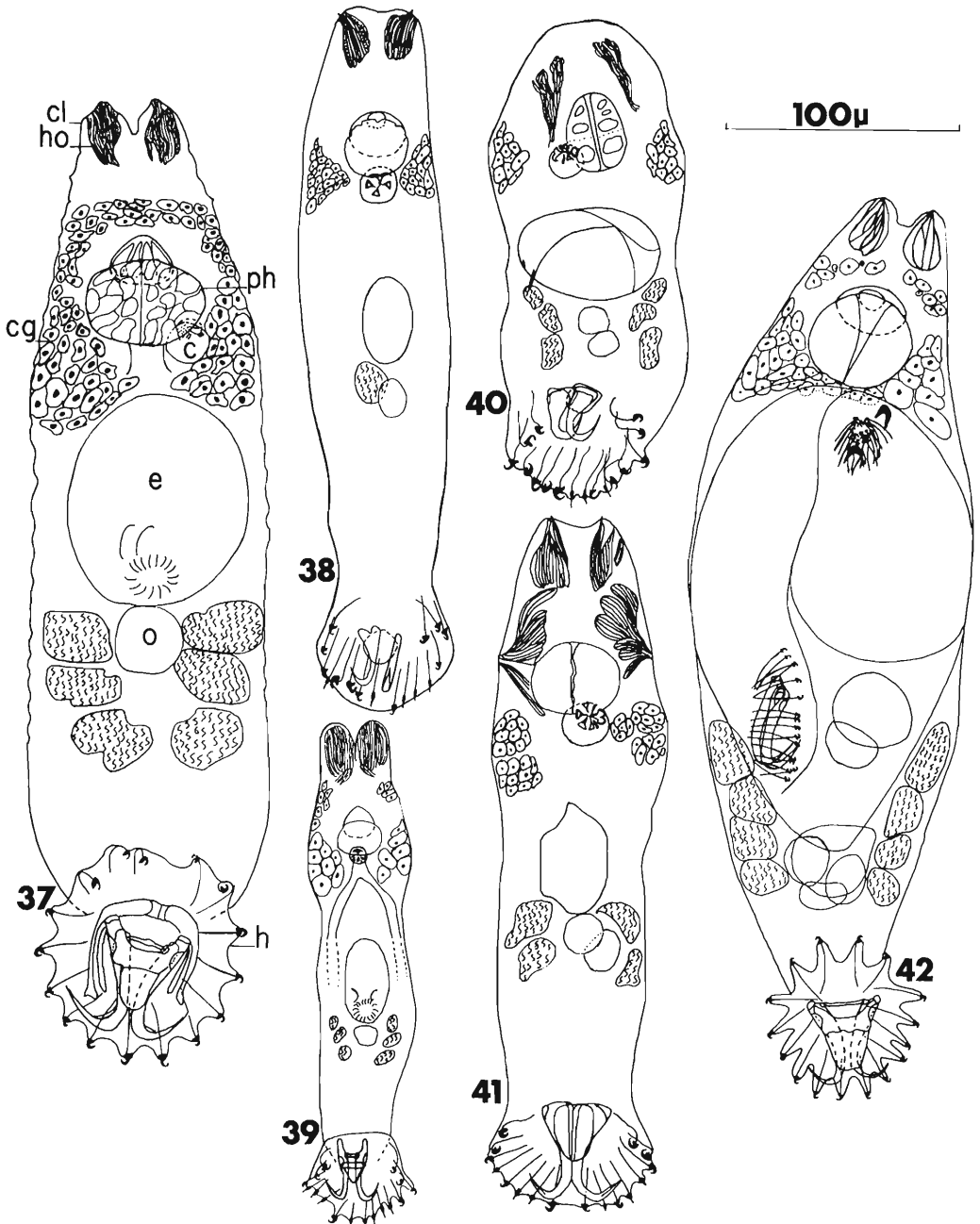


Figure 37. *Gyrodactylus vanclaevei* sp. n. Figure 38. *Archigyrodactylus archigyrodactylus* sp. n. Figure 39. *Gyrodactylus aggregata* sp. n. Figure 40. *Archigyrodactylus atherinops* sp. n. Figure 41. *Archigyrodactylus leiby* sp. n. Figure 42. *Gyrodactylus bodegensis* sp. n.

Abbreviations: C, Cirrus. CG, Cephalic gland. CL, Cephalic Lobe. E, Embryo. H, Haptor. HO, Head Organs. O, Ovary. Ph. Pharynx.

fan shaped, width 53 (43–62), length 41 (36–53). Superficial anchor roots short, often bent mesiad. Anchor folds present; arc membrane double, inconspicuous (Fig. 18); anchor length 29 (27–32), base width 7 (6–8). Ends of superficial bar enlarged, anterior parts secured under anchor folds (Fig. 20); bar length 15 (12–16). Superficial bar shield tapered, extending beyond midlength of anchor shafts. Deep bar slender, bent posteriorly in middle, ends secured within anchor knobs (Fig. 19); length 11 (10–13). Proximal enlargement of hook shank minimal or absent. Hooklet base low, heel truncate, toe shelfless. Lamella ends undiminished external to junction of hooklet shaft and point (Fig. 22). Hook length 20 (19–24); length of hooklet 6 or 7. Three pairs subovate testes; ovary subovate, located between testes. One embryo in each specimen, oriented as inverted U. Cirrus in 12 specimens; with 5–8 spinelets (Fig. 21).

Sequential development of sclerotized haptor parts normal except that the hooklet bases become visible prior to appearance of the hook shanks (Mizelle and Kritsky, 1967). The closest relative of *Gyrodactylus aggregata* is *G. wagneri cernuae* Malmberg, 1956. It differs from this species in being less than 25% as long, and the lamella is single instead of double and extends to the junction of the hooklet shaft and point rather than to the hooklet base. Further, the morphology of the bars and cirrus is different (Figs. 18–22; and Malmberg, 1956: 53).

*Gyrodactylus bodegensis* sp. n.  
(Figs. 23–28, 42)

This species is based on 17 specimens (Holotype, USNM Helm Coll. No. 61690) from the sharp nose sculpin (skin), *Clinocottus acuticeps* (Gilbert).

With characters of the genus as emended by Mizelle and Kritsky (1967). Cuticle thickened in areas, striated in anterior trunk half; length 456 (337–579), width 124 (82–195). Cephalic lobes moderate to well developed, spicules absent. Cephalic glands confluent posterior to pharynx. Anterior part of pharynx subovate to acorn shaped; posterior larger, width 55 (42–77). Gut indefinite. Peduncle narrow to moderate; haptor subovate, width 77 (62–90), length 69 (61–80). Superficial anchor root short and "capped."

Horseshoe-shaped mass of tissue between and around anchor bases extends to proximal anchor shafts. Anchor folds well developed; arc membrane thin, double, inconspicuous (Fig. 23). Anchor length 46 (43–49), base width 13 (12–14). Superficial-bar rami secured under anchor folds, shield extensive (Fig. 25); bar length 32 (31–34). Deep bar usually irregular, ends secured within anchor knobs (Fig. 24), length 15 (13–21). Hook shank attached within toe of hooklet base, proximal end inconspicuously or not enlarged. Hooklet heel globose, toe with short shelf. Lamella terminates bluntly near junction of hooklet shaft and point (Fig. 28). Hook length 29 (25–30); hooklet length 6 or 7. Four pairs ovate testes; ovary of loosely associated cells between testes. Seven specimens with two embryos each, others one. Older embryo invariably in inverted U-shape. Cirrus in 12 specimens; with 7–9 spinelets (Figs. 26, 27).

Sequential development of sclerotized haptor structures normal; second embryos develop early (Mizelle and Kritsky, 1967). The closest relative of *Gyrodactylus bodegensis* is *G. minimus* Malmberg, 1956. It differs from this species by the presence of a relatively long lamella, the presence of a cirrus, and general morphology of bars and anchors (Figs. 23–28; and Malmberg, 1956: 59).

*Gyrodactylus vanleavei* sp. n.  
(Figs. 29–37)

This species is based on six specimens (Holotype, USNM Helm. Coll. No. 61695) from the sea trout (skin), *Hexagrammos superciliosus* (Pallas).

With characters of the genus as emended by Mizelle and Kritsky (1967). Cuticle infrequently striated transversely in trunk mid-region; lateral body margins undulate from cephalic glands to area of gonads; length 367 (265–499), width 102 (82–124). Cephalic lobes exaggerated, spicules not observed. Head organs occupy principal volume of cephalic lobes, extend posteriorly into cephalic area. Cephalic glands extend as inverted crescentic band between head organs and pharynx. Anterior part of pharynx conical; posterior larger, width 40 (23–48). Gut not observed. Peduncle short and broad; haptor subovate, width 77 (64–88), length 80 (72–92). Superficial

anchor roots short; acutely recurved anchor points frequently extend near level of anchor base. Broad, conspicuous, horseshoe-shaped tissue mass between ends of superficial anchor roots, on each side of anchor bases, and part of anchor shafts (Fig. 32). Narrow band of tissue present subterminally between superficial anchor roots (Fig. 33). Anchor folds present, accessory fold on tips of superficial anchor roots; arc membrane conspicuous, double (Fig. 29). Anchor length 47 (43–51), base width 12 (11–14). Superficial bar rami extend beyond anchor knobs, partly secured under anchor folds. Bar shield extending to distal area of anchor shafts (Fig. 31); bar length 25 (24–26). Attenuated ends of deep bar secured in anchor knob cavities (Fig. 30); length 18 (15–23). Proximal part of hook shank not enlarged. Posterior hooklet border straight; toe with shelf, transversely truncate. Lamella fragile, terminates acutely beyond hooklet point (Fig. 36). Hook length 27; hooklet length 6. Ovary loosely organized mass, between two or three pairs of testes. Two specimens with two embryos each, one with three, others one. Cirrus in five specimens, dextromedian; numerous spinelets in three principal rows (Figs. 34, 35).

Embryos insufficient to determine sequential development of sclerotized haptor armament. Younger embryos develop early (Mizelle and Kritsky, 1967). The superficial bar of *Gyrodactylus vancleavei* resembles that of *G. atratuli* Putz and Hoffman (1963, Fig. 1; and *nobis*, Fig. 31). The anchors however resemble those of *G. salaris* Malmberg (1956: 55; and *nobis*, Fig. 29). The cirrus is very different from that of any described species.

#### SUMMARY

*Archigyrodactylus* gen. n. is proposed and six new species of Gyrodactylinae are described from marine fishes from Bodega Bay (Sonoma Co.) California as follows: *Archigyrodactylus archigyrodactylus* from *Cymatogaster aggregata* Gibbons; *A. atherinops* from *Atherinops affinis* (Ayres); *A. leiby* from

*Hypocritichthys analis* (Agassiz) (type host), *Hyperprosopon ellipticum* (Gibbons) and *H. argentium* Gibbons; *Gyrodactylus aggregata* from *Cymatogaster aggregata* Gibbons; *G. bodegensis* from *Clinocottus acuticeps* (Gilbert); and *G. vancleavei* from *Hexagrammos superciliosus* (Pallas). *Archigyrodactylus* gen. n. differs from *Gyrodactylus* conspicuously by the presence of a primitive type of haptor poorly or not at all set off from the body; anchors with wide bases frequently divided into superficial and deep roots subequal in length; and the absence of anchor knobs, folds, membranes, and haptor bars. A thin, lightly sclerotized plate apparently replaces the superficial bar. The cirral spinelets are more conspicuous than those in *Gyrodactylus* species.

#### ACKNOWLEDGMENTS

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## Cuticle Ultrastructure of the *Meloidogyne hapla* Larva<sup>1</sup>

I. K. A. IBRAHIM<sup>2</sup> AND J. P. HOLLIS<sup>3</sup>

The cuticle of adult females of the genus *Meloidogyne* has been the subject of several investigations (Elsea, 1951; Bird, 1958; Bird and Rogers, 1965; Ibrahim, Hollis and Birchfield, 1966). The present study extends the scope of such investigations to the second stage larva of *M. hapla*. The results show that the external cuticle of this nematode is non-cellular and multilayered; consisting of three main layers: cortex, matrix and fiber layer.

### MATERIALS AND METHODS

Second stage larvae of *M. hapla* were obtained from infected roots of tomato plants grown in the greenhouse and from hatching eggs. Specimens were fixed at room temperature in small vials for 2 hr in 6% glutaraldehyde in 0.05 M phosphate buffer at pH 6.8, then they were exposed for 2 hr in 2% osmium tetroxide in the same buffer for final fixation. The fixed specimens were washed thoroughly with distilled water, dehydrated in a graded series of ethanol-water solutions, washed twice with propylene oxide, soaked in a 1:1 v/v mixture of propylene oxide plus Epon mixture and then embedded in Epon mixture in Beem plastic capsules. The Epon mixture consisted of ml proportions of Epon 812 15, Dodecenyl succinic anhydride 25, Araldite 506 20, Dibutyl phthalate 1.5, and Benzyl dimethyl amine 1.0 (Luft, 1961; Mollenhauer, 1964). The capsules were incubated at 60 C for 48 hr; then the blocks were trimmed and sections were cut to give a bright gold to silver interference color with a glass knife in a Sorvall MT-2 Porter-Blum Ultra-Microtome. The sections were stained with 2% aqueous lead oxide for 15 min and with 2% aqueous uranyl acetate for 30 min, and then were viewed under a HU-11A Hitachi electron microscope at 50 kv.

### RESULTS

Electron micrographs of the infective larval stage of *M. hapla* showed a noncellular and multilayered cuticle about 1.6  $\mu$  thick. The external cuticle consisted of three main layers: cortex, matrix and fiber layer (Fig. 1). The cortex had an electron-dense structure about 0.06  $\mu$  in thickness and was deeply striated. Beneath the cortex was an electron-transparent layer, the matrix, which was dissected by the striae of the cortex. It was about 0.25  $\mu$  thick. The innermost layer, the fiber layer, showed two zones: an outer zone about 0.35  $\mu$  thick which appeared in longitudinal sections as a palisadelike array and an inner zone about 0.85  $\mu$  thick containing four to six dark bands separated from each other by light-colored areas. The cuticle was marked by both transverse and longitudinal striae. Average length of the interstrial regions (annules) was approximately 0.35  $\mu$ .

Cuticular thickening of the wall of the esophageal lumen was evident and in addition there was a pair of toothlike cuticular thickening along both sides of each lumen radius (Fig. 2). Anteriorly, the cuticular thickening of the esophageal lumen appeared homogenous without lamellation (Fig. 3). In the posterior region of the esophagus, however, the thickening was lamellated and composed of individual platelets arranged in longitudinal rows parallel to each other or arranged in scissor-shaped structures (Fig. 4). A cavity in the dorsal radius of the esophageal lumen can be seen in the posterior part of the esophagus (Fig. 4). The tip of this radius appears to be branched, forming small tubules near the intestinal tissue (Fig. 5).

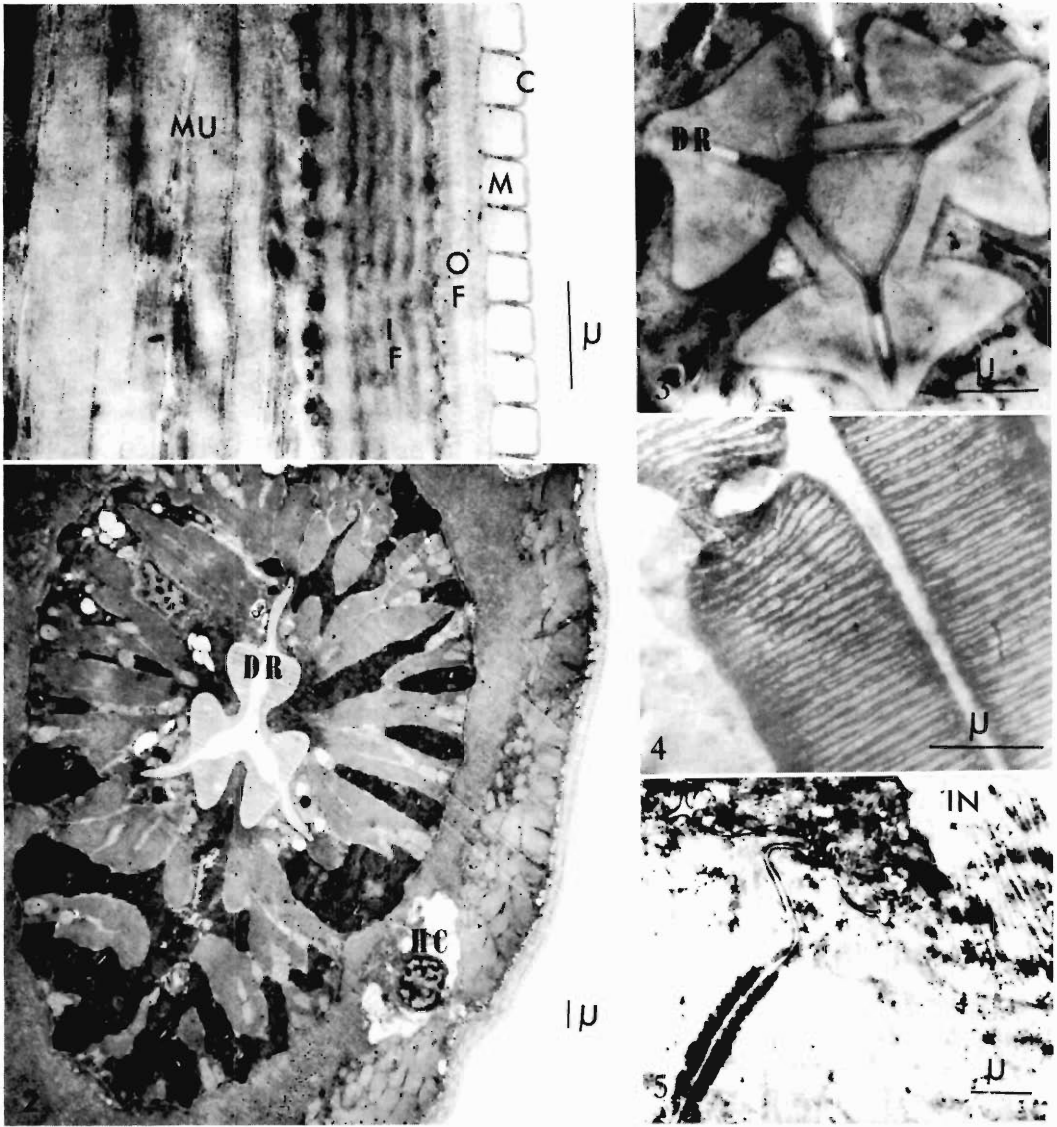
The results show that the cuticular morphology of the infective larval stage of *M. hapla* embraces the general concept of the nematode cuticle as it is understood at the present time.

The clarity with which the esophageal tissue has been resolved in this study provides more precise information on the structure and function of this important part of the nematode than could be obtained with light microscopy.

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Figures 1-5. Electron micrographs of *Meloidogyne hapla*, second stage larva. 1. Longitudinal section through body wall. 2. Cross section through the esophageal region. 3. Cross section of esophageal lumen, anteriorly. 4. Enlarged portion of esophageal lumen, posteriorly. 5. Branching tip of dorsal radius of esophageal lumen near the intestinal tissue.

Abbreviations: C, cortex; DR, dorsal radius; H, hypodermis; HC, hypodermal cord; IF, inner fiber zone; IN, intestine; M, matrix; MU, muscles; OF, outer fiber zone.

The esophagus is essentially a muscular organ, probably its primary function is pumping food materials from the host tissues into the intestine.

#### SUMMARY

The external cuticle of the infective larval stage of *Meloidogyne hapla* consists of three main layers: cortex, matrix and fiber layer. The fiber layer evidently consists of two zones: an outer zone appearing in longitudinal sections as a palisadelike array and an inner zone containing four to six dark bands separated from each other by light-colored areas.

Cuticular thickening of the wall of the esophageal lumen is evident and in addition there is a pair of toothlike cuticular thickenings along both sides of each lumen radius.

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### Studies on Echinostomatidae (Trematoda) in Malaya. XV. The Life History of *Echinostoma murinum* (Tubangui, 1931)<sup>1</sup>

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This paper describes the life cycle of *Echinostoma murinum* (Tubangui, 1931), as well as the morphology of the parasite's various stages. The first descriptions were from the Philippines (Tubangui, 1931 and 1932) and from China (Wu, 1951) but were incomplete. The parasite is now found in Malaysia. This paper emphasizes those aspects not covered by the two earlier investigators.

#### MATERIALS AND METHODS

*Gyraulius convexiusculus* (Hutton) were collected from a pond near Kuala Lumpur, Malaysia and flown to San Francisco. Several of one batch shed cercariae with 45 collar spines. The cercariae encysted in various freshwater

snails and, when the cysts were fed to rats, adult worms identical with *Echinostoma murinum* (Tubangui, 1931) were obtained. Eggs from these worms were the starting point for this study. Laboratory-raised *Gyraulius convexiusculus* served as first intermediate hosts, various freshwater snails as second intermediate hosts, and white rats, white mice, and hamsters as experimental definitive hosts. Infected snails were kept in small aquaria at room temperatures ranging from 25-29 C. The same techniques as those reported in previous studies of Malayan echinostomes (Lie, 1963, 1965, 1966a and b) were used. All measurements are in microns.

#### RESULTS

EGG AND MIRACIDIUM (Figs. 1, 2): Eggs appeared in stools 6 days after infection, in uncleaved condition, yellowish-brown, 86-101 by 53-62, with thickening at nonoperculated end of shell.

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Miracidia 57–73 by 32–39 fixed in hot, 2% silver nitrate. Retractable anterior papilla with two pairs of long hairs (Fig. 1). Body covered with four rows of ciliated epidermal plates (Fig. 2): first (anterior) row with six trianguloid plates, two ventral, two dorsal, and two lateral, 13 long and 11–14 wide at base; second row with six squarish plates, three dorsal and three ventral, 14–19 long and wide; third row with four plates also squarish, one dorsal, one ventral, and two lateral, 19–25; and fourth row with two trianguloid plates, one ventral and one dorsal, 18–22 long and 23–27 wide at base. Cilia 12 long. Two fingerlike processes, 3.5 long, one situated at base of each lateral anterior epidermal plate, with short bristle immediately anterior to each process. Primitive gut filled with refractile granules with opening at tip of apical papilla. Penetration gland cells not visible. Eyespots shown in Fig. 1. Two flame cells: the ventro-posterior cell with excretory pore ventrolateral, and the dorsoanterior cell with excretory pore dorsolateral; both pores between third and fourth rows of epidermal plates. Several germ cells in body cavity.

REMARKS: Newly hatched miracidia swim rapidly through the water, often passing snail hosts without changing direction. When they strike a host, they start to penetrate any exposed part of the snail, at the same time shedding their epidermal plates. The penetration process is usually completed in about 30 min. Attempts to infect *Biomphalaria glabrata* (Say) with large numbers of miracidia were unsuccessful.

#### SPORO CYST (Fig. 3)

Sporocysts develop as contractile, elongated sacs in the ventricle of the heart, grey or yellowish-grey, with many small refractile granules measuring 1.5. Mature sporocysts are 72–150 by 50–90 and contain several rediae and germ balls. Birth pore is probably present, located near that portion of sporocyst attached to the heart muscle. Sporocysts become smaller after production of rediae, which takes about 2 weeks, and ultimately die.

#### REDIA (Figs. 4, 5)

Rediae of the first generation released 6 or more days after exposure. Newly released

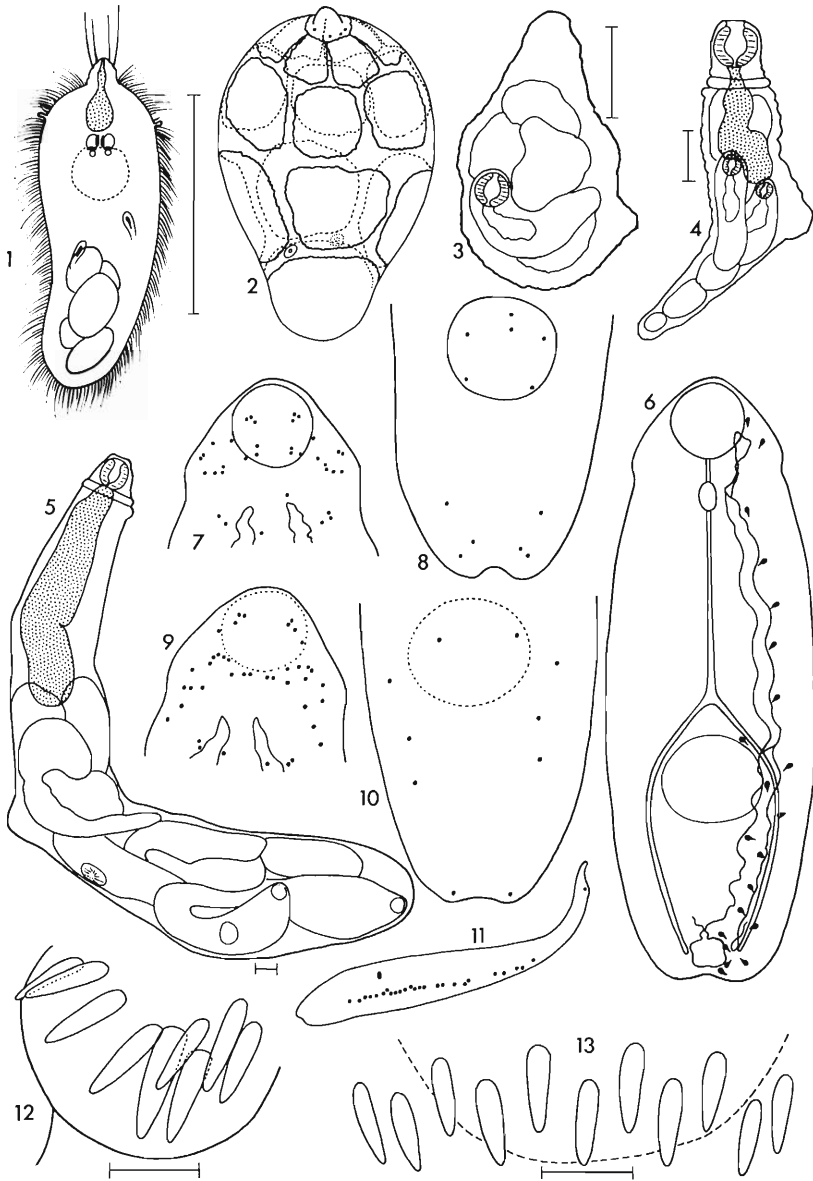
rediae colorless, 130 long by 55 wide, with distinct locomotor organs and continuous collar; pharynx 38 by 32; intestine 40; distance anterior end to collar 45; distance posterior end to locomotor organs 35. Migration of rediae mainly to ovotestis; some to liver, albumen gland, lung, kidney, and mantle edge. Mature first-generation rediae orange, producing rediae 2–3 weeks after exposure, 404–480 long, 76–90 wide; pharynx 36–48 wide; intestine 80–140 long with dark brown contents; distance anterior end to collar 80–112; distance posterior end to locomotor organs 85–160. Birth pore dorsal, immediately posterior to collar. Second-generation rediae produced about 2 weeks or more after exposure.

Mature rediae producing cercariae 3 or more weeks after exposure, 790–1,690 long, 126–269 wide; pharynx 52–68 wide; intestine 205–632; distance anterior end to collar 52–100 and distance posterior end to locomotor organs 426–1,027.

REMARKS: In field-collected snails rediae are often found infected with microsporidia. Whether more than two generations of rediae develop and whether first-generation rediae produced cercariae, as in *Hypoderaeum dingeri* Lie, 1964 and *Paryphostomum segregatum* Dietz, 1909 (Lie and Basch, 1967) could not be determined because of difficulties in rearing the snails in San Francisco.

#### CERCARIA (Figs. 6–11)

Cercaria released from the snail 22 or more days after exposure. Measurements based on 30 specimens fixed in hot water. Body 280–336 by 80–100; oral sucker subterminal, 31–40 long by 36–43 wide; prepharynx 11–25 long; pharynx 19–25 long by 16–19 wide; esophagus solid, 76–111 long, and consisting of about 12 cells; intestinal ceca solid, extending to level of excretory bladder (Fig. 6). Protrusible acetabulum posterior to midbody, 44–52 long by 47–54 wide. Collar conspicuous, 70–87 in diameter, with 45 spines 12.5 long arranged as in adult (see description of adult). Body covered with small spines extending ventrally to midbody, laterally over two-thirds of body, and dorsally to posterior end. Patterns of integumentary seta-bearing papillae (see Lie, 1966a) shown in Figs. 7–11. Cystogenous cells numerous, fewer near oral sucker and pharynx,



Figures 1-13. *Echinostoma murinum*. (Camera lucida drawings, unless otherwise stated. Projected scales are 50  $\mu$ ). 1. Miracidium showing apical papilla, gut, eyespots, flame cells, and lateral processes. 2. Miracidium in silver nitrate solution showing epidermal cells and excretory duct outlets. 3. Sporocyst containing rediae. 4. Mature first-generation redia containing rediae. 5. Mature redia containing cercariae. 6. Freehand drawing of cercaria showing arrangement of flame cells. 7-11. Patterns of seta-bearing papillae. 7. Anteroventral body surface. 8. Posteroventral body surface. 9. Anterodorsal body surface. 10. Posterodorsal body surface. 11. Tail, lateral view. 12. Corner, lateral and one pair of dorsal spines. Arrangement of corner spines somewhat disturbed due to pressure; the two lateral spines arranged in a single row; the first pair of the dorsal spines alternating. 13. Collar spines on dorsal surface with alternating arrangement.

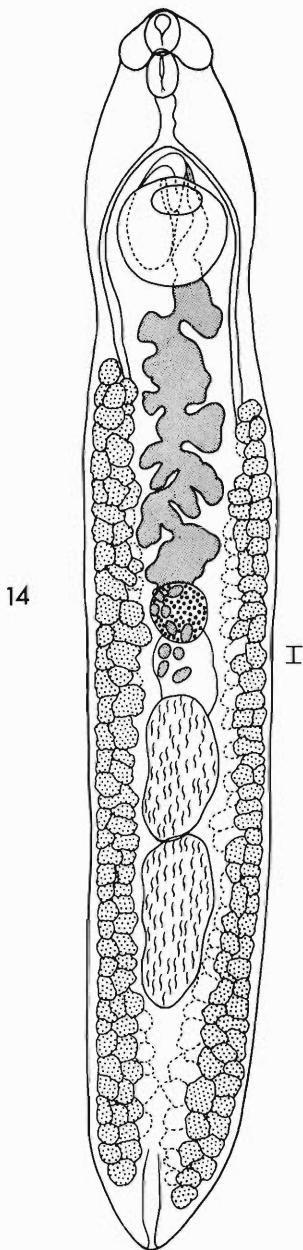


Figure 14. Adult *Echinostoma murinum*.

with granular contents. Penetration gland cells along esophagus with six duct openings on dorsal lip of oral sucker. Paraesophageal gland cells, characterized by intense staining in neutral red, Nile blue sulfate or Bismarck brown

(see Lie, 1966b) absent. Genital primordia two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, connected by a string of cells passing dorsal to acetabulum. Tail 292–348 by 28–40, without fins.

Excretory system stenostomate with main tubes extending from small bipartite bladder to sides of oral sucker; main tubes dilated between pharyngeal and acetabular levels and filled with many yellowish-brown refractile granules. Flame cells difficult to see, probably 21 pairs arranged as in Fig. 6. Caudal branch of excretory system entering anterior fourth of tail where it bifurcates and opens laterally at pair of primary pores.

REMARKS: The cercaria lives for several hours in water, curving its body ventrally while swimming. It resembles the cercaria of *E. murinum* described by Tubangui (1932) and by Wu (1951). Tubangui counted 22 pairs of flame cells, Wu 21–22 pairs. The cercariae they described were slightly larger, but this may have been due to the fixative used.

#### METACERCARIA

Metacercarial cysts usually spherical, 134–152 in diameter, may be obtained experimentally in the pericardial sac and posterior portion of the kidney in the following snails: *Gyraulus convexiusculus*, *Lymnaea rubiginosa* (Michelin), *Indoplanorbis exustus* (Deshayes), *Biomphalaria glabrata* and *B. tenagophila* (D'Orbigny). Cercariae reach the pericardial sac through the urinary orifice, kidney and ciliated renopericardial duct. In *G. convexiusculus* harboring rediae of *E. murinum*, cysts may also be found in the liver, ovotestis, lung, kidney, or within the rediae, particularly in those infected with microsporidia. Such cysts may be formed by cercariae that have never left the snail host. In snails not harboring rediae, cysts are found only in the pericardial sac and posterior portion of the kidney.

#### ADULT (Figs. 12–14)

Measurements based on 30 specimens varying in age from 23–90 days. Body elongate, 3,723–4,626 by 470–686, attaining maximum width in uterine region (Fig. 14). Body scales posterior to collar, covering anterior sixth of body dorsally, anterior half laterally, and all but posterior sixth ventrally. Collar well de-

veloped, 285–334 wide, with 45 conspicuous collar spines in 29 specimens and 43 in one specimen. Arrangement of spines characteristic: five corner spines on each side, three oral and two aboral, varying in length in unfixed specimens from 45 to 65, the latero-aboral one usually the longest and latero-oral one the shortest (Fig. 12); two laterals on each side arranged in single rows 41–54 long; 31 dorsals, 41–49 long, 16 oral and 15 aboral, in specimens with 45 spines; and 29 dorsals, 15 oral and 14 aboral, in the specimen with 43 spines (Fig. 13). Oral sucker subterminal, 140–172 long by 120–143 wide. Acetabulum within anterior fourth of body, 354–442 long by 354–410 wide. Prepharynx 20–32; pharynx 136–168 long by 108–144 wide; esophagus bifurcating anterior of acetabulum; ceca extending almost to posterior end of body. Genital pore immediately preacetabular followed by shallow genital atrium. Testes posterior to midbody, intercecal, tandem, more or less oblong with wavy margins in fixed unflattened specimens; anterior testis 355–647 by 158–304; posterior testis 411–711 by 173–304. Cirrus sac ovoid, almost extending to posterior margin of acetabulum and containing a seminal vesicle, pars prostatica, and unspined cirrus, 500 long when protruded. Ovary spherical or subspherical, pretesticular at midbody, 164–274 in diameter. Mehlis' gland complex between ovary and anterior testis, similar to that of other Malayan echinostomes (see Lie, 1965) with small ovicapt 40 wide, ciliated oviduct, Laurer's canal opening on dorsal surface of the worm with ciliated proximal part, no seminal receptacle. Uterine seminal receptacle posterior to ovary, containing numerous spermatozoa. Uterus intercecal, pretesticular, with 8–11 coils and containing 80–100 eggs, connecting genital atrium through metraterm. Vitellaria extending from below posterior margin of acetabulum to posterior end of body, overlapping ceca, not united ventrally but possibly merging dorsally distal to testis. Excretory bladder extending from near hind testis to excretory pore at posterior tip of worm.

REMARKS: Worms develop in the duodenum and the first part of the small intestine in white rats, mice and hamsters and live for about 7 months in these animals. They did not develop in chicks, ducklings or pigeons obtainable in California.

## DISCUSSION

Tubangui (1931, 1932) and Wu (1951) described the arrangement of the collar spines as follows: five corner spines on each side and 35 marginal spines in a double row. Their drawings, however, clearly show the arrangement described in this paper.

Wu infected Chinese ducklings and chicks, but the California ducklings and chicks used in this study were not susceptible.

*E. murinum* is apparently widespread in Asia and has now been reported from three countries.

## SUMMARY

*Echinostoma murinum* is reported for the first time in Malaysia and its life cycle is described. The first intermediate host is *Gyraulax convexiusculus* (Hutton). Adult worms were obtained experimentally from rats, hamsters, and mice in California.

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***Proterodiplostomum intermedium* n. sp. (Trematoda: Digenea) from the Crocodile *Caiman crocodilus crocodilus* (L.) in Venezuela**

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All of the species under the genus *Proterodiplostomum* Dubois (1936; 1938) are parasites of crocodiles and until now have been described only from Brazil. There are only three species and these are: *Proterodiplostomum longum* (Brandes, 1888) Dubois, 1936, from the intestine of *Melanosuchus niger* (Spix), *P. tumidulum* Dubois, 1936, from the intestine of *Caiman crocodilus* (L.) and *P. medusae* (Dubois, 1936) Caballero, Hidalgo and Grocott, 1957, syn. *Diplostome medusae* Dubois, 1936, from the intestine of *Caiman crocodilus* (L.). Insofar as the body length is concerned, *Proterodiplostomum intermedium*, is a form, as the name suggests, intermediate between *P. longum* on one side *P. tumidulum* and *P. medusae* on the other side. As a result of comparative study, to be discussed later, *P. intermedium* has proved to be a new species and the first member of the genus ever recorded outside Brazil.

Five specimens were recovered from the small intestine of a crocodile, *Caiman crocodilus crocodilus* (L.). Two of these individuals were stained with diluted Harris' hematoxylin (one part of hematoxylin and nine parts of distilled water) as recommended by Cable, 1964, and the other two with Semichon's acetocarmine while the fifth one was sectioned and stained with eosin-hematoxylin procedure.

The measurements are in mm.

*Proterodiplostomum intermedium* n. sp.  
(Figs. 1-2)

Anterior end of hindbody dorsally attached to posterior end of forebody. Prepharynx absent. Part of pharynx lying dorsally over posterior region of oral sucker. Posterior ends of intestinal ceca extending more than halfway in posttesticular region. Tribocytic organ anteroposteriorly elongated. Cavity of tribocytic organ bordered with 18-21 papillae. Dorsal surface of tribocytic organ, in sectioned material, provided with deeply staining unicellular

structures, possibly glands associated with this organ, not to be confused with vitelline follicles. Ovary unlobed, spherical or subspherical, pretesticular, median or slightly submedian. Testes not lobed, tandem, anteroposteriorly elongated. Posterior testis invariably notched into two anteroposterior divisions. Intertesticular space variable in length. Ootype complex between two testes. Laurers' canal present. Vitelline follicles, in all specimens, extending from immediately posterior to anterior border of anterior testis and encroaching upon forebody slightly anterior to its posterior border with a considerable concentration in certain specimens. Median region of forebody, between its posterior border and posterior border of tribocytic organ, occupied by irregularly scattered vitelline follicles. In certain specimens, a few follicles present on one or both sides, between two testes. In still other individuals, a few follicles, on both sides, extending about halfway in posttesticular region. Maximum number of eggs in a specimen 15. Terminal parts of genitalia, posterior to posterior testis, very prominent. Paraprostate markedly developed, lateral. Vesicula seminalis coiled, lying between paraprostate and terminal part of uterus. Posterior part or efferent duct of paraprostate joining with posterior uncoiled part of seminal vesicle with resultant formation of a common ejaculatory duct. This common ejaculatory duct opening, into bursa copulatrix, at tip of a muscular genital cone. A muscular suckerlike structure, called by Dubois (1953) "atrial sucker," associated with bursa copulatrix. Uterus opening independently, in cavity of bursa copulatrix, between "atrial sucker" and genital cone. Cavity of bursa copulatrix variable in shape and opening exteriorly by a wide subterminal genital pore. Measurements of 4 adult specimens: total body length, 2.332-2.620; forebody, 0.605-0.640 by 0.275-0.300; hindbody, 1.727-1.980 by 0.16-0.176; ratio of two segments

of body, 2.854–3.093; oral sucker, 0.042–0.055 by 0.045–0.050; pharynx, 0.045–0.050 by 0.030–0.043; esophagus, 0.017–0.067 long; ventral sucker, 0.037–0.045 by 0.045–0.055; tribocytic organ, 0.150–0.155 by 0.090–0.125; ovary, 0.067–0.087 by 0.072–0.090; anterior testis, 0.135–0.180 by 0.100–0.125; posterior testis, 0.125–0.157 by 0.095–0.125; intra-uterine eggs, 0.090–0.115 by 0.050–0.067; distance of anterior border of ventral sucker from anterior end of forebody, 0.242–0.248; distance of anterior border of tribocytic organ from anterior end of forebody, 0.297–0.308; distance of anterior border of tribocytic organ from posterior border of ventral sucker, from nil–0.017; distance of anterior border of ovary from anterior end of hindbody, 0.803–1.155; distance of posterior border of ovary from anterior border of anterior testis, 0.005–0.012; intertesticular space, 0.057–0.074 long; post-testicular extent, 0.429–0.484 long.

TYPE HOST: *Caiman crocodilus* (L.).

HABITAT: Small intestine.

TYPE LOCALITY: San Bonifacio, about 174 km southeast of Cumaná, Edo. Monagas, Venezuela.

SPECIMEN DEPOSITED: USNM Helm. Coll. No. 61765, *Proterodiplostomum intermedium* (holotype).

DISCUSSION

*Proterodiplostomum intermedium* is readily distinguished from other species of the genus by the smaller length of its forebody, smaller size of oral sucker, pharynx, esophagus, ventral sucker, and tribocytic organ. Moreover, the anterior limits of its vitelline follicles extend only to the posterior border of the tribocytic organ whereas in all the other species, *P. longum*, *P. tumidulum*, and *P. medusae*, the vitelline follicles always extend anterior to the tribocytic organ. The distance between the posterior border of the ventral sucker and the

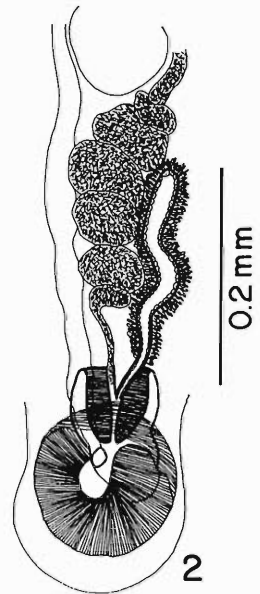
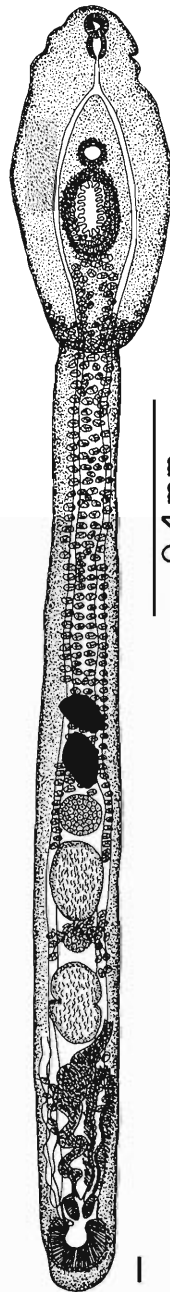


Figure 1. *Proterodiplostomum intermedium* n. sp., ventral view.

Figure 2. Reconstruction of terminal genitalia of *P. intermedium*, showing paraprostate, seminal vesicle, uterus, genital cone, opening of common ejaculatory duct at tip of genital cone, uterine orifice, cavity of bursa copulatrix and atrial sucker.

anterior border of the tribocytic organ in *P. intermedium* varies from nil-0.017 in contrast with a similar distance of *P. longum*. *P. tumidulum*, and *P. medusae* where the ventral sucker occupies a place considerably anterior to the tribocytic organ. In addition, there are other characters differential enough for the separation of *P. intermedium* and these are detailed below.

The ratio of the two segments of *P. intermedium* is smaller than that of *P. longum*; the length of its hindbody is smaller; its ovary and testes are also smaller; and finally the eggs of *P. intermedium* are larger than those of *P. longum*. *P. intermedium* is also a distinct species from *P. tumidulum* and *P. medusae* due to the possession of a greater ratio of its two segments and a longer hindbody. It further differs from *P. tumidulum* in having a larger ovary, in greater length of its anterior testis and larger eggs. In *P. medusae* and *P. intermedium* the ovaries and the eggs are almost equal in size but *P. intermedium* has a larger anterior testis and its posterior testis is smaller.

Ruiz and Rangel (1954) described *Pseudoneodiplostomum brasiliense* from a species of *Caiman* in Brazil but the details of its terminal genital system are lacking. Sudarikov (1960, in Skrjabin, 1960) removed it from *Pseudoneodiplostomum* and put it under the genus *Proterodiplostomum* whereas Yamaguti, 1958, correctly reallocated it to *Pseudoneodiplostomum*. As suggested by Dubois in a personal communication, the species of Ruiz and Rangel might belong to *Proterodiplostomum* but, since, the details of the terminal parts of its genital system are unknown, which are a determining

factor from the diagnostic standpoint, the species should be treated as originally described.

#### ACKNOWLEDGMENTS

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## Two New Species of *Platynosomum* (Trematoda: Dicrocoeliidae) from South American Monkeys<sup>1</sup>

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Since 1960, New World monkeys used in studies in laboratories of the Oak Ridge National Laboratory and Oak Ridge Associated Universities have been examined for parasites. Recoveries of dicrocoeliid trematodes were made from the gall bladders, bile ducts, and intestines of some of these monkeys (Cosgrove, 1966). The monkeys are from indefinite localities in the upper Amazon basin. Nine of 20 *Callimico goeldii* and 35 of 441 *Saguinus nigricollis* (= *Tamarinus nigricollis*) were found to harbor a new species we assign to the genus *Platynosomum* Looss, 1907. Two of 441 *S. nigricollis* harbored another new species of this same genus. All measurements are in mm.; data outside parentheses are measurement of holotype, measurement within brackets is average measurement of 14 specimens; other data indicate range of measurements.

### *Platynosomum amazonensis* sp. nov.

DESCRIPTION: (Fig. 1) Body of mature specimens elongate, ellipsoidal, anterior end blunt, expanding toward widest point at level of testes, posterior part of body tapering behind vitellaria, body length 3.30 ([3.70] 3.02–4.93), body width 1.08 ([1.28] 0.99–1.72). Ratio of body width/length 1:3.05. Cuticle without spines, papillae, or scales. Oral sucker subterminal, round-oval 0.306 ([0.392] 0.300–0.465), mouth opens ventrally. Acetabulum round-oval 0.360 ([0.451] 0.360–0.660), distance between suckers (middle of oral to middle of acetabulum) 0.80 ([0.771] 0.570–1.100). Oral sucker: acetabulum ratio: 1:1.17 ([1.15] 0.97–1.26). Prepharynx absent. Pharynx globular, 0.120 ([0.141] 0.120–0.150) located at posterior margin of oral sucker, sometimes overlapping margin. Oesophagus narrow, thin-walled, sometimes curving, bifurcates approxi-

mately midway between oral and ventral suckers to form intestinal caeca which extend toward lateral margins of body turning posteriorly at level of acetabulum and extending posteriorly in sinuous curves, terminate 0.390 ([0.390] 0.300–0.540) from posterior end of body. Male genital pore submedian, opens at level of posterior margin of pharynx, 0.384 ([0.470] 0.380–0.540) from anterior end of worm. Cirrus pouch elongate, 0.300 in length by 0.090 in width ([0.297 by 0.122] 0.150–0.450 by 0.090–0.135) contains an unarmed, eversible cirrus and a coiled seminal vesicle, posterior bulbous portion of cirrus pouch in contact with anterior margin of acetabulum or lies anterior to acetabulum. Testes symmetrical, round to elongate oval, 0.300 by 0.228 ([0.247 by 0.199] 0.135–0.345 by 0.135–0.345) with indented margins lateral to and in zone of acetabulum. Ovary transversely elongate, lobulate or round, 0.192 by 0.300 ([0.213 by 0.252] 0.200–0.400 by 0.130–0.274) posterior to posterior margin of testes, submedian. Seminal receptacle submedian, globular, posterior and dorsal to ovary. Mehlis' gland lateral to seminal receptacle. Laurer's canal not seen. Vitellaria follicular, largely extra-caecal, commence in posterior testicular zone, composed of large sized follicles, extend 0.64–0.76 ([0.515] 0.220–0.825) posteriorly beyond mid-body. Paired vitelloducts arise laterally in middle or, rarely posterior region, of vitelline field, extend to vitelline reservoir on midline of body. Uterus mostly intercaecal contains light, undeveloped eggs in portion going posteriorly and dorsally from ovarian region, descends in wide loops to posterior region of hindbody where it turns and ascends in coils ventrally to region of ovary, passes dorsally to acetabulum and cirrus pouch and opens to exterior through female genital pore at level and next to opening of male genital pore. Excretory pore posterior, terminal; excretory bladder cylindrical, elongate, bifurcates

<sup>1</sup> Research at the Oak Ridge National Laboratory sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

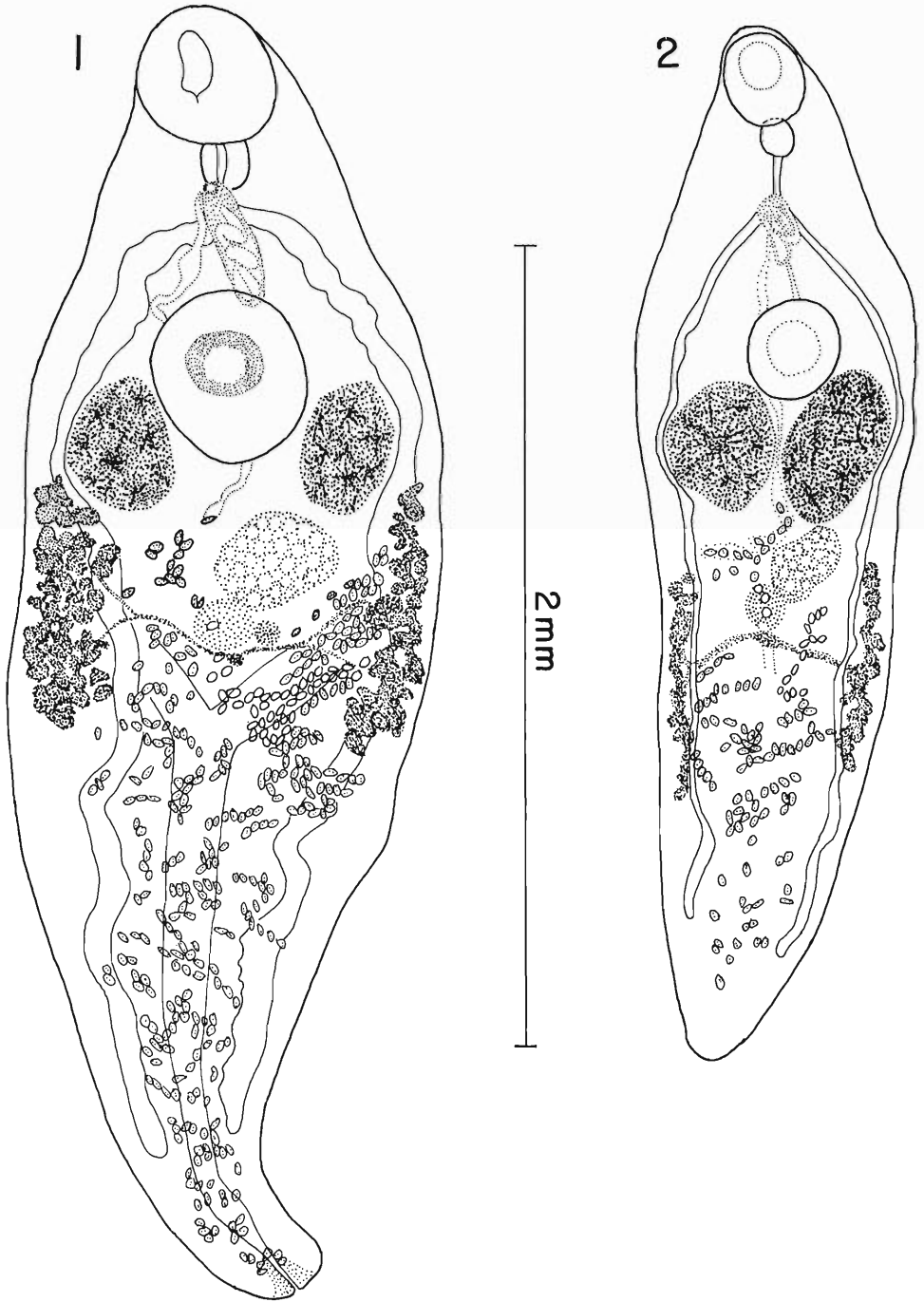


Figure 1. *Platynosomum amazonensis* sp. nov. Holotype.  
Figure 2. *Platynosomum marmoseti* sp. nov. Holotype.

immediately posterior to ovary; primary excretory branches extend laterally to level of testes. Ova from terminal uterus dark-brown, thick-shelled, operculate, containing fully developed miracidia, each with two large oval posterior vesicles; 0.037 by 0.026 (0.028 by 0.021–0.043 by 0.030).

HOSTS: *Callimico goeldii* (Thomas, 1904). *Saguinus nigricollis* (Spix, 1823).

HABITAT: Biliary ducts.

LOCALITY: South America, Amazon Headwaters.

TYPE SPECIMEN: Holotype and four paratypes in USNM Helminthological Collection. Nos. 61760, 61761, and 61762, respectively. The name of this species refers to the area of origin of the host.

*Platynosomum marmoseti* sp. nov.

DESCRIPTION (Fig. 2). Body of mature specimens elongate 2.72 ([2.58] 2.15–2.88), subcylindrical, tapering gradually towards anterior and posterior ends, rounded terminally, widest 0.74 ([0.73] 0.52–0.86) at level of testes. Ratio of body width/length 1:3.6. Cuticle lacking papillae, scales, or spines. Oral sucker 0.210 by 0.192 ([0.250] 0.210–0.270) subterminal, elongate-oval, mouth opening ventrally, subequal to acetabulum. Acetabulum elongate round to oval 0.222 by 0.228 ([0.288] 0.222–0.315) distance between sucker 0.740 ([0.744] 0.691–0.795). Oral sucker: acetabulum ratio 1:1.11 ([1:1.15] 1:1.05–1:1.3). Prepharynx absent. Pharynx globular 0.072 ([0.093] 0.072–0.110). Oesophagus narrow, thin-walled, bifurcates between oral and ventral suckers. Intestinal caeca pass laterally around ventral sucker and terminate 0.240 ([0.270] 0.120–0.380) from posterior extremity. Male genital pore median or slight submedian opening at level of esophageal bifurcation, 0.378 ([0.410] 0.280–0.450) from anterior end of worm. Cirrus pouch elongate, 0.360 by 0.072 ([0.31 by 0.089] 0.27 by 0.11–0.36 by 0.075) containing an unarmed eversible cirrus and a coiled seminal vesicle, located wholly anterior to ventral sucker. Testes symmetrical, large, with smooth margins, elongate-oval, 0.300 in length ([0.393] 0.300–0.510) by 0.240 ([0.250] 0.195–0.270) in width, located immediately posterior to ventral sucker, rarely, anterior

margin of one testis intrudes into acetabular zone. Ovary small with smooth margins, or slightly indented 0.168 by 0.120 ([0.163] 0.135–0.194), submedian, posterior to testes' field. Seminal receptacle, median, globular, posterior and dorsal to ovary. Mehlis' gland posterior to ovary. Laurer's canal not seen. Vitelline follicles, largely extracaecal, composed of moderate number of large size follicles, commence at anterior margin of ovarian zone, extend 0.48–0.55 ([0.540] 0.420–0.750) into, and almost exclusively confined to hind-body. Common vitelloducts arise at midlevel of vitelline field and extend to midline of body and terminate in a vitelline reservoir. Descending limb of uterus mostly intercaecal, contains light undeveloped eggs, goes dorsally and posteriorly from ovarian zone in wide loops; a little anterior to posterior termination of body, uterus turns and ascends, passes dorsally to ventral sucker to open to exterior through female genital pore at level of and next to opening of male genital pore. Excretory pore posterior, terminal. Ova from terminal uterus, dark-brown, thick-shelled, operculate, contain fully developed miracidia, each with two large posterior oval granular vesicles; 0.036 by 0.022 ([0.034 by 0.022] 0.031 by 0.021–0.039 by 0.025).

HOST: *Saguinus nigricollis* (Spix, 1823).

HABITAT: Biliary ducts.

LOCALITY: South America, Amazon headwaters.

TYPE SPECIMEN: Holotype and six paratypes in USNM Helm. Collection, Nos. 61763 and 61764, respectively. The name of this species derives from "marmoset," common name of this group of monkeys.

DISCUSSION

Nineteen genera of Dicrocoeliidae are wholly or in part found in mammals (Yamaguti, 1958). Species from seven of these genera are found in primates; five genera, *Brodenia* Gedoelst, 1913; *Concinnum* Bhalariao, 1936, *Dicrocoelium* Dujardin, 1845, *Eurytrema* Looss, 1907, and *Leipertrema* Sandosham, 1951, are found in primates in Africa, Japan, and Borneo; two genera, *Athesmia* Looss, 1899 and *Controrchis* Price, 1929, are found in New World primates.

The new species of dicrocoeliids described above, *Platynosomum amazonensis* sp. nov.

and *P. marmoseti* sp. nov. recovered from *Callimico* and *Saguinus*, are not placed in *Brodedia* since they have longer intestinal caeca and lack the serrate margins of the body characteristic of that genus; they are not placed in *Concinnum* or *Leipertrema* since they have longer intestinal caeca and more extensive vitelline fields; they are not placed in *Dicrocoelium* since they do not possess tandem or obliquely placed testes; and they differ from species of *Eurytrema* by different body shape, more extensive vitelline fields, and by having the genital opening bi- or prebifurcal. They clearly do not belong to the New World genera *Athesmia* or *Controrchis*, the former genus being distinguished by unilateral postovarian vitellaria and the latter genus by the testes being separated longitudinally by the acetabulum.

*Platynosomum amazonensis* sp. nov. and *P. marmoseti* sp. nov. are placed in the genus *Platynosomum* Looss, 1907, owing to shape, acetabular or postacetabular position of symmetrical testes, and origin of the vitellaria in the testicular field or immediately post-testicular.

Seven species have been described as belonging to this genus: six from birds and one from mammals. The species from birds are *P. semifuscum* Looss, 1907 (type), *P. deflextens* (Rudolphi, 1819), *P. illiciens* (Braun, 1901), *P. proxilliciens* (Canavan, 1937), *P. reficiens* (Braun, 1901), and *P. ventricosum* (Rudolphi, 1809); while one species, *P. fastosum* Kossack, 1910, has been described from mammals (Yamaguti, 1958). Travassos (1944) included *P. alectoris* Noeller and Enigk, 1933 from birds and then placed it in the genus *Conspicuum* (placed in *Lyperosomum* by Shtrom, 1940 and concurred in by Skrjabin and Evranova, 1952). Skrjabin and Evranova, 1952, include *P. brauni* Freitas and Lent, 1937 (considered a synonym of *P. illiciens* by Travassos, 1944 and Yamaguti, 1958), *P. fallax* Heidigger and Mendheim, 1938 (considered a synonym of *P. proxilliciens* by Travassos, 1944 and Yamaguti, 1958), *P. muris* Stscherbakova, 1942 (reassigned to the genus *Skrjabinus* (Bhalerao, 1936) Shtrom, 1940 by Yamaguti, 1958) and *P. voluptarium* (Braun, 1901) (considered a synonym of *P. illiciens* by Tra-

vassos, 1944, and Yamaguti, 1958). With the exception of *S. muris* the hosts of the above species are birds; *S. muris* is found in a vole, *Sylvaemus sylvaticus*.

*P. amazonensis* sp. nov. and *P. marmoseti* sp. nov. differ from *Platynosomum* species found in birds in host distribution, and in the following: They are smaller than *P. semifuscum* and have different sizes and ratios between the suckers. They differ from *P. deflextens* by having larger testes and by a different vitelline: testes distribution. They differ from *P. illiciens* by having smaller testes and ovary and a less extensive uterine development. They differ from *P. reficiens* in having less extensive vitelline distribution. They differ from *P. ventricosum* by having smaller, not tandem, testes and by having smaller eggs.

*P. amazonensis* sp. nov. and *P. marmoseti* sp. nov. differ from *P. fastosum* found in mammals in body size and proportions, in having smaller testes and ovary, in the lesser extent of the vitellaria, smaller size of ova, and kind of host. They differ from *S. muris* found in a vole in the markedly lesser anterior extent of the vitellaria.

*Platynosomum amazonensis* sp. nov. recovered from *C. goeldii* and *S. nigricollis* differs from *P. marmoseti* sp. nov. recovered from *S. nigricollis* in body length and width and in the ratio of these measurements; testes position and size; origin, position, and extent of vitellaria; position of the male genital opening; relative positions of oral and ventral suckers; and extent of uterine development. These two species (while both occur in *Saguinus*) have not been found simultaneously in the same individual host. It is considered most likely that the tamarins are from different localities in South America.

The genus *Platynosomum* thus contains species from a wider host range than has hitherto been reported. Further examination of the livers and gall bladders of South American primates (now being used in many different types of study in this country) should reveal much more of interest to the student of dicrocoeliid trematodes.

#### SUMMARY

Parasitologic examination of New World

primates, *Callimico goeldii* (Thomas, 1904) and *Saguinus nigricollis* (Spix, 1823) revealed two new species of dicrocoeliid trematodes: *Platynosomum amazonensis* sp. nov. in 9 of 20 *C. goeldii* and in 35 of 441 *S. nigricollis*; and *Platynosomum marmoseti* sp. nov. in 2 of 441 *S. nigricollis*. The differentiating characteristics which distinguish these species are described.

#### ACKNOWLEDGMENTS

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## Morphological Changes During the Molt of *Diploscapter coronata* (Nematoda: Rhabditidae)

HELEN CAROL HECHLER<sup>1</sup>

#### INTRODUCTION

The genus *Diploscapter* is unusual among nematodes because of the heavy sclerotization of its lips. Development of the sclerotization, and of other cephalic structures, can be followed in molting specimens. The progress of the molt in *Diploscapter coronata* (Cobb, 1893) Cobb, 1913 is reported here.

#### MATERIALS AND METHODS

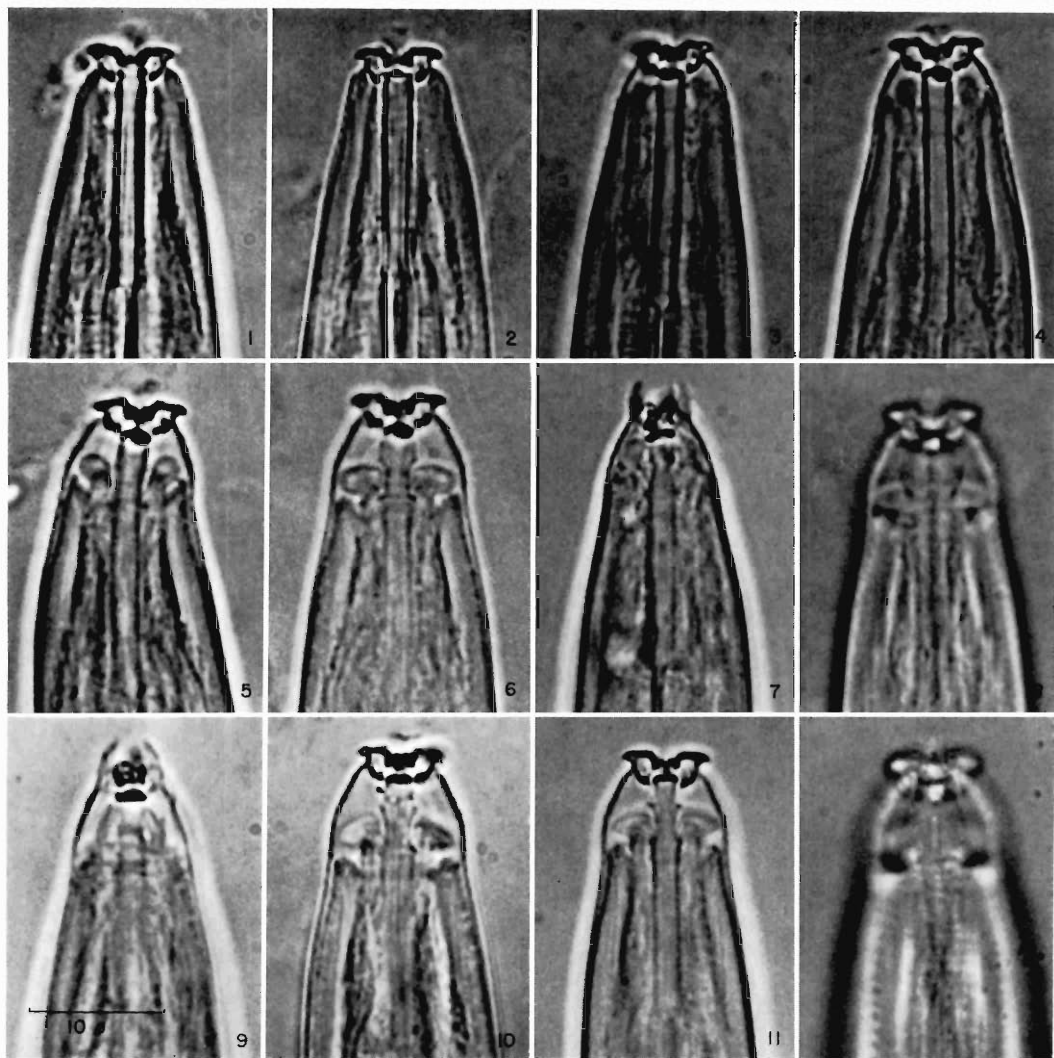
Females and larvae of *D. coronata* collected from a golf green at Cincinnati, Ohio, were established in petri dish culture using as a food source the bacterium, *Aerobacter aerogenes* (Kruse) Beijerinck, growing on a medium originally developed by Chang (1958), but modified to consist of: sucrose, 2.5 g; tryptose, 0.5 g; Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1.5 g; KH<sub>2</sub>PO<sub>4</sub>, 0.1 g; agar, 15.0 g; distilled water, 1 liter. When the culture was well established,

several subcultures were started from single females. Only one of these cultures was saved for maintenance. Subcultures were made daily and stored at room temperature. Nematodes in the late preadult stage were mounted, and progress of the final molt was observed and photographed, as described by Hechler and Taylor (1966). Following the terminology of Steiner (1942), the names "hamuli" for the submedian lips, and "laciniae" for the lateral lips, are used here.

#### RESULTS

**FINAL MOLT:** In the preadult *Diploscapter coronata* highly refractive structures include: the hamuli, the head framework at the base of the lips, the lining of the stomatal cylinder, and, less conspicuous, the lining of the esophageal lumen (Fig. 1). The presence of a very narrow nonsclerotized band surrounding the stomal cylinder a short distance from its anterior end causes the sclerotized ring at the anterior margin to appear in cross section as

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Figures 1-12. Micrographs of *Diploscapter coronata*. Fig. 1. Preadult head. Figs. 2-12. Stages in final molt. 2. Anterior ring retracted. 3. Vacuolate tissue surrounding stoma. 4. Lips retracted, rings surrounding stoma visible. 5. Hamuli beginning to elongate. 6. Rotation of hamuli complete. 7. Dorso-ventral view, same stage as figure 4, lacinae early in development. 8. Lateral view, focus on disc-shaped lacinia. 9. Dorso-ventral view, same stage as Fig. 8, showing connecting strands from papillae and amphids. 10. Stomatal sclerotization completely lost. 11. New stomatal sclerotization beginning, hamuli becoming hook-shaped. 12. Focus on lacinia with fringed margins.

two round, highly refractive dots. This ring appears somewhat thicker than the remainder of the stomatal lining, except for a similar narrow, thicker section at its base.

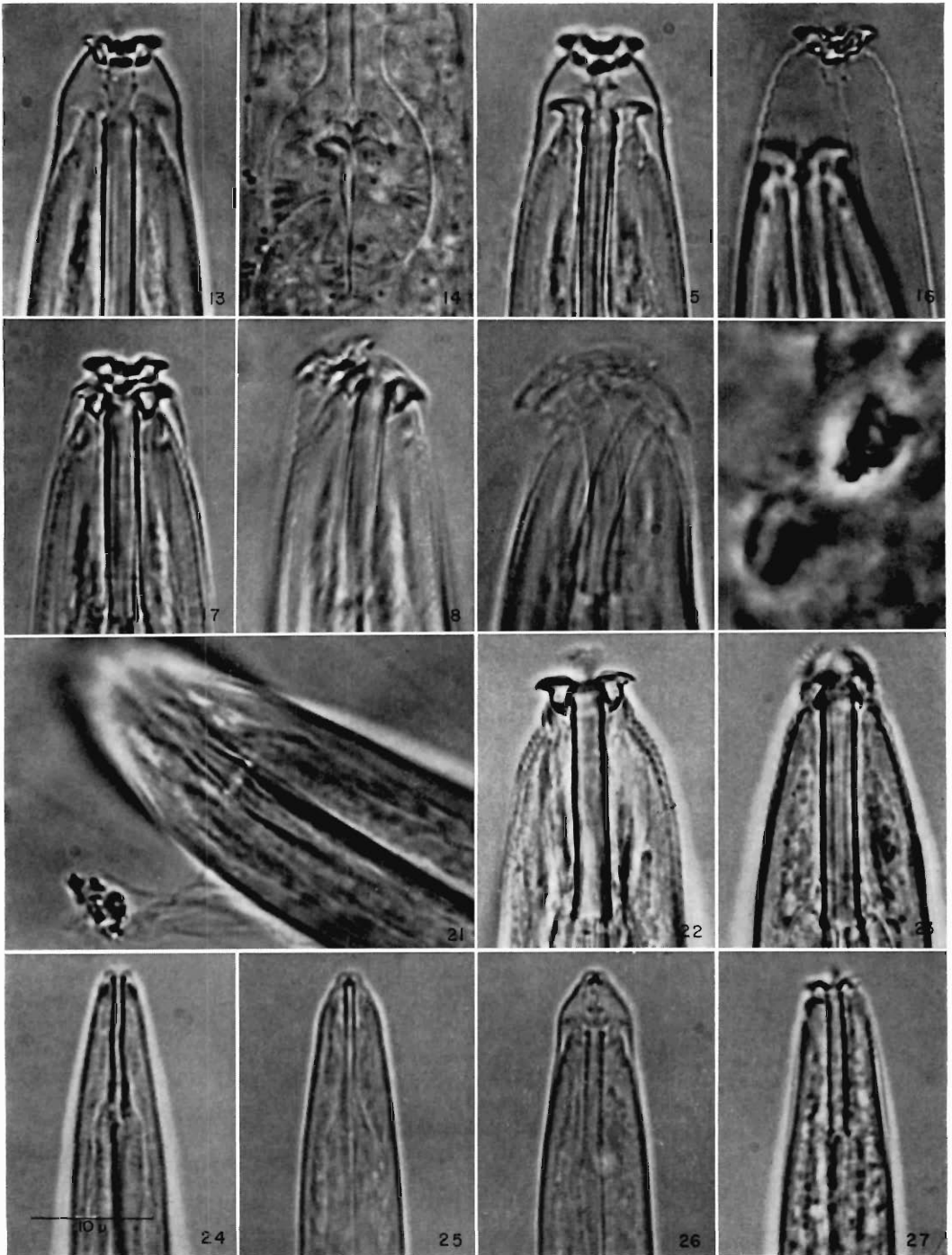
When the final molt is about to begin, locomotion becomes progressively sluggish and

feeding is less frequent. The stoma retracts posteriad and the anterior ring appears conspicuous at its anterior margin (Fig. 2). Two moderately refractive lateral strands, which connect it to the oral aperture, are occasionally visible. Feeding activity continues until the

conspicuous ring reaches the level of the lip base, and although occasional further spasmodic contractions of the procorpus and valve flaps occur, no food particles are ingested. Two narrow rings, which become increasingly conspicuous, appear surrounding the anterior third of the stomatal cylinder, and the surrounding tissue becomes vacuolate (Fig. 3). A hyaline cavity with its base at the level of the posterior ring appears surrounding the stoma (Fig. 3) and gradually the vacuoles disappear. Meanwhile the lining of the stomatal cylinder, except for the anterior ring, gradually becomes less refractive and the adult lips begin to separate from the preadult cuticle. Inconspicuous strands, which connect papillae on the developing hamuli to corresponding structures on the preadult cuticle, are occasionally visible as the separation widens. At first the adult hamuli are spherical (Fig. 4), but as the head continues to retract the hamuli elongate and their long axes gradually rotate from parallel to perpendicular to the long axis of the nematode (Figs. 5, 6). Their bases are at the level of the posterior surrounding ring, which occupies a position at the anterior margin of the stoma in the adult. The anterior ring appears as a ridge surrounding the preadult anterior stomatal cavity, which remains attached to the exuvia. Early in their development the laciniae are spherical, except for the flat to concave surface adjacent to the stoma (Fig. 7). Later they become disc-shaped (Fig. 8). Strands are present which connect the labial papillae and amphids to the corresponding locations on the preadult cuticle (Fig. 9). The hamuli continue to elongate and the refractiveness of the linings of the stoma and esophagus continues to decrease until it is similar to that of the surrounding tissue (Fig. 10). By this time there is neither any locomotion nor any activity of the esophagus. The developing hamuli become increasingly hook-shaped and sclerotization of the stomatal lining begins (Fig. 11). Meanwhile the laciniae become oval in shape and fringes appear on their margins (Fig. 12). After sclerotization of the stomatal lining is well advanced, it begins to appear on the hamuli beginning at the points of the hooks (Fig. 13). Meanwhile the preadult esophageal lumen lining separates from the walls of the lumen

and is easily visible anterior to the adult bulb flaps (Fig. 14). The nematode head begins to move from side to side within the preadult cuticle and the esophagus begins to perform as in feeding, but more slowly. This esophageal activity occasionally creates a pressure deficit which pulls the walls of the preadult lumen together (Fig. 15). Sclerotization of the hamuli continues to advance toward the oral aperture and to extend posterior within the oral cavity (Fig. 15), and meanwhile the two cuticles are separated by contractile twitching movements throughout the length of the body. The nematode then begins to move back and forth within the preadult cuticle, and the preadult stomatal lining is pulled out through the adult oral aperture (Fig. 16). Contractions of the esophagus cause the preadult esophageal lining, including that of the valve flaps, to move into the intestine, and also cause alternate replacement and regurgitation of the stomatal lining. Sclerotization advances to the head framework at the lip bases, and also posterior in a shallow, conical structure which extends between the oral aperture and the base of the lips (Figs. 17, 22, 23). The nematode begins alternately to press with its lips against the preadult cuticle (Fig. 18) and to move its head rapidly back and forth in a dorso-ventral plane (Fig. 19). Finally the exuvia breaks just behind the lips and the nematode emerges (Fig. 21). No evidence of a flow of esophageal gland material, no presence of a narrow, softened, refractive band surrounding the anterior region of the nematode, and no extensive softening of the exuvia has been seen. A few minutes after the nematode emerges, the preadult esophageal lining is expelled through the anus (Fig. 20). The final molt is complete in 8 to 10 hours under the conditions of these studies. The structures shed attached to the exuvia include: the highly refractive hamuli, head framework, anterior margin of the stomatal lining, excretory pore lining, and rectal lining; as well as the less refractive laciniae, linings of the amphids and papillae, and the lining of the stomatal cylinder. The esophageal lining is shed through the intestine and anus.

**POST-HATCHING MOLT:** The stoma of the newly hatched *D. coronata* is similar to that





of the preadult. However, all the lips are rounded and nonsclerotized (Fig. 24). Early in the posthatching molt the stoma moves posterior and the sclerotized ring at the anterior margin of the stoma becomes conspicuous, as in the final molt (Fig. 25). Further progression of the molt is similar to the final molt, with formation of hamuli and laciniae (Fig. 26), except for omission of formation of the shallow, conical, sclerotized structure at the anterior end of the stoma (Fig. 27).

#### DISCUSSION

There are three major morphological patterns in the head region of *D. coronata* during its life cycle: first, the hatching stage has rounded, nonsclerotized lips; second, the subsequent larval stages possess hamuli and laciniae; and third, in the adult the shallow, conical, sclerotized structure is present just behind the oral aperture. All stages feed effectively, and it is not known how these morphological changes benefit the nematode.

In the description of the molts the terminology of the cephaloboid stomatal structures was not used. The sclerotized ring at the anterior stomatal margin in larval forms, as well as the anterior conical structure in adults, because of their position, could correspond to the cheilorhabdions. Likewise, the narrow thickenings at the base of the stoma could be called telorhabdions. A thorough investigation of molting in many other genera within the Rhabditidae, with special attention to which structures retain or lose sclerotization during the molt and which remain attached to the

exuviae, may help to clarify the identity of stomatal structures in the various genera.

The tissue surrounding the stoma becomes vacuolate just before sclerotization of the lining begins to decrease. Possibly the vacuoles are formed by chemical changes which result in dissolution of the stomatal lining.

The passage of the esophageal lining into the intestine and out through the anus in molting *Seinura* spp. was not reported by Hechler and Taylor (1966). Molting *Seinura* specimens were studied further with attention to this detail, and although occasionally amorphous material of low refractiveness was seen within the intestinal lumen, none of it could be positively identified as esophageal lining. Possibly in this genus the lining is completely dissolved by the esophageal gland secretions, and is either expelled through the stylet or is passed into the intestine.

Molting *D. coronata* seems to emerge from the exuvia by mechanical means, either by the rasping action of the hamuli, or pressure of the head, or both. No evidence of other types of emergence, discussed by Hechler and Taylor (1966), has been seen.

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Figures 13-27. 13-19. Final molt. 13. Sclerotization of stomatal cylinder nearly complete. 14. Basal bulb with separated bulb flap lining anterior to bulb flaps. 15. Sclerotization complete in hamuli, preadult stomatal lining collapsed due to action of esophagus. 16. Preadult stomatal lining pulled out of oral aperture. 17. Sclerotization of head region complete. 18. Nematode pressing with head against exuvia. 19. Nematode moving head from side to side within exuvia. 20. Preadult bulb flaps in agar after expulsion from anus. 21. Nematode emerging from exuvia, preadult lips at left. 22. Adult head, lateral view. 23. Adult head, dorso-ventral view. 24-26. Hatching stage. 24. Head. 25. Beginning of molt. 26. Advanced molt, hamuli well developed. 27. Second stage, head.

**A Trematode, *Macrolecithus indicus* n. sp. from the Intestine of a Freshwater Fish, *Puntius sophore* (Ham.), from Lucknow, India**

S. P. GUPTA AND VINOD AGRAWAL<sup>1</sup>

INTRODUCTION

Hasegawa and Ozaki (1926) proposed the genus *Macrolecithus*, with *M. gotoi* Hasegawa and Ozaki, 1926 as type species, in the family Allocreadiidae Stossich, 1904. Park (1939) added two more species, *M. elongatus* Park, 1939 and *M. phoxinusi* Park, 1939 from the alimentary canal of *Phoxinus lagowskii* (Dybawski) from Sensen, North Tyosen (Korea). He considered the genus *Macrolecithus* an intermediate form between the Allocreadiidae and Plagiorchiidae. Yamaguti (1958) placed the genus in a new subfamily Wallininae of the family Allocreadiidae. The authors are in complete agreement with this proposal as the excretory bladder is tubular, a characteristic feature of the family Allocreadiidae.

*Macrolecithus indicus* n. sp.  
(Figs. 1-5)

Numerous specimens were collected from the intestine of a freshwater fish, *Puntius sophore* (Ham.) from the Gomti River at Lucknow. All measurements are in mm.

**DESCRIPTION:** Based on 15 specimens. Body elongate, aspinose, rounded anteriorly and bluntly pointed posteriorly, 3.95-7.22 in length by 0.98-2.32 in width at level of ootype. Oral sucker subterminal, globular, 0.32-0.49 in length by 0.32-0.53 in width. Ventral sucker globular, larger or equal to oral sucker, 0.33-0.60 in length by 0.36-0.63 in width at 1.70-2.21 from anterior extremity. Prepharynx very short, 0.04-0.08 in length; pharynx, 0.11-0.22 in length by 0.12-0.27 in width; esophagus tubular, straight or slightly curved, 0.45-0.65 in length bifurcating midway between pharynx and ventral sucker terminating a little anterior to posterior end of body.

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

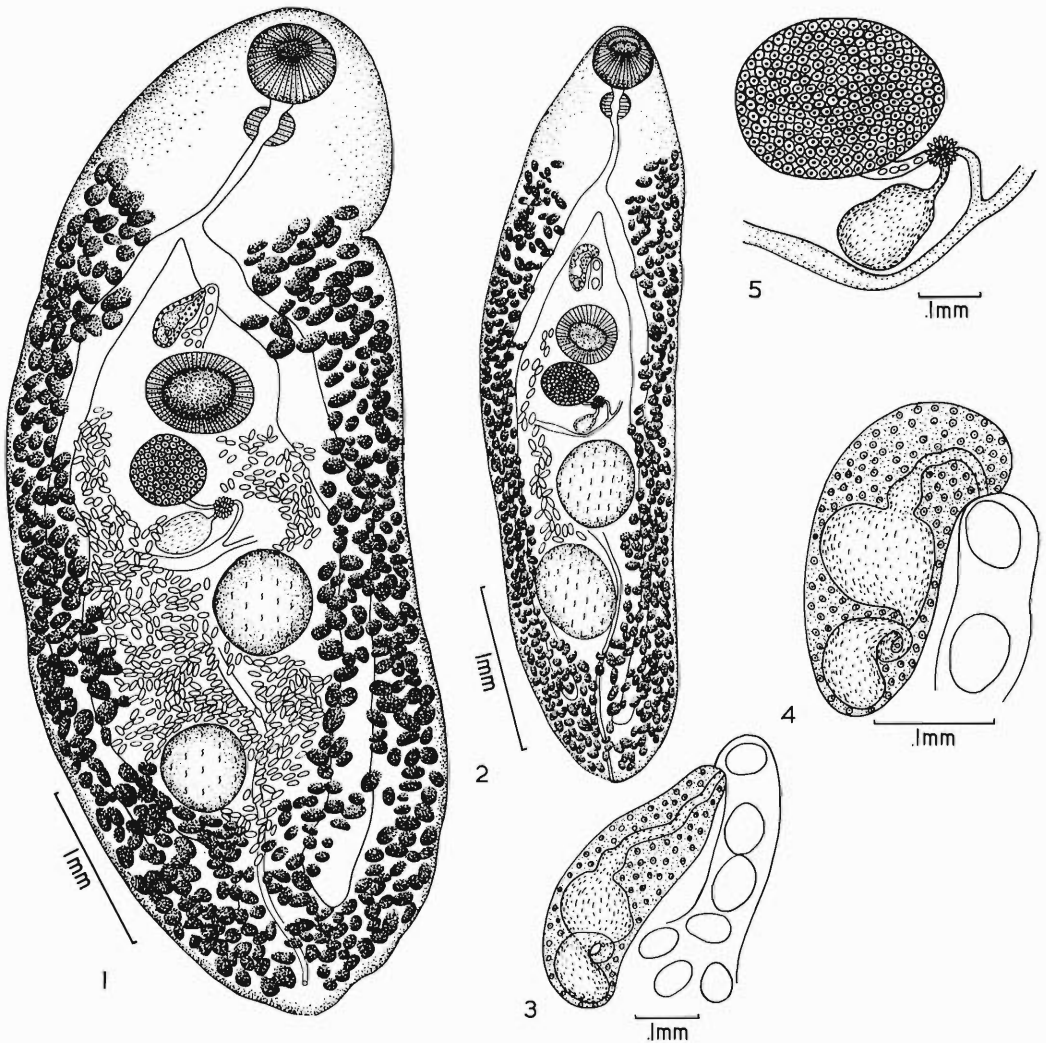
Genital pore median, intercecal, about midway between cecal bifurcation and ventral sucker at 1.40-1.83 from anterior extremity.

Testes entire, oval, postequatorial, intercecal, directly or obliquely tandem, close or apart from each other. Anterior testis 0.29-0.63 in length by 0.30-0.58 in width at 2.24-3.58 from anterior extremity. Posterior testis larger, smaller or equal to anterior testis, 0.30-0.73 in length by 0.32-0.52 in width at 2.76-5.07 from hind end. Cirrus pouch claviform, submedian, intercecal, extending from genital pore up to a little anterior to ventral sucker, 0.20-0.43 in length by 0.11-0.21 in width lying at 1.18-2.18 from anterior extremity. Vesicula seminalis large, divided into two or three parts by a tubular coil filling most basal part of cirrus sac; pars prostatica globular, 0.04-0.06 by 0.035-0.07; ejaculatory duct long and tubular, 0.07-0.19 in length; cirrus muscular and nonspiny. A large number of prostate gland cells fill entire space in cirrus pouch around ejaculatory duct and pars prostatica.

Ovary subspherical, oval, smooth, median, or submedian, 0.25-0.43 in length by 0.31-0.50 in width immediately posterior to ventral sucker or slightly overlapping it. Receptaculum seminis, flask shaped, postovarian, 0.08-0.32 in length by 0.09-0.27 in width at 2.0-3.31 from anterior extremity. From hind end of ovary arises oviduct which opens at ootype. Vitellaria follicular, extending from pharynx or level of intestinal bifurcation or midway between pharynx and intestinal bifurcation to posterior end mainly lateral and ventral to ceca but extending into intercecal space caudad of posterior testis. Vitelline ducts unite in front of ovary to form a yolk reservoir that opens into ootype. Uterine coils numerous, intercecal and extracecal passing between the testes extending up to anterior border of posterior testis or a little posterior to hind end of posterior testis, running forward to level of ventral sucker. Metraterm short located right

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The paratype and holotype specimens of the form described in this paper will be deposited in Dr. G. S. Thapar's Helminthological Collection, Lucknow, U.P., India.



Figures 1-5. *Macrolecithus indicus* n. sp. 1. Entire. Dorsal view. Uterus extending beyond hind testis; ventral sucker larger than oral sucker and anterior testis larger than posterior. 2. Entire. Dorsal view. Uterus extending up to hind end of anterior testis; suckers equal and anterior testis smaller than posterior. 3-4. Cirrus pouch enlarged. Dorsal views. 5. Ootype enlarged. Dorsal view.

of cirrus sac. Eggs oval and operculated, 0.04-0.10 in length by 0.03-0.05 in width.

HOST: *Puntius sophore* (Ham.).

LOCATION: Intestine.

LOCALITY: Lucknow.

DISCUSSION

The new form differs from previously described species of *Macrolecithus* Hasegawa

and Ozaki, 1926 in having testes separated by uterine coils. The present form differs from *M. elongatus* and *M. phoxinusi* in having genital pore median and located at the posterior region of the intestinal bifurcation instead of being located towards the left of the esophagus, in having cirrus pouch intercecal instead of towards the left of the body, in having testes entire rather than with irregular or slightly

lobed margins, and in the structure of vesicula seminalis. The new form closely resembles *M. gotoi* in the position of genital pore but differs from it in having a long tubular esophagus. Park (1939) distinguished *M. phoxinusi* from *M. elongatus* from the same host, *Phoxinus logowskii*, by the character of eggs. In *M. elongatus* the uterus is pretesticular and the eggs are 78 to 81 by 36 to 43  $\mu$  with thick shells; in *M. phoxinusi* the uterus reaches the posterior edge of the posterior testis and the eggs are 70 to 84 by 45 to 48  $\mu$  with thin shells. In the opinion of authors the extension of uterus is a variable character as the posterior extent of the uterus in *M. indicus* showed equal variation and the relative size of eggs is a minor character and has no importance. Accordingly *M. phoxinusi* is considered a synonym of *M. elongatus*.

KEY TO THE SPECIES OF *Macrolecithus*  
HASEGAWA ET OZAKI, 1926

1. Genital pore intercecal ..... 2  
Genital pore extracecal ..... *M. elongatus*
2. Esophagus long and testes separated by  
uterine coils ..... *M. indicus* n. sp.  
Esophagus short and testes not separated  
by uterine coils ..... *M. gotoi*

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**Studies on the Genus *Alaimus* de Man, 1880, with Description of Six New Species from India**

MOHAMMAD RAFIQ SIDDIQI AND ZAHID HUSAIN<sup>1</sup>

The genus *Alaimus* de Man, 1880, belongs, besides the related genus *Amphidelus* Thorne, 1939, in the family Alaimidae Micoletzky, 1922. Thorne (1939) considered this family under Dorylaimoidea. Chitwood and Chitwood (1937) regarded it as a member of Enoplida. Clark (1961) proposed a new suborder, Alaimina, under Enoplida in view of the presence of seven esophageal glands and on the reported opening of the dorsal esophageal gland anterior to the nerve ring (Chitwood, 1937) which obviously is not true. Therefore, Clark (1962) modified the concept of Alaimina. However, this time, Clark brought the superfamily Diphtherophoroidea (Micoletzky, 1922) Clark, 1961, under Alaimina. This opinion was concurred by Goodey (1963) who referred the suborder to Dorylaimida

(de Man, 1876) Pearse, 1942. Goodey, in the same publication, proposed and recognized Alaimoidea in place of Alaimidae. In our view, Diphtherophoroidea does not belong to Alaimina but to Dorylaimina where Belonenchidae and Basirotyleptidae have members with somewhat similar buccal armature and occasionally an excretory pore is found in members of Leptonchidae.

THE GENUS *Alaimus* DE MAN, 1880

DIAGNOSIS: Alaimidae: Lip region rounded to conical; outer cirlet of papillae easily seen, inner obscure. Excretory pore variable in position on neck but usually near head end. Amphidial aperture a minute pore, amphid pouch lacking, sensillae close to amphidial apertures. Stoma appearing conical, triradiate. Esophagus enlarging in posterior third or less. Esophageal glands reported as seven but usu-

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ally three seen. Esophago-intestinal valve small, discoidal, or conoid. Prerectum absent. Vulva transverse, usually pre-equatorial, with thick labia. Ovary single except in *A. macer* Andrassy, 1958. Ventro-median series of supplements present.

TYPE SPECIES: *Alaimus primitivus* de Man, 1880.

KEY TO SPECIES OF *Alaimus*  
(BASED ON FEMALES)

- |  |                             |  |                              |
|--|-----------------------------|--|------------------------------|
| 1. Ovary single .....  | 2                           | Length of tail less than 8 times anal<br>body width .....      | 19                           |
| Ovaries paired .....   | <i>macer</i> Andrassy, 1958 | 15. Length of tail more than 10 times anal<br>body width ..... | 16                           |
| 2. Body length 4 mm .....  |                             | Length of tail less than 10 times anal<br>body width .....     | 18                           |
| ..... <i>elongatus</i> de Man, 1906                                |                             | 16. Lip region abruptly narrowed .....                         | 17                           |
| Body length under 3 mm .....                                       | 3                           | Lip region not abruptly narrowed .....                         |                              |
| 3. Postrectal intestinal sac present .....                         |                             | ..... <i>meyli</i> Andrassy, 1961                              |                              |
| ..... <i>thamugadi</i> Maupas, 1900                                |                             | 17. Lip region broadly truncated .....                         |                              |
| Postrectal intestinal sac absent .....                             | 4                           | ..... <i>leptus</i> n. sp.                                     |                              |
| 4. Body length about 1 mm or more .....                            | 5                           | Lip region narrow, not truncated .....                         |                              |
| Body length about 0.8 mm or less .....                             | 12                          | ..... <i>thrixus</i> n. sp.                                    |                              |
| 5. Esophagus enlarging just behind nerve<br>ring .....             | <i>glissus</i> Thorne, 1939 | 18. Tail hooked, terminus rounded .....                        |                              |
| Esophagus enlarging more posterior ..                              | 6                           | ..... <i>medius</i> n. sp.                                     |                              |
| 6. Tail over 7 times anal body width .....                         | 7                           | Tail not hooked, terminus pointed .....                        |                              |
| Tail under 7 times anal body width .....                           | 10                          | ..... <i>parvus</i> Thorne, 1939                               |                              |
| 7. Body length 2 mm; a = 90 .....                                  |                             | 19. Tail end pointed .....                                     | 20                           |
| ..... <i>simplex</i> Cobb, 1914                                    |                             | Tail end rounded ..  | <i>arcuatus</i> Thorne, 1939 |
| Body length less than 2 mm; a = less<br>than 70 .....              | 8                           | 20. Cuticle with prominent longitudinal<br>striae .....        | <i>striatus</i> Loof, 1964   |
| 8. Tail end abruptly conoid ..                                     | <i>hamulus</i> n. sp.       | Cuticle without longitudinal striae .....                      | 21                           |
| Tail end not abruptly conoid .....                                 | 9                           | 21. Tail terminus abruptly narrowed; L =<br>0.52-0.62 .....    | <i>editorus</i> n. sp.       |
| 9. Excretory pore 3 head widths or less<br>from anterior end ..... | <i>jaulasali</i> n. sp.     | Tail terminus not abruptly narrowed;<br>L = 0.8 .....          | <i>acutus</i> Thorne, 1939   |
| Excretory pore more than 5 head<br>widths from anterior end .....  |                             |  |                              |
| ..... <i>primitivus</i> de Man, 1880                               |                             |  |                              |
| 10. V = 36; esophagus 1/5 as wide as body<br>at nerve ring .....   | <i>similis</i> Thorne, 1939 |  |                              |
| V = 42-43; esophagus not so slender<br>at nerve ring .....         | 11                          |  |                              |
| 11. Body length 2.5 mm; C = 24 .....                               |                             |  |                              |
| ..... <i>tenuis</i> Thorne, 1939                                   |                             |  |                              |
| Body length 1.3 mm; C = 17 .....                                   |                             |  |                              |
| ..... <i>proximus</i> Thorne, 1939                                 |                             |  |                              |
| 12. Vulva almost equatorial .....                                  | 13                          |  |                              |
| Vulva pre-equatorial .....   | 14                          |  |                              |
| 13. Tail end pegged .....  |                             |  |                              |
| ..... <i>mucronatus</i> Altherr, 1950                              |                             |  |                              |
| Tail end not pegged ..   | <i>minor</i> Cobb, 1893     |  |                              |
| 14. Length of tail more than 8 times anal<br>body width .....      | 15                          |  |                              |

SPECIES INQUIRENDAE: (1) *Alaimus fili-*  
*formis* Daday, 1894. (2) *A. modestus* Schuur-  
mans-Stekhoven and Teunissen, 1938. (3) *A.*  
*multipapillatus* Wu and Hoeppli, 1929. (4)  
*A. papillatus* (Daday, 1899) Micoletzky, 1922.

*Alaimus jaulasali* n. sp.<sup>2</sup>  
(Fig. 1, A-P)

MEASUREMENTS: Eight females (paratypes):  
L = 1.1-1.2 mm; a = 55-60; b = 4.8-5.3; c =  
7.0-8.6; V = 41-44.

Male (paratype): L = 1.06 mm; a = 70;  
b = 4.5; c = 11; T = 46.

Female (holotype): L = 1.15 mm; a = 60;  
b = 4.9; c = 8; V = 43<sup>14</sup>.

DESCRIPTION: Female: Body elongate-cy-  
lindrical, regularly tapering towards both ends,  
assuming a "C" form when relaxed in hot  
water; tail occasionally becoming considerably  
ventrally arcuate. Cuticle smooth, with exceed-  
ingly fine transverse striae. Lip region rounded,  
continuous with body contour, approximately

<sup>2</sup>Type specimens of all the new species reported here  
have been deposited in the Zoology Museum of the Aligarh  
Muslim University and Rothamsted Exp. Sta., Harpenden,  
England.

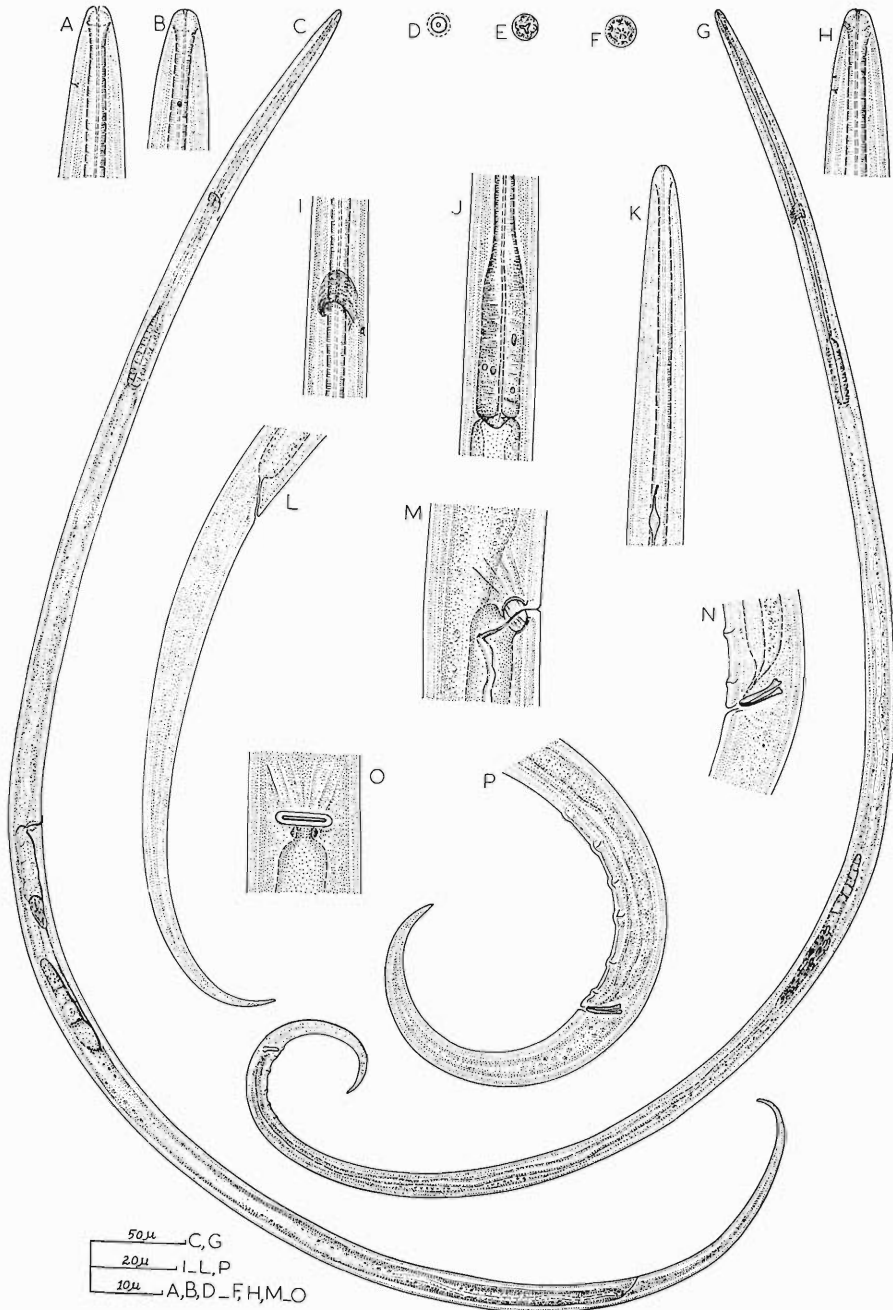


Figure 1. A-P. *Alaimus jaulasali* n. sp. A. Head end of female, lateral. B. Head end of female, ventral. C. Female (holotype). D. En face view. E. Cross section through stoma. F. Cross section through base of lip region. G. Male. H. Head end of male. I. Nerve ring and hemizonid. J. Basal region of esophagus. K. Anterior end of male. L. Female tail. M. Vulva, lateral. N. Spicular region. O. Vulva, ventral. P. Tail end of male.

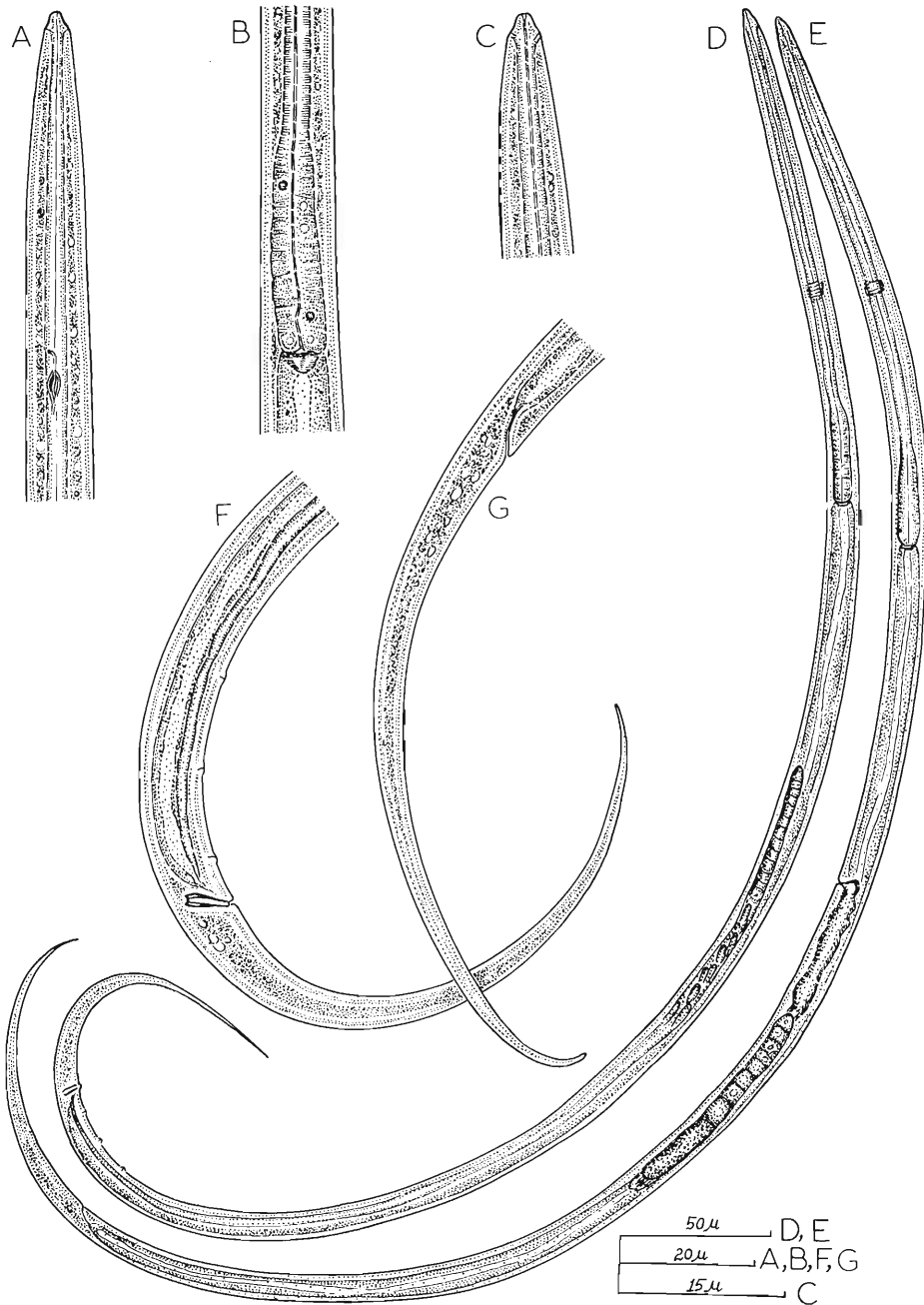


Figure 2, A-G. *Alaimus thrixus* n. sp. A. Anterior end of male. B. Esophageal base of female. C. Head end of female. D. Male. E. Female. F. Tail end of male. G. Tail end of female.

$\frac{1}{3}$  as wide at outer papillae as body at neck base. Tail elongate, regularly tapering to a pointed terminus, 11–12 times anal body width long. Amphids porelike, about  $85 \mu$  from anterior end; sensillae about  $25 \mu$  anterior to nerve ring. Mouth a small circular aperture, leading to a short, triradiate stoma behind which esophageal lumen appearing to be collapsed in a hexaradiate pattern (Figs. 1, D–F).

Excretory pore  $16\text{--}19 \mu$  or  $2\frac{1}{2}$  to 3 head widths from anterior end of body. Esophagus slender,  $\frac{2}{3}$  body width at nerve ring, expanding in its last fifth. Esophagus-vulva distance  $20\text{--}45 \mu$  longer than the length of esophagus. Nerve ring prominent, at about midesophagus, prominent ventral offshoot of nerve ring forming a hemizonid a little posteriorly (Fig. 1, I). Esophago-intestinal valve small, broadly rounded.

Vulva a transverse slit about  $\frac{2}{3}$  as long as body width. Vagina directed posteriorly, anterior branch of reproductive system absent. Uterus fairly elongate, with an elongate-oval spermatheca with sperms at its distal end. Posterior ovary well developed. Intestine with few spherical granules. Rectum about anal body width.

Male: Testis single. Spicules short, almost straight,  $9 \mu$  or  $\frac{3}{4}$  anal body width long. Six ventro-median supplementary papillae present (Fig. 1, P). Tail ventrally curved in a semi-circle, about 7 times anal body width.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of sal trees, *Shorea robusta*, in Jaulasal Forest Range, Haldwani Forest Division, U.P., India.

RELATIONSHIP: *Alaimus jaulasali* n. sp. comes close to *A. primitivus* de Man, 1880 and *A. parvus* Thorne, 1939. From *A. primitivus* it differs in having a more slender body, more anteriorly placed excretory pore, a shorter basal enlargement of esophagus, a more pointed tail, and a greater number of supplementary papillae in male.

From *A. parvus* it can be differentiated in having a more slender and longer body, more robust anterior part of esophagus, esophagus-vulva distance  $30\text{--}45 \mu$  longer than neck length (much shorter in *A. parvus*) and tail over 10 times anal body width long.

*Alaimus thrixus* n. sp.

(Fig. 2, A–G)

MEASUREMENTS: Three females (paratypes): L =  $0.71\text{--}0.72$  mm; a =  $53\text{--}58$ ; b =  $4.0\text{--}4.2$ ; c =  $6.2\text{--}6.6$ ; V =  $40\text{--}41$ .

Two males (paratypes): L =  $0.70\text{--}0.76$  mm; a =  $60\text{--}67$ ; b =  $4.0\text{--}4.1$ ; c =  $6.6\text{--}7.6$ ; T =  $50\text{--}51$ .

Female (holotype): L =  $0.72$  mm; a =  $58$ ; b =  $4.1$ ; c =  $6.4$ ; V =  $40^{17}$ .

DESCRIPTION: Female: Body ventrally arcuate, regularly tapering at both ends (Fig. 2, E). Lip region elevated, conoid-rounded, abruptly narrowing anteriorly from level of outer labial papillae. Cuticle marked by very fine transverse striae. Esophagus  $\frac{1}{3}$  as wide as body near nerve ring, gradually enlarging in its basal  $\frac{2}{3}$  to become three body widths long. Nerve ring a little behind middle of esophagus. Esophago-intestinal valve conoid-rounded.

Vulva transverse, vagina leading inward and backward. Ovary posterior, with a single row of oocytes. Esophagus-vulva distance shorter than length of esophagus and equal to or longer than tail length. Rectum a little over anal body width long. Tail elongate-conoid,  $14\text{--}16$  anal body widths long; tail terminus finely rounded; tail end appearing filiform.

Male: Body sharply curved in posterior region. Excretory pore seen on one individual at  $35 \mu$  from anterior end of body; amphids  $15 \mu$  behind excretory pore. Testis with few spermatocytes arranged in tandem, followed by elongate, spindle-shaped sperms filling the vas deferens. Spicules slender, almost straight,  $8 \mu$  or less than anal body width long. Supplements consisting of 3 ventromedian, preanal papillae located  $8$ ,  $12$ , and  $36 \mu$  anterior to cloaca. Tail about 11 times anal body width long; tail end finely rounded.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of banana plants in Vishakhapatnam, Andhra Pradesh, South India.

RELATIONSHIP: *Alaimus thrixus* n. sp. comes close to *A. parvus* Thorne, 1939 and *A. meylli* Andrassy, 1961. It differs from the former species in having a more slender body, a narrow, conical head and a longer tail measuring over ten times anal body width. From the later species it can be easily differentiated in having conical-narrow lip region, esophagus-



vulva distance equal to or longer than tail length and a shorter tail ( $c = 5.4$  in *A. meylli*).

*Alaimus hamulus* n. sp.  
(Fig. 3, A-H)

MEASUREMENTS: Ten females (paratypes): L = 0.95–1.20 mm; a = 54–64; b = 4.2–4.8; c = 11–15; V = 42–46.

Two males (paratypes): L = 1.0–1.2 mm; a = 58–62; b = 4.3–4.6; c = 10–11; T = 40–48.

Female (holotype): L = 1.0 mm; a = 60; b = 4.3; c = 12; V = 45.

DESCRIPTION: Female: Body ventrally arcuate, more so in posterior region, slightly spiral. Cuticle marked by fine transverse striae. Lateral hypodermal chords  $\frac{2}{3}$  as wide as body. Lip region conoid-rounded, smooth, continuous with body contour. Esophagus enlarging in its posterior fifth to become  $\frac{2}{3}$  as wide as body. Nerve ring a little posterior to middle of neck. Esophago-intestinal valve small, conoid. Excretory pore 25–28  $\mu$  from anterior end of body. Amphidial apertures about five body-widths from anterior end.

Vulva a transverse slit; vagina extending  $\frac{1}{3}$  into body. Anterior branch of reproductive system absent. Esophagus-vulva distance a little less than length of esophagus. Ovary elongate, reflexed. Rectum about anal body width long. Tail ventrally hooked, regularly tapering except at terminus where it abruptly narrows to a sharp point; 7–8 anal body widths long.

Male: Esophagus enlarging in its last quarter. Excretory pore 25  $\mu$  from anterior end. Spicules strongly cephalated, 10  $\mu$  long; a longitudinal partition wall dividing its lumen into two equal chambers (Fig. 3, E and G). Supplementary papillae five, anteriormost lying 75  $\mu$  and the posteriormost 7  $\mu$  in front of cloaca (Fig. 3, E). Tail cuticle in ventrolateral region swollen to give an impression of a bursa. Cloacal aperture oval, transverse, 4  $\mu$  long. Tail ventrally arcuate, about eight times anal body width long; terminus abruptly narrowed, pointed.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of *Pinus excelsa* at Simla (Punjab State), North India. Paratypes also collected from soil around roots of jack

tree at Rampur (District headquarters), U.P. and Apricot soil in Dehra Dun, U.P., India.

RELATIONSHIP: *Alaimus hamulus* n. sp. comes close to *A. glissus* Thorne, 1939 and *A. similis* Thorne, 1939. From the former it can be differentiated in having a more posterior amphid, esophagus not enlarging immediately behind nerve ring, a more posterior vulva and a ventrally hooked tail. It differs from *A. similis* in having a more robust anterior part of esophagus, a more posterior vulva, an abruptly pointed tail terminus and in being bisexual.

*Alaimus leptus* n. sp.  
(Fig. 3, I-L)

MEASUREMENTS: Five females (paratypes): L = 0.55–0.63 mm; a = 46–54; b = 3.7–4.2; c = 5.2–6.5; V = 43–45.

Female (holotype): L = 0.63 mm; a = 52; b = 3.7; c = 6; V = 44.

DESCRIPTION: Female: Body spirally coiled when relaxed by gentle heat. Cuticle smooth, striae not marked out. Lip region marked off from body by a depression, not much narrowed, anteriorly truncated. Amphids about 80  $\mu$  from anterior end of body. Esophagus about  $\frac{1}{3}$  as wide as body, enlarging at its last  $\frac{1}{6}$  to  $\frac{1}{4}$ ; enlarged part 20–24  $\mu$  by 6–8  $\mu$ . Nerve ring prominent, 100  $\mu$  from anterior end of body. Esophago-intestinal valve small.

Vulva a transverse slit, 6  $\mu$  or a little less than half the body width long; labia thick. Vulva-esophagus distance 48–58  $\mu$  less than the neck length and 12–15  $\mu$  more than tail length. Anterior branch of reproductive organs absent, posterior normal. Rectum cuticularized, about anal body width long, anus prominent. Tail cylindrical with little tapering, ventrally arcuate in a semicircle, 15 times anal body width long; tail terminus conoid-rounded.

Male: Not found.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of *Pinus excelsa* at Simla, Punjab State, North India.

RELATIONSHIP: *Alaimus leptus* n. sp. comes close to *A. parvus* Thorne, 1939 and *A. thrixus* n. sp. from both of which it differs in having an offset broadly truncated lip region and more cylindroid tail showing slight tapering. It has a wider head and smaller esophageal enlargement than *A. thrixus* n. sp.

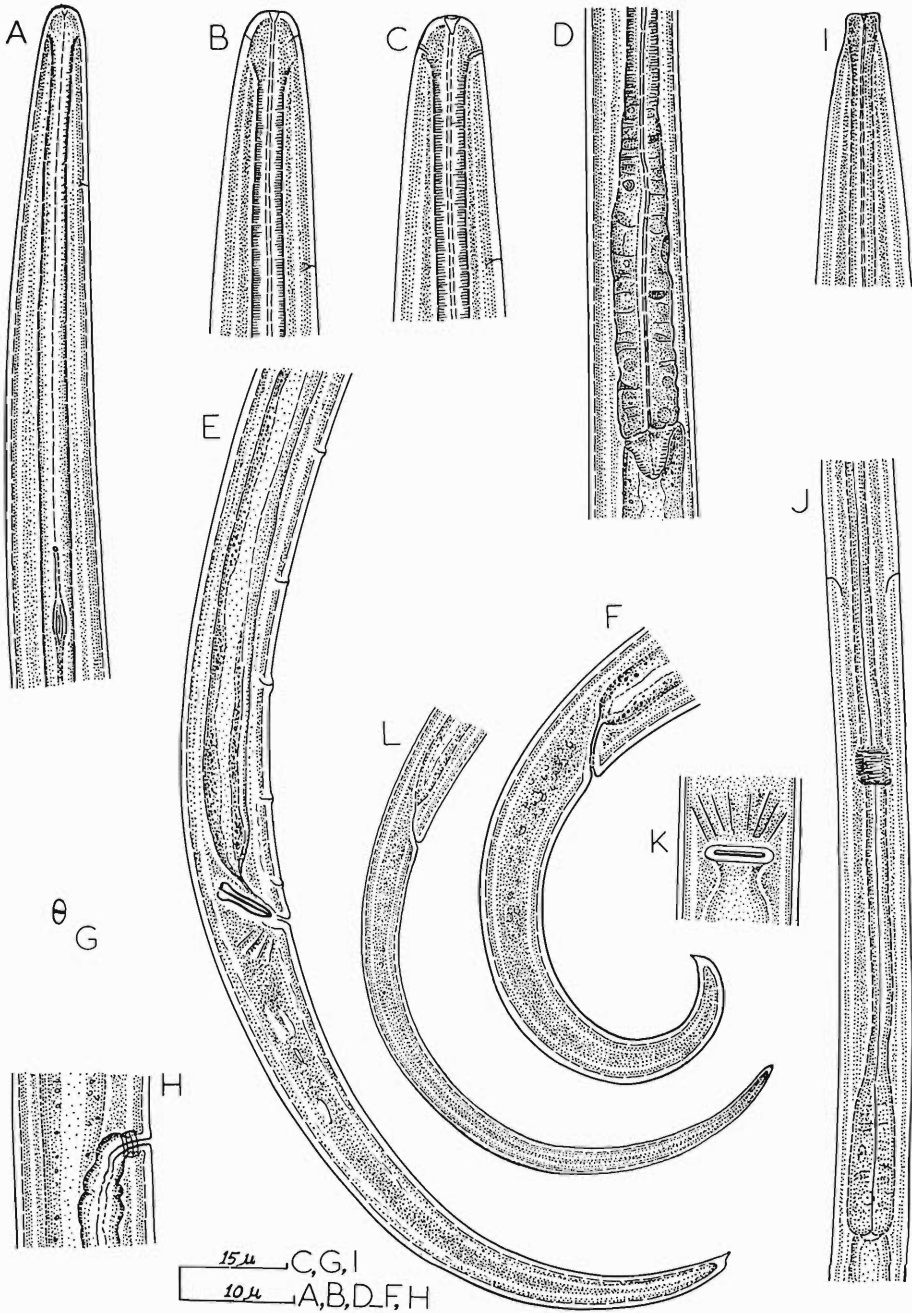


Figure 3, A-H. *Alaimus hamulus* n. sp. A. Anterior end of female. B. Head end of female. C. Head end of male. D. Esophageal base of female. E. Tail end of male. F. Tail end of female. G. Cross section through middle of spicule. H. Vulva, lateral. I-L. *Alaimus leptus* n. sp. I. Head end of female. J. Posterior portion of esophagus of female, ventral. K. Vulva, ventral. L. Tail end of female.

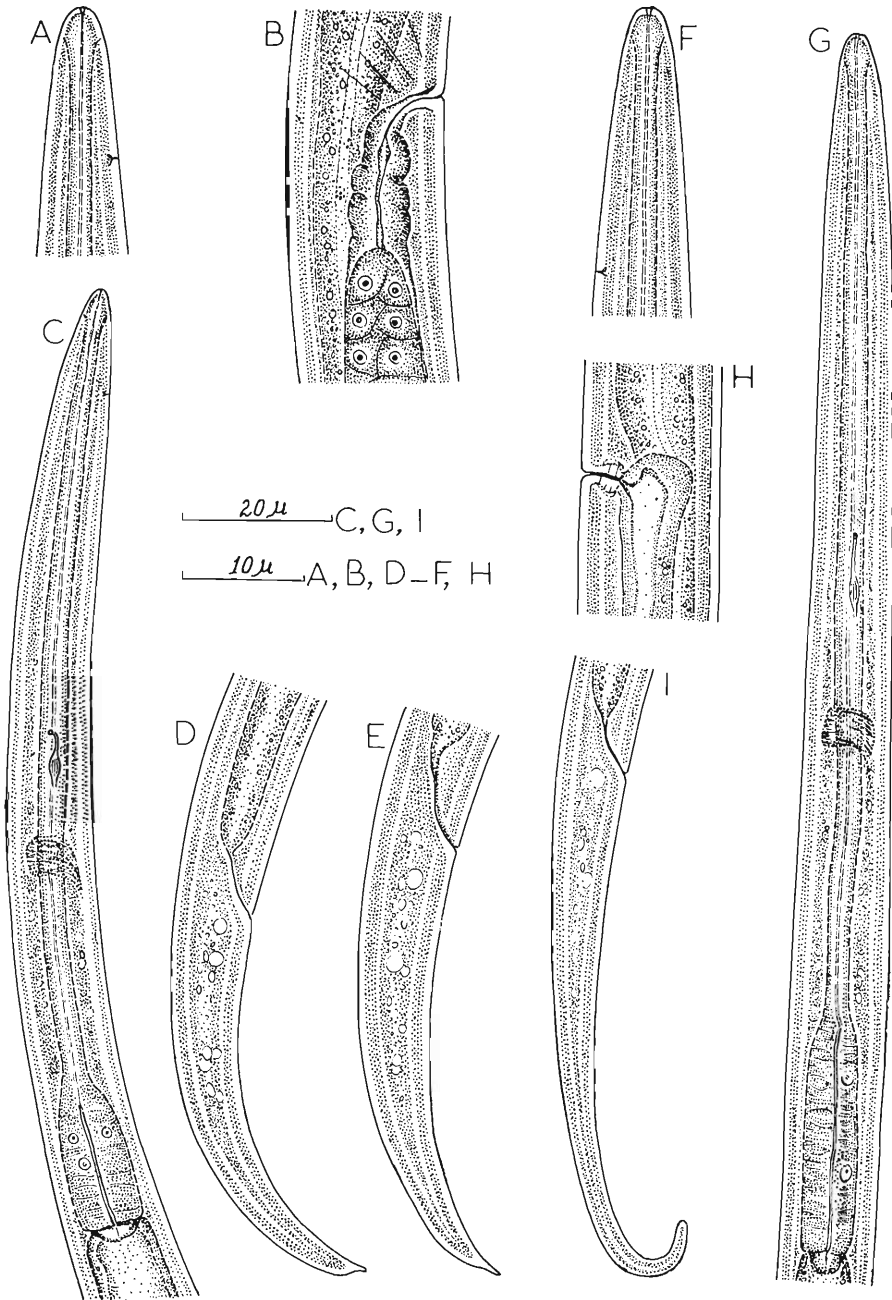


Figure 4, A-E. *Alaimus editorus* n. sp. A. Head end of female. B. Vulva, lateral. C. Esophagus of female. D, E. Tails of female. F-I. *A. medius* n. sp. F. Head end of female. G. Esophagus of female. H. Vulva, lateral. I. Tail of female.

*Alaimus editorus* n. sp.  
(Fig. 4, A-E)

MEASUREMENTS: Seven females (paratypes): L = 0.52-0.62 mm; a = 33-38; b = 4.3-4.5; c = 10-13; V = 45-47.

Female (holotype): L = 0.61 mm; a = 38; b = 4.3; c = 13; V = 47.

DESCRIPTION: Body curved ventrally to assume a "C" form. Cuticle moderately thick, marked by fine transverse striae. Lip region smoothly rounded, slightly tapering anteriorly, continuous with body contour. Excretory pore far forward, about three lip region widths from anterior end of body. Esophagus about  $\frac{2}{3}$  as wide as body near middle of neck, enlarging in its last  $\frac{1}{4}$  to  $\frac{1}{2}$ ; enlargement 18-23  $\mu$  by 10-12  $\mu$ . Nerve ring a little posterior to middle of neck. Amphidial pores about a body width anterior and hemizonid a little behind nerve ring. Intestine with wide lumen and small refractive granules.

Vulva transverse, leading into thick-walled vagina directed inward and backward. Reproductive organs single, posterior; ovary reflexed more than halfway back to vulva. Rectum about anal body width long, not prominently cuticularized, anus sometimes difficult to see. Tail uniformly tapering, only slightly ventrally arcuate, 5.5-6.5 times anal body width, tail end asymmetrical, rather abruptly narrowed to a pointed terminus.

Male: Not found.

TYPE HABITAT AND LOCALITY: Collected from soil around roots of mango trees, *Mangifera indica* L. at Bahraich, Eastern U.P., India.

RELATIONSHIP: Species related to *Alaimus editorus* n. sp. are *A. minor* Cobb, 1893; *A. acutus* Thorne, 1939 and *A. striatus* Loof, 1964. From *A. minor* it differs in having a shorter and narrower esophagus (b = 3 in *A. minor*) and longer tail. From *A. acutus* it can be differentiated in having a shorter body, more posteriorly located vulva and less sharply tapering tail. It also differs from *A. striatus* in the absence of longitudinal striae and the tail having an asymmetrical, pointed terminus.

*Alaimus medius* n. sp.  
(Fig. 4, F-I)

MEASUREMENTS: Five females (paratypes): L = 0.64-0.72 mm; a = 43-47; b = 3.7-3.9; c = 7-8; V = 41-43.

Female (holotype): L = 0.7 mm; a = 44; b = 3.8; c = 7.7; V = 42.

DESCRIPTION: Body only slightly ventrally arcuate, a little more in the posterior region. Cuticle smooth, with fine, faint transverse striae. Lateral hypodermal chords granulated,  $\frac{1}{4}$  as wide as body. Lip region rounded, slightly tapering anteriorly, continuous with body contour, labial papillae indistinct. Esophagus about  $\frac{1}{2}$  as wide as body near midneck, enlarging in its last fifth to a cylindrical bulb about twice body width long. Nerve ring prominent, a little behind middle of esophagus. Amphidial apertures at  $\frac{2}{3}$  neck length. Excretory pore 23  $\mu$  or about six head widths from anterior end of body.

Vulva a transverse slit, vagina short, with thick cuticular lining. Only posterior branch of reproductive organs present. No spermatozoa in uterus. Rectum anal body width long, not prominently cuticularized. Tail regularly tapering to a rounded terminus, distal end hooked ventrally (Fig. 4, I).

Male: Not found.

TYPE HABITAT AND LOCALITY: Soil around roots of *Pinus longifolia* at Simla, Punjab State, North India.

RELATIONSHIP: *Alaimus medius* n. sp. is related to *A. glissus* Thorne, 1939, *A. arcuatus* Thorne, 1939, and *A. parvus* Thorne, 1939. From *A. glissus* and *A. arcuatus* it differs in having a shorter body, longer neck, a shorter enlargement of esophagus and longer tail (esophagus enlarging behind nerve ring, c = about 12 in *A. glissus* and *A. arcuatus*). From *A. parvus* it can be differentiated in having a relatively longer neck, more posterior excretory pore and a hooked, round-ended tail.

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### On the Status of the Genera of the Superfamily Aphelenchoidea (Fuchs, 1937) Thorne, 1949 with the Descriptions of Six New Species of Nematodes from India<sup>1</sup>

S. ISRAR HUSAIN AND ABRAR M. KHAN

The superfamily Aphelenchoidea (Fuchs, 1937) Thorne, 1949 is represented by four families. The family Aphelenchidae with the only representative, *Aphelenchus* Bastian, 1865 Anomyctidae with *Anomyctus* Allen, 1940; Paraphelenchidae with *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925 and *Metaphelenchus* Steiner, 1943, and the family Aphelenchooididae is represented by sixteen genera. The latter includes extremely varied groups of nemas, among them are obligate plant parasites, mycophages, predators and insect parasites. The genera *Seinura* Fuchs, 1931 and *Paraseinura* Timm, 1961 of the family Aphelenchooididae are quite distinctive by virtue of having long, attenuated to filiform tails without a terminal mucron and hence a new subfamily Seinurinae is proposed for the accommodation of these two genera. Besides the genus *Rhadinaphelenchus* Goodey, 1960, is highly distinctive because of very slender body, massive sclerotization of the labial arches, elongated median bulb, wide vulvar-flap, unusually curved vagina, shape of the spicules and sclerotized spadelike extension of the male tail. In view of these characters a new subfamily Rhadinaphelenchinae in the family Aphelenchooididae is proposed for the reception of the genus *Rhadinaphelenchus*.

#### A KEY TO THE SUBFAMILIES OF APHELENCHOIDIDAE

1. Body exceedingly slender; labial arches with massive sclerotization; vagina unusually curved, vulvar-flap wide ..... Rhadinaphelenchinae n. subfam.  
Body not exceedingly slender; labial arches without massive sclerotization; vagina not much curved ..... 2
2. Tail long, attenuated to filiform without a terminal mucron .....  
..... Seinurinae n. subfam.  
Tail short, conical with or without a terminal mucron .....  
.... Aphelenchooidinae Skarbilovich, 1947

#### Subfamily Rhadinaphelenchinae n. subfam.

DIAGNOSIS: Aphelenchooididae: very slender nematodes. Labial arches strongly sclerotized. Median bulb elongated. Vulvar-flap wide. Vagina unusually curved. Male tail extended into sclerotized spadelike extension. Spicules slightly arcuate with prominent rostrum, ventral element of spiculum extended back over posterior portion, an arrangement which differs from that found in other genera of the family.

TYPE AND ONLY GENUS: *Rhadinaphelenchus* Goodey, 1960. Subfamily Aphelenchooidinae Skarbilovich, 1947.

<sup>1</sup> Contribution from the Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

DIAGNOSIS: Aphelenchoididae: Skarbilovich, 1947.

DIAGNOSIS: Aphelenchoididae: Tails short, conical, never long and filiform, with or without a terminal mucron. Esophageal glands forming long lobes overlapping the intestine, lying separate, dorsally in the body. Male tail without bursa. Spicules more or less rose-thorn-shaped. Gubernaculum absent.

TYPE GENUS: *Aphelenchoides* Fischer, 1894. Syn. (*Pathoaphelenchus*) Cobb, 1927.

OTHER GENERA: *Megadorus* Goodey, 1960; *Bursaphelenchus* Fuchs, 1937; *Cryptaphelenchus* (Fuchs, 1915) Ruhm, 1956; *Cryptaphelenchoides* Goodey, 1960; *Laimaphelenchus* Fuchs 1937; *Tylaphelenchus* Ruhm, 1956; *Ektaphelenchus* (Fuchs, 1937) Skrjabin et al., 1954; *Parasitaphelenchus* Fuchs, 1929; *Peraaphelenchus* Wachek, 1955; *Entaphelenchus* Wachek, 1935, *Schistonchus* Cobb, 1927 and *Ruehmaphelenchus* Goodey, 1963.

*Aphelenchoides absari* n. sp.

(Fig. 1, A-C)

FEMALES (10): L = 0.39-0.45 mm; a = 30-33; b = 4.0-4.5; c = 16-20; V = 69-77%; spear = 11-13  $\mu$ .

MALES (10): L = 0.32-0.43 mm; a = 30-35; b = 4.3-5.2; c = 12-17; spear = 11-13  $\mu$ ; spicules = 15-19  $\mu$ .

DESCRIPTION: Body cylindrical, ventrally arcuate when relaxed by gentle heat, tapering on both extremities. Cuticle finely annulated. Lateral field marked by four incisures. Lip region distinctly set off by a constriction, cap-like. Spear without basal knobs, measuring 11-13  $\mu$  in length. Procorpus a slender tube ending in an oval valvulated median bulb. Isthmus short, encircled by a nerve ring, just below the median bulb. Excretory pore at the level of nerve ring. Hemizonid not seen. Esophageal glands overlapping the intestine ventrolaterally. Intestine granular. Rectum short. Anus distinct. Tail three times the anal-body width long, convex-conoid with an obscure mucron.

Vulva postequatorial. Ovary single, prodelphic, outstretched with oocytes arranged in a single file except for a short region of multiplication. Postuterine sac absent.

Males similar to females in appearance. Testis single, outstretched. Spermatocytes

serially arranged. Spicules paired, typically aphelenchoid type, 15-19  $\mu$  in length. Three pairs of post anal subventral papillae present. One pair adanal, one pair midway along the tail and the third pair near the tail terminus. Tail as in females but with distinct mucron, 3-4  $\mu$  long.

HOLOTYPE: Female, slide No. 991, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

ALLOTYPE: Male, slide No. 991-A, collected with the female; other data same as for holotype.

PARATYPE: Twelve females and nine males with the authors.

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

TYPE LOCALITY: Bulandshahr, U.P., India.

DIAGNOSIS AND RELATIONSHIP: *Aphelenchoides absari* n. sp. comes closer to *A. singhi* Das, 1960, *A. brevionchus* Das, 1960, *A. brevicaudatus* Das, 1960 and *A. parietinus* Bastian, 1865. It differs from all the four species in possessing spear without knobs and further from *A. brevicaudatus* and *A. brevionchus* in the size of spear. It differs from *A. parietinus* in the presence of males, absence of postuterine sac and more posteriorly located vulva in females (V = 66-70, Franklin, 1955).

*Aphelenchoides andrassyi* n. sp.

(Fig. 1, D, E)

FEMALES (3): L = 0.39-0.44 mm; a = 23-28; b = 3.2-3.9; c = 6-12; V = 61-67%; spear = 9-10  $\mu$ .

DESCRIPTION: Body cylindrical, ventrally arcuate on death, tapering on both extremities. Cuticle finely annulated. Lateral field marked by three incisures. Lip region distinctly set off by a constriction. Spear short, without basal knobs, 9-10  $\mu$  long. Procorpus a slender tube. Median bulb oval with well-developed valvular apparatus. Isthmus short, encircled by nerve ring. Excretory pore and hemizonid not observed. Esophageal glands overlapping intestine. Intestine granular. Anus distinct. Rectum half the vulvar-body width long. Tail long elongate-conoid, ventrally curved, nearly seven times the anal-body width long, regularly tapering to a rounded terminus provided with star-shaped mucron.

Vulva postequatorial. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Oval spermatheca present. Postuterine sac long nearly three times the vulvar-body width long.

Males not found.

**HOLOTYPE:** Female, Slide No. 993, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**PARATYPE:** Two females with the authors.

**TYPE HABITAT:** Soil around the roots of *Hibiscus rosasinensis* L.

**TYPE LOCALITY:** University Campus, Aligarh Muslim University, Aligarh.

**DIAGNOSIS AND RELATIONSHIP:** *Aphelenchoides andrassyi* n. sp. comes closer to *A. asterocaudatus* Das, 1960 and *A. nonvielleri* Andrassy, 1959. It differs from both of them in the smaller body size, unknobbed spear and in the position of vulva. It further differs from *A. nonvielleri* in the shape of tail mucron.

*Aphelenchoides chinensis* n. sp.

(Fig. 1, F-H)

**FEMALES (8):** L = 0.38–0.55 mm; a = 25–30; b = 7.9–11.4 (measured up to median bulb); c = 14–19; V = 67–70%; spear = 12–15  $\mu$ .

**MALES (2):** L = 0.44–0.50 mm; a = 26; b = 7.9–8.9; c = 13–16; spear = 13–14  $\mu$ ; spicules = 19–22  $\mu$ .

**DESCRIPTION:** Body cylindrical, ventrally arcuate when relaxed by gentle heat, tapering on both extremities. Cuticle finely annulated. Lateral field marked by six incisures, outer ones being crenate, inner ones faint. Lip region distinctly set off by a constriction. Spear 12–15  $\mu$  long, without basal knobs. Procorpus a slender tube ending in a hemispherical median bulb, valvular apparatus of the median bulb distinctly well developed. Isthmus short, encircled by a nerve ring just after the median bulb. Excretory pore nearly one median bulb-width behind the median bulb. Esophageal glands overlapping the intestine dorsally. Rectum more than the anal-body width long. Tail convex-conoid, usually ventrally curved, slightly less than four times the anal-body width long, regularly tapering to a rounded terminus with a single ventral mucron.

Vulva postequatorial. Ovary single, pro-

delphic, outstretched with oocytes arranged in a single row except for a short region of multiplication, spermatheca not seen. Postuterine sac nearly three times the vulvar-body width long.

Males similar to females in appearance. Testis single, outstretched. Spermatocytes serially arranged. Spicules paired, typically aphelenchoid in shape, sharply arcuate with a conspicuous ventral apex. Three pairs of postanal subventral papillae present. One pair adanal, one pair midway along the tail and a third pair near the tail terminus.

**HOLOTYPE:** Female, Slide No. 990, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**ALLOTYPE:** Male, Slide No. 990-A, collected with the female, other data same as for holotype.

**PARATYPE:** Seven females and one male with the authors.

**TYPE HABITAT:** Soil around the roots of *Litchi chinensis* Sonner.

**TYPE LOCALITY:** Dehra Dun, U.P., India.

**DIAGNOSIS AND RELATIONSHIP:** *Aphelenchoides chinensis* n. sp. comes closer to *A. subtenuis* Cobb, 1926, but differs from it in the much smaller body size, position of vulva (V = 78% in *A. subtenuis*) more slender and attenuated body and longer tail (c = 22–23 in *A. subtenuis*).

*Aphelenchoides jacobi* n. sp.

(Fig. 2, A-D)

**FEMALES (5):** L = 0.36–0.49 mm; a = 27–32; b = 3.4–4.7; c = 9–12; V = 65–71%, spear = 12–14  $\mu$ .

**MALES (2):** L = 0.35–0.41 mm; a = 27–32; b = 3.6–4.5; c = 11–15; spear = 13–14  $\mu$ ; spicules = 13–15  $\mu$ .

**DESCRIPTION:** Body cylindrical, ventrally curved when relaxed by gentle heat, tapering on both extremities, cuticle finely annulated. Lateral field marked by three incisures. Lip region set off from the body contour by a distinct constriction, caplike, faintly annulated. Spear without basal knobs, 13–14  $\mu$  in length. Procorpus a slender tube. Median bulb oval with well-developed valvular apparatus. Isthmus short, encircled by a nerve ring. Excretory pore just below the level of nerve ring. Hemi-

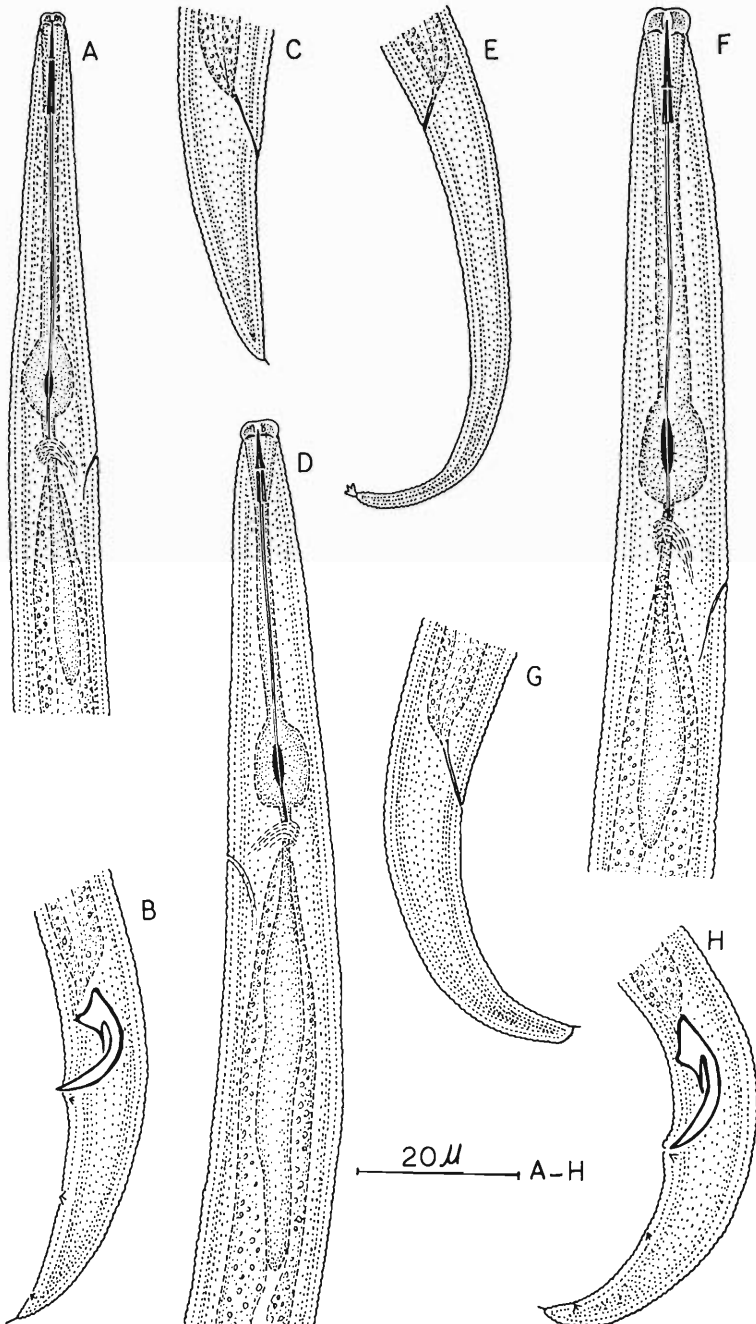


Figure 1, A-C. *Aphelenchoides absari* n. sp. A. Esophageal region; B. Male tail; C. Female tail; D-E. *Aphelenchoides andrassyi* n. sp. D. Esophageal region; E. Female tail; F-H. *Aphelenchoides chinensis* n. sp. F. Esophageal region; G. Female tail; H. Male tail.



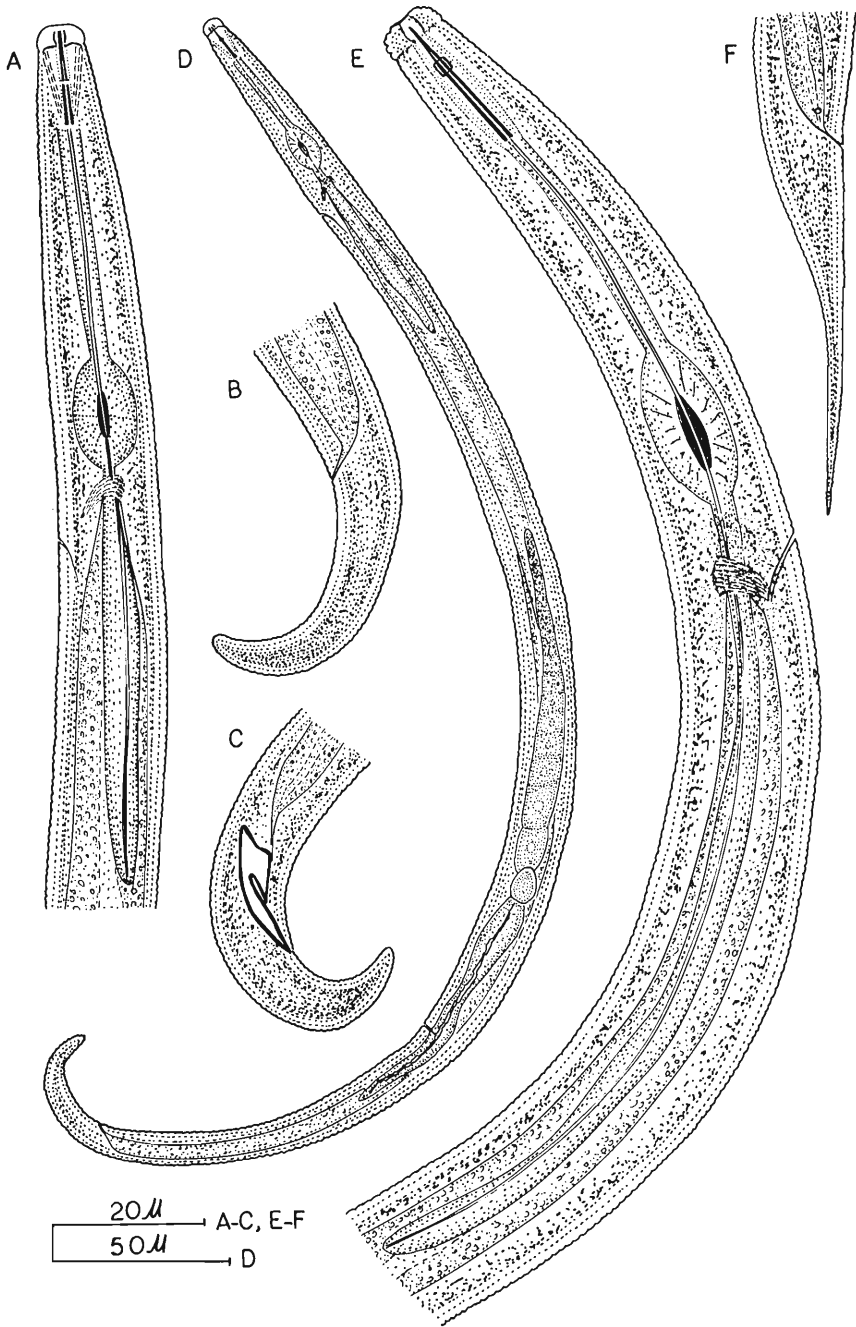


Figure 2, A-D. *Aphelenchoides jacobi* n. sp. A. Esophageal region; B. Female tail; C. Male tail; D. Entire female; E-F. *Seinura oostenbrinki* n. sp. E. Esophageal region; F. Female tail.

zonid not observed. Esophageal glands overlapping intestine. Intestine packed with granules. Anus prominent. Rectum short, half the anal body width long. Tail nearly four anal-body widths long, dorsally convex-conoid, ventrally curved with a rounded terminus lacking a mucron.

Vulva postequatorial. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Elongate pouchlike spermatheca present. Postuterine sac slightly less than two anal-body widths long.

Males similar in appearance to females with shorter tail which is less than three anal-body widths long. Testis single, outstretched spermatocytes serially arranged. Spicules paired, typically aphelenchoid type. Two pairs of postanal subventral papillae present. One pair adanal and the second at midtail length.

HOLOTYPE: Female, slide No. 992, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

ALLOTYPE: Male, slide No. 992-A, collected with the female, other data same as for holotype.

PARATYPE: Four females and one male with the authors.

TYPE HABITAT: Soil around the roots of *Hibiscus-rosa sinensis* L.

TYPE LOCALITY: University Campus, Aligarh Muslim University, Aligarh.

DIAGNOSIS AND RELATIONSHIP: In view of the tail shape *Aphelenchoides jacobii* n. sp. comes closer to *A. abyssinicus* Filipjev, 1931 and *A. goeldi* Steiner, 1914, but it differs from the former in much smaller body size and more posteriorly located vulva while from the latter in longer more slender body with longer tail and in more posteriorly located vulva ( $V = 66\%$  in *A. goeldi*).

#### Subfamily Seinurinae n. subfam.

DIAGNOSIS: Aphelenchoididae: Tail long, elongate to filiform without a terminal mucron. Head continuous or set-off. Stylet usually long and slender, anterior pointed part is jointed in *Paraseinura*; lumen of spear relatively wide. Median bulb elliptical, oblong or long oval with prominent valve. Ovary single, prodelphic. Postuterine sac present or absent. The spicules are somewhat differently shaped from

those of *Aphelenchoides*; the proximal end of the transverse bar prolonged with dorsal limb into a prominent apex, and there is always a prominent rostrum at the other end of the transverse bar. Male tail with papillae. Gubernaculum present in *Paraseinura*.

TYPE GENUS: *Seinura* Fuchs, 1931.

OTHER GENUS: *Paraseinura* Timm, 1961.

#### *Seinura oostenbrinki* n. sp.

(Fig. 2, E-F)

FEMALES (15): L = 0.51–0.70 mm; a = 30–35; b = 8.0–10.0 (measured upto median bulb); c = 9–12; V = 73–83%; spear = 19–22  $\mu$ .

DESCRIPTION: Body cylindrical, slightly ventrally curved when relaxed by gentle heat, tapering on both extremities, finely annulated. Lip region with three to four annules, distinctly set off by a constriction, caplike, measuring 8 by 4  $\mu$ . Cephalic framework obscure. Spear 19–22  $\mu$  long, without basal knobs but with distinct and broad lumen. Small spear guide present just above the middle of spear. Corpus a slender tube ending in a median bulb twice as long as wide with a well-developed valve. Nerve ring about half a median bulb length behind the median bulb. Esophageal glands forming long lobes. Excretory pore near the anterior edge of the nerve ring, 70–95  $\mu$  from the anterior end. Hemizonid prominent, 15–20  $\mu$  behind the excretory pore. Intestinal lumen broad and distinct. Intestine granular. Anus distinct. Rectum short. Tail long and attenuated, nearly five times the anal-body width long, abruptly narrowing after slightly more than  $\frac{1}{3}$  of its length from the anterior end to filiform shape terminating into a finely pointed terminus. Phasmids slightly preanal.

Vulva prominent, vagina at right angles to the body axis, short, extending only  $\frac{1}{3}$  of the vulvar-body width. Ovary single, prodelphic, outstretched. Oocytes arranged in two rows. Spermatheca absent. Postuterine sac  $\frac{1}{3}$  to slightly more than  $\frac{1}{2}$  vulvar-body width long.

Males not found.

HOLOTYPE: Female, Slide No. 642, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

PARATYPE: Thirty females in the collection of the authors.

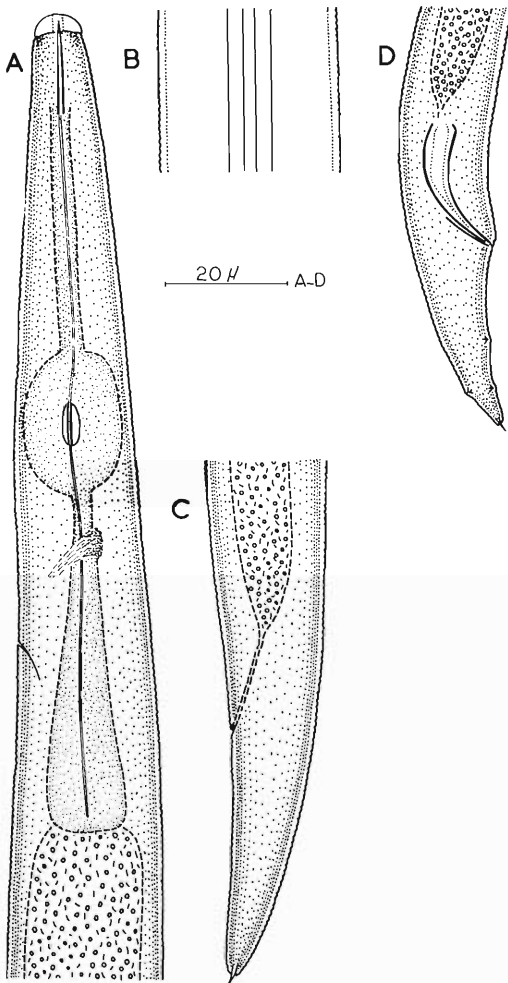


Figure 3, A-D. *Paraphelenchus sacchari* n. sp. A. Esophageal region; B. Lateral field; C. Female tail; D. Male tail.

**TYPE HABITAT:** Soil around the roots of *Allium cepa* L.

**TYPE LOCALITY:** Aligarh.

**DIAGNOSIS AND RELATIONSHIP:** *Seinura oostenbrinki* n. sp. comes closer to *Seinura diversa* (Paesler, 1957) Goodey, 1960 but differs from it in the smaller and more slender body and in tail length less than vulva-anus distance (tail length = 1½ times the vulva-anus distance in *S. diversa*).

*Paraphelenchus sacchari* n. sp.  
(Fig. 3, A-D)

**FEMALES** (5): L = 0.59–0.88 mm; a = 30–39; b = 5.1–6.6; c = 20–21; V = 68–77%; spear = 11–16 μ.

**MALES** (2): L = 0.61–0.66 mm; a = 39; b = 5.1–5.2; c = 21; spear = 14 μ; spicules = 25 μ; gubernaculum = 10–11 μ.

**DESCRIPTION:** Body cylindrical, assuming ventrally arcuate shape when relaxed by gentle heat. Cuticle distinctly annulated, subcuticle finely annulated. Lateral field marked by four crenate incisures, occupying 1/5 of the body width. Head flat, caplike offset by a constriction, measuring 7 μ in width and 3 μ in height. Spear short, 11–16 μ long, without basal swellings. Corpus a slender tube joining well-developed ovoid median bulb with strongly developed crescentic valves. Basal bulb elongate-pyriform, obscure. Nerve ring crossing the isthmus above the level of excretory pore. Excretory pore 95–105 μ from the anterior end. Intestine granular. Rectum distinct, nearly one anal-body width long. Tail dorsally convex-conoid with a distinct mucron. Tail tip round. Phasmid 8 μ anterior to the level of anus.

Vulva a transverse slit. Ovary single, prodelphic, outstretched. Oocytes arranged in a single file except for a short region of multiplication. Postuterine sac distinct, nearly three times the vulvar-body width long, extending 1/3 to nearly 1/2 the vulva-anus distance.

Males similar to females in general shape and appearance. Testis single, outstretched. Spermatocytes serially arranged. Spicules paired, separate, arcuate, and cephalated, 25 μ long. Gubernaculum simple, 10–11 μ in length. Papillae as shown in Figure 3, D.

**HOLOTYPE:** Female, slide No. 972, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**ALLOTYPE:** Male, slide No. 972-A, collected with the females. Other data same as for holotype.

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

**TYPE LOCALITY:** Bulandshahr, U.P., India.

**DIAGNOSIS AND RELATIONSHIP:** *Paraphelenchus sacchari* n. sp. comes closer to *P. basili*

Das, 1960 but differs from it in the larger body size and shorter tail.

#### SUMMARY

Two new subfamilies Seinurinae and Rhadinaphelenchinae are proposed in the family Aphelenchoididae and a key to the subfamilies of the family Aphelenchoididae is presented. Four new species of *Aphelenchoides* Fischer, 1894, one each of *Seinura* Fuchs, 1931 and *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925 are described and figured. They are *Aphelenchoides absari* n. sp., *A. andrusstyi* n. sp., *A. chinensis* n. sp., *A. jacobi* n. sp., *Seinura oostenbrinki* n. sp., and *Paraphelenchus sacchhari* n. sp.

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## A New Subfamily, A New Subgenus and Eight New Species of Nematodes from India Belonging to Superfamily Tylenchoidea<sup>1</sup>

S. ISRAR HUSAIN AND ABRAR M. KHAN

The genera belonging to the family Tylenchidae differ greatly with respect to sclerotization of the cephalic framework. In *Tylenchus*, *Psilenchus*, and *Macrotrophurus* cephalic sclerotization is absent; in *Trophurus*, *Pseudhalenchus*, and *Ditylenchus*, weak sclerotization is present; and in *Tylenchorhynchus* and *Telotylenchus* it is quite conspicuous. It therefore appears that there is a gradual evolution of the cephalic sclerotization in the family Tylenchidae leading to the genera of the families Hoplolaimidae and Pratylenchidae with well-developed sclerotization. Moreover, the presence of two ovaries, large enveloping bursa, large size of phasmids, and conspicuous head sclerotization of the genera *Tylenchorhynchus* and *Telotylenchus* show affinities with the members of Hoplolaimidae.

Siddiqi (1960) described the genus *Telotylenchus*, which he placed along with *Pseudhalenchus* Tarjan, 1958 in a new subfamily Telotylenchinae. Loof (1963), rightly questioned the creation of this subfamily as the two genera in question exhibit resemblance with respect to basal esophageal region only, a character not enough to warrant the creation of a separate subfamily. *Telotylenchus* resembles *Tylenchorhynchus* more than it resembles *Pseudhalenchus* with regard to the shape of head and spear, relative dimensions of the various body parts, number and form of ovaries, tail shape in both sexes, details of male reproductive organs and the large size of phasmids. Therefore, *Telotylenchus* is better placed with *Tylenchorhynchus* and *Pseudhalenchus* with *Ditylenchus* which it closely resembles.

Recently Eliava (1964) created a new subfamily Tylenchorhynchinae under Hoplolaimidae to accommodate *Tylenchorhynchus* because of its conspicuous head sclerotization, well-developed stylet with parallel protractor muscles, raylike phasmids, and large envelop-

ing bursa. From the recent account on the morphology of 68 species of the genus *Tylenchorhynchus* given by Tarjan (1964), 45 species possess inconspicuous head sclerotization, 8 species moderately conspicuous head sclerotization and 11 species very conspicuous head sclerotization. Furthermore the genera having two ovaries like *Psilenchus* and *Macrotrophurus*, well-developed stylet like *Macrotrophurus* and *Tetylenchus*, and large enveloping bursa like *Trophurus*, *Macrotrophurus*, *Neoditylenchus*, and *Sychnotylenchus* are found in Tylenchidae also as in Hoplolaimidae. Although the majority of the species of the genus *Tylenchorhynchus* possess relatively conspicuous phasmids *T. microphasmis* Loof, 1959 possesses a relatively small phasmid, a characteristic feature of Tylenchidae.

Moreover, Eliava (1964) failed to include *Telotylenchus*, a closely related genus to *Tylenchorhynchus* under Tylenchorhynchinae. In view of these facts the subfamily Tylenchorhynchinae should better be placed under Tylenchidae as a link between the families Tylenchidae, Hoplolaimidae, and Pratylenchidae. An amended diagnosis of Tylenchorhynchinae is therefore presented.

Linford and Oliveira (1940) proposed the genus *Rotylenchulus* which along with *Nacobus* Thorne and Allen, 1944 was placed in the newly created subfamily Nacobbinae under the family Pratylenchidae. Allen (1960) on the other hand suggested that *Rotylenchulus* should be placed near *Helicotylenchus* Steiner, 1945 in the family Hoplolaimidae and that *Nacobbus* in the subfamily Nacobbinae of the family Pratylenchidae. Similar views were expressed by Siddiqi (1963) with respect to the genus *Nacobbus*. The authors while generally agreeing with Allen (1960) and Siddiqi (1963) feel that in view of the saccate body of the mature female of the genus *Rotylenchulus* and the nature of its parasitism, this genus does not fit in the existing subfamilies of the family Hoplolaimidae and therefore a new subfamily Rotylenchulinae is proposed under Hoplolaim-

<sup>1</sup>Contribution from the Section of Plant Pathology, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

idae to include the genus *Rotylenchulus*. A key to the subfamilies of Hoplolaimidae is presented.

SUBFAMILY TYLENCHORHYNCHINAE  
ELIAVA, 1964.

DIAGNOSIS: Tylenchidae: Lip region continuous or set off by a constriction. Head skeleton fairly well developed. Lateral field with four to six incisures. Cuticle distinctly annulated. Phasmids large; deirids indistinct; amphids porelike near the lateral lips. Spear well developed and knobbed. Terminal portion of esophagus glandular, overlapping the intestine or set-off bulb. Gonads paired, opposed, outstretched. Female tail short and rounded. Bursa enveloping male tail.

TYPE GENUS: *Tylenchorhynchus* Cobb, 1913.

OTHER GENUS: *Telotylenchus* Siddiqi, 1960.

SUBFAMILY TYLENCHINAE DE MAN, 1876

DIAGNOSIS: Tylenchidae: Lip region smooth or striated. Head skeleton slight or absent. Lateral field marked with incisures. Cuticle finely or coarsely annulated. Amphids usually porelike, located near the lateral lips or sometimes elliptical slits placed below the level of lateral lips. Terminal portion of esophagus a definite bulb, sometimes lobed behind and extending slightly over the intestine (glandular, overlapping the intestine in *Pseudhalenchus*). Gonad single or paired; if single, short postuterine sac present. Tail long filiform, convex-conoid to broadly rounded or sometimes with clavate terminus. Bursa adanal, subcaudal or caudal.

TYPE GENUS: *Tylenchus* Bastian, 1865.

OTHER GENERA: *Ditylenchus*, *Neoditylenchus*, *Sychnotylenchus*, *Psilenchus*, *Macrotyrophurus*, *Anguina*, *Paranguina*, *Trophurus*, *Chitinytylenchus*, *Tetylenchus*, *Tylodorus*.

The authors have proposed a new subgenus *Ottolenchus* under the genus *Tylenchus* Bastian, 1865.

*Tylenchus* subgenus  
*Ottolenchus* n. subgen.

DIAGNOSIS: *Tylenchus*: Small sized nematodes. Body cuticle strongly annulated. Lateral field with only two crenate incisures, running parallel to the body till anus or cloaca.

Head rounded with a slight depression at the base of lip region, without clear annulations. Median bulb oval. Males with moderately developed distinctly crenate bursa. Spicules and gubernaculum tylenchoid. Tail long and filiform with acute terminus which is often recurved.

TYPE SPECIES: *Tylenchus* (*Ottolenchus*) *equisetus* n. subgen., n. sp.

*Tylenchus* (*Ottolenchus*) *equisetus*  
n. subgen., n. sp.  
(Fig. 1, A-D)

FEMALES (12): L = 0.38–0.47 mm; a = 27–28; b = 5.4–6.5; c = 4.4–4.6; V = 58–62%; spear = 12–14  $\mu$ .

MALES (8): L = 0.40–0.48 mm; a = 30–35; b = 5.3–6.3; c = 3.5–4.7; spear = 12–14  $\mu$ ; spicules = 15–17  $\mu$ ; gubernaculum = 5–6  $\mu$ .

DESCRIPTION: Body cylindrical, open C-shaped when relaxed by gentle heat, tapering on both extremities. Cuticle and subcuticle strongly annulated. Head flat and rounded with a slight depression at the base of the lip region. Spear with rounded basal knobs, 12–14  $\mu$  in length. Orifice of the dorsal esophageal gland close to spear base. Procorpus a slender tube ending in an oval valvulated median bulb. Isthmus long and slender, encircled by a nerve ring. Excretory pore situated at 68–72  $\mu$  from the anterior end of the body. Basal esophageal bulb pyriform with three gland nuclei, set off from the intestine. Cardia rounded. The distance from the anterior end of the body to the center of the median bulb is slightly less than the distance from the latter to the base of esophagus.

Vulva postequatorial with reduced lateral cuticular flap. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Oval spermatheca present. Postuterine sac short, half the vulvar-body width long. Tail long and filiform with acute terminus, 12–14 times the anal-body widths long, tip often recurved. Lateral field marked by two strongly crenate incisures, running parallel to the body upto the anus or cloaca.

Males similar to females in shape and appearance. Body more slender than females. Testis single, outstretched. Spicules paired, tylenchoid 15–17  $\mu$  in length when measured along the curved median line, ventrally curved

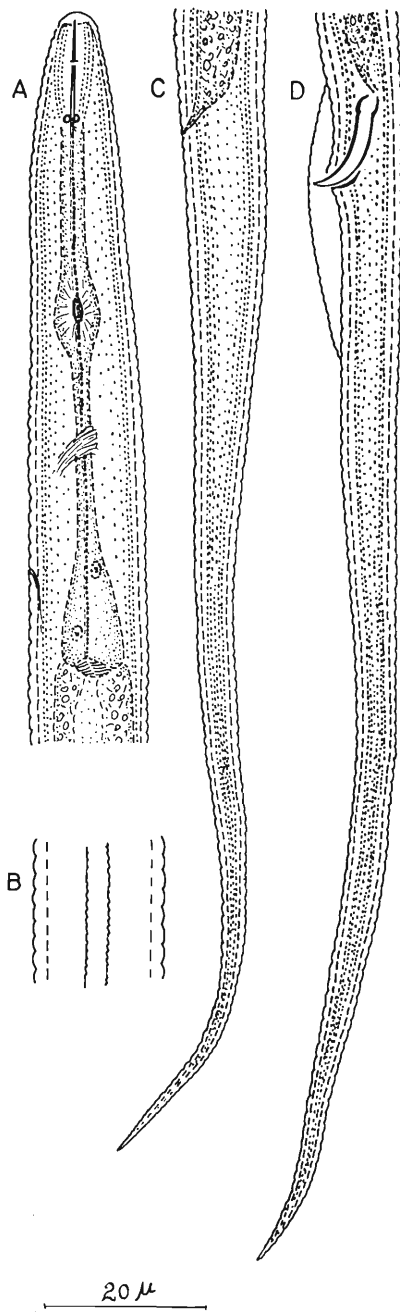


Figure 1, A-D. *Tylenchus (Ottolenchus) equisetus* n. subgen., n. sp. A. Esophageal region of female; B. Lateral field; C. Female tail; D. Male tail.

and cephalated, Gubernaculum simple, 5-6  $\mu$  long. Bursa moderately developed, distinctly crenate, originating at the level of the head of the spicules and terminating at more than two cloacal-body diameters behind the cloaca, nearly four times the cloacal-body width long. Tail 14-16 times the cloacal-body widths long with acutely pointed terminus. Phasmids post-anal, nearly one cloacal-body width behind the cloaca.

HOLOTYPE: Female, Slide No. 101, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

ALLOTYPE: Male, Slide No. 101-B, collected with the females; other data same as for holotype.

PARATYPES: Twenty-five females and 15 males in the collection of the authors.

TYPE HABITAT: Soil around the roots of *Casuarina equisetifolia* Forst.

TYPE LOCALITY: University Campus, Aligarh Muslim University, Aligarh, U.P., India.

DIAGNOSIS AND RELATIONSHIP: *Tylenchus* subgen., *Ottolenchus* n. subgen. resembles *T.* subgen. *Miculenchus* Andrassy, 1959 and *T.* subgen. *Aglenchus* Andrassy, 1954. It differs from the former in possessing moderately developed bursa in males (bursa absent in *Miculenchus*) and lip region not clearly annulated while from the latter in possessing oval median bulb (median bulb round in *Aglenchus*) and only two strongly crenate incisures in the lateral field.

*Tylenchus (Lelenchus) mirus* n. sp.  
(Fig. 2, A-D)

FEMALES (4): L = 0.36-0.42 mm; a = 32-35; b = 5.4-6.9; c = 3.7-4.8; V = 63-64%; spear = 10-11  $\mu$ .

MALES (3): L = 0.35-0.38 mm; a = 32-39; b = 4.6-5.0; c = 3.5-4.1; spear = 10-12  $\mu$ ; spicules = 11-13  $\mu$ ; gubernaculum = 3-4  $\mu$ .

DESCRIPTION: Body cylindrical, ventrally arcuate when relaxed by gentle heat, tapering on both extremities. Cuticle finely annulated. Lateral field marked by four incisures. Lip region rounded, continuous with the body contour, annulated, annulations faint. Spear short with rounded basal knobs, 10-11  $\mu$  long. Orifice of the dorsal esophageal gland close to spear base. Corpus a slender tube ending in

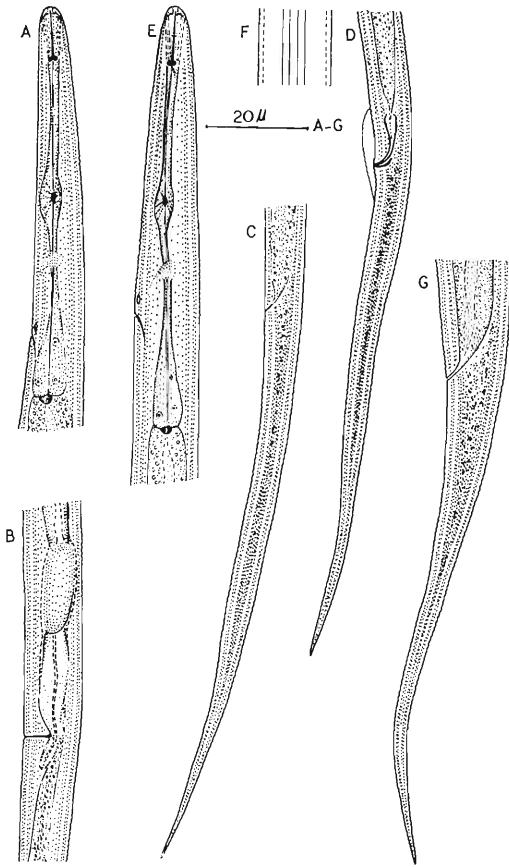


Figure 2, A-D. *Tylenchus (Lelenchus) mirus* n. sp. A. Esophageal region of female; B. Portion of female reproductive organs; C. Female tail; D. Male tail. Figure 2, E-G. *Tylenchus (Lelenchus) cynodontus* n. sp. E. Esophageal region of female; F. Lateral field; G. Female tail.

a fusiform valvulated median bulb. Isthmus slender, encircled by a nerve ring near its middle. Basal esophageal bulb pyriform. Cardia round. Excretory pore  $58-63 \mu$  from the anterior end. Hemizonid distinct, situated just anterior to the excretory pore. Distance from the anterior end of the body to the center of the median bulb is almost equal to the distance from the latter to the base of the esophagus. Intestine granular. Rectum very short. Tail long and filiform with acutely pointed terminus, nearly fifteen times the anal-body widths long. Phasmids could not be seen.

Vulva postequatorial. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Elongate pouchlike spermatheca present. Postuterine sac short, nearly half the vulvar-body width long.

Males similar to females in shape and appearance. Testis single, outstretched. Spicules paired, arcuate,  $11-13 \mu$  long when measured along the curved median line. Gubernaculum short and simple,  $3-4 \mu$  in length. Bursa rudimentary,  $2\frac{1}{2}$  times the cloacal-body widths long.

**HOLOTYPE:** Female, Slide No. 103, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**ALLOTYPE:** Male, Slide No. 103 B, collected with the females; other data same as for holotype.

**PARATYPES:** Three females and two males in the collection of the authors.

**TYPE HABITAT:** Soil around the roots of *Hibiscus rosa-sinensis* L.

**TYPE LOCALITY:** University Campus, Aligarh Muslim University, Aligarh, U.P., India.

**DIAGNOSIS AND RELATIONSHIP:** *Tylenchus (Lelenchus) mirus* n. sp. comes closer to *T. (L.) discrepans* Andrassy, 1954, and *T. (L.) infirmus* Andrassy, 1954. It differs from the former in the position of vulva, size of the spear and the tail length (tail ten times the anal-body widths long in *T. (L.) discrepans*), and from the latter in the larger size of the body and position of vulva ( $V = 57.9\%$  in *T. (L.) infirmus*).

*Tylenchus (Lelenchus) cynodontus* n. sp.  
(Fig. 2, E-G)

**FEMALES (6):**  $L = 0.39-0.48$  mm;  $a = 26-32$ ;  $b = 4.7-5.2$ ;  $c = 4.3-5.2$ ;  $V = 60-65\%$ ;  $spear = 9-11 \mu$ .

**DESCRIPTION:** Body cylindrical, ventrally arcuate when relaxed by gentle heat, tapering on both extremities. Cuticle finely annulated. Lateral field marked by four incisures, occupying nearly  $\frac{1}{3}$  the corresponding body width. Lip region continuous with the body contour, flat and rounded, narrower than front end of body. Spear short with small rounded basal knobs,  $9-11 \mu$  long. Orifice of the dorsal esophageal gland close to spear base. Procorpus a slender tube ending in a fusiform median bulb.



Isthmus long encircled by a nerve ring. Hemizonid distinct, situated 2-3 body annules anterior to the excretory pore. Basal esophageal bulb spindle-shaped. Cardia rounded. The distance from the anterior end of the body to the center of the median bulb is slightly less than the distance from the latter to the base of esophagus. Rectum short, nearly  $\frac{1}{3}$  of the anal-body width long. Tail long and filiform with acute terminus, measuring nearly 8-9 times the anal-body widths. Nearly last  $\frac{1}{4}$  of the tail dorsally bent.

Vulva postequatorial. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Elongate pouchlike spermatheca present, measuring 20 by 7  $\mu$ . Postuterine sac short, nearly half the vulvar-body width long.

HOLOTYPE: Female, Slide No. 102, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

PARATYPES: Five females in the collection of the authors.

TYPE HABITAT: Soil around the roots of *Cynodon dactylon* (L.) pers.

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

DIAGNOSIS AND RELATIONSHIP: *Tylenchus* (*Lelenchus*) *cynodontus* n. sp. resembles *T. (L.) discrepans* Andrassy, 1954 but differs from it in the absence of males, size of the spear (spear = 7.0-7.7  $\mu$  in *T. (L.) discrepans*), length of the tail (c = 4.0-4.4 in *T. (L.) discrepans*), continuous head and comparatively more posteriorly located vulva.

*Ditylenchus minutus* n. sp.

(Fig. 3, A-C)

FEMALES (15): L = 0.35-0.48 mm; a = 20-27; b = 4.5-4.7; c = 10-13; V = 72-80%; spear = 8-9  $\mu$ .

MALES (5): L = 0.32-0.38 mm; a = 23-25; b = 4.2-4.7; c = 9-10; spear = 8-9  $\mu$ ; spicules = 10-12  $\mu$ ; gubernaculum = 4-5  $\mu$ .

DESCRIPTION: Body cylindrical, regularly tapering towards both extremities. Cuticle finely annulated. Lateral field marked by four incisions. Lip region slightly set off, striated. Buccal spear weak, with basal knobs. Orifice of the dorsal esophageal gland close to the spear base. Procorpus a slender tube ending in an oval valvulated median bulb. Isthmus long, encircled by a nerve ring pos-

terior to its middle. Basal esophageal bulb distinctly set off from the intestine. Excretory pore 71  $\mu$  apart from the anterior end of the body.

Vulva a transverse slit. Ovary single, prodelphic, outstretched with oocytes arranged in a single file. Postuterine sac twice or slightly more than twice the vulvar-body widths long, extending half way from vulva to anus. Rectum half or slightly less than half the anal-body width long. Tail elongate-conoid with sub-acute terminus.

Males similar in appearance to females. Testis single, outstretched spermatocytes serially arranged. Spicules paired, ventrally arcuate, cephalated, 10-12  $\mu$  long. Gubernaculum simple, trough-shaped, 4-5  $\mu$  in length. Bursa crenate, enveloping less than  $\frac{1}{3}$  of the tail length. Tail ventrally arcuate, regularly tapering and ending in a subacute terminus.

HOLOTYPE: Female, collected in October 1964, Slide No. 115, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

ALLOTYPE: Male, Slide No. 115-B, collected with the female; other data same as for holotype.

PARATYPES: Twenty females and six males in the collection of the authors.

TYPE HABITAT: Soil around the roots of *Punica granatum* L.

TYPE LOCALITY: Ghazipur, U.P., India.

DIAGNOSIS AND RELATIONSHIP: *Ditylenchus minutus* n. sp. resembles *D. misellus* Andrassy, 1958 as regards body size but can at once be differentiated by the almost straight body shape on being relaxed (spiral in *D. misellus*); more posteriorly located vulva (V = 68.2% in *D. misellus*); more robust body and short tail.

*Ditylenchus cyperi* n. sp.

(Fig. 3, D-G)

FEMALES (10): L = 0.50-0.66 mm; a = 18-29; b = 5.2-6.3; c = 17-18; V = 75-83%; spear = 10-11  $\mu$ .

MALES (2): L = 0.46-0.50 mm; a = 22-33; b = 4.5-4.9; c = 15-17; spear = 10-11  $\mu$ ; spicules = 15-18  $\mu$ ; gubernaculum = 7-9  $\mu$ .

DESCRIPTION: Body cylindrical, tapering on both extremities, slightly arcuate on death.

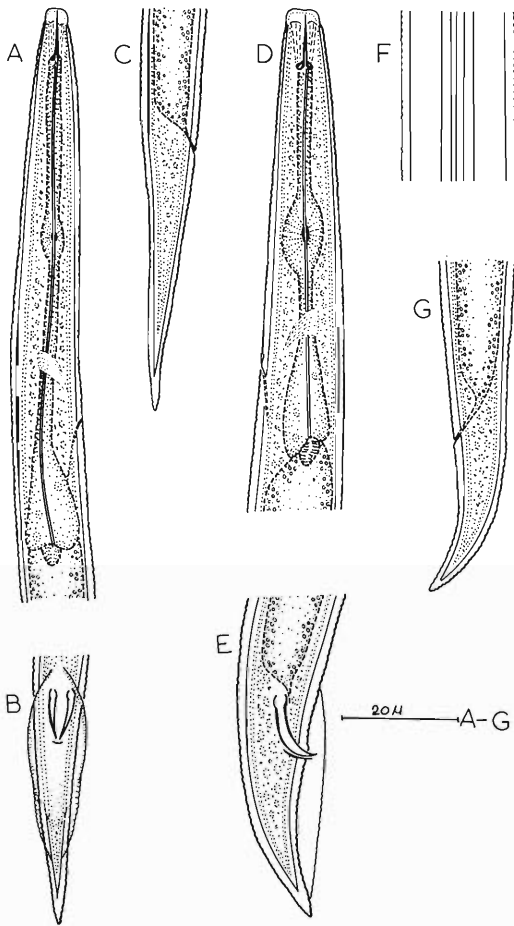


Figure 3, A-C. *Ditylenchus minutus* n. sp. A. Esophageal region of female; B. Male tail (ventral view); C. Female tail. Figure 3, D-G. *Ditylenchus cyperi* n. sp. D. Esophageal region of female; E. Male tail; F. Lateral field; G. Female tail.

Cuticle finely annulated. Lateral field marked by five incisures. Lip region continuous with the body contour, flat and rounded. Spear weakly developed with knobs, 10-11  $\mu$  long. Orifice of the dorsal esophageal gland close to spear base. Corpus a slender tube with oval valvulated median bulb. Basal esophageal bulb slightly overlapping the intestine ventrally. Isthmus encircled by a nerve ring. Excretory pore situated near the beginning of the basal esophageal bulb. Hemizonid 2-3 body an-

nules long, situated just anterior to the excretory pore.

Vulva a transverse slit. Ovary single, prodelphic outstretched with oocytes arranged in a single file. Rounded spermatheca present. Postuterine sac extending halfway to the vulvanus distance. Tail convex-conoid ending in a subacute terminus.

Males similar to females in general appearance. Testis single, outstretched. Spermatozoocytes serially arranged. Spicules paired, ventrally arcuate, cephalated, 15-18  $\mu$  long. Gubernaculum simple, 7-9  $\mu$  in length. Bursa subcaudal, originating at the level slightly anterior to the head of the spicules.

HOLOTYPE: Female, collected in December, 1964, Slide No. 116, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

ALLOTYPE: Male, Slide No. 116-B, collected with the female; other data same as for holotype.

PARATYPES: Nine females and one male in the collection of the authors.

TYPE HABITAT: Soil around the roots of *Cyperus rotundus* L.

TYPE LOCALITY: University Campus, Aligarh Muslim University, Aligarh.

DIAGNOSIS AND RELATIONSHIP: *Ditylenchus cyperi* n. sp. resembles *D. nannus* Siddiqi, 1963, *D. mirus* Siddiqi, 1963, *D. procerus* (Bally and Raydon, 1931) Filipjev, 1936, and *D. dipsecoideus* (Andrássy, 1952) Andrássy, 1956, but differs from (i) *D. nannus* in the position of vulva, size of the spear, and spicules and in the tail shape; (ii) *D. mirus* in the position of vulva, size of the spear and gubernaculum and in tail shape; (iii) *D. procerus* in the body width and tail length ( $c = 14$  in *D. procerus*) and (iv) *D. dipsecoideus* in the longer postuterine sac, lateral field with five incisures and more terminal bursa in males.

*Ditylenchus ausafi* n. sp.

(Fig. 4, A-E)

FEMALES (8): L = 0.51-0.61 mm; a = 24-29; b = 5.7-6.6; c = 9-10; V = 72-75%; spear = 10-11  $\mu$ .

MALES (2): L = 0.45-0.47 mm; a = 32-34; b = 4.9-5.2; c = 9-10; spear = 10-11  $\mu$ ; spicules = 12-15  $\mu$ ; gubernaculum = 6-7  $\mu$ .

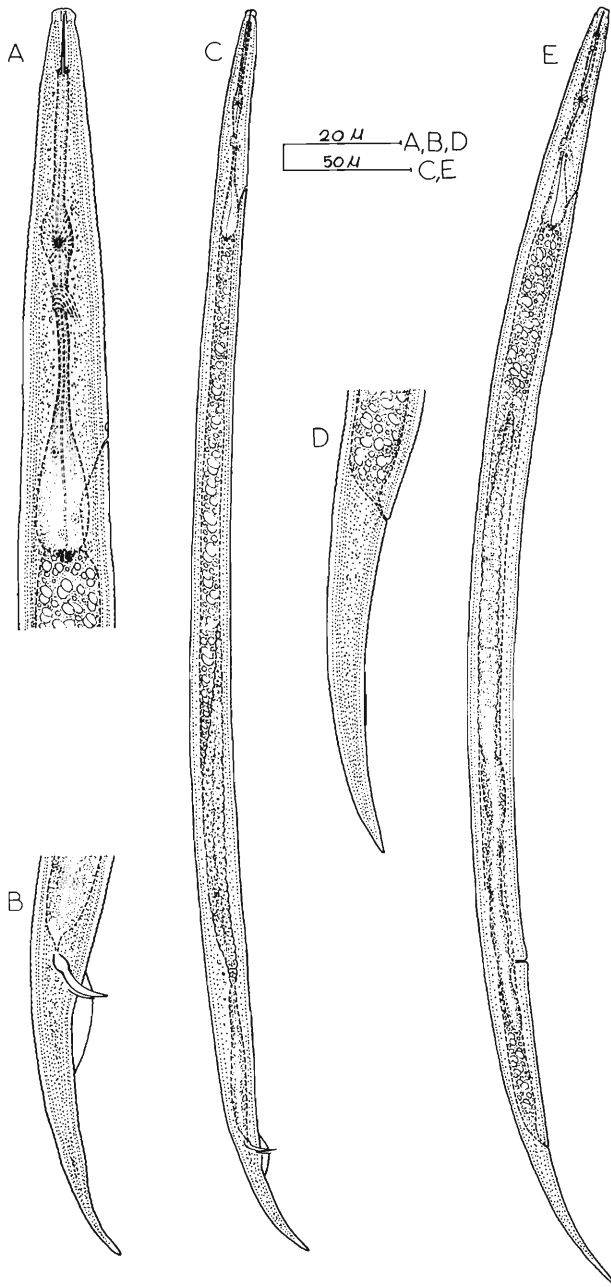


Figure 4, A-E. *Ditylenchus ausafi* n. sp. A. Esophageal region of female; B. Male tail; C. Entire male; D. Female tail; E. Entire female.

**DESCRIPTION:** Body cylindrical, tapering on both extremities, slightly arcuate on death. Cuticle finely annulated. Lateral field marked by four incisures. Lip region set off from the body contour, annulated. Spear weak, 10–11  $\mu$  long. Orifice of the dorsal esophageal gland close to spear base. Corpus a slender tube ending in an oval valvulated median bulb. Isthmus long, encircled by a nerve ring. Basal esophageal bulb pyriform. Excretory pore 74–82  $\mu$  apart from the anterior end of the body. Hemizonid prominent, 2–3 body annules long, located just anterior to the excretory pore.

Ovary single, prodelphic, outstretched with oocytes arranged in a single file, sometimes reaching near the basal esophageal bulb. Post-uterine sac slightly more than vulvar-body width long. Tail long elongate-conoid with subacute terminus.

Males similar in appearance to females. Testis single, outstretched. Spermatocytes serially arranged. Spicules paired, ventrally arcuate cephalated, 14–15  $\mu$  long. Gubernaculum simple, 6–7  $\mu$  in length. Bursa enveloping slightly less than  $\frac{1}{3}$  of the tail length. Tail shape as in females.

**HOLOTYPE:** Female, collected in December, 1964, Slide No. 117, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**ALLOTYPE:** Male, Slide No. 117-B, collected with the females; other data same as for holotype.

**PARATYPES:** Seven females and one male in the collection of the authors.

**TYPE HABITAT:** Soil around the roots of *Rosa* sp.

**THE LOCALITY:** University Campus, Aligarh Muslim University, Aligarh.

**DIAGNOSIS AND RELATIONSHIP:** *D. ausafi* n. sp. comes closer to *D. cyperi* n. sp., *D. nannus* Siddiqi, 1963, *D. mirus* Siddiqi, 1963, and *D. dipsecoideus* (Andrássy, 1952) Andrassy, 1956, but differs from (i) *D. cyperi* in the number of incisures in the lateral field, long tail, short bursa and anteriorly located vulva; (ii) *D. nannus* and *D. mirus* in possessing long tail, short bursa, shape of the tail terminus and more anteriorly located vulva; and (iii) *D. dipsecoideus* in short bursa and longer body.

*Pseudhalenchus minutus* Tarjan, 1958

**MALE (1):** L = 0.44 mm; a = 36.6; b = 4.2; c = 9.3; spear = 8  $\mu$ ; spicules = 14  $\mu$ ; gubernaculum = 5  $\mu$ .

Although the specimens of *Pseudhalenchus minutus* Tarjan, 1958, isolated from around the roots of *Brassica oleracea* L. differ from the original measurements as given by its author, but in view of the fact that only one specimen was isolated, it has, therefore, been considered as geographical variant of *P. minutus*. This is the first report of this species from India.

Subfamily Rotylenchulinae n. subfam.

**DIAGNOSIS:** Hoplolaimidae: Sexual dimorphism a prominent feature, female being swollen to kidney shape, male vermiform. Orifice of the dorsal esophageal gland about half way between spear base and median esophageal bulb. Females with two ovaries and post median vulva. Esophageal glands forming long lobe overlapping the intestine. Male with rather weak head sclerotization, weak spear, and reduced esophagus and with or without bursa.

**TYPE AND ONLY GENUS:** *Rotylenchulus* Linford and Oliveira, 1940.

A KEY TO THE SUBFAMILIES OF  
HOPLOLAIMIDAE

1. Mature females vermiform ..... 2  
Mature females swollen, kidney shaped  
..... Rotylenchulinae n. subfam.
2. Basal part of esophagus bulbar .....  
..... Dolichodorinae Chitwood and  
Chitwood, 1950  
Basal part of esophagus glandular ..... 3
3. Ovaries paired ..... 4  
Ovary single .....  
..... Rotylenchoidinae Whitehead, 1958
4. Phasmids absent .....  
..... Aphasmatylenchinae Sher, 1965  
Phasmids present ..... 5
5. Heads show sexual dimorphism, cephalic framework usually with strong sclerotization, spear 2–4½ times head-width long .....  
..... Hoplolaiminae Filipjev, 1934  
Head four lobed; spear considerably long .... Belonolaiminae Whitehead, 1958

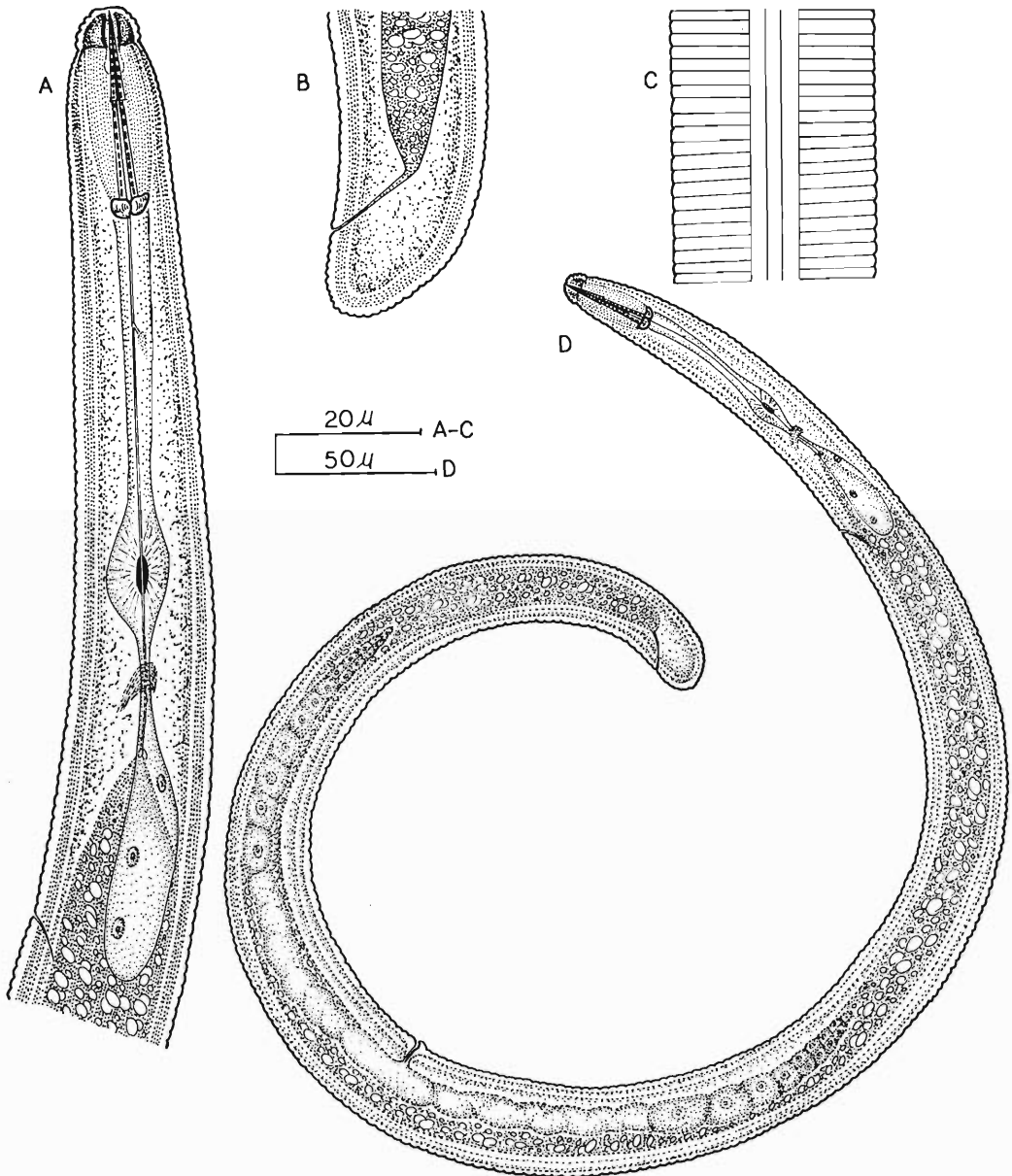


Figure 5, A-D. *Rotylenchus helicus* n. sp. A. Esophageal region; B. Female tail; C. Lateral field; D. Entire female.

*Rotylenchus helicus* n. sp.

(Fig. 5, A-D)

FEMALES (20): L = 0.66–0.86 mm; a = 26–30; b = 4.9–6.7; c = 56–93; V = 20–28.55–70<sup>18–25</sup>%; spear = 28–32  $\mu$ .

DESCRIPTION: Body spirally curved when relaxed by gentle heat. Cuticle distinctly annulated, annules 2  $\mu$  apart at midbody. Lateral field marked by four crenate incisures, measuring  $\frac{2}{3}$  of the corresponding body width, outer incisures more distinct than the inner ones. Lip region hemispherical, with five labial annules, slightly set off, if at all. Labial sclerotization moderate. Spear well developed with anteriorly concave and posteriorly convex knobs, anterior part of the spear slightly shorter than the posterior part. Orifice of the dorsal esophageal gland more than half the spear length behind the spear base, measuring 15–18  $\mu$  from the spear base. Procorpus cylindrical ending in an oval median bulb with well-developed valvular apparatus. Nerve ring just behind the median bulb. Excretory pore near the end of the glandular esophagus. Esophageal glands overlapping the intestine more than four body annules dorsally, laterally and ventrally, typically the greatest overlap dorsally. Intestinal portion granular. Rectum distinct, slightly less than the anal-body width long. Phasmids 3–5 annules posterior to anus. Tail convex-conoid with broadly rounded terminus,  $\frac{1}{2}$ – $\frac{3}{4}$  anal-body width long. Tail annules numbering 8–10 ventrally.

Ovaries paired, opposed and outstretched. Spermatheca not seen. Spermogonium present, epiptygma distinct. Oocytes arranged in a single file except for a short region of multiplication.

Males not found.

HOLOTYPE: Female, Slide No. 361, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

PARATYPES: Fifty females in the collection of the authors.

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

TYPE LOCALITY: Rampur, U.P., India.

DIAGNOSIS AND RELATIONSHIP: *Rotylenchus helicus* n. sp. resembles *R. quartus* (Andrássy, 1958) Sher, 1961, *R. buxophilus* Golden, 1956, *R. unisexus* Sher, 1965, *R. incultus* Sher 1965,

and *R. orientalis* Siddiqi and Husain, 1964, but differs from all except the last named species in possessing the orifice of the dorsal esophageal gland at more than half the spear length behind the spear base and shorter tail. It further differs from (i) *R. quartus* in head shape, position of vulva (V = 54–59% in *R. quartus*) and the number of tail annules; (ii) *R. buxophilus* in possessing smaller body, size of the spear and the position of vulva (V = 52–58% in *R. buxophilus*); (iii) *R. unisexus* in the number of lip region annules, positions of excretory pore, phasmid and vulva; (iv) *R. incultus* in the positions of excretory pore, phasmid and vulva, absence of males and in the presence of distinct epiptygma in females; (v) *R. orientalis* in the size of the spear, position of phasmid, presence of distinct epiptygma and normally developed ovaries (posterior ovary reduced in *R. orientalis*).

*Hemicyclophora dhirendri* n. sp.

(Fig. 6, A-E)

FEMALES (6): L = 0.64–0.77 mm; a = 15–24; b = 5.2–6.3; c = 11–12, V = 80–85%; spear = 67–70  $\mu$ ; Body annules = 100–240.

MALES (2): L = 0.56–0.64 mm; a = 20–27; b = ?; c = 7.0–7.2; spicules = 40–41  $\mu$ ; gubernaculum = 10  $\mu$ .

DESCRIPTION: Body remains almost straight on death or assumes a slightly ventrally arcuate shape; cylindrical, almost of the same diameter up to vulva from where it narrows uniformly into an elongate conical tail. Fifth cuticle of the body not very loose. Cephalic framework moderately sclerotized. Lip region continuous with the body contour, flat and rounded with one or two annules. Lateral field a single line, crossed by transverse annulations and forming rectangular blocks. Spear well developed extending up to 18–20 annules of the body; slightly arcuate with posteriorly backward flattened knobs, spear tip 45–48  $\mu$  long. Orifice of the dorsal esophageal gland 7  $\mu$  from the spear base. The whole spear is enclosed by the procorpus of the esophagus which is fused with the metacorpus. Isthmus short, expanding to form a pyriform basal bulb. Nerve ring encircles the isthmus. Hemizonid not seen. Excretory pore 4–6 annules posterior to the base of the esophagus, i.e., on 38th to 42nd body annule.

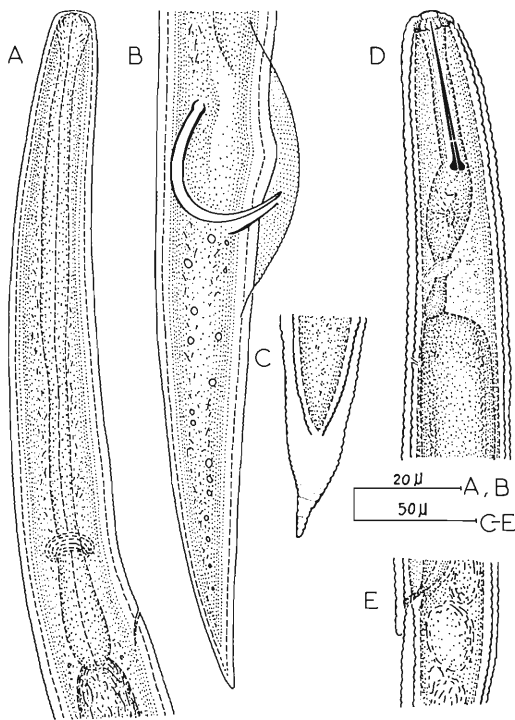


Figure 6, A-E. *Hemicyclophora dhirendri* n. sp. A. Esophageal region of male; B. Male tail; C. Female tail region; D. Female esophageal region.

Ovary monodelphic, prodelphic and outstretched with prominent spherical spermatheca filled with sperms; spermatheca set off. Oocytes arranged in a single row except for a short region of multiplication. Vulva opens under a folded skirt of second cuticle. Tail conical with rounded terminus.

Male cuticle marked by fine transverse striae. Lateral field marked by two crenate incisures becoming invisible after cloaca. Lip region slightly expanded. Spear absent. Pharynx with slight sclerotization forming a chamber. Testis single, outstretched. Spicules sickle-shaped. Bursa crenate, less than two times as long as body-width. Tail conoid with a rounded terminus.

**HOLOTYPE:** Female, collected in October, 1964, Slide No. 661, deposited with the Plant Pathology Section, Department of Botany, Aligarh Muslim University, Aligarh, U.P., India.

**ALLOTYPE:** Male, Slide No. 661-B, collected with the females, other data same as for holotype.

**PARATYPES:** Five females and one male in the collection of the authors.

**TYPE HABITAT:** Soil around the roots of *Cyperus rotundus* L.

**TYPE LOCALITY:** University Campus, Aligarh Muslim University, Aligarh.

**DIAGNOSIS AND RELATIONSHIP:** *Hemicyclophora dhirendri* n. sp. comes closer to *H. oostenbrinki* Luc, 1958; *H. typica* De Man, 1921 and *H. uniformis* Thorne, 1955. It differs from *H. oostenbrinki* in the absence of longitudinal lines, hemizonid and the tubular sheath covering the spicules. It also differs in the tail shape and size of the spicules. It differs from *H. typica* in the size of the spear, lateral field, male body-width and in having the vulva under a folded skirt. From *H. uniformis*, it differs in lesser number of body annules and smaller size of the females.

#### SUMMARY

Interrelationship of the genera of the family Tylenchidae with special reference to *Tylenchorhynchus* and *Telotylenchus* is discussed. The subfamily Tylenchorhynchinae Eliava, 1964 is shifted to the family Tylenchidae for the last two named genera, which can serve as a bridge between the families Tylenchidae, Hoplolaimidae and Pratylenchidae. The systematic position of the genus *Rotylenchulus* is also discussed with the erection of a new subfamily Rotylenchulinae (Hoplolaimidae) for it. A new subgenus *Ottolenchus* is proposed under the genus *Tylenchus* Bastian, 1865. Two new species under the subgenus *Lelenchus* of the genus *Tylenchus*, three new species of the genus *Ditylenchus* and a new species each of *Rotylenchus* and *Hemicyclophora* are described from Indian soils. *Pseudohalenchus minutus* Tarjan, 1958, is reported for the first time from this country.

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\* Original not seen.



## Helminth Parasitism in Gars from South Texas with a Description of *Dichelyne lepisosteus* n. sp. (Nematoda: Cucullanidae)<sup>1</sup>

S. CASTO<sup>2</sup> AND B. MCDANIEL<sup>3</sup>

Certain anatomical, physiological, and ecological circumstances in the gar family Lepisostidae allow these fish to survive where others sometimes perish. These same factors undoubtedly influence the helminth fauna present in gars. The available literature on gar helminths is widely scattered and fails to represent the helminth parasites infecting the Lepisostidae from a relatively local area. This paper reports the composition and intensity of helminth parasitism found among several species of gars collected in southern Texas.

Host nomenclature used herein follows Knapp's (1953) study of freshwater fish in Texas. The species and subspecies, listed in decreasing order of abundance in Texas, follow:

- Lepisosteus osseus leptorhynchus* Girard  
(Southern Longnose Gar)
- Lepisosteus osseus oxyurus* Rafinesque  
(Northern Longnose Gar)
- Lepisosteus productus* Cope (Spotted Gar)
- Lepisosteus spatula* Lacépède (Alligator Gar)
- Lepisosteus platostomus* Rafinesque  
(Shortnose Gar)

Another species, the Florida spotted gar (*Lepisosteus platyrhincus*) occurs elsewhere in North America. However, Blair et al. (1957) recognized *L. platyrhincus* as "similar to and possibly conspecific with *L. productus*"; if so, then all species of gar are present in Texas.

Twenty-three helminth parasites have been recorded previously from gars in Canada and the United States, i.e., ten trematode, six nematode, six cestode, and one acanthoceph-

alan species. Table 1 lists these parasites and their gar hosts.

### MATERIALS AND METHODS

Fish were caught with trout lines and netted between 1 March 1964 and 11 August 1964 in five regions in southern Texas (Fig. 1). These areas included (a) Lake Mathis on the lower Nueces River drainage, (b) the upper Nueces River drainage, (c) the upper Frio River drainage, (d) an impoundment on Los Olmos Creek, and (e) the upper Rio Grande near Laredo.

A total of 27 spotted, 10 alligator, and 10 longnose gars were collected from these locations. No shortnose gars were included in the field collections.

### RESULTS

All individuals, with the exception of two spotted gars, were infected with one or more helminth parasites (Table 2). The average numbers of helminths recovered from *L. spatula*, *L. productus*, and *L. osseus* were 21.9, 86.9, and 52.2, respectively.

Over 100 *Dichelyne lepisosteus* n. sp. were recovered from the intestines of alligator and spotted gars; longnose gars were not infected (Table 3). Infection was greatest in alligator gars; 86 adult parasites were taken from four infected hosts. Average infection among alligator gars was 21.5 parasites, which was in considerable excess of the 2.8 helminths per infected spotted gar.

Ten adult *Contracaecum* sp. (unidentified) were taken from the stomachs of three longnose gars collected from the upper Nueces River, and one adult from a single spotted gar from Lake Mathis (Table 3).

Larval *Eustrongylides* sp. were recovered from only 2 of the 47 hosts examined. Infection was slight (Table 3). A 15½ pound alligator gar contained two encysted larvae in the stomach wall near a larval *Contracaecum spiculigerum* infection. A 6 cm larva was also

<sup>1</sup> Adapted from a thesis presented by the senior author to the Department of Biology, Texas College of Arts and Industries, in partial fulfillment of the requirements for the degree of Master of Science.

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<sup>3</sup> Department of Biology, Texas College of Arts and Industries, Kingsville. (Present address: Department of Entomology-Zoology, South Dakota State University, Brookings.)

TABLE 1. Summary of known helminth parasites from Lepisosteidae of North America.

Gar helminths	Locale	Authority
Longnose gar		
<i>Bucephalopsis gracilenscens</i>	North Carolina	Tennet (1909)
<i>Proteocephalus ambloplitis</i>	Wisconsin	Pearse (1924)
<i>P. singularis</i>		
<i>Leptorhynchoides thecatus</i>		
<i>Macroderoides spiniferus</i>	Mississippi	Simer (1929)
<i>M. parvum</i>	Lake Champlain, New York	Hunter (1932)
<i>Proteocephalus australis</i>	Galveston Bay, Texas	Chandler (1935)
<i>P. elongatus</i>		
<i>Apophallus venustus</i>	Canada	Cameron (1945)
<i>Proteocephalus singularis</i>	Lake Erie	Bangham and Hunter (1939)
<i>P. ambloplitis</i>		
<i>Bothriocephalus</i> sp.		
<i>Macroderoides spiniferus</i>		
<i>Leptorhynchoides thecatus</i>		
<i>Cystidicola lepisostei</i>		
Shortnose gar		
<i>Proteocephalus singularis</i>	Wisconsin	Pearse (1924)
<i>Macroderoides spiniferus</i>		
<i>Contracaecum spiculigerum</i>	Reelfoot Lake, Tennessee	Bangham and Venard (1942)
<i>Proteocephalus singularis</i>		
<i>Leptorhynchoides thecatus</i>		
<i>Paramacroderoides echinus</i>		
<i>Macroderoides spiniferus</i>		
<i>Proteocephalus ambloplitis</i>		
Spotted gar		
<i>Contracaecum spiculigerum</i>	Florida	Bangham (1941)
<i>Dichelyne</i> sp.		
<i>Camallanus</i> sp.		
<i>Eustrongylides</i> sp.		
<i>Agamonema</i> sp.		
<i>Clinostomum marginatum</i>		
Gyrodactylidae		
<i>Proteocephalus singularis</i>		
<i>Leptorhynchoides thecatus</i>		
Alligator gar		
<i>Rhipidocotyle lepisostei</i>	Louisiana	Hopkins (1954)

taken from the body cavity of a longnose gar. Spotted gars were not infected with *Eustrongylides* sp.

Adult *Macroderoides spiniferus* was recovered from the intestines of the spotted gars; no other body region was infected. Only spotted gars from the upper Frio River drainage were infected with this parasite despite the fact that eight additional hosts were collected at Lake Mathis. Incidence of this parasite among the 11 infected fish was slight (Table 3).

The intestines of two longnose gars from the Nueces River yielded 12 specimens of the trematode, *Paramacroderoides echinus*. Intensity of infection was low (Table 3). Hosts from all other locations were free of infection.

*Clinostomum* sp. were encysted in the mesenteries and ovaries of two female longnose gars from the Nueces River (Table 3).

These cysts yielded 13 large metacercariae up to 7 mm in length. A single larva from the stomach of an alligator gar was considered an accidental infection because of its abnormal location in the host.

A total of 345 *Contracaecum spiculigerum* larvae were recovered from 17 of 47 gars examined (Table 4). Alligator gars were 90% infected and averaged 31.7 larvae per host. Spotted gars were less frequently infected (25.9%), whereas, only a single larva was recovered from one of ten longnose gars. The stomach wall was commonly infected (88%), followed by the mesenteries (7%), intestinal wall (5%), and fatty tissues (0.3%). The greatest single infection occurred in a 14½ pound alligator gar which contained 101 larvae.

*Proteocephalus ambloplitis* infection was intense among all species of gars. Over 900

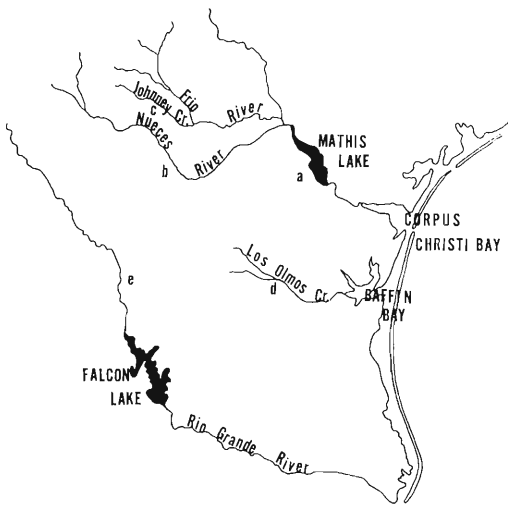


Figure 1. Map of southern Texas showing collection sites of hosts. (a) Lake Mathis on the lower Nueces River drainage, (b) the upper Nueces River drainage, (c) the upper Frio River drainage, (d) an impoundment on Los Olmos Creek, and (e) the upper Rio Grande near Laredo.

larvae and 500 adults were recovered from 45 of 47 fish examined. Adults were found in both alligator and spotted gars. Of these, alligator gars were the more heavily infected, whereas these same hosts were lowest in plerocercoid infections (Table 5). Ten different body locations harbored plerocercoids. Of these, the muscular walls of the digestive tract were most commonly infected (Table 6).

One acanthocephalan was recovered from the intestine of a longnose gar from the Nueces River. However, specific identification proved impossible because of a partially invaginated proboscis.

*Dichelyne lepisosteus* n. sp.  
(Fig. 2)

**DESCRIPTION:** Characters of family Cucullanidae Cobbold, 1864: slender nematodes, body elongated, cylindrical, tapering evenly in both sexes from esophageal region to tail. Cephalic region with thickening characteristic of genus. Cephalic glands present. Anterior end of esophagus forming false buccal capsule. Female 12.5–13.2 mm long, maximum diameter at posterior end of esophagus, 0.3–0.45

mm; head end bluntly rounded, posterior end tapering to pointed tail. Esophagus 1.00–1.5 mm long, 0.5 mm broad behind mouth, narrowing to 0.2 mm in center region, posterior region 0.4 mm and club-shaped. Intestine ribbon-shaped, bent and folded, with a single folded anterior diverticula reaching approximately to nerve ring. Anus 0.5–0.8 mm from posterior end; tail spear-shaped. Vulva situated posterior to middle of body. Post anal region with three pairs of lateral micropapillae. Rectal glands prominent. Two ovaries present. Male 9.3–10.5 mm long. Esophagus 1.0–1.2 mm long, 0.1–0.2 mm broad at expanded anterior end, 0.25–0.3 mm broad at neck, and 0.1–0.2 mm broad through bulb. Cloaca 0.1–0.2 mm from tip of tail. Caudal papillae arranged in pairs characteristic of genus. Two pairs of papillae postanal, two pairs adanal and three pairs preanal. Posterior pair of postanal papillae ventral near tip of tail, next pair dorsal-lateral, next pair lateral and most anterior pair ventral. One pair of adanal papillae, these large and ventral, placed on sides of genital prominence in front of and behind cloacal passage; one pair small and situated laterally. First pair of reanal papillae situated close to anterior pair of adanal papillae, remaining two pairs spaced evenly from cloaca. Ventral sucker absent. Spicules 2.3–2.6 mm long, tip beveled and pointed. Gubernaculum present.

**DISCUSSION:** Tornquist (1931), Baylis (1939), Yamaguti (1941 and 1961), Skrjabin et al. (1954), Ali (1956), Campana-Roguet (1957), and Agrawal (1965) have discussed the family Cucullanidae, Cobbold, 1864, in respect to the corrected placement of the genera assigned within the family. The treatment here recognizes the genus *Dichelyne* as a part of the subfamily. Dacnitoidinae in agreement with Yamaguti (1961) and Agrawal (1965). However, due to the establishment of a species with only three preanal papillae a revised description of the genus *Dichelyne* must be made. The description of the genus *Dichelyne* Jagerskiold (1902) given by Yamaguti (1961) should be amended to read "Preanal papillae from 3–5 pairs."

*D. lepisosteus* n. sp. may be separated from all other members of the genus *Dichelyne* by the reduction of the number of preanal papillae in the male.

TABLE 2. Summary of helminth parasitism in 3 species of gars from southern Texas.

	Alligator gar ( <i>L. spatula</i> )	Spotted gar ( <i>L. productus</i> )	Longnose gar ( <i>L. osseus</i> )	All
Number hosts examined	10	27	10	47
Number hosts infected	10	25	10	45
Per cent hosts infected	100.0	92.6	100.0	95.7
Number species helminth parasites	5	5	6	8
Number individual parasites recovered	869	547	522	1,938
Number parasites/infected host	21.9	86.9	52.2	43.1

HOST: *Lepisosteus spatula* Lacépède.

LOCATION: Intestine.

LOCALITY: San Patricio County, Texas, Mathis Lake.

TYPE SPECIMEN: Deposited in United States National Museum (USNM No. 61491).

#### DISCUSSION

The heavy infections of *Proteocephalus ambloplitis* and *Contracaecum spiculigerum* reported herein and by previous authors (Table 1) indicates that these parasites are widespread and common in gars. The recovery of *P. ambloplitis* plerocercoids and adults from the same individual host substantiates the work of Pearse (1924). The discovery of large numbers of *C. spiculigerum* larvae and *P. ambloplitis* plerocercoids encysted within the walls of the digestive tract suggests that these parasites exhibit a marked preference in their area of encystment. The recovery of the genera *Dichelyne* and *Eustrongylides*, previously recorded by Bangham (1941) from Florida, extends their distribution to South Texas.

The common occurrence of larval *Contracaecum spiculigerum* in gars leads to interesting speculation on the nature of the life history of the parasite as related to the feeding habits of the hosts. The life cycle involves a larval stage in either one or two fish hosts followed by the adult stage in a fish-eating bird (Thomas, 1937). Cormorants and pelicans usually serve as definitive hosts for members of the genus *Contracaecum*. Oglesby (1960), Owre (1962), and McDaniel and Patterson (1966) have recently reported the recovery of *Contracaecum* from pelicans in the gulf of Mexico. Gars serve as dead-end hosts for the larvae since few fish-eating birds feed on any but the smallest gars. However, because of their own piscivorous habits, gars continue to accumulate the larvae during their entire life. It is also probable that the encysted larvae will live for many years within the body of the host. This presumably accounts for the large numbers of larvae found encysted within gars.

The present literature (cf. tables) does not reveal the effects of heavy larval *C. spiculigerum* infections on their hosts. No definite

TABLE 3. Incidence and intensity of parasites in three species of gars.<sup>1</sup>

No. fish examined	No. fish infected	Per cent infected	No. parasites	Parasites/infected host	Parasites found in species
Alligator gar— <i>L. spatula</i>					
10	4	40	86	21.5	<i>Dichelyne lepisosteus</i> n. sp (adults)
10	9	90	285	31.7	<i>Contracaecum spiculigerum</i> (larvae)
10	1	10	2	2.0	<i>Eustrongylides</i> sp. (larvae)
10	1	10	1	1.0	<i>Clinostomum</i> sp. (larvae)
Spotted gar— <i>L. productus</i>					
27	10	37.0	28	2.5	<i>Dichelyne lepisosteus</i> n. sp. (adults)
27	7	25.9	59	8.4	<i>Contracaecum spiculigerum</i> (larvae)
27	1	3.7	1	1.0	<i>Contracaecum</i> sp. (adults)
27	11	40.7	23	2.0	<i>Macroderoides spiniferus</i> (adults)
Longnose gar— <i>L. osseus</i>					
10	1	10.0	1	1.0	<i>Contracaecum spiculigerum</i> (larvae)
10	3	33.3	10	3.3	<i>Contracaecum</i> sp. (adults)
10	1	10.0	1	1.0	<i>Eustrongylides</i> sp. (larvae)
10	2	20.0	12	6.0	<i>Paramacroderoides echmus</i> (adults)
10	2	20.0	13	6.5	<i>Clinostomum</i> sp. (larvae)

<sup>1</sup> Incidence and intensity of *Proteocephalus ambloplitis* are given in Table 5.

TABLE 4. Incidence and intensity of *Contracaecum spiculigerum* larvae in anatomical regions of three species of gar. Numbers in parenthesis indicate the number of infected fish.

Body region	Alligator gar ( <i>L. spatula</i> ) (9)	Spotted gar ( <i>L. productus</i> ) (7)	Longnose gar ( <i>L. osseus</i> ) (1)	All species (17)	Per cent
Wall of stomach and esophagus	257 (9)	46 (4)	0	303 (13)	87.8
Wall of intestine	6 (3)	11 (3)	0	17 (6)	5.0
Mesenteries	23 (3)	1 (1)	1 (1)	24 (5)	6.9
Fatty tissue	0	1 (1)	0	1 (1)	0.3
Totals	285	59	1	345	100.0

correlation between the condition of the host and the number of parasites recovered was indicated in this study. The only observed evidence of host injury was the occasional presence of what appeared to be an abscess surrounding the encysted larva.

*Proteocephalus ambloplitis* infections offer another example of how the feeding habits of the hosts affect the acquisition of a parasitic fauna. Gars feed either upon (a) smaller fish containing mature plerocercoids which in turn develop into adult worms in the intestine of the host, or (b) upon fish which had recently fed upon copepods infected with the proceroid. In the latter case the proceroids develop into plerocercoids within the body of the gar. Plerocercoids may also be acquired by young gars feeding directly upon copepods infected with the proceroid. In the event that large gars become infected with plerocercoids it is probable that the life cycle ends for that parasite, because no fish is known to prey upon large gars. These mechanisms explain the presence of both adults and plerocercoids in the same host.

Hughhins (1959) states that the plerocercoid of *Proteocephalus ambloplitis* destroys the reproductive potential in game fishes. However, there was no evidence from our work

that the infection was in any way damaging to the host, even though plerocercoids were found in the gonads of eight individuals.

Species of the genus *Eustrongylides* have been reported from one of 82 Florida spotted gars (Bangham, 1941). Other hosts include the barred killifish, *Fundulus* sp., (Mueller, 1934), and the white sucker, *Catostomus* sp. (Meyer, 1954).

Adult *Contracaecum* are reported from gars for the first time in this study. Individuals were recovered from a spotted gar from Mathis Lake and three longnose gars from the Nueces River.

Two species of Macroderodiidae were recovered from gars examined in this study. *Macroderoides spiniferus* is a common parasite of gars (Table 1). Leigh (1958) determined the life cycle of the Florida spotted and demonstrated that the gar was the definitive host.

*Paramacroderoides echinus* is recorded exclusively from gars. Originally described as a new genus and species of the family Macroderoidieae from shortnose gars in Reelfoot Lake, Tennessee, it has since been found to be common in Florida spotted gars by Leigh and Holliman (1956). *Macroderoides spiniferus* was restricted to gars collected from the

TABLE 5. Incidence and intensity of *Proteocephalus ambloplitis* in three species of gar.

	Alligator gar ( <i>L. spatula</i> )	Spotted gar ( <i>L. productus</i> )	Longnose gar ( <i>L. osseus</i> )	All species
Number hosts examined	10	27	10	47
Per cent infected	100.0	92.6	100.0	95.7
Number hosts infected with:				
Plerocercoid only	0	17	10	27
Adults only	9	0	0	9
Plerocercoid and adults	1	8	0	9
Number hosts infected	10	25	10	45
Number plerocercoid parasites	6	419	485	910
Number adult parasites	489	17	0	506
Parasites/infected host	49.5	25.6	48.5	31.4

TABLE 6. Incidence and intensity of plerocercoid *Proteocephalus ambloplitis* in anatomical regions of three species of gar. Numbers in parentheses indicate the number of infected fish.

Body region	Alligator ( <i>L. spatula</i> ) (1)	Spotted gar ( <i>L. productus</i> ) (25)	Longnose gar ( <i>L. osseus</i> ) (10)	All species	Per cent
Wall of stomach	0	115 (7)	236 (7)	351 (14)	38.6
Wall of intestine	5 (1)	132 (12)	102 (4)	239 (16)	26.3
Mesenteries	0	33 (7)	67 (6)	100 (13)	11.0
Liver	0	43 (5)	22 (4)	65 (9)	7.1
Gonads	0	14 (8)	0	14 (8)	1.5
Body cavity	0	46 (12)	20 (3)	66 (15)	7.3
Lumen of intestine	0	16 (3)	7 (1)	23 (4)	2.5
Lumen of stomach	0	0	26 (1)	26 (1)	2.9
Swim-bladder	0	3 (2)	0	3 (2)	0.3
Spleen	1 (1)	17 (2)	5 (1)	23 (4)	2.5
Totals	6	419	485	910	100.0

upper Frio River while *Paramacroderoides echinus* was recovered from the Nueces River.

The metacercariae of *Clinostomum* recovered from the mesenteries and ovaries of two female longnose gars are probably an undescribed species. It is not *Clinostomum marginatum*, the only species of this genus recorded from gars (Bangham, 1941). A comparison of the metacercariae morphology showed that the well-developed reproductive primordium and marginated intestine characteristic of *C. marginatum* were not present in the specimens collected from the longnose gars from Texas. A close relationship probably exists between the metacercariae found in Texas and the related genus *Odhneriotrema* (Clinostomidae) from the gonads, mesenteries, and fatty tissues of gars in Florida (Leigh, 1960).

Adult digenetic trematodes found in gars collected from Mathis Lake appear to be undescribed in the literature. Two fairly complete adults were studied, but they were nonetheless unsuitable for a description of new taxa. Additional material is now being secured for descriptive purposes.

#### SUMMARY

Three species of the gar family Lepisostidae were examined for helminth parasites. Host samples included 27 spotted gars, 10 alligator gars, and 10 longnose gars collected from five locations in southern Texas.

Helminth infection was widespread; eight species of parasites were recovered from 45 of the 47 hosts examined. One or more species of parasites was noted in all three host species and from each of the five localities sampled.

*Proteocephalus ambloplitis* and *Contracaecum spiculigerum* were the parasites most frequently encountered. Both adults and plerocercoids of the former were found in infected individuals, whereas only the larvae of the latter were recovered. *P. ambloplitis* plerocercoids were most frequently encysted in the wall of the stomach, the wall of the intestine, and in the mesenteries. *C. spiculigerum* larvae demonstrated an affinity for the same locations.

*Macroderoides spiniferus* and *Paramacroderoides echinus* were restricted in their geographic distribution in southern Texas.

The single metacercaria of *Clinostomum* recovered from the digestive tract of an alligator gar was judged an atypical case. This parasite had likely only recently excysted from the flesh of a fish upon which the host had fed. The infection of *Clinostomum* metacercariae from the ovaries and mesenteries of longnosed gars has not previously been reported among lepisostids.

The discovery of unidentified *Contracaecum* sp. within the stomach of longnose and spotted gars is the first time mature forms of this genus have been recorded from these hosts.

The distribution of the genus *Dichelyne*, previously recorded only from Florida, now extends to South Texas with the finding of *D. lepisosteus* n. sp.

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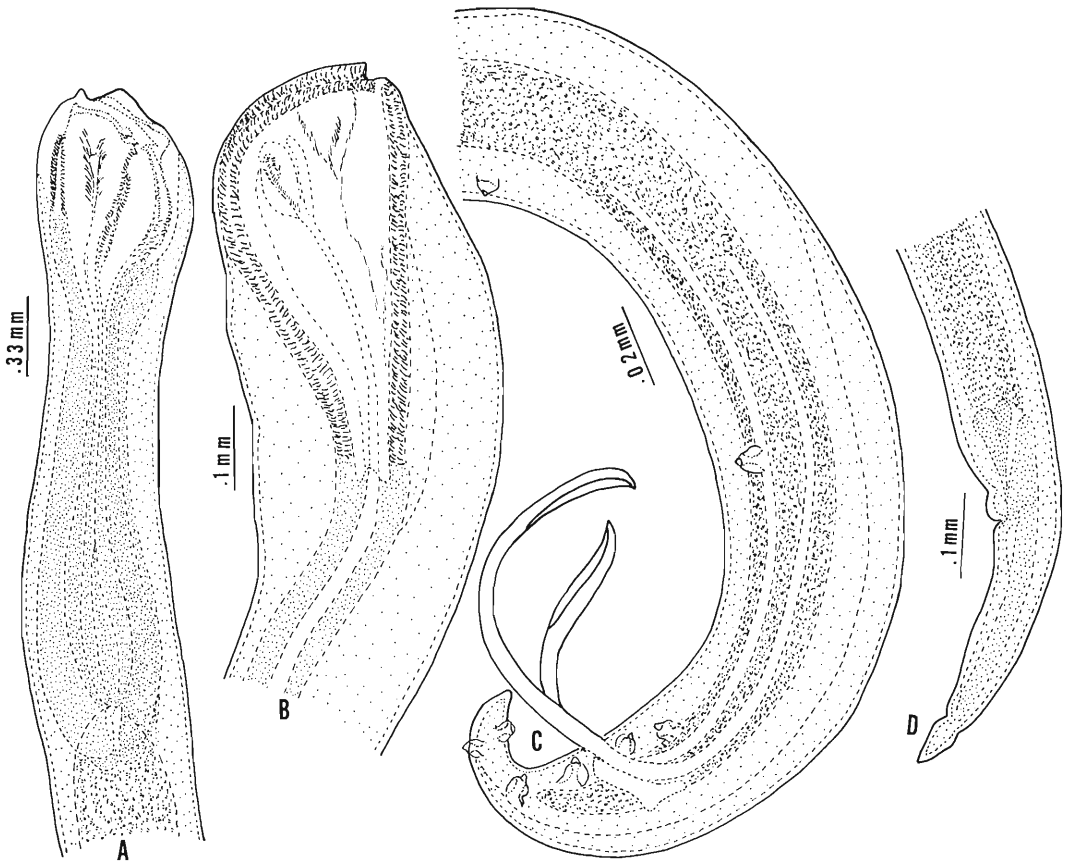


Figure 2. *Dichelyne lepisosteus* n. sp. (a) Anterior extremity of head of holotype male showing esophageal bulb, (b) anterior extremity of head of allotype female, (c) posterior extremity of holotype male showing spicules, gubernaculum, and papillae, and (d) posterior extremity of allotype female.

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## Trials with Yomesan and Other Selected Chemicals Against *Thysanosoma actinioides*, the Fringed Tapeworm of Sheep

R. W. ALLEN, F. D. ENZIE, AND K. S. SAMSON

### INTRODUCTION

The fringed tapeworm, *Thysanosoma actinioides*, is responsible for substantial losses from liver condemnations among sheep in the western states. Until recently, no drug had any significant effect against the parasite. In 1962, we reported (Allen et al., 1962) that bithionol [2,2'-thiobis (4,6-dichlorophenol)] removed appreciable numbers of *Thysanosoma* from sheep and was reasonably well tolerated in effective dosages. We also reviewed the pertinent literature on the chemotherapy of fringed tapeworm infections and described our experiences with several other compounds.

The present report summarizes results obtained with several additional compounds in tests against *Thysanosoma* infections in aged ewes and lambs. The trials were conducted at irregular intervals from 1961 to 1965 as circumstances allowed and as suitable test animals became available.

### EXPERIMENTAL PROCEDURES

The 139 sheep, comprising 129 aged ewes and 10 lambs, were obtained from range flocks in central New Mexico. All were passing *Thysanosoma* segments when randomly assigned to principal and control groups in a series of controlled anthelmintic tests. About equal numbers of treated and untreated animals were used in each trial. Feed was not withheld prior to administration of the test chemicals<sup>1</sup> by capsule, drench, or tablet. After

treatment, the animals were held in drylot until necropsy. The number and volume (water displacement) of fringed tapeworms were determined for principals and controls at the end of the post-treatment holding periods, which ranged from 4 to 99 days. Data on weight changes were obtained following treatment for all animals.

Yomesan (50% wettable powder) was administered by drench or as tablets containing 500 mg of active agent. Bayer 9015 (tablets containing 150 mg of active agent) and hexachlorophene were given in gelatin capsules. Hetol and G 27384 (50% wettable powder) were given by drench. Freon 112 was mixed with mineral oil and given by drench or in gelatin capsules; Freon 113 was given similarly without mineral oil.

### RESULTS

Yomesan had marked anthelmintic action against *Thysanosoma* infections in aged ewes, particularly when given at dose rates ranging from approximately 400 to 600 mg/kg of body weight (Table 1). In trials at the highest dose level, 14 of 15 sheep (93%) were free of fringed tapeworms at necropsy 4 to 10 days after treatment, whereas *Thysanosoma* were found in 13 of 14 untreated controls. Moreover, 369 tapeworms were recovered from the 13 infected control sheep and only 9 from the 1 infected principal, an indicated efficacy of 97% on the basis of comparative worm counts. *Thysanosoma* infections were substantially reduced also in tests at the 400- and 500-mg levels. In these trials, only five *Thysanosoma* were recovered at necropsy from one of eight treated ewes, whereas 141 specimens were found in seven of nine controls. Tenuicidal action was clearly shown at dose rates of 200 and 300 mg/kg as well, but the drug was not uniformly effective at these levels.

Yomesan was well tolerated. The only sign of toxicity was a transient softening of the feces for about 3 days in sheep that were given the drug at the 600-mg level. A number of

<sup>1</sup>From the Animal Disease and Parasite Research Division, ARS, USDA, New Mexico State University, Las Cruces. Dr. Enzie is with the Division's Beltsville Parasitological Laboratory, Beltsville, Md. This work was carried out in cooperation with the New Mexico Agricultural Experiment Station.

<sup>1</sup>Tetrachlorodifluoroethane [Freon 112] and trichlorotrifluoroethane [Freon 113] were supplied by E. I. du Pont de Nemours & Co., Wilmington, Del.; 2,2'-methylenebis (3,4,6-trichlorophenol) [hexachlorophene] was provided by Dr. Salsbury's Laboratories, Charles City, Iowa; 1,4-bis-trichloromethyl benzene [Hetol] was furnished by the National Laboratories, Kansas City, Mo.; 1-(3-trifluoromethyl-4-chlorophenyl)-3-(3,4-dichlorophenyl)urea [G 27384] was supplied by Geigy Chemical Corp., Ardsley, N. Y.; 3,3'-dichloro-5,5'-dinitro-*o,o'*-biphenol [Bayer 9015, formerly ME 3625], and 2',5-dichloro-4'-nitrosalicylanilide [Yomesan; Bayer 2353] were provided by Chemagro Corp., Kansas City, Mo.

TABLE 1. Effect of Yomesan on *Thyosanoma* in Sheep.

Test no.	Approx. dose rate (mg/kg)	Animal weight (kg)	Total dose (active agent) (g)	Weight change		Tapeworms at necropsy <sup>1</sup>			
				(lbs.)	(days)	No.	Vol. (ml)		
1	200	54.1	10.8	+1.0	8	0	0		
		48.6	9.7	+1.0	8	0	0		
		50.5	10.3	+3.5	8	3 (3)	0.4 (0.4)		
		Control	50.5	—	+3.5	8	3	1.4	
		Control	61.4	—	+3.5	8	8 (11)	1.6 (3.0)	
2	200	61.4	12.3	-8.5	6	2	0.1		
		54.1	10.8	+7.0	6	0	0.0		
		50.0	10.1	+12.0	6	0	0.0		
		56.4	11.3	+1.0	6	19	3.6		
		45.0	9.0	-2.0	6	1	0.2		
		50.0	10.0	-2.5	6	0 (22)	0.0 (3.9)		
		Control	58.6	—	+7.0	6	14	3.8	
		Control	56.8	—	+3.0	6	34	12.0	
		Control	55.0	—	-1.0	6	4	5.4	
		Control	48.6	—	+10.5	6	14	6.4	
		Control	55.0	—	+5.0	6	11	5.0	
		Control	46.8	—	+9.5	6	9 (86)	2.8 (35.4)	
3 <sup>2</sup>	200	36.8	7.4	+10.5	19	46	5.0		
		37.7	7.6	+2.0	19	14	2.8		
		34.5	6.9	+8.0	19	56	8.2		
		26.8	5.4	+5.0	19	5	0.8		
		32.7	6.6	+8.0	19	61 (182)	5.4 (22.2)		
		Control	38.2	—	+7.0	19	42	7.6	
		Control	25.0	—	+1.5	19	16	5.2	
		Control	35.5	—	-4.0	19	9	4.0	
		Control	34.5	—	+11.0	19	12	5.0	
		Control	34.1	—	+10.0	19	21 (100)	5.2 (27.0)	
		4	300	48.2	14.5	+4.0	6	14	1.8
				50.5	15.2	+7.0	6	14	1.1
52.3	15.7			+5.0	6	2	0.1		
56.4	16.9			+6.0	6	0 (30)	0.0 (3.0)		
Control	51.4			—	+6.0	6	29	5.2	
Control	46.4			—	0.0	6	20	5.6	
Control	53.6			—	+1.0	6	37	8.4	
Control	52.3			—	+1.5	6	13	6.4	
Control	64.1			—	+2.0	6	20 (119)	8.2 (33.8)	
5	400			40.5	17.5	+1.0	5	0	0.0
		45.5	27.8	+9.0	5	5	0.6		
		39.5	19.8	+3.5	5	0 (5)	0.0 (0.6)		
		Control	40.5	—	+3.5	5	20	15.0	
		Control	49.1	—	+14.5	5	27	18.0	
		Control	47.3	—	+11.5	5	30	12.5	
6	500	45.5	—	+11.5	5	20 (97)	17.0 (52.5)		
		62.3	32.0	+1.0	6	0	0.0		
		48.6	29.0	+2.0	6	0	0.0		
		57.3	25.0	-4.0	6	0 (0)	0.0 (0.0)		
		Control	62.7	—	+2.0	6	26	13.0	
		Control	51.8	—	-2.0	6	9	2.0	
7	500	53.2	—	-2.0	6	0 (35)	0.0 (15.0)		
		54.5	27.5	-7.0	4	0	0.0		
		55.5	28.0	-13.0	4	0 (0)	0.0 (0.0)		
		34.1	20.5	-5.0	4	0	0.0		
		Control	44.5	—	-4.0	4	9	1.0	
		Control	55.9	—	-9.0	4	0 (9)	0.0 (1.0)	
8	600	55.9	33.5	-12.0	4	0	0.0		
		56.8	34.0	-3.5	4	0 (0)	0.0 (0.0)		
		Control	55.9	—	-4.5	4	15 (15)	7.2 (7.2)	
		54.5	27.5	+2.0	4	0	0.0		
9	600	57.7	34.5	-7.0	4	0	0.0		
		52.7	31.5	+3.0	4	0 (0)	0.0 (0.0)		
		Control	51.4	—	-5.0	4	6	2.3	
		Control	50.0	—	+2.0	4	6	2.4	
		Control	55.0	—	+5.0	4	7 (19)	7.4 (12.1)	
		45.5	27.5	-13.0	4	0	0.0		
10	600	52.7	32.0	-14.0	4	0	0.0		
		42.7	26.0	-6.0	4	0	0.0		
		50.5	30.5	-7.0	4	0	0.0		
		48.2	29.1	-15.0	4	0 (0)	0.0 (0.0)		

<sup>1</sup> Numbers in parentheses are totals.<sup>2</sup> Lambs; all others aged ewes.<sup>3</sup> Necropsy 10 days after treatment.<sup>4</sup> Necropsy 8 days after treatment.

TABLE 1. *Continued.*

Test no.	Approx. dose rate (mg/kg)	Animal weight (kg)	Total dose (active agent) (g)	Weight change		Tapeworms at necropsy <sup>1</sup>	
				(lbs.)	(days)	No.	Vol. (ml)
11	Control	44.5	—	-4.0	4	74	10.0
	Control	53.6	—	-7.0	4	28	13.0
	Control	45.0	—	-5.0	4	89	15.0
	Control	51.4	—	-3.0	4	21	10.0
	Control	51.4	—	-8.0	4	24 (236)	9.0 (57.0)
	600	47.3	28.0	0.0	7	0	0.0
		45.0	27.0	+5.0	7 <sup>2</sup>	0	0.0
		47.7	29.0	-4.0	7	0	0.0
		44.1	26.5	+2.0	7	9 (9)	0.4 (0.4)
	Control	47.7	—	+4.0	7 <sup>1</sup>	31	5.6
	Control	40.9	—	0.0	7 <sup>1</sup>	27	5.6
	Control	44.1	—	+3.0	7 <sup>1</sup>	32 (90)	7.2 (18.4)

sheep lost weight after treatment, but weight losses also occurred among the controls. Indeed, analysis of variance of the weight changes showed that losses among principals in the several groups were not significantly different from those of their respective controls.

Freon 112, Freon 113, hexachlorophene, G 27384, Hetol, and Bayer 9015 showed no promise of useful action against *Thysanosoma* in trials with 20 ewes and 11 lambs (Table 2). Freon 112 was apparently well tolerated, but Freon 113 caused marked distress in each of three lambs. Dosing was followed by the rapid onset of dyspnea, incoordination, and excessive salivation. One lamb died within 30 minutes, but the others recovered rapidly and were apparently normal during the remainder of the 15-day post-treatment holding period. The G 27384 apparently removed all *Thysanosoma* from one of two ewes that were dosed at the rate of about 150 mg/kg of body weight, and no tapeworms were found in one ewe that was given 300 mg/kg. The latter dosage, however, elicited a severe diarrhea which persisted after 4 days and was accompanied by a weight loss of 7 pounds. Hexachlorophene, Hetol, and Bayer 9015 were well tolerated but not demonstrably effective when given in dosages up to 31, 152, and 10 mg/kg, respectively.

#### DISCUSSION

In an earlier paper (Allen et al., 1962), we noted that *Thysanosoma* is especially unresponsive to chemical attack. We suggested, therefore, that in the search for effective thysanosomacides consideration should be given to any chemical that shows teniacidal action. Moreover, because the fringed tapeworm in-

habits the biliary system, we also suggested that attention should be given to chemicals that are eliminated in the bile, particularly those with teniacidal or fasciolacidal properties. This rationale is supported and encouraged by the antiparasitic activity of bithionol and Yomesan.

Yomesan was selected for testing against *Thysanosoma* because it has shown teniacidal action against several tapeworm species in domestic animals and poultry (Boisvenue and Hendrix, 1965; Forbes, 1963; Nugara, 1963; Stampa and Terblanche, 1961). At dose rates of 50 to 80 mg/kg of body weight, the drug was well tolerated and very effective against *Moniezia*, *Avitellina*, and *Thysaniezia* (Stampa and Terblanche, 1961), common intestinal cestodes of ruminants in South Africa. It was ineffective, however, against *Stilesia hepatica*, a tapeworm which, like *Thysanosoma*, is found in the biliary system. The tests with *Stilesia* involved somewhat larger dosages than those employed against the intestinal species; however, specific toxicity trials with lambs showed that no ill effects were induced when Yomesan was given at levels up to 500 mg/kg of body weight. For these and other reasons, we elected to initiate our trials with Yomesan at the comparatively high dose rate of 200 mg/kg to establish its thysanosomacidal potential as soon as possible. The preliminary results showed that the fringed tapeworm was responsive to Yomesan, and investigations were continued with larger doses which were well tolerated as well as highly effective. It is interesting, however, although possibly coincidental, that equivalent dosages seemed to be less effective in small animals than in large

TABLE 2. Anthelmintic trials with selected compounds against *Thysanosoma* in sheep.

Chemical	Ewes	Lambs	Approx. dosage <sup>1</sup> (mg/kg)	Post-treatment period (days)	Sheep infected at necropsy	Tapeworms at necropsy			
						Number		Volume (ml)	
						Avg	Range	Avg	Range
Freon 112	—	3	330	6	3	6.3	2-14	1.3	0.6- 2.6
Controls	—	3	—	6	3	6.3	4- 8	2.1	2.0- 2.2
Freon 112	—	3	660	15	3	11.3	4-23	3.9	2.2- 6.5
Freon 113	—	3 <sup>2</sup>	660	15	2	8.0	7- 9	3.8	1.0- 6.5
Controls	—	3	—	15	3	6.0	1-12	3.4	1.0- 6.2
Hexachlorophene	—	2	31	14	2	6.0	1-11	0.6	0.1- 1.0
Controls	—	3	—	14	3	7.3	2-14	1.5	1.0- 2.4
G 27384	2	—	50	4	2	29.0	25-33	6.5	6.0- 7.0
Controls	5	—	—	4	5	47.2	21-89	11.4	9.0-15.0
G 27384	2	—	150	5	1	23.0	—	2.0	—
G 27384	1	—	300	5	0	—	—	—	—
Controls	2	—	—	5	2	4.5	4- 5	3.1	1.0- 5.2
Hetol	4	—	140	7	4	28.0	13-49	6.0	2.0- 9.0
Bayer 9015	4	—	6	7	4	12.3	3-23	1.4	0.2- 2.8
Controls	5	—	—	7	5	23.8	13-37	6.8	5.2- 8.4
Bayer 9015	2	—	5	99	0	—	—	—	—
Controls	1	—	—	99	1	2.0	—	1.6	—
Bayer 9015	2	—	5	4	1	28.0	—	2.0	—
Controls	2	—	—	4	1	16.0	—	6.4	—
Bayer 9015	3	—	9	5	3	4.7	1-10	0.4	<0.1- 1.0
Controls	4	—	—	5	4	24.3	20-30	15.6	12.5-18.0

<sup>1</sup> Active agent.<sup>2</sup> One lamb died within 30 minutes of treatment. (See text.)

(see tests 1 to 3, Table 1), a circumstance similar to that noted by the South African workers.

Although Yomesan, as used by Stampa and Terblanche (1961), showed no promise of effective action against *Stilesia hepatica*, the marked efficiency of the drug against *Thysanosoma* in our trials and the apparent safety of the chemical at substantially higher levels than those used heretofore against *Stilesia* suggest that larger doses might well be used in further trials with the latter parasite.

Most of the other compounds used in this work, namely, hexachlorophene, Hetol, Bayer 9015, and Freon 112 are known to be effective fasciolacides (Kendall and Parfitt, 1962; Knapp et al., 1965; Kuttler et al., 1963). All were given at dose rates approximating or exceeding levels employed against liver flukes, but none showed significant action against *Thysanosoma*. Freon 113 was used because of its close relationship with Freon 112 and because it had anthelmintic action in dogs (Mohler, 1933).

The G 27384 was reported to be active against certain cestodes (Petschulat, 1964). The chemical apparently removed *Thysano-*

*soma* from some of the test animals, but its toxicity would seem to militate against it.

#### SUMMARY

Yomesan [2',5-dichloro-4'-nitrosalicylanilide] substantially reduced infections of the fringed tapeworm, *Thysanosoma actinioides*, when given to sheep at dose rates ranging from approximately 400 to 600 mg/kg of body weight. In trials at the maximum level, only 9 tapeworms were recovered from 1 of 15 principals, whereas 369 tapeworms were recovered from 13 of 14 untreated controls. The numbers of *Thysanosoma* were appreciably reduced also at dose rates of about 400 to 500 mg/kg. In these trials, 5 tapeworms were found at necropsy in 1 of 8 treated ewes and 141 in 7 of 9 controls.

Yomesan was well tolerated in these dosages. A temporary softening of the feces, in ewes treated at the 600-mg level, was the only evidence of toxicity. Differences in weight changes between treated and control groups were not statistically significant.

In controlled tests involving 39 ewes and 20 lambs, the following showed no promise

of useful action against the fringed tapeworm: Tetrachlorodifluoroethane [Freon 112], trichlorotrifluoroethane [Freon 113], 2,2' methylenebis (3,4,6-trichlorophenol) [hexachlorophene], 1,4-bis-trichloromethyl benzene [Hetol], 3,3'-dichloro-5,5'-dinitro-o,o'-biphenol [Bayer 9015], and 1-(3-trifluoromethyl-4-chlorophenyl)-3-(3,4-dichlorophenyl)urea [C 27384].

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## Description and Taxonomic Position of the DD-136 Nematode (Steinernematidae, Rhabditoidea) and Its Relationship to *Neoalectana carpocapsae* Weiser

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## INTRODUCTION

In 1954, nematodes isolated from diseased codling moth larvae in Virginia were examined by the late Dr. G. Steiner, who considered them as belonging to the Steinernematidae (anonymous, 1955) and very close to *Neoalectana chresima* Steiner (Dutky and Hough, 1955). However they were never described and have been known since mainly by the code number, DD-136. Moreover, the original diagnosis given *N. chresima* by Steiner is very scant and, although from the available information similarities with the DD-136 nematode are apparent (Glaser et al. 1942), a description of *N. chresima* Steiner was never published

and this species should be designated as a nomen nudum.

The lack of a binomial and accompanying description of the DD-136 nematode has caused no little confusion with nematologists and other scientists working with these forms. The nematodes from the original sample were maintained in the laboratory and, because of their wide host range and potential as a biological control agent of insects, were distributed to a number of insect pathologists throughout the world.

Accounts of the biology of this nematode have already been presented (Anonymous, 1955, 1956; Dutky, 1959; Schmiege, 1962,

1963; Welch and Briand, 1961; Poinar, 1966; Poinar and Thomas, 1966; Poinar and Hims-worth, in press).

In 1953, Weiser found and later described *Neoplectana carpocapsae* Weiser from diseased codling moth larvae in Czechoslovakia (Weiser, 1955).

The relationship between these two nematodes has never been clear and while Schmiede (1964) felt that they were probably the same species (but apparently did not examine living material of *N. carpocapsae* Weiser), Dutky et al. (1964) state that "it appears from the description given by Weiser that the two nematodes are not the same species." Later Weiser (1966) mentioned in regards to the DD-136 nematode (translated from Czech) "a number of differences show that it is a species distinguishable from *N. carpocapsae* which was isolated in Europe from the same host." But he does not mention any specific differences and goes on to say "lack of more detailed data in Dutky's study hinders comparison of both." Moreover, Jackson (1965) found several types of differences between them and treated the two as separate species, regarding the DD-136 nematode as *N. dutkii*. However, this name is invalid and remains a nomen nudum since there was no formal publication of the specific epithet nor an accompanying description.

The present study was undertaken to characterize the DD-136 nematode and reexamine the relationship between this form and *N. carpocapsae*. This study also focuses attention on the problems of variation associated with such groups as the neoplectanid nematodes.

#### MATERIALS AND METHODS

Axenic populations of *Neoplectana carpocapsae* Weiser were received from Dr. J. Weiser of Prague. Infective juveniles were removed from the culture tubes, and then injected with a suspension of *Achromobacter nematophilus* Poinar and Thomas into larvae of *Galleria mellonella*. The resulting infective juvenile progeny later emerged from these insects and contained cells of the above bacterium in their intestine, as is characteristic for the DD-136 nematode (Poinar, 1966). Cultures of the DD-136 nematode were sent to this laboratory in 1963 from the USDA

Insect Pathology Laboratory, at Beltsville, Md. Thus both *N. carpocapsae* and DD-136 were maintained in the laboratory on *Galleria mellonella* larvae in the presence of *A. nematophilus*, and any other naturally occurring microorganisms from the insect. This was done to insure that environmental conditions were similar during the development of both nematodes.

For comparative measurements, care was taken to introduce approximately equal numbers of infective juveniles into the oral cavity of last instar insect larvae. After holding the diseased insects for 4 days at 70 F, first generation adult nematodes were removed, suspended in saline, killed with gentle heat and placed in 3% formalin for measurements. Some were later placed in lactophenol for detailed studies of the mouth and caudal region. Measurements were made on heat-killed infective juveniles that emerged naturally from *Galleria* larvae about 15 days after infection.

Detailed examinations of the lateral line of the third-stage infective nematodes were made with the electron microscope. Juveniles were fixed with 1% osmium in veronal acetate buffer (pH 7.2), embedded in maraglas and ultra-thin sections examined with an RCA-F-3 electron microscope.

Squash mounts were made for chromosome counts. The gonads were removed and stained with aceto-orcein.

For controlling matings, infective-stage juveniles of both nematodes were placed individually in hanging drops of insect blood with *Achromobacter nematophilus*. After reaching the adult stage, males and females of one nematode were transferred to drops containing females and males of the other nematode, respectively. As a control, nematodes were transferred to the opposite sex of the same nematode and females of both nematodes were held isolated in separate drops.

#### RESULTS

All of a total of 18 paired matings attempted were successful, showing that the DD-136 nematode and *N. carpocapsae* could interbreed, producing viable, normal progeny, which in turn produced repeated generations when introduced into *Galleria* larvae. Similar results were obtained whether males or females

TABLE 1. Comparative measurements of females of the Czechoslovakian and DD-136 strains of *N. carpocapsae* Weiser.<sup>1</sup>

Character	DD-136 (N = 25)			Czechoslovakian (N = 21)		
	X	Range		X	Range	
Total length (mm)	3.68	2.80	5.16	3.31	1.97	5.81
Greatest width	148.0	123.2	184.8	156.3	123.2	192.5
Stoma length	6.5	4.7	9.3	5.9	3.1	7.8
Stoma width	8.3	6.2	9.3	9.3	6.2	12.4
Length head to excretory pore	61.1	46.5	74.4	56.7	34.1	86.8
Width at excretory pore	76.9	65.1	87.0	82.2	65.1	108.5
Length head to nerve ring	137.3	117.8	161.2	140.1	108.5	170.5
Width at nerve ring	103.2	86.8	124.0	116.9	91.0	133.3
Length head to base of esophagus	191.0	161.2	217.0	201.5	155.0	241.8
Width at base of esophagus	112.0	99.2	133.3	131.4	111.6	145.7
Length tail	36.3	27.9	46.5	34.7	18.6	55.8
Width tail	69.1	49.6	86.8	63.9	46.5	77.5
Per cent vulva	54.1	51.5	56.0	52.0	50.0	58.0

<sup>1</sup> All measurements in microns unless otherwise specified.

of the DD-136 nematode were mated with *N. carpocapsae*. These experiments establish that the nematodes are conspecific. Isolated females produced ova, but did not deposit viable eggs, indicating that these nematodes are dioecious and zygotenic.

No consistent qualitative morphological characters were found which could be used to separate the two nematodes. Structurally, both nematodes are very similar and the variation that occurred in the shape of the tail, stoma, spicules, gubernaculum, and position of the male anal papillae restricted the use of these structures as diagnostic characters. Jackson (1965) used some of the above structures for differentiating DD-136 from *N. carpocapsae*, however, after having examined several hundred adults of both nematodes, the present author feels that the variability is too great for definite conclusions to be drawn. However, it should be pointed out that Jackson examined axenic nematodes maintained on artificial media and thus possibly with less overall variation.

Quantitative data obtained for comparison of the sexual stages and infective juveniles of both nematodes are presented in Tables 1, 2, and 3. These data are comparative and, since they include only measurements of the first generation adults, do not represent the complete variability which would also cover the smaller succeeding generations in the insect. Differences between the measurements of *N. carpocapsae* presented here and those presented by Weiser (1955, 1956) reflect the

variation within this species. While first generation adults were measured in this study, Weiser probably measured adults of the succeeding generations, thus obtaining significantly smaller values. Further variation would probably arise from the host parasitized, associated microorganisms and physical factors of the environment.

Tests of significance (*t*-test) were made on the quantitative data presented in Tables 1, 2, and 3. Even under these relatively stable conditions, values obtained for the females were too variable to be significant at the 5% level. A similar condition existed in the male populations, although a significant difference in length and reflection of testis was recorded. This may be due to the quicker maturation of DD-136, since populations measured 5 or 6 days after infection showed no significant difference in length or reflection of testis. Variation was less in the infective juveniles as shown in Table 3, and several values were found to be significantly different, especially the distance from the head to the excretory pore. This value remained significantly different over several samples taken, even though further sampling showed that variation in length and distance from head to nerve ring was too great for these characters to be used for diagnostic purposes.

Measurements of the distance from the head to excretory pore made on the infective juveniles resulting from the crossing experiments usually fell between those obtained for the parental populations (Table 4).

TABLE 2. Comparative measurements of males of the Czechoslovakian and DD-136 strain of *N. carpocapsae* Weiser.<sup>1</sup>

Character	DD-136 (N = 25)			Czechoslovakian (N = 12)		
	$\bar{X}$	Range		$\bar{X}$	Range	
Total length (mm)	1.45	1.09	1.71	1.32	1.25	1.40
Greatest width	101.6	77.0	130.9	117.0	107.8	130.9
Stoma length	5.6	2.6	7.8	5.6	5.2	7.8
Stoma width	4.9	3.9	6.5	4.6	2.6	6.5
Length head to excretory pore	61.4	46.5	74.4	71.3	55.8	74.4
Width at excretory pore	47.7	37.2	58.9	52.1	46.5	58.9
Length head to nerve ring	110.1	93.0	124.0	127.7	108.5	155.0
Width at nerve ring	58.9	46.5	71.3	68.5	62.0	86.8
Length head to base of esophagus	154.7	136.4	167.4	151.9	145.7	155.0
Width at base of esophagus	64.5	49.6	77.5	70.7	65.1	77.5
Length bent portion of testis to base of esophagus	128.6	53.9	284.9	136.3	84.7	192.5
Length tip of testis to tail	1,150.0	780.0	1,560.0	1,000.0	780.0	1,090.0
Reflection testis	563.6	400.4	808.5	385.0	284.9	477.4
Length tail	30.4	23.4	39.0	31.2	22.1	35.1
Width anus	42.6	32.5	54.6	44.5	39.0	52.0
Length spicula	64.6	58.5	71.5	66.3	62.4	70.2
Width spicula	11.1	9.1	13.0	12.5	10.4	13.0
Length gubernaculum	47.1	39.0	55.9	47.1	42.9	52.0
Width gubernaculum	5.2	3.9	6.5	6.4	5.2	7.8

<sup>1</sup> All measurements in microns unless otherwise specified.

The infective third-stage juveniles of both nematodes possess conspicuous lateral fields. The number and shape of the folds and striae constituting these fields are sometimes characteristic and used in the differentiation of nematode species. Electron micrographs show that these fields are similar in both nematodes and at midbody consist of six longitudinal ribs, comprising two pairs of pronounced outer ribs on either side of a pair of finer inner ribs (Fig. 1, A, B).

Smears of testicular tissue from both nematodes showed a chromosomal condition of four bivalents and a single univalent. This indicates a 2N condition of nine chromosomes for the males and ten for the females (Fig. 1, C, D).

A qualitative description of the DD-136 nematode follows.

Steinernematidae Chitwood and Chitwood 1937, 1950; *Neoalectana* Steiner 1929.

Adult forms (Figs. 2 and 3). Cuticle smooth, head truncate to slightly rounded, lips united, setae obscure; two circles of anterior papillae, six inner labial papillae and six outer cephalic papillae. Amphids small, porelike, near level of cephalic papillae. Stoma partially collapsed with only an anterior vesibule remaining. Collar lacking, esophageal tissue close to mouth opening, reaching to the base of the vesibule. Cheilorhabdions represented as

lightly sclerotized areas lining the inside of the lip region anterior to the esophagus. A small sclerotized area just beneath the cheilorhabdions probably represents the modified prorhabdions. Meso-, meta-, and telorhabdions vestigial, although sometimes rarely represented as refractive edges lining the collapsed walls of the stoma.

Esophagus muscular, the anterior portion of the procorpus slightly expanded just behind the vesitubule, then extending into a slightly enlarged nonvalvulated metacarpus, followed by an isthmus and terminating in a basal bulb containing a small haustum with three bulb flaps lined with refractive ridges. Base of esophagus often inserted into the anterior portion of the intestine. Nerve ring surrounding isthmus just anterior to the basal bulb. Excretory pore usually anterior to nerve ring. Lateral field and phasmids inconspicuous.

Female amphidelphic with opposed reflexed ovaries. Variable in size, some giant forms reaching 10 mm. Vulva a transverse slit, bearing two prominent ventral protuberances. Vagina short with muscular walls leading into a prouterus which serves as an egg chamber—a small constriction separates this from the remainder of the uterus, where fertilization occurs. Well developed glandular oviduct leads into the growth zone of the ovary and finally into the elongate germinal zone. Fe-



TABLE 3. Comparative measurements of the 3rd stage infective juveniles of the Czechoslovakian and DD-136 strain of *N. carpocapsae* Weiser.<sup>1</sup>

Character	DD-136 (N = 25)			Czechoslovakian (N = 25)		
	$\bar{X}$	Range		$\bar{X}$	Range	
Total length	547.0	438.0	625.0	572.0	488.0	613.0 <sup>2</sup>
Greatest width	24.0	22.0	28.0	26.0	25.0	28.0
Head to excretory pore	35.7	34.0	40.0	42.1	39.0	56.0 <sup>3</sup>
Head to excretory pore (sample 2)	38.6	36.4	40.3	43.2	39.0	58.5 <sup>3</sup>
Head to nerve ring	85.0	81.0	90.0	88.0	84.0	93.0 <sup>2</sup>
Length tail	53.0	50.0	59.0	53.0	47.0	59.0

<sup>1</sup> All measurements in microns.

<sup>2</sup> Means significantly different at 5% level.

<sup>3</sup> Means significantly different at 1% level.

male tail bluntly conical to dome shaped—with or without a short spine on the tip. Second and succeeding generation females in the host are correspondingly smaller in size. Pigmy forms or swollen miniature females were never found.

Male with single reflexed testis consisting of a germinal and growth zone leading into a seminal vesicle containing spermatophores. Vas deferens conspicuous, with glandular walls. Spicules paired, symmetrical, curved and bearing a more or less pronounced arch on their ventral surface (Fig. 3 C). Shape of capitulum variable—from slightly pointed to round or flat. Surface and edge of calamus and lamina bearing ridges. A thin velum present. The gubernaculum is also variable, ranging from completely flattened to bow shaped in lateral view, with the proximal portion bent at various angles and sometimes even bluntly bifurcate (Fig. 3, D, E). In dorsal view, the distal portion consists of two lateral projections with a thin sclerotized spine between them. The area between the lateral projections is connected by a thin membrane. Male tail with a complement of 23 anal papillae (11 pairs and a single median adanal) comprising two rows of six ventrolateral papillae and five paired post anal papillae. Of the latter group, two pair are situated laterally on the tail, one near the terminus and the other in the vicinity of the gubernaculum. This latter pair is variable in position and often difficult to observe. Tip of tail conical with a small appendage. Bursa absent.

Infective-stage juveniles (3rd stage) (for illustration, see Poinar, 1966) much narrower than corresponding parasitic juvenile. Mouth

and anal opening closed, esophagus and intestine collapsed; tail pointed; lateral fields distinct.

The development and bionomics of both nematodes were studied and compared under laboratory conditions. The observations made here on *N. carpocapsae* were similar to those Weiser (1966) reported earlier and the infection pattern of this nematode is similar to that previously reported for the DD-136 nematode (Poinar and Himsforth, in press). Aside from a quicker development of the DD-136 nematode to the adult stage in the insect, no major differences were discerned between the two nematodes during the course of this investigation and it is concluded that both belong to a single species.

#### DISCUSSION AND CONCLUSIONS

Although breeding studies establish that the nematodes are conspecific, there is evidence indicating that they are not identical. Morphological variability prevents the use of structural characters for differentiating between the two nematodes. However, Jackson (1965, 1966) detected differences in response to axenic growth media and in serological reactions. Although both nematodes behaved similarly on solid media, in fluid environments DD-136 normally developed to the adult stage, while *N. carpocapsae* usually did not develop at all. In further investigations along this line, Hansen and Yarwood (1967) found that in axenic liquid media distributed as a film over glass wool (for method, see Hansen and Cryan, 1966), very few of the infective juveniles of *N. carpocapsae* exsheathed and reached

TABLE 4. Distance from head to excretory pore in infective juveniles of the Czechoslovakian and DD-136 strain of *N. carpocapsae* Weiser and their cross F<sub>1</sub> progeny.<sup>1</sup>

Date	DD-136	F <sub>1</sub>	Czechoslovakian
1 March 1966	37.7	38.4 <sup>2</sup>	40.8
12 March 1966	37.0	39.0	42.0
20 March 1966	37.7	38.4	40.8

<sup>1</sup> Each figure represents the average of 50 individuals.

<sup>2</sup> Means not flanked by lines are significantly different at the 5% level.

the adult stage where reproduction would occur, while most of the DD-136 nematodes reached the adult stage and reproduced. However, when developing stages of *N. carpocapsae* were placed in the same environment, reproductive cultures were readily established, suggesting that a major difference between the two nematodes is the ability of the infective juveniles to exsheath and initiate development when placed in liquid media.

Do these differences warrant subspecific status for these organisms? Subspecies are populations capable of interbreeding, yet which differ from each other taxonomically and are isolated ecologically or geographically. These nematodes interbreed, but the great degree of variability rules out morphological separation on any practical basis. The distance from the head to the excretory pore in the infective juveniles was the only consistent difference found in this study. Yet it should be pointed out that a relatively small population of both nematodes was examined and this difference may represent only a relative one between isolated populations. Both nematodes were discovered in geographically isolated areas, DD-136 from Virginia and *N. carpocapsae* from Czechoslovakia. However, there is no way of knowing the precise range of either nematode, especially since DD-136 has now been introduced into Europe for field tests.

Host specificity here probably is not as important as in other groups, since both nematodes do not develop on host tissue alone, but on a mixture of host tissue and bacteria. This association with certain bacteria permits them to parasitize a wide range of insects. The DD-136 nematode was found to be associated with a characteristic bacterium, *Achromobacter*

*nematophilus* Poinar and Thomas. A closer association between these nematodes would have been established if the bacterial flora of *N. carpocapsae* could have been examined for the presence of *A. nematophilus*, or a closely related form. Unfortunately, natural xenic colonies of this nematode were recently lost (Weiser, personal communication).

It is possible that adaptation to hosts in a particular physical environment occurs, however since both nematodes were originally found in codling moth larvae, differentiation along these lines may not yet have developed.

It is the author's impression that this and related species are composed of a complex of separate populations throughout a major part of the world, all modified to a greater or lesser extent and perhaps adapted to a particular environmental or host "niche."

The author feels that taxonomic (morphological and physiological) differentiation between these two nematodes has not yet reached the point where they can be called distinct subspecies. On this basis, these nematodes should be assigned to an infrasubspecific rank. Although they may be considered populations from the zoological standpoint, a more appropriate term might be strain. This is an infrasubspecific category which is used in microbiology and is defined in the International code of nomenclature of bacteria and viruses (1958) as being "made up of the descendants of a single isolation in pure culture." This may be especially appropriate here since both nematodes can be maintained in the laboratory continuously on living insects or artificial media, similar to bacterial cultures. It is realized that this term has no official standing in the zoological code of nomenclature.

It is proposed that the DD-136 nematode now be considered as the DD-136 strain of *N. carpocapsae* Weiser and what was originally *N. carpocapsae* Weiser be known as the Czechoslovakian strain of *N. carpocapsae* Weiser.

In studying the DD-136 nematode, Schmiege (1962, 1963) obtained a wide range of deMan values when he compared adults of the first and second generation, finding that during growth of the female, the size of the body organs did not vary proportionally to the total body size, and he concluded that the deMan

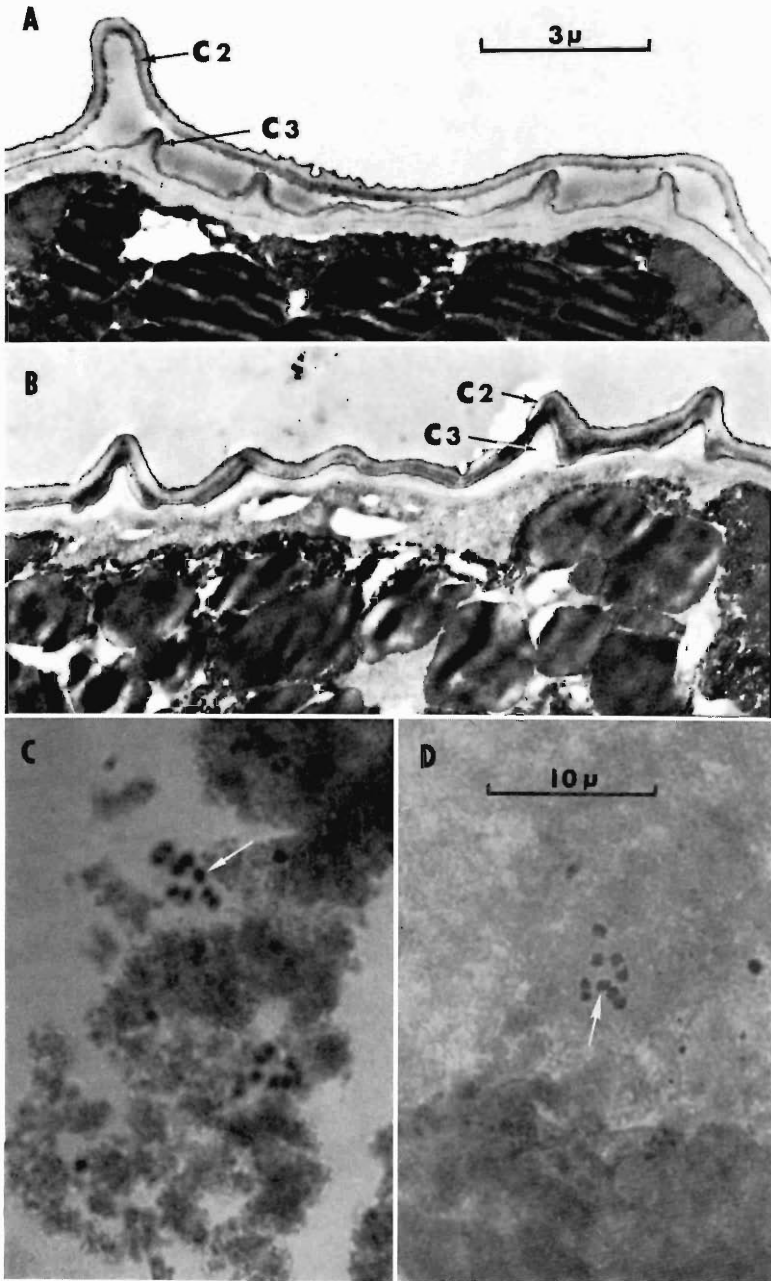


Figure 1. *Neoplectana carpocapsae* Weiser. A. Electron micrograph of the lateral field of the infective juvenile of the Czechoslovakian strain. B. Same of the DD-136 strain (magnification same as A). C. Male chromosomes of the DD-136 strain (magnification same as D). D. Same for the Czechoslovakian strain. (Arrow points to the univalent sex chromosome.) C2—second stage cuticle, C3—third stage cuticle.

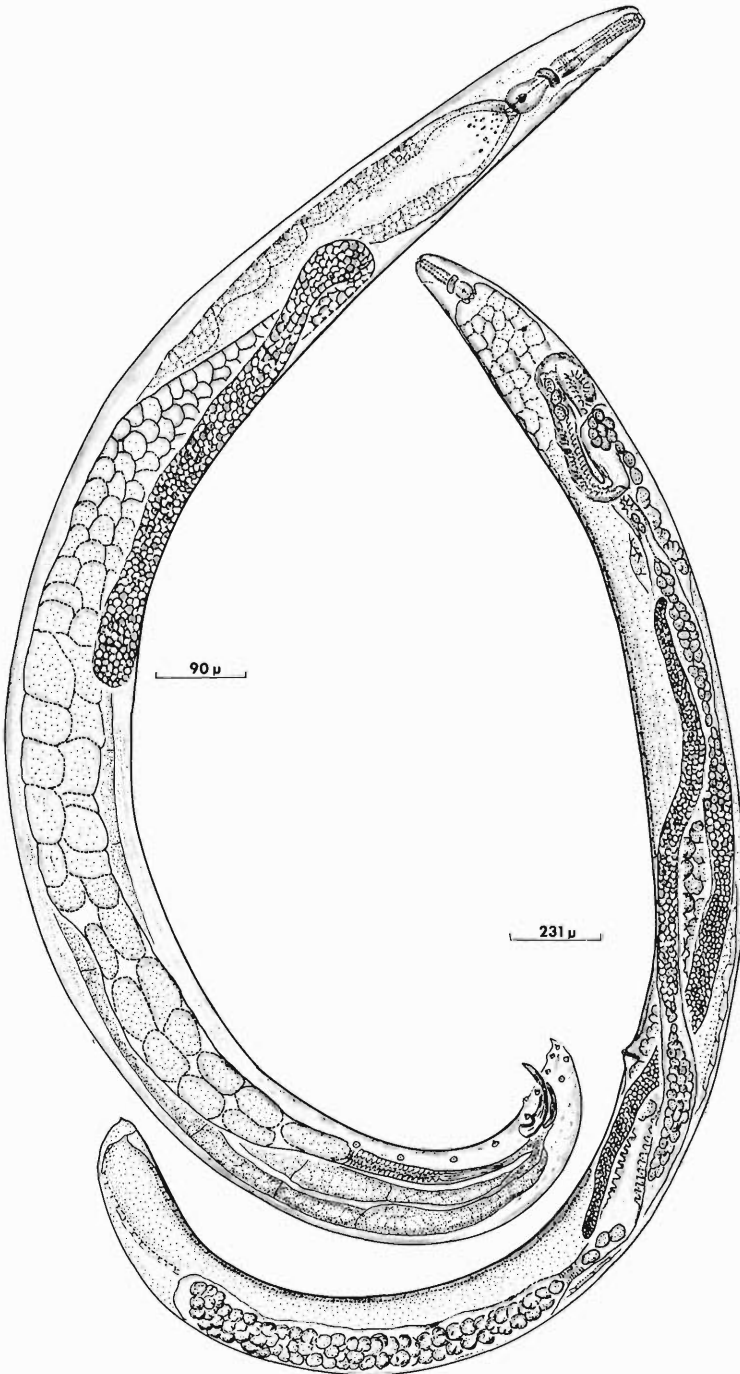


Figure 2. Adult forms of the DD-136 strain of *Neoplectana carpocapsae* Weiser.

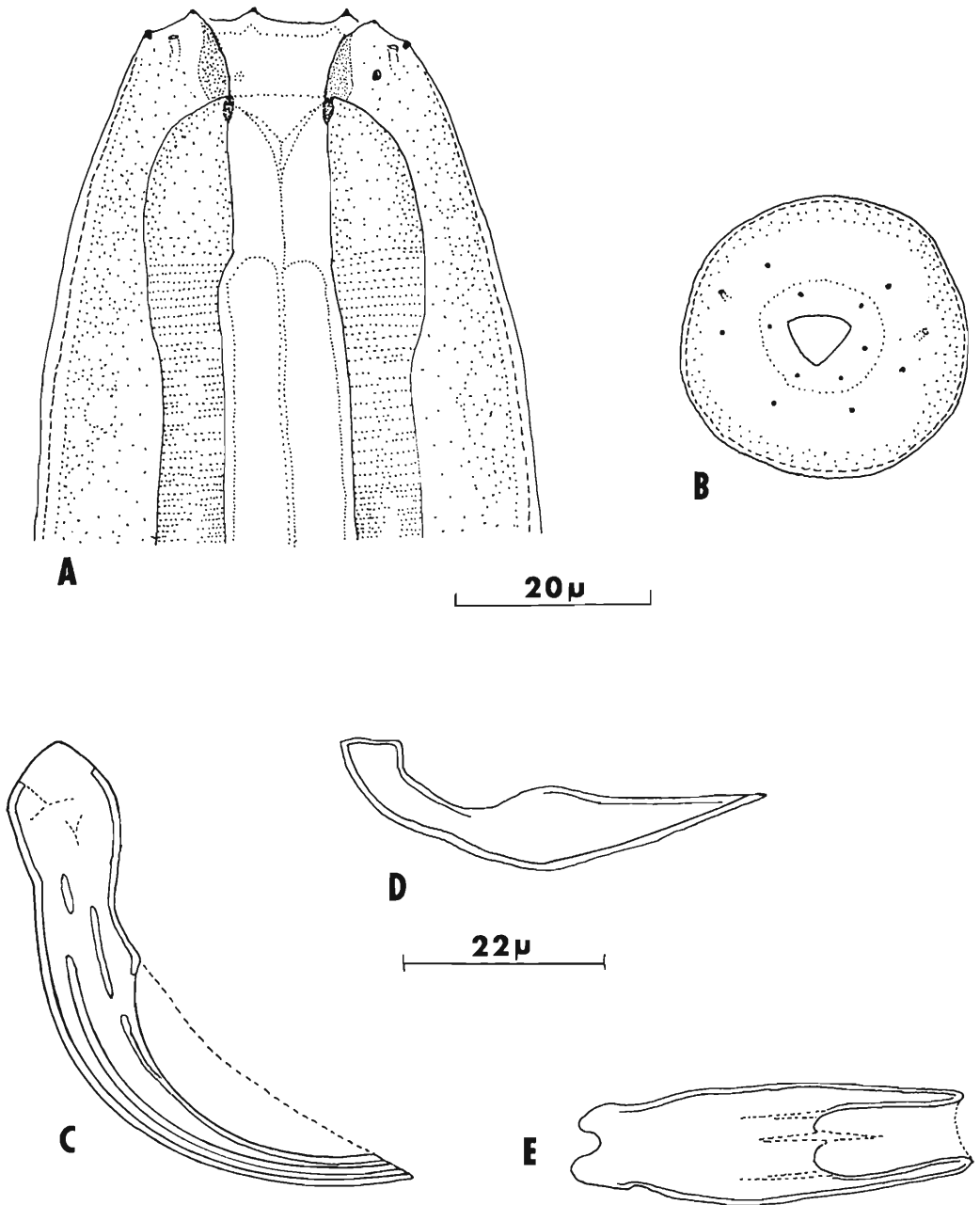


Figure 3. DD-136 strain of *Neoplectana carpocapsae* Weiser. A. Dorsal view of head region of the female. B. "En face" view of female. C. Lateral view of spicule. D. Lateral view of gubernaculum. E. Dorsal view of gubernaculum.

index was not a good character to distinguish species of this group. Weiser (1966) also mentioned the extreme size variation in the Czechoslovakian strain, and results in the present study support these observations. The variable index values occur because of differential growth rates of various body areas. When there is ample nourishment, usually the condition for the first generation adults, the nematodes, especially the females, enlarge after reaching the adult stage. The amount of growth that occurs in the body between the base of the esophagus and anus is proportionally greater than that which occurs in the esophageal and caudal region, resulting in variable indices. Added variability arises from the diminishing adult size with each new generation in the host.

With the limited value of measurements for diagnosis we see that distinguishing *N. carpocapsae* Weiser from previously described members of the genus is difficult. Weiser (1955) listed no diagnostic characters with the original description of *N. carpocapsae* and in a later publication (Weiser and Kohler, 1955) used various morphological characters, both quantitative and qualitative, for the separation of species. However, many of these characters have never been evaluated, and since many of the earlier descriptions gave no reference to variation, the validity of these characters for diagnosis is questionable. The manner of treating the nematode prior to measurement is also of considerable importance. The structure of the stoma for instance varied considerably depending on how the nematodes were killed and fixed. Thus before *N. carpocapsae* Weiser can be clearly separated from previously described species, some knowledge of the variability and breeding potential of the latter should be acquired. When living material is available, further hybridizing studies will be conducted to determine the biological relationship between *N. carpocapsae* and other neoplectanid nematodes.

The diagnostic value of biological characters such as the presence of pigmy females in the 2nd and 3rd generation, and the retention of eggs in the uterus resulting in viviparity should be evaluated.

In general, nematode species have been defined mainly on a morphological and host

preference basis, thus establishing morphological rather than biological species. Although many morphological species are probably also true biological species, when clear-cut differences are not apparent, reproductive isolation can serve as the final decision for specific identity. Neoplectanid and free living nematodes lend themselves well for this type of study, while other forms can be examined when adequate methods of handling and rearing are developed.

Studies involving hybridization have already been done with some of the biological races of *Ditylenchus dipsaci* (Kühn). Races occur which are morphologically indistinguishable and restricted to certain host plants. This host specificity breaks down on callus tissue and interbreeding occurs, indicating that the races are conspecific (Eriksson, 1965). Roberts et al. (1954) used this criterion in determining if the ovine and bovine strains of *Haemonchus contortus* were distinct species and Augustine (1939) held the same view when comparing *Strongyloides* from different animal hosts. Duke (1964) recently established conspecificity between the simian and human strains of *Loa loa* and found that the characters of size and periodicity of microfilariae, which varied in both strains, segregated out according to a simple Mendelian pattern in the F<sub>1</sub> and F<sub>2</sub> generations.

Further study on the strains of *N. carpocapsae* Weiser may reveal other differences between them, however it appears that both nematodes diverged relatively recently from a common stock and the differences found so far (mainly physiological) reflect the state of continued isolation under different environmental conditions.

#### ACKNOWLEDGMENTS

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#### SUMMARY

A description of the DD-136 nematode is given. Detailed qualitative and quantitative comparative studies were made on *Neoplectana carpocapsae* Weiser and the DD-136 nematode. These nematodes are able to inter-

breed and on this basis are considered conspecific. After a discussion of the similarities and differences between these nematodes and the variation associated with them, it was concluded to assign them to the infrasubspecific rank of strain. It is proposed that the DD-136 nematode be considered as the DD-136 strain of *N. carpocapsae* Weiser and what was originally *N. carpocapsae* be known as the Czechoslovakian strain of *N. carpocapsae* Weiser.

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**Life History Studies on *Cardicola klamathensis* (Wales, 1958)  
Meade and Pratt, 1965 (Trematoda: Sanguinicolidae)<sup>1</sup>**

THOMAS G. MEADE

INTRODUCTION

*Cardicola klamathensis* (Wales, 1958) Meade and Pratt, 1965, was recovered from the efferent renal veins of Lahontan cutthroat trout fingerlings, *Salmo clarkii* Richardson, by Wales (1958) and placed into genus *Sanguinicola*. Subsequent studies by Meade and Pratt (1965) resulted in a transfer of the blood fluke from genus *Sanguinicola* to *Cardicola*. No life history studies were conducted by Wales (1958) although cercariae of the lophocercous, brevifurcate, apharyngeate type were present in local snails.

This paper redescribes the sporocyst and cercaria of *C. klamathensis* and supplies experimental evidence which shows the cercaria to be the correct one for *C. klamathensis*.

MATERIALS AND METHODS

One thousand one hundred twenty snails, *Flumenicola seminalis* Hinds, were collected at the Klamath Fish Hatchery, Klamath County, Oregon, and isolated into glass fingerbowls where about 2% shed cercariae spontaneously. The larvae were probably the same type found earlier by Wales although several aspects of their morphology differed from his description.

Infective cercariae were placed into aquaria with young fingerling trout obtained from the Alsea Hatchery, Benton County, Oregon. More than 100 cutthroat trout from the Alsea Hatchery examined for other types of fish blood flukes were free of infection with *C. klamathensis*. Ten fish removed from this same lot of fish were exposed to cercariae during the summer of 1965. Trout were found to be difficult to keep for extended periods in aquaria under usual laboratory conditions as noted by Meade and Pratt (1965). To increase survival rates, no more than five fingerlings were placed in each 10 gallon aquarium. The water, maintained at a constant cold room temperature

of 12.8 C, was well filtered, aerated, and changed every 5 days. By carefully following the above procedure, two fish were maintained for 6 weeks and one for 9 weeks. The total number of fish exposed experimentally to infective cercariae was limited due to the small quantity of larvae obtained from snails.

Larvae and adults were studied intensively before fixing. Supravital staining with neutral red was employed for these initial studies. Observation of living material proved far superior to fixed preparations. Following live study, both larvae and adults were fixed with hot 5% formalin, stained with Semichon's acetocarmine, cleared in oil of cloves, and mounted in balsam.

All measurements are in microns unless otherwise indicated.

RESULTS AND DISCUSSION

Six weeks following exposure to cercariae, two fingerling trout were sacrificed and both were infected. A total of 21 young flukes with an average measurement of 460 long by 73 wide were taken from all areas of the circulatory system. The fish maintained for 9 weeks was infected with five flukes. Two were mature adult worms measuring 1.74 mm long by 0.43 mm wide and were removed from the efferent renal vein. The three remaining worms, removed from the gills and liver, were only partially mature and averaged 643 long by 98 wide. The flukes recovered matched the description given by Wales for *C. klamathensis*.

Periodic cracking of snails was undertaken to check the soft parts for sporocysts and rediae. Unlike the report by Wales, only sporocysts were found. There was no evidence of pharynx and gut. Sporocysts were thin-walled, ovoid to elongate in shape, and were present in the visceral mass. No birth pore was evident. Between 15 and 20 cercariae were usually present in various stages of development with only two or three mature cercariae in each sporocyst. The flame cell pattern of cercariae was best studied during this develop-

<sup>1</sup> Contribution of Department of Biology, Sam Houston State College, Huntsville, Texas.

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mental period. On examination of snails collected in September and early October, cercariae appeared to be free in the visceral mass and no sporocysts could be found.

Cercariae were examined both living and following fixation with hot formalin. Heat killed larvae were 795 long with the body 305 long by 87 wide. The tail measured 490 long and 45 wide, with furcae 109 long. Along the top of the body was a dorsal fin which extended somewhat more than one-half the body length. An apical papilla with eight rows of short black spines was set off from the remainder of the body by a prominent constriction. A small mouth opening was posteroventral to the rows of spines. Eight penetration glands extended into the body of the cercaria to little more than one-half of the body length. No pharynx or esophageal musculature was present. Termination of the delicate gut could not be determined with certainty due to the enlarged ends of the penetration glands. Small light colored spines extended randomly over the surface of the cercarial body. A small, thin-walled, highly contractile excretory bladder was present. The flame cell count 2 [2 + 2] was derived by studying the developing cercaria. No flame cells were observed in the tail. A single excretory duct ran the length of the tail to about three-fourths its total length where it formed a forklike branch with each tube passing to an arrowhead-shaped furcal tip. Small round cells of variable numbers were loosely scattered throughout the tail. Prominent membranes were along both margins of the furcae. No bulbs were present at the furcal tips (Fig. 1).

Living cercariae were shed in largest numbers in the early morning. When immotile, the cercarial body was positioned at right angles to the tail with the posterior half of the tail often being curved so the body of the larva came to rest in the angle of the furcae (Fig. 2). During activity, due to stiffening of the organism, the body came to a thirty degree angle with the tail. The usual pattern of behavior was for larvae to move to the top of the water and gradually sink to the bottom. Larvae were attracted to light. Cercarial response could be initiated by almost any object which was suddenly placed among them and moved about. Contact with fish appeared to be by accident.

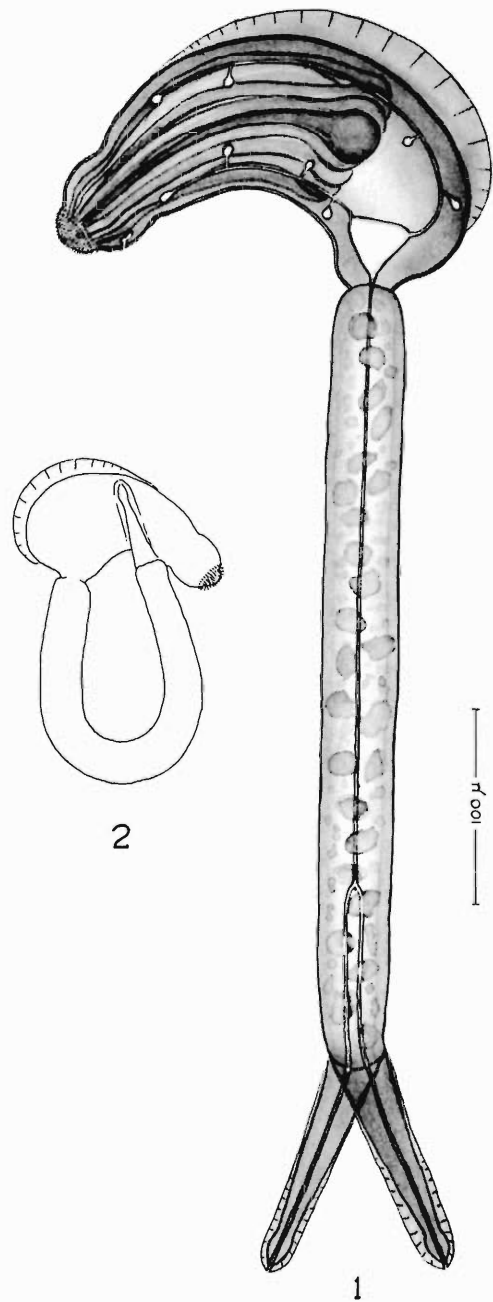


Figure 1. Cercaria of *Cardicola klamathensis* under light cover slip pressure. Camera lucida drawing. To scale.

Figure 2. Cercaria of *C. klamathensis* in natural position. Not to scale.

No special response was observed when cercariae were placed with the fingerlings. However, on physical contact, penetration was initiated; the process started with the tail which moved actively pushing the apical papilla of the cercaria against the host. Within 15 minutes cercariae were usually under the skin of the fingerlings with the detached forked tails lying along side. Success of cercariae in penetration of fishes was noticeably better when less heavily scaled areas were attacked.

Adults recovered from experimental and natural infections of fingerling trout were essentially the same as those described by Wales (1958). Mature adult worms recovered from both types of infections were always present in the efferent renal vein. Immature worms were removed from all parts of the blood circulatory system. The location of the mature fluke in the more spacious efferent renal vessel might possibly be the result of the worms large size in relation to the size of the fish. *Cardicola klamathensis* was the largest blood fluke collected by the author from fishes in the Pacific Northwest.

No miracidial stages were recovered during the course of the study.

#### SUMMARY

Larval stages of *Cardicola klamathensis*

(Wales 1958) Meade and Pratt, 1965, were obtained from snails and the adult fluke recovered from experimental infections of Lahontan cutthroat trout fingerlings, *Salmo clarkii* Richardson. From a lot of ten fish exposed to cercariae, three survived, two for 6 weeks and one for 9 weeks. Immature flukes were removed from all parts of the blood circulatory system; mature forms from the efferent renal vein only.

In addition to life history studies, two larval stages are redescribed. Sporocysts were located in the host snail, *Flumenicola seminalis* Hinds. Contrary to the report by Wales, rediae were absent. Cercariae were furcocercous, brevifurcate, and apharyngeate, possessed a fin along the dorsal body surface, and anteriorly displayed an apical papilla with eight rows of spines. Eight penetration glands, a small contractile excretory bladder, and a flame cell formula of 2 [2 + 2] are described. Arrowhead-shaped furcal tips were uniformly present.

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## Two New Species and Further Notes on *Criconemoides* Taylor, 1936 (Criconematidae: Nematoda)

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In the course of surveying the forest soils of New Mexico to determine the nature and distribution of plant-parasitic nematodes in those soils, the junior author has found seven species of *Criconemoides*. Two of these are judged to be new species, the descriptions of which follow. The specific limits of other

described species are extended by these collections, which also represent new distribution records.

Through the courtesy of Dr. G. Hartwich of the Zoological Museum of the Humboldt-University, Berlin, type specimens of *Criconemoides demani* (Micoletzky, 1925) Taylor 1936, and *Criconemoides sphagni* (Micoletzky, 1925) Taylor, 1936, were made available for study. These were in sufficiently good condi-

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tion to add to our knowledge of these species and to designate type specimens.

Type specimens of *Criconemoides hispalensis* Arias, López, and Jiménez, 1963, and *Criconemoides montserratii* Arias, Jiménez, and López, 1965, also were kindly sent by F. Jiménez-Millán for study, a report of which is given below.

*Criconemoides dividus* n. sp.  
(Fig. 1, C-G)

FEMALES (4) (paratypes): L = 0.33 mm (0.32-0.36); a = 10.8 (10-11); b = 3.4 (3.3-3.6); c = 41-43; vulva = 96 (96-97); stylet = 52  $\mu$  (50-53); prorhabdion = 41  $\mu$  (39-42); total body annules = 82 (81-84) dorsal, 71 (70-73) ventral.

MALE: Unknown.

LARVA: Unknown.

FEMALE (holotype): L = 0.32 mm; a = 11; b = 3.4; c = 41; vulva = 96; stylet = 50  $\mu$ ; prorhabdion = 39  $\mu$ ; total body annules = 81 dorsal, 70 ventral.

Body tapers slightly anteriorly to bluntly rounded head. Lip region not set off. Sublateral lobes absent. First labial annule narrower and thinner than succeeding ones, second and third successively wider and thicker; all three similar to rest of body annules in outline. Longitudinal markings on first annule and continue over entire body. Stylet robust, knobs about 6-7  $\mu$  across with forward directed processes. Excretory pore on 24th annule, 97  $\mu$  from anterior end (on 23rd-25th annule in paratypes, 98-103  $\mu$  from anterior end). Gonad outstretched and occupies 33% of body length (45-49% in paratypes). Spermatheca absent, spermatozoa not evident. Vulva with simple oval outline, on fifth annule from posterior end of body. Lips of vulva rounded, protruding slightly beyond outline of body shape as formed by adjacent annules. Anus on third annule from terminus. Terminus bluntly rounded in outline, last annule an irregular fringed or convoluted edge. Body annules average 4.3-4.7  $\mu$  (4.1-5.2) each distinctly rounded and retrorse. Anastomoses fairly common, some intervening annules have a distinct jog or break anteriorly directed forming a semblance of a lateral line but not over entire length of body.

HOLOTYPE: Female collected by J. W. Riffle, 9 July 1963, slide number 784, University of California Nematode Survey Collection, Davis.

PARATYPES: Two females, same data as holotype; deposited: one female, slide number 785 (also various sections of one paratype on slides 786-789), University of California Nematode Survey Collection, Davis; one female, slide 7, Rocky Mountain Forest and Range Experiment Station Nematode Slide Collection, Albuquerque, New Mexico.

TYPE HOST: Soil about roots of *Pinus ponderosa* Laws. and *Juniperus monosperma* (Engelm.) Sarg., at 7,100 feet elevation.

TYPE LOCALITY: Two miles southeast on Burnt Mesa Road, Bandelier National Monument, near Los Alamos, New Mexico.

DIAGNOSIS: This species keys to and is most closely related to *Criconemoides caelatus* Raski and Golden, 1966, from which it differs in the shorter stylet (50-53  $\mu$  vs. 61-75  $\mu$  *caelatus*); fewer body annules (70-84 vs. 91-103); and definitely less pronounced bead-like markings on cuticle of *dividus*.

FEMALE (1) (Manzano, New Mexico): L = 0.29 mm; a = 11; b = 3.3; c = ?; vulva = 95; stylet = 50  $\mu$ ; prorhabdion = 39  $\mu$ ; total body annules = 78 dorsal, 66 ventral.

This single female from soil about *Pinus ponderosa* and *Juniperus deppeana* Steud. has the excretory pore on the 22nd annule (89  $\mu$  from anterior end) and vulva on 4th annule from terminus, ovary outstretched, spermatheca lacking.

FEMALE (1) (Corona, New Mexico): L = 0.37 mm; a = 12; b = 3.9; c = ?; vulva = 95; stylet = 47  $\mu$ ; prorhabdion = 36  $\mu$ ; total body annules = 78.

Another single female from soil about the roots of *Pinus edulis* Engelm. and *Juniperus monosperma* has outstretched ovary with a prominent spermatheca in anterior ventral lobe of uterus, full of spermatozoa. Both these single females from widely separated localities very closely resemble the type specimens, which confirms the identity of the species while contributing slightly wider range to length, size of stylet, and presence of spermatheca.

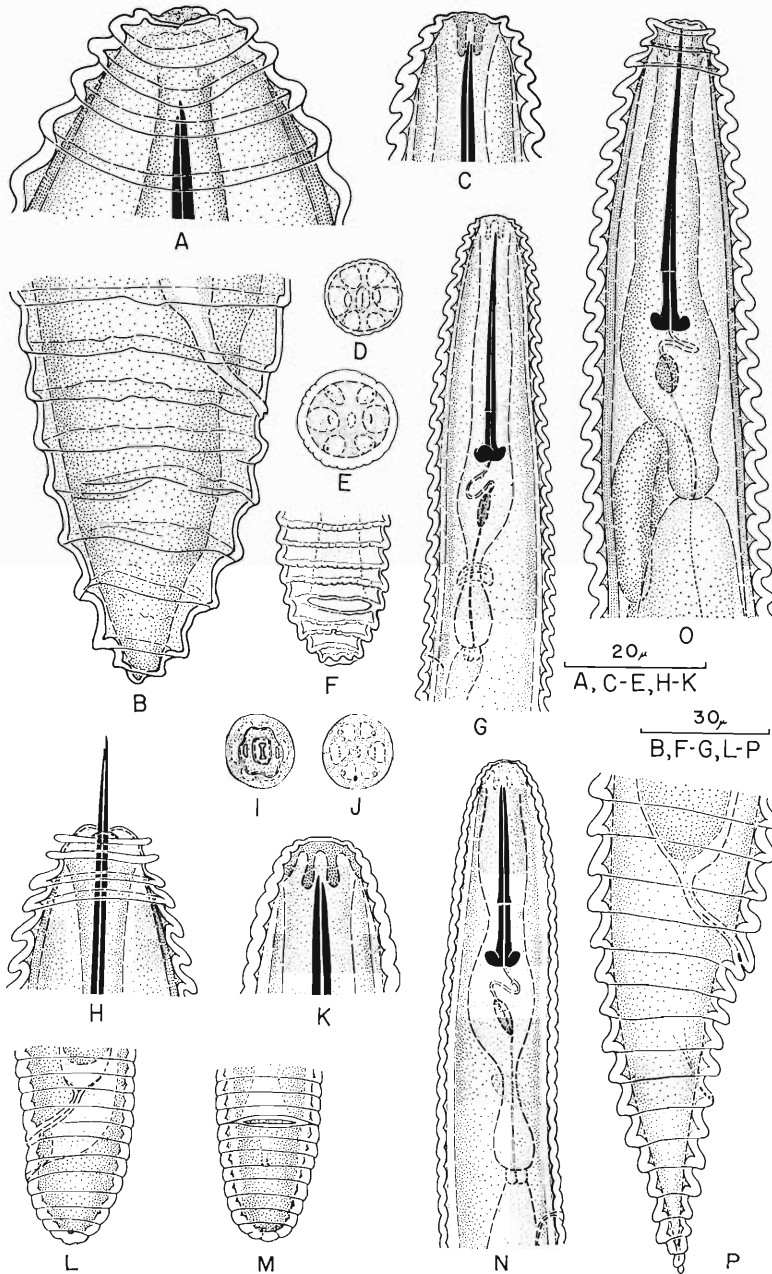


Figure 1. *Criconemoides montserratii*. Female: A—head; B—tail. *Criconemoides dividus* n. sp. Female: C—head; D—en face view; E—cephalic framework; F—tail; G—esophageal region. *Criconemoides sphagni*. Female: H—head. *Criconemoides humilis* n. sp. Female: I—en face view; J—cephalic framework; K—head; L—tail, lateral view; M—tail, ventral view; N—esophageal region. *Criconemoides demani*. Female: O—esophageal region; P—tail, lateral view.

*Criconemoides humilis* n. sp.

(Fig. 1, I-N)

FEMALES (18) (paratypes): L = 0.45 mm (0.36–0.51); a = 15.5 (13–18); b = 4.6 (3.9–5.6); c = 41.0 (33–48); vulva = 95 (94–96); stylet = 43  $\mu$  (38–46); prorhabdion = 30  $\mu$  (27–32); total body annules = 116 (106–122) dorsal, 113 (105–118) ventral.

MALE: Unknown.

LARVAE (5) (paratypes): L = 0.33–0.39; a = 12–17; b = 4.0–5.0; c = 24–29; stylet = 31–36; prorhabdion = 21–25  $\mu$ ; total body annules = 119–122 dorsal, 114–124 ventral.

FEMALE (holotype): L = 0.46 mm; a = 16; b = 4.4; c = 39; vulva = 95; stylet = 46  $\mu$ ; prorhabdion = 31  $\mu$ ; total body annules = 116 dorsal, 111 ventral.

Body tapers only slightly anteriorly to bluntly rounded shape, tail tapers less to hemispherical outline. Labial annules not set off, but the first annule is markedly narrower and thinner than the second; each succeeding body annule wider and thicker with maximum size at 10th–11th annule. Labial disc prominent, rounded to rectangular in en face view with I-shaped oral aperture. Submedian lobes only vaguely rounded and not clearly set off; the two ventral lobes fuse to a bilobed outline as do the other dorsal pair. Amphids elongate, slitlike near labial disc. Stylet well developed with massive knobs. Isthmus elongate, slender with spatulate posterior bulb bearing a lobed, non-muscular valve at junction with intestine. Excretory pore on 30th (28th–34th) annule, 111  $\mu$  (101–132) from anterior end. Anastomosis occurs 6–7 times, but no other markings are present on intervening annules to create the semblance of a lateral line or field. Annules about 4  $\mu$  thick, smooth, without markings, not strongly retrorse. Gonad outstretched 46% (37–84) of body length. Spermatheca prominent with large rounded spermatozoa. Vulva on 7th–8th annule from terminus (one anastomosis occurs on tail and count differs between dorsal and ventral sides), 7th–10th annule in other paratypes. Vulva opening a simple oval in ventral outline, laterally it shows narrow, close vulvar lips not exceeding the outline formed by adjacent annules. Anus on 5th (4th–6th) annule from terminus. Tail hemispherical with several finely indented lobes making definition of last annule difficult.

LARVA: Body shape similar to adult female except head region tapers more sharply anteriorly. Tail bluntly rounded, terminal lobes not quite so bluntly rounded as in female. Stylet and esophagus similar to female only slightly smaller. Excretory pore on 31st–34th annule (83–99  $\mu$ ) from anterior end. Anastomosis occurs randomly over body. Annules about 3–5  $\mu$  thick. Cuticle not as coarse as in adult giving annules a more retrorse outline. Edge of annules appears to have an irregular series of exceedingly fine refractive elements too fine to be certain of their nature. Anus on 6th–7th annule from terminus. Developing gonad 57–101  $\mu$  long indicating these are probably fourth-stage larvae.

HOLOTYPE: Female collected 5 March 1963, by J. W. Riffle, slide number 790, University of California Nematode Survey Collection, Davis.

PARATYPES: Fifty-five females, 19 larvae, same data as holotype, deposited: 41 females, 16 larvae, slide numbers 550–558 and 576–582, University of California Nematode Survey Collection, Davis; 13 females, 3 larvae, slide numbers 4, 4a, and 4b, Rocky Mountain Forest and Range Experiment Station Nematode Slide Collection, Albuquerque, New Mexico.

TYPE HOST: Soil about roots of *Pinus ponderosa* at 7,400 feet elevation.

TYPE LOCALITY: Near Tijeras, New Mexico. Ten miles south on N.M. 10 from its intersection with U.S. 66, and 0.1 mile east on Anaya Road at southern boundary of Cibola National Forest.

DIAGNOSIS: This species is most closely related to *Criconemoides annulatus* Taylor, 1936, from which it differs in the short stylet (38–46  $\mu$  for *humilis*; 65–108  $\mu$  for *annulatus*) and fewer body annules, average 116 (106–122) vs. 113–153. The inclusion of *C. hemisphaericaudatus* Wu, 1965, and *C. rotundicaudatus* Wu, 1965, in the taxon, *C. annulatus*, gives a very wide range to the stylet size and number of body annules of that species. Because of the strong similarities in other characters there is a possibility these specimens merely extend the range of *annulatus*. However, the stylet of *humilis* is consistently so much shorter, only slightly more than half the smallest measurements of *annulatus*. It is judged they represent a separate entity.

The collection of two females with more typical characteristics of *annulatus* from a separate locality, as reported below, gave further credence to the identity of *humilis*.

*Criconemoides informis* (Micoletzky, 1922)  
Taylor, 1936

FEMALES (11): L = 0.44 mm (0.37–0.50); a = 10.5 (9–12); b = 4.1 (3.8–4.5); c = 28.9 (19–44); vulva = 91 (90–92); stylet = 62  $\mu$  (53–67); prorhabdion = 48  $\mu$  (41–53); total body annules = 60 (55–64) dorsal, 58 (53–62) ventral.

These females closely resemble the collections from Colorado reported by Raski (1952). Sublateral lobes prominent with the two ventral ones united to a bilobed structure and the two dorsal ones likewise. Gonad outstretched or with two flexures, spermatozoa not observed in spermatheca.

LARVAE (3): L = 0.30–0.31 mm; a = 9–10; b = 3.1–3.5; c = ?; stylet = 47–53  $\mu$ ; prorhabdion = 36–42  $\mu$ ; total body annules = 54–61.

These resemble the females closely, but the first annule of the head is somewhat set off. Annules with a slightly roughened margin but no obvious spinations or markings.

ADDITIONAL HOSTS AND LOCALITIES: Soil about roots of *P. ponderosa* and *J. monosperma* in Bandelier National Monument near Los Alamos, New Mexico; *P. ponderosa* near Tijeras, New Mexico; *P. ponderosa* and *P. edulis* near Tajique, New Mexico.

*Criconemoides lamellatus* Raski and  
Golden, 1966

FEMALES (13): L = 0.59 mm (0.49–0.70); a = 10 (8–13); b = 3.8 (3.3–4.7); c = 38 (25–71); vulva = 92 (90–93); stylet = 95  $\mu$  (84–105); prorhabdion = 72 (63–79); total body annules = 56 (51–60) dorsal, 57 (53–60) ventral.

These specimens are very much like the type specimens from South Carolina, but are somewhat larger with a longer tail (c value smaller). The vulva on the New Mexico specimens is more consistently on the 5th–7th annule from the terminus. This also gives a slightly smaller vulva percentage. Anus mostly on 3rd–4th annule from terminus. The stylet also is larger, the minimum 84  $\mu$  barely overlaps the longest measured in the type speci-

mens. The excretory pore is located on the 18th–21st annule (194–227  $\mu$ ) from the anterior end. The annules are also slightly more gross averaging 9.2–13.9  $\mu$  in thickness. No spermatozoa were observed in the ventral swelling of the anterior part of the uterus which commonly serves as the spermatheca. Gonad usually outstretched but in one instance reflexed 21  $\mu$  posteriorly.

LARVAE (2): L = 0.40–0.44 mm; a = 9.1; b = 3.3–3.4; c = ?; stylet = 72–77  $\mu$ ; prorhabdion = 53–58  $\mu$ ; total body annules = 55–57 dorsal, 59–61 ventral.

These specimens have 11 rows of spines on 3rd annule, increasing to 14–16 at midbody. The developing gonad is 72–101  $\mu$  long indicating they are probably fourth-stage larvae.

ADDITIONAL HOSTS AND LOCALITIES: Soil about the roots of *Pinus ponderosa* and *Juniperus deppeana* near Manzano, New Mexico; *P. ponderosa* and *Juniperus monosperma* in Bandelier National Monument near Los Alamos, New Mexico; *Pinus edulis*, *P. ponderosa*, *Juniperus scopulorum* Sarg., and *J. deppeana* near Tajique, New Mexico.

*Criconemoides annulatus* Taylor, 1936

FEMALES (2) (Corona, New Mexico): L = 0.49–0.50 mm; a = 12; b = 3.8–4.0; c = 23–30; vulva = 93–94; stylet = 70–75  $\mu$ ; prorhabdion = 52–59  $\mu$ ; total body annules = 136–138 dorsal, 131–136 ventral.

LARVA (1) (Corona, New Mexico): L = 0.40 mm; a = 14; b = 4.6; c = 24; stylet = 36  $\mu$ ; prorhabdion = 24  $\mu$ ; total body annules = 135.

These specimens fit very closely the characteristics of *C. annulatus* as proposed by Raski and Golden (1966) which would include a stylet range of 65–108  $\mu$ .

ADDITIONAL HOSTS AND LOCALITY: Soil about the roots of *P. edulis* and *J. monosperma* near Corona, New Mexico.

*Criconemoides macrodorus* Taylor, 1936

FEMALES (4): L = 0.30 mm (0.29–0.30); a = 11.6 (11–13); b = 2.7 (2.4–3.3); c = 21.9 (16–29); vulva 91 (89–92); stylet 89  $\mu$  (85–94); prorhabdion = 81  $\mu$  (77–84); total body annules = 95–109 dorsal, 93–108 ventral.

These are very much like other collections of *macrodorus* in general aspect, head region

as well as in measurements above. Excretory pore on 34th–36th annule (87–97  $\mu$ ) from anterior end. Vulva on 11th–14th, anus on 7th–9th annule from terminus. In one female the spermatheca was prominent and full of spherical spermatozoa.

ADDITIONAL HOSTS AND LOCALITIES: Soil about roots of *P. ponderosa* and *J. deppeana* near Manzano, New Mexico; *P. ponderosa* and *J. monosperma* in Bandelier National Monument near Los Alamos, New Mexico.

*Criconemoides xenoplax* Raski, 1952

FEMALES (6): L = 0.58 mm (0.46–0.70); a = 12.7 (11–15); b = 4.6 (3.9–5.0); c = 29 (24–34); vulva = 94 (93–95); stylet = 62  $\mu$  (58–67); prorhabdion = 49 (41–51); total body annules = 92 (90–94).

These specimens resemble very closely *C. xenoplax* with a stylet at the lower range of previously reported collections. The excretory pore is at 24th–26th annule (131–160  $\mu$ ) from the anterior end. Vulva on 7th–8th, anus on 5th annule from terminus. Spermatheca absent. Annules with very slightly irregular edge.

ADDITIONAL HOSTS AND LOCALITIES: Soil about the roots of: *P. ponderosa* and *J. monosperma* in Bandelier National Monument near Los Alamos, New Mexico; *P. ponderosa*, *P. edulis*, *J. deppeana*, *J. scopulorum* near Taji-que, New Mexico; *P. edulis* and *J. monosperma* near Corona, New Mexico.

DISCUSSION: Recent advancements in our knowledge of morphological characteristics and variations in the genus *Criconemoides* have complicated the usefulness of earlier descriptions especially when type specimens are not available for study and comparison. Judgments of synonymy and identification are increasingly unreliable when based only on published descriptions. The last report on this group (Tarjan, 1966) placed *C. xenoplax* and *Criconemoides tenuiannulatus* (Tulaganov, 1949) in synonymy with *C. beljaevae* (Kirjanova, 1948). Objection to this action is based on incomplete knowledge of the last two species. Without additional studies and redescrptions from type specimens or additional specimens from type localities, including accurate and detailed reports on the lip regions of each, the identifi-

cation and distinction of *C. beljaevae* and *C. tenuiannulatus* remain in doubt. They properly belong in species inquirendae and are so placed here.

*Criconemoides demani* (Micoletzky, 1925)

Taylor, 1936

(Fig. 1, O–P)

LECTOTYPE (female): L = 0.50 mm; a = 11; b = 4.5; c = 13; vulva = 85; stylet = 71  $\mu$ ; prorhabdion = 58  $\mu$ ; total body annules = 69.

A single female, the only specimen available for study, was considerably flattened but was sufficiently well preserved to merit designation as type of this species.

Head with two labial annules distinctly set off from succeeding body annules, smaller not retrorse (first annule turned anteriorly). Sublateral lobes absent or apparently so. Isthmus short, barely setting off posterior bulb. Excretory pore not seen. Body annules 7–8  $\mu$  thick, rounded, not retrorse but become slightly retrorse in tail region. Detritus packed in between almost all annules as illustrated by Micoletzky. Gonad 66% of body length, appears to have one flexure posteriorly, 58  $\mu$  long. No spermatheca discernible. Vulva on 13th annule from terminus. Lips of vulva protrude slightly beyond body outline, anterior lip large, projecting beaklike, posteriorly. Anus on 8th annule from terminus. Tail tapers regularly into a distinctly conical outline.

LECTOTYPE: Female collected June 1924 on slide number 8815 of the Zoological Museum of the Humboldt-University, Berlin.

TYPE HOST AND LOCALITY: The type slide is labeled as *Carex-Ufermeim*, Tjustrupösö, Denmark, LXXXVI.

DISCUSSION: Tarjan (1966) transferred *C. demani* to species inquirendae because of inadequate descriptions by which it could be distinguished from other species. The specimen described here permits this deficiency to be corrected. *C. demani* can now be reorganized as a distinct species and is hereby removed from species inquirendae. *C. demani* is considered most closely related to *C. ravidus* Raski and Golden, 1966, from which it is distinguished in the original description of *C. ravidus*.



*Criconemoides sphagni* (Micoletzky, 1925)  
Taylor, 1936  
(Fig. 1, H)

LECTOTYPE (female): L = 0.43 mm; a = 10; b = 2.8; c = 15; vulva = 4688; stylet = 116  $\mu$ ; prorhabdion = 105  $\mu$ ; total annules = 97.

This species was well described in detail by Micoletzky in 1925; however, the head region shows some characteristics that should be discussed. These specimens taper slightly anteriorly to a rounded head bearing two narrow annules set off from the rest of the body annules and are not retrorse. The first annule is slightly narrower in diameter than the second. The third annule appears to be retrorse and similar to the other succeeding body annules. The lip region is low rounded with sublateral lobes lacking. Other characteristics as described by Micoletzky.

LECTOTYPE: Female (one of three females on one slide designated by circle of ink on slide) on type slide number 8824 deposited in the Zoological Museum of the Humboldt-University, Berlin.

*Criconemoides hispalensis* Arias, López,  
and Jiménez, 1963

FEMALE: L = 0.41 mm; a = 10; b = ?; c = 26.3; vulva = 92; stylet = 63  $\mu$ ; prorhabdion = 48  $\mu$ ; total body annules = 76.

This single specimen has a bluntly rounded head with a rather obvious and protruding labial disc. There are sublateral lobes apparently present but en face sections are needed to confirm these and to determine their structure. These could possibly be sublateral plates. The tail tapers slightly to a bluntly rounded outline. Vulva on 6th and anus on 4th annule from terminus.

The illustration of *C. hispalensis* appears to have 57 body annules. This specimen has more but the difference could be within a specific range. There is also a discrepancy in measurement of total length of body and stylet. The original description reports L = 196.4  $\mu$  with a stylet of 35  $\mu$ .

From the characters visible on the single whole mount this would key to *C. maritimus* De Grisse, 1964, *C. rotundicauda* Loof, 1964, or *C. rosae* Loof, 1964. Without more details on the structure of the sublateral lobes it is not possible to determine its exact relationship,

and it is correctly placed in species inquirendae (Tarjan, 1966).

*Criconemoides montserrati* Arias,  
Jiménez, and López, 1965  
(Fig. 1, A-B)

FEMALE: L = 0.68 mm; a = ?; b = 4.1; c = ?; vulva = 90; stylet = 85  $\mu$ ; prorhabdion = 62  $\mu$ ; total body annules = 69.

This single female was badly flattened, and the distended annules seem to be caused by poor fixation, which probably gives a greater total length than normal. Head end shows a bluntly rounded outline with a prominent labial disc and a suggestion of sublateral lobes, the nature of which cannot be determined without en face sections. Tail is bluntly conoid in outline, but the flattened condition leaves this questionable.

The discernible characters suggest relationship to *C. insignis* Siddiqi, 1961, but there is not sufficient information to permit an accurate determination of relationship. It is therefore transferred to species inquirendae.

#### CORRECTION

The designation of slide numbers 542-543 as paratypes of *Criconemoides permistus* Raski and Golden, 1966, should be corrected and changed to slide numbers 200-201, University of California Nematode Survey Collection, Davis.

#### SUMMARY

*Criconemoides dividus* and *Criconemoides humilis* are described as new species; additional collections are reported for *C. informis*, *C. lamellatus*, *C. annulatus*, *C. macrodorus*, and *C. xenoplax*; additional descriptions are made from type specimens of *C. demani* and *C. sphagni* for which lectotypes and paralectotypes are designated; *C. montserrati*, *C. heljaevae*, and *C. tenuiannulatus* are transferred to species inquirendae.

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### Preliminary Observations on the Anthelmintic Activity of Methyridine Against *Capillaria contorta* in Quail

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*Capillaria contorta* is a helminth that occurs naturally in the crop of domestic and wild turkeys, ducks, pheasants, quail, and Hungarian partridges (Cram *et al.*, 1931). The parasite inhabits the mucosa of the esophagus and crop. It has a direct life cycle, with the embryonated egg as the infective stage. Birds acquire infection by ingesting embryonated eggs in contaminated feed or water.

Wehr (1948) and Wehr and Coburn (1943) reported that heavy infections of *C. contorta* were pathogenic, particularly among game

birds reared in captivity. In severe infections, the mucosa of the esophagus and crop is thickened, inflamed, and covered with a flocculent exudate. Clinical signs include droopiness, weakness, and emaciation. Fatal infections occur frequently. (Several birds from a naturally infected flock of quail (*Colinus virginianus*), submitted to this laboratory from the New York State Game Farm, Ithaca, New York, died from severe capillariasis caused by *C. contorta*.)

Numerous workers, including Geeraerts (1964), Granville *et al.* (1964), Grattan (1963), Hassan and Ahmed (1963), Hendriks (1962,

<sup>1</sup> Retired 30 November 1965.

TABLE 1. The anthelmintic action of methyridine against *Capillaria contorta* when given to quail by subcutaneous injection. (Worms in dead birds not counted.)

Experiment no.	No. of quail	Dosage (mg/quail)	Treatment repeated	Live worms recovered at necropsy		Efficacy (per cent)	Remarks
				Total	Range		
1	5	11		553	64-160	0	—
	5	23		444	1-153	0	—
	5	35		109	1-63	68	ataxia
	5	45		160	1-134	53	ataxia
	4	—	—	275	37-97	—	—
2	5	56		64	3-21	85	ataxia
	5	68		12	0-9	97	ataxia
	5	79		23	0-16	93	ataxia; 1 bird died
	5	90		27	0-21	92	ataxia; 1 bird died
	5	—	—	440	13-197	—	—
3	5	35	Day 2	77	0-49	68	ataxia
	5	45	Day 2	2	0-2	99	ataxia
	5	56	Day 2	34	1-15	86	ataxia
	5	68	Day 2	9	0-21	96	ataxia
	5	—	—	241	23-71	—	—
4	24	45	Day 2	173	0-64	87	ataxia; 2 birds died
	16	56	Day 2	64	0-22	94	ataxia
	4	68	Day 2	1	0-1	99	ataxia
	6	—	—	375	12-127	—	—
5	12	35	Days 4 and 9	13	0-11	97	slight ataxia
	6	45	Days 4 and 9	0	0-0	100	slight ataxia
	5	—	—	172	19-47	—	—

1963), Litjens (1963), and Thienpont and Mortelmans (1962), have reported that methyridine [2-(B-methoxyethyl) pyridine] was highly effective in poultry against another capillarid, *C. obsignata*. An opportunity was presented to test this systemic drug against *C. contorta*, when the authors received the infected quail from New York. The results of these tests are described in this report.

#### MATERIALS AND METHODS

About 400 mature bobwhite quail were received<sup>2</sup> in May 1965. The majority of the quail had naturally acquired infections of *C. contorta*, and were weak and emaciated. One hundred and forty-seven of these quail, determined by fecal examination to have infections of *C. contorta*, were used in controlled anthelmintic tests from 21 June to 19 August 1965 (Table 1). Infected quail were randomly assigned to principal and control groups. The medicated groups contained 4 to 24 quail each, and the control groups contained 4 to 6 quail each. All groups were held in separate wire cages throughout the test period.

Methyridine, as a 5% aqueous solution of

the proprietary Promintic,<sup>3</sup> was administered subcutaneously in three separate regimens: (a) as single injections of 11 to 90 mg (0.25 to 2.0 ml of the test solution); (b) as injections of 35 to 68 mg (0.75 to 1.5 ml) on each of 2 successive days; and (c) as three separate injections of 35 or 45 mg (0.75 or 1.0 ml) spaced at intervals of 4 or 5 days.

All surviving quail were necropsied 7 or 8 days after treatment, and, with the use of a dissecting microscope, the crop and esophagus of each were examined for capillarids. All capillarids found were removed with forceps and immersed in warm water to determine if they were alive. Worms not stimulated into noticeable activity by the warm water were considered to have been killed by the drug. Efficacy of treatment was calculated using the following formula:

$$\frac{\text{avg no. worms in controls} - \text{avg no. worms in treated quail}}{\text{avg no. worms in controls}} \times 100 = \% \text{ efficacy.}$$

#### RESULTS AND DISCUSSIONS

The pertinent data are summarized in Table 1. The results indicate that methyridine is effective against *C. contorta* in quail when administered in single doses of at least 68 mg/

<sup>2</sup> The authors thank Mr. R. E. Reynolds, New York State Game Farm, Ithaca, New York for giving these quail to the Beltsville Parasitological Laboratory for experimental use.

<sup>3</sup> Supplied through the courtesy of Fort Dodge Laboratories, Ft. Dodge, Iowa.

quail. Toxicosis, characterized by ataxia and resulting in some mortality, was evident at these single dose levels. The 2-dose regimen of 45, 56, and 68 mg resulted in comparable efficacy and intoxication. Three separate doses of 35 or 45 mg, spaced at 4- or 5-day intervals, were highly efficacious, and there was less intoxication and mortality. Handling each quail three times over a 9- or 10-day period, however, militates against the use of this regimen for game birds.

#### SUMMARY

In controlled anthelmintic tests, methyridine, 2-(B-methoxyethyl) pyridine, was efficacious against *Capillaria contorta* in quail (*Colinus virginianus*). The drug was administered subcutaneously in three regimens: (1) as single injections of 11 to 90 mg; (2) as injections of 35, 45, 56, and 68 mg on each of 2 successive days; and (3) as three injections of 35 or 45 mg spaced at 4- or 5-day intervals. Some ataxia was noticed in most treated quail, and a few died. Maximal intoxication was produced by the single dose regimen of 79 and 90 mg and 20% died. Single doses of 35 or 45 mg were relatively ineffective; however, three separate injections, at these same levels, and spaced at intervals of 4 or 5 days, were 95 to 100% effective. Although only mild toxicosis occurred, the necessity of handling each quail three times reduces the practicality of this regimen for use with game birds.

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**Notes on the Genus *Ascarophis* Beneden, 1871, in Antarctic Fishes<sup>1</sup>**

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The first representative of the nematode genus *Ascarophis* Beneden, 1871, described from the Antarctic and Subantarctic regions was *A. campbelli* collected by Filhol from fish at Campbell Island. His material taken from *Notothenia microlepidota* Hutton, 1876, was studied by Chatin (1885) and described as *Spiroptera campbelli*. Johnston and Mawson (1943) considered the worm a species of *Ascarophis* and provisionally listed it as *A. campbelli*. *Ascarophis lycodichthys*, *A. chalinuræ*, and *A. nototheniæ* were described by Johnston and Mawson (1945) in the reports of the British, Australian, and New Zealand Antarctic Research Expedition of 1929–31. However, with the exception of a few specimens of *A. nototheniæ*, collections of these three species were made during the Australasian Antarctic Expedition (1911–14).

*A. lycodichthys* was recovered from *Lycodichthys antarcticus* Pappenheim, 1911, at Western Base (66°18' S, 94°58' E) and *A. chalinuræ* was recovered from *Chalinuræ ferrieri* Regan, 1913, collected at Station V (64°34' S, 127°17' E). *A. nototheniæ* was collected in both of the mentioned expeditions but was more commonly found in fishes taken by the Australasian Antarctic Expedition than in those collected by the BANZAR Expedition. It was found in 18 different species of Antarctic and Subantarctic fishes. Extreme egg size and extreme length of long spicule given in the description of this species indicate that another worm species must have been included or that the authors erred in the metric analyses of these structures.

This report presents the results of further investigations of *Ascarophis nototheniæ* from the Antarctic and Subantarctic regions and a reconsideration of the previously reported *Ascarophis* species from these regions.

**MATERIALS AND METHODS**

Fishes were collected by traps and hook and line during the periods 1959–60, 1960–61, and 1964–65. They were collected from the southeastern corner of McMurdo Sound (1959–60 and 1964–65), near McMurdo Station (77°51' S, 166°40' E) and near Wilkes Station (66°15' S, 110°32' E) (1960–61). During 1964–65 fishes were examined immediately upon capture and when this was not possible, they were retained in laboratory aquaria containing aerated seawater in the temperature range of -2 to 8 C or, in the case of those caught by hook and line, in a refrigerator at 10 C until examined. Fishes remaining in the refrigerator for 72 hours were preserved in formalin (10 cc commercial formol to 80 cc of seawater), after a ventral longitudinal slit had been made in the body wall. These specimens were examined at Roanoke College. A 0.7% solution of sodium bicarbonate or 0.7% sodium chloride was employed in handling or examining living worms. The alimentary tract was removed and immersed in sodium bicarbonate solution. The stomach and intestine were separated and placed in petri dishes. The organs were then opened longitudinally and handpicked under a dissecting microscope. The parasites encountered were removed. The stomach and intestine were scraped, placed in separate 8 to 16 ounce bottles and shaken vigorously to further liberate the helminths from mucous. The bulk was strained through a wire sieve to remove the coarser material and remaining larger parasites. The sieve was then examined under the dissecting microscope and the adhering helminths removed. The filtrate was set aside to permit sedimentation. After decanting the uppermost layers of the supernatant, the remainder was centrifuged, in several installments where necessary. The sediment was removed and examined under the binocular microscope. In other instances, after preliminary examination with the dissecting microscope, the separated portions of the intestine were placed in sieve pans (U. S.

<sup>1</sup> Supported in part by Grants GA 146 and 228 under the United States Antarctic Research Program of the National Science Foundation.

Series Equivalent 80), scraped, thoroughly sprayed with tap water, and the residue examined with a dissecting microscope. In other cases the intestine and stomach were placed on separate Baermann apparatus containing warm 0.7% sodium chloride or 0.7% sodium bicarbonate for 8 hours. The sediment was then drawn off and examined with the dissecting microscope.

The nematodes recovered were killed and fixed in warm Looss agent (Morgan and Hawkins, 1949), or warm AFA.

The worms recovered during 1959–60 and 1961–62 from McMurdo Sound and Wilkes Station were removed from preserved hosts.

Specimens used for study were cleared and mounted in lactophenol or were stained lightly in Delafield's hematoxylin and mounted in Yetwin's medium. The denticulate ridge associated with the mouth structures was more clearly defined when the latter method of preparation was applied.

The type specimens of *Ascarophis nototheniae* were not available from the South Australian Museum at the time of this study. However, Dr. P. M. Mawson (Mrs. P. M. Thomas), Zoology Department, University of Adelaide, provided alternative material which she considered to be more satisfactory. These specimens (Table 1, Column 5) are from the type host although not the type locality; material from their locality was identified at the time the types were described.

Measurements of internal structures of the worms were made with an ocular micrometer or with camera lucida drawings and scales derived from a stage micrometer. Gross measurements were made using drawings of specimens and a micrometer scale, both projected with an American Optical "Tri-Simplex" microprojector.

#### RESULTS AND DISCUSSION

Of a total of 89 *Trematomus bernacchii* Boulenger, 1902, examined at McMurdo Sound during 1964–65, 35% were infected with *Ascarophis nototheniae*. The rate of infection was light; the range being one to nine parasites per host, while the mean rate of infection was 2.2 worms per animal. Seventy-three per cent of the worms were recovered from the stomach while only 27% were obtained from the

intestines. The sex distribution in this species is 38% males and 62% females. In this respect, this species differs significantly from the type species of the genus, *A. morrhuae* Beneden, 1871, of which the male was only recently described (Gordon, 1951).

Twenty-seven *T. hansonii* Boulenger, 1902, examined from McMurdo Sound during 1964–65 were negative for *A. nototheniae* infection, although this was the only host in collections available to us from Wilkes Station (1961–62). During 1959–60 *A. nototheniae* was recovered from *T. hansonii* and *T. bernacchii* from McMurdo Sound. Other fish hosts collected from McMurdo Sound (1964–65) which were non-infected with *A. nototheniae* were: *Trematomus borchgrevinki* Boulenger, 1902, 56; *T. centronotus* Regan, 1914, 12; *T. lepidorhinus* Pappenheim, 1912, 4; *T. loennbergi* Regan, 1913, 1; *Rhigophila dearborni* DeWitt, 1962, 82; *Pleurogramma antarctica* Boulenger, 1902, 5; and *Dissostichus mawsoni* Norman, 1937, 2.

Family Spiruridae Oerley, 1855

*Ascarophis nototheniae* Johnston  
and Mawson, 1945

(Figs. A–E)

**DESCRIPTION** (all measurements in mm): Small, filiform worms. Males smaller than females. Anterior end slightly rounded. Lateral margins of dorsoventrally elongate mouth surrounded by two trilobed lips, each containing a spine measuring up to 0.013 in length (Fig. C). Dorsal and ventral curvature of mouth formed by toothlike processes. Head bears two pairs of sublateral cephalic papillae (Fig. B) and the amphids. Buccal capsule (Fig. A) tubular, thick walled and lined with cuticula; dorsoventrally widened toward mouth but laterally compressed. Long esophagus occupies up to ½ entire body length. Separation of muscular and glandular regions not clearly defined but anterior esophagus narrower and shorter. Nerve ring (Fig. A) and excretory pore situated in region of anterior esophagus. Cuticle transversely striated (Fig. A) except at extreme tips of worm. Striations more conspicuous in anterior half, sometimes resembling posteriorly directed spines.

**MALE:** Body 6.4–10.8 long; 0.021–0.028 wide

TABLE 1. Comparison of Antarctic and Subantarctic species of the genus *Ascarophis* Beneden, 1871. Dimensions in mm.<sup>1</sup>

<i>A. campbelli</i> (Chatin, 1885) Johnston and Mawson, 1943	<i>A. lycodichthys</i> Johnston and Mawson, 1945	<i>A. chalinurac</i> Johnston and Mawson, 1945	<i>A. nototheniae</i> Johnston and Mawson, 1945	<i>A. nototheniae</i> alternative material S. Australian Museum (4 specimens)	<i>A. nototheniae</i> McMurdo Sound (1959-60) and Wilkes Station (1961-62) (19 specimens)	<i>A. nototheniae</i> McMurdo Sound (1964-65) (22 specimens) (mean)
L ♂		7	3.7-8	4.32	6.4-8.9	6.7-10.8 (8.3)
L ♀	25	4.6	9.4-10.5	5-12.6	3.8-6.6	8.7-19.3
B:V		1:1♂-1.5♀	1:3.6	1:3.0-4.5	1:2.6-3.9	1:2.7-4.4 (1:3.3)
V:Oa	1:1.9	1:3.4	1:2-3	1:1.9-4.0	1:1.8-2.8	1:1.8-3.8 (1:2.6)
Oa:Op	1:7.7	1:4.2	1:6-8	1:3.6-5.0	1:7.6-11.3	1:5.9-12.7 (1:8.5)
Ot:L	1:1.7	1:3.1	1:2.4-7	1:2.3-2.9	1:2.1-4.1	1:1.6-3.9 (1:2.7)
P-V:L	1:2.3	1:1.7-1.8	1:2.6-3	1:2.8-2.9	1:2.2-3.6	1:2.2-2.8 (1:2.6)
Eggs		0.047-0.050	(extreme 0.018) 0.040-0.045	0.041	0.042-0.048	0.040-0.047 (0.043)
L		0.018	(extreme 0.013) 0.022-0.025	0.024	0.023-0.027	0.025-0.029 (0.027)
W		0.34	(extreme 0.59) 0.410-0.450	0.439	0.300-0.426	0.322-0.395 (0.351)
Spicule		0.12	0.060-0.080	0.085	0.085-0.118	0.082-0.117 (0.085)

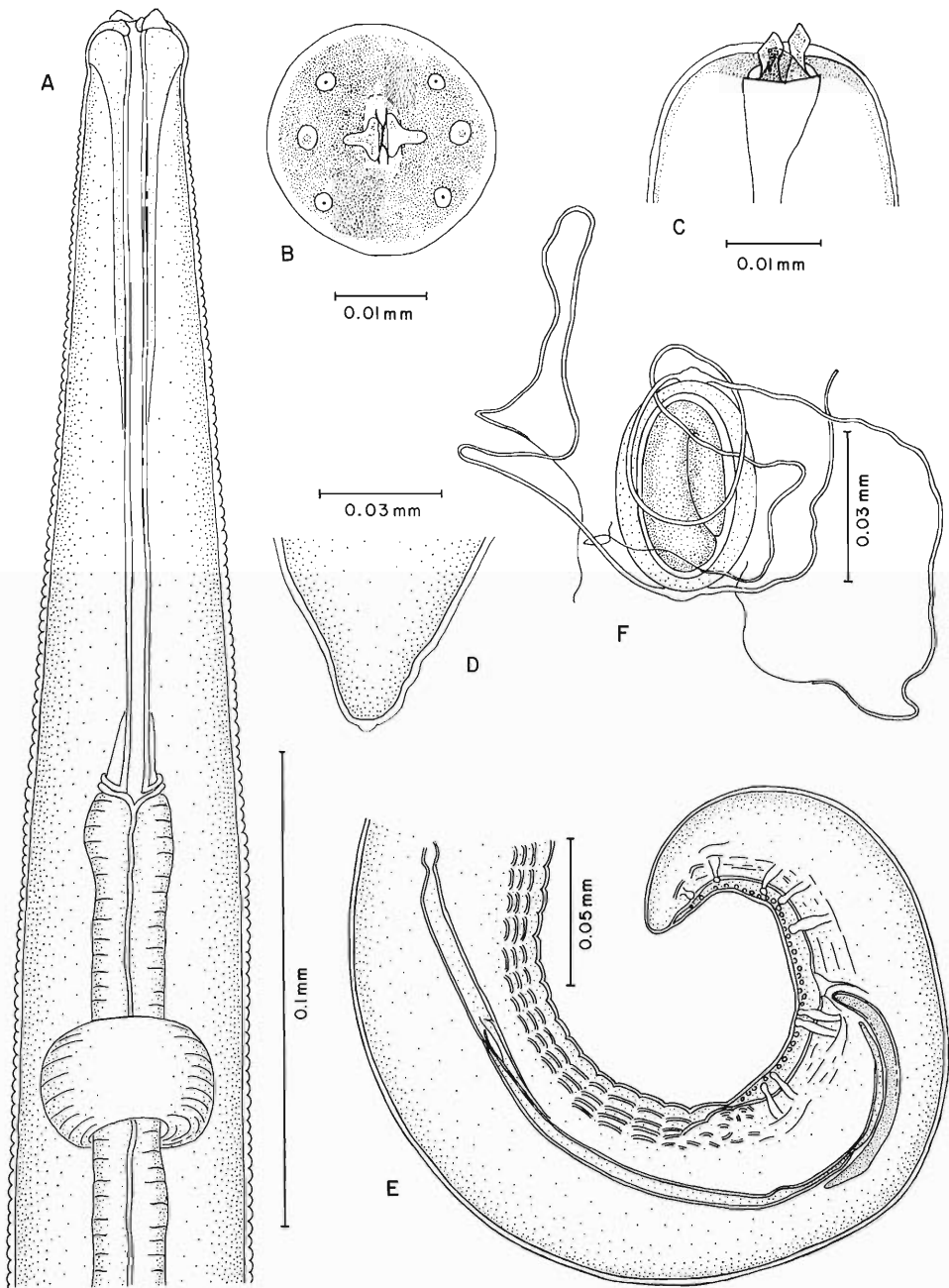
<sup>1</sup> The abbreviations used are: L, length; V, length of the vestibule; Oa, length of the anterior, and Op, of the posterior parts of the esophagus; Ot, combined lengths of V, Oa and Op; P-V, distance of the vulva from the posterior end of the body; Sp, spicule; B, breadth of the body at the level of the posterior end of the vestibule; W, width of the egg.

at head end; 0.042-0.052 wide at juncture of buccal capsule (vestibule) and esophagus, and 0.056-0.085 wide at anterior end of long spicule. Buccal capsule 0.135-0.185 long by 0.011-0.014 wide laterally at anterior end and 0.005-0.007 at posterior end. Anterior muscular esophagus 0.283-0.517 long and 0.013-0.018 wide at level of excretory pore. Posterior esophagus 2.240-3.900 long and 0.031-0.047 wide near esophagointestinal juncture. Entire esophagus 2.620-4.420 long. Nerve ring 0.17-0.24 and excretory pore 0.28-0.35 from anterior end. Tail bluntly rounded at tip, 0.205-0.266 long. Caudal end of male (Fig. E) curled ventrally forming one to three spiral turns. Caudal alae narrow (0.027 wide) containing small tubercles at their outer edges. Four pairs of preanal papillae arranged in two sets of sublateral pairs, and five pairs of post-anal papillae arranged in single sublateral pairs, more or less equidistant from each other. All papillae pedunculate, measuring up to 0.021 in length, except most posterior pair which is considerably smaller than others and close to end of body. Hollowed ventral region on tail preceded by a conspicuous longitudinal field

of well-defined scalelike projections. Spicules very unequal in length, 0.322-0.395 and 0.082-0.117. Gubernaculum absent.

FEMALE: Body 8.7-19.3 long; 0.024-0.027 wide at head end; 0.043-0.057 wide at juncture of buccal capsule and esophagus, and 0.092-0.122 at region of vulva. Buccal capsule 0.142-0.191 long by 0.013-0.020 wide laterally at anterior end and 0.005-0.008 wide at posterior end. Anterior muscular esophagus 0.30-0.55 by 0.014-0.016 at level of excretory pore and posterior esophagus 3.08-4.48 by 0.036-0.054 near esophagointestinal juncture. Entire esophagus 3.57-4.98 long. Nerve ring 0.17-0.25 and excretory pore 0.28-0.34 from anterior end. Anus 0.044-0.062 from posterior extremity; tail short, bluntly rounded at tip terminating in a small conical projection (Fig. D) measuring up to 0.004 long. Postequatorial vulva, 4.2-7.1 from posterior end of body, a transverse slit surrounded by distinct lips. Vagina directed anteriorly, uterus amphidelphic with eggs aligned in single rows.

Eggs (Fig. F) thick shelled, 0.040-0.048 long by 0.023-0.029 wide. Mature eggs contain developed larvae. Two polar caps, each



Figures A-F. *Ascarophis nototheniae* Johnston and Mawson, 1945. A. Approximate dorsoventral view of anterior region of female. B. En face view. C. Lateral view of anterior end. D. Posterior tip of female. E. Lateral view of posterior end of male. F. Fully developed egg from uterus in region of vulva.

with two conspicuous filaments, present on embryonated eggs from region of vulva. Size of caps variable to 0.005 by 0.01 (height by width). Filaments 0.312–0.440 long.

HOSTS: *Trematomus bernacchii* and *T. han-soni*.

LOCATION: Stomach and intestines.

LOCALITY: Wilkes Station and McMurdo Sound, Antarctica.

SPECIMENS DEPOSITED: USNM Helm. Coll., No. 61364.

Taxonomic data of other authors on the four species of *Ascarophis* reported from the Antarctic and Subantarctic regions have been summarized in Table 1, Columns 1, 2, 3, and 4: results obtained from the present study are shown in Table 1, Columns 5, 6, and 7. Body measurements and proportions simulated in this table are those originally employed by Johnston and Mawson (1945) and are adhered to in this paper for purposes of comparison.

*A. nototheniae* described by Johnston and Mawson (1945) (Table 1, Column 4) compare favorably with specimens in our study (Table 1, Columns 4, 5, and 6) except for several significant deviations. The extreme egg dimensions of 0.018 by 0.013 do not fit within the range of 0.040–0.048 by 0.022–0.029. Another major discrepancy is their report of no filaments in the eggs which they observed. It was found in our specimens that only embryonated eggs contained filaments, and in view of the small size of their female specimens, the possibility is great that they were dealing with sexually immature worms. They also include an extreme length for spicule No. 1 as 0.59. Their short spicule length of 0.060 is below the range encountered in any of the three groups of specimens in our study. Zhukov (1960) pointed out that several species, possibly relating to various genera, may have been included in the original description of *A. nototheniae*. The spicule lengths given by Johnston and Mawson (1945) appear to substantiate this conclusion or indicate that they erred in the metric analyses of these structures and the ova.

The range of all measurements in the current study falls within a reasonably consistent pattern. The total lengths of the one male and three female worms examined from the alterna-

tive materials appear to be low, but only one of the female worms contained embryonated eggs. The remaining data resulting from the metric analyses of this material, except the proportion of the anterior esophagus to posterior esophagus, fall within the simulated proportions of our material or are continuous with them. The lengths of the posterior esophagi are less than the minimum lengths of these organs in our materials and the reductions correlate with the smaller total body lengths, thus the differences may be a matter of development. The egg lengths and widths fall within a range of 0.040–0.048 by 0.023–0.029. The long spicules of male worms fall within a range of 0.300–0.439, and the short spicules fall within a range of 0.085–0.118.

The presence or absence of filaments in the eggs of members of the genus *Ascarophis* has long been one of the distinguishing features of the different species. Structural characteristics of *A. nototheniae* are closely related to those of *A. morrhuae* and it has been suggested by various authors that they are conspecific. *A. morrhuae*, however, contains eggs with one polar plug bearing two long filaments; whereas mature eggs of *A. nototheniae* contain two polar plugs (caps), each with two long, conspicuous filaments.

One male *A. nototheniae* specimen from the McMurdo Sound (1964–65) collection contained a cuticular fold around the neck. Another male specimen from the same host had a single fold surrounding only  $\frac{1}{2}$  of the neck region. The internal structure of these two worms was slightly distorted and it appears probable that the presence of the anterior folds may be attributed to fixation, as has been suggested by other authors.

The only specific measurement given for *Ascarophis campbelli* (Table 1, Column 1) was an average female length of 25.0. Chatin's (1885) description was incomplete, but since no specimens are available for reevaluation, it must remain provisionally in the genus *Ascarophis* where it was placed by Johnston and Mawson (1943). These authors believe, however, that in view of the wide known distribution of *A. nototheniae* in the Subantarctic (Crozetts, Heard Island, Kerguelen, and Macquarie Island), it is likely that an examination of material from Campbell Island would show



that this parasite is a synonym of *A. campbelli*.

Measurements in Table 1, Column 3, given for *A. lycodichthys* fall within the range of *A. nototheniae* specimens in this study. *A. lycodichthys* was described from one female and a fragment of a male (or immature female). This species was poorly defined and although the fragment agreed with *A. nototheniae*, the female had a stouter build and occurred in a different host. However, we feel that *A. lycodichthys* should be considered a synonym of *A. nototheniae*, due to the lack of specific differentiating features.

The description of *A. chalinuræ* was based on three females and one male specimen. The recent redescription of the genus *Ascarophis* by Poljanski (1952) renders the generic assignment of *A. chalinuræ* untenable. It deviates in the absence of two "sticklike" lips or indistinctly trilobed pseudolabia, possession of six pairs of postanal papillae rather than five pairs, and an apparent lack of polar plugs and well-developed filaments on the eggs.

#### ACKNOWLEDGMENTS

The authors acknowledge with thanks the gift of the specimens collected at McMurdo Sound, 1959-60, and Wilkes Station, 1961-62, by Messrs. W. S. Wilson and W. J. Saunders under Grant GA 13853 from the United States Antarctic Research Program of the National Science Foundation to Dr. W. J. Hargis, Jr., Virginia Institute of Marine Science. We appreciate the arrangements made by Dr. H. M. Laws, Curator of Marine Invertebrates, South Australian Museum, for the loan of available *Ascarophis nototheniae* material from the collection of Dr. P. M. Mawson, Zoology Department, University of Adelaide.

#### SUMMARY

*Ascarophis campbelli* (Chatin, 1885) Johnston and Mawson, 1943; *A. lycodichthys* Johnston and Mawson, 1945; *A. chalinuræ* Johnston and Mawson, 1945; and *A. nototheniae* Johnston and Mawson, 1945, have been recognized from Antarctic piscine hosts. The examination of the Antarctic cods *Trematomus bernacchii* Boulenger, 1902, and *Trematomus hansonii* Boulenger, 1902, trapped near Wilkes Station and in McMurdo Sound, produced specimens of *Ascarophis nototheniae* which, in conjunc-

tion with alternative type material from the South Australian Museum, have enabled us to reexamine the species. As suggested by Zhukov (1960), Johnston and Mawson (1945) may have combined two or more species under this single concept. However, they (Johnston and Mawson, 1945) may have erred in the metric analyses of ova and spicules, as evidenced by the extreme ova dimensions and length of spicule No. 1 and the smaller lengths of spicule No. 2. The head bears two pairs of papillae and the amphids, two pseudolabia at right angles to the dorsoventrally elongated mouth, and two small "teeth." Johnston and Mawson (1945) observed no terminal filaments on the eggs but our examination of 50 eggs revealed the majority to have two polar plugs, each giving rise to two filaments. The recent redescription of the genus *Ascarophis* by Poljanski (1952) renders the generic assignment of *A. chalinuræ* doubtful; since it lacks pseudolabia, possesses six pairs of postanal papillae and lacks ovarian polar plugs and filaments. *A. campbelli* is very inadequately known. *A. lycodichthys* is synonymized with *A. nototheniae*.

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## Activity of Three Anthelmintics Against Mixed Infections of Two *Trichostrongylus* Species in Gerbils, Sheep, and Goats

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In any screening program to test anthelmintics, a suitable host-parasite system is of primary importance. Since this laboratory<sup>2</sup> plans to conduct such a screening program on a long-range basis, we tried to determine the best host-parasite system for that purpose.

In connection with gastrointestinal nematodes, Standen (1963) listed three natural host-parasite systems involving small laboratory animals, which were then employed as primary anthelmintic screens, viz. the rat—*Nippostrongylus brasiliensis*, mouse—*Nematospiroides dubius*, and rabbit—*Trichostrongylus retortaeformis*. According to the literature, improved primary screens that aid in the discovery of new anthelmintics may be developed by using species of *Trichostrongylus*, normally parasitic in domestic animals, in susceptible small laboratory animals. Such species are useful because of their low host specificity, high pathogenic potential, and because certain species occur in both the stomach and small intestine of susceptible hosts.

Drudge *et al.* (1955) and Leland and Drudge (1957) reported that rabbits could be infected with *Trichostrongylus axei* from ruminants and equines, and possibly also with *Trichostrongylus colubriformis*. Wood (1958) standardized infections of *T. colubriformis* in rabbits, and concluded that this system was satisfactory for evaluating potential livestock anthelmintics. However, in this system phenothiazine and Trolene (Ronnel) were inactive, whereas carbon tetrachloride and diethylcarbamazine were active. Cauthen (1958) reported that in the rabbit-*T. axei* system, phenothiazine, piperazine citrate, and "Cu-nic" (1.75% copper sulfate plus 0.8% Black Leaf 40) showed only minimal activity.

Although guinea pigs can be infected experimentally with *T. colubriformis* (Herlich *et al.*, 1956; Herlich, 1958; Gordon *et al.*, 1960; Sturrock, 1963; Williams and Palmer, 1964), and apparently with *T. axei* (El-Rawi, 1961), their susceptibility seems to vary widely and the system may be of limited value.

Leland (1961, 1963a) reported that Mongolian gerbils (*Meriones unguiculatus*) could be infected with *T. axei* and that the system showed promise as a primary anthelmintic screen (1963b). It successfully detected activity of thiabendazole at 200 mg/kg, but not at 12.5 to 100 mg/kg; however, certain other anthelmintics showed no activity.

Williams and Palmer (1964) used *T. colubriformis* infections in rats, mice, guinea pigs, and rabbits for primary anthelmintic screens. They found that young male rats (50 g) and mice (20 g) were satisfactory hosts for *T. colubriformis*, and that Libyan gerbils (*Meriones libycus*) could be infected with *T. axei* and *T. colubriformis*.

The reports of Leland (1961, 1963a, b) stimulated us to investigate further the utility of the gerbil-trichostrongyle system in anthelmintic research. We found that Mongolian gerbils could be infected with both *T. axei* and *T. colubriformis*. Data are reported in this paper on mixed infections of these two nematodes. Limited trials were also conducted with similar experimental infections in sheep and goats, primarily to check the efficiency of the anthelmintics used. We have also reported some unexpected changes that occurred over about 17 months in the main *Trichostrongylus* spp. isolate used in this work. Part of this work was previously reported by the authors in abstract (1965).

### MATERIALS AND METHODS

**ANIMALS EMPLOYED:** Young gerbils (*Meriones unguiculatus*) of both sexes were purchased from Tumblebrook Farm, Brant Lake, New York. The gerbils were kept in small wire cages and given a standard pelleted feed

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and water ad lib. At the start of the tests the gerbils were 6 to 12 months old, and the mean weight of 112 gerbils was 77 g. However, the mean weights of 26 experimental groups of gerbils varied from 70 to 96 g. Most of the experimental groups comprised equal numbers of both sexes.

The 6 goats and 18 sheep used in this work were raised helminth free and were 1 to 2 years old when infected. In Test 5 the mean weight of the animals varied from 31 to 50 kg per group. The sheep and goats were females and wethers, and were assigned to the test groups without regard to sex.

**ORIGIN, CULTURE, AND ENUMERATION OF INFECTIVE LARVAE:** Two isolates of *Trichostrongylus* spp. were used, viz., KH (Kentucky Horse) and BB (Beltsville Burro).

**KH ISOLATE:** This was supplied by Dr. S. E. Leland, then of the University of Kentucky. It consisted of several thousand *T. axei* larvae of Equine Isolate "A" (Leland, 1963a), but Dr. Leland informed us that he was not sure this isolate was pure *T. axei*. About 2,000 of these larvae were inoculated orally into each of several rabbits, and larvae from the rabbit fecal cultures were inoculated in Lamb 85 (Table 1). Only *T. axei* adults were recovered at necropsy from the rabbits originally inoculated with the KH isolate, but a small number (0.4%) of *T. colubriformis* were recovered from the small intestines of gerbils in Test 1. Thereafter, the KH isolate was transferred successively to two goats and another lamb (207) to obtain enough larvae for later tests. Table 1 shows that by January 1965 the species configuration of the KH isolate had changed from the supposedly pure *T. axei* to an isolate with only about 36% *T. axei*, the remainder being *T. colubriformis*. The origin of the latter species and the cause of this quantitative shift in numbers of the two species are obscure, but the *T. colubriformis* may have been present in the original isolate. Nevertheless, because this isolate consisted of both a stomach and an intestinal species of *Trichostrongylus*, it was feasible to test anthelmintics against both species in gerbils and in domestic ruminants.

**BB ISOLATE:** We attempted to obtain a pure *T. axei* isolate by inoculating a helminth-free lamb (221, Table 1) with infective larvae

obtained from culture of burro (*Equus asinus*) feces containing trichostrongyle eggs. Upon necropsy, the same two species were recovered as the two which comprised the KH isolate. The reaction of the two trichostrongyle isolates, consisting of the same two species, to three anthelmintics in gerbils was compared (Test 8, Tables 1, 2).

Infective larvae for the tests were obtained from fecal cultures of stock animals (see Table 1). The larvae per ml of stock suspension were estimated by averaging counts of four 1 ml samples. Because the gerbil stomach is small, each stock suspension was adjusted so that the appropriate number of larvae was delivered in 0.5 ml or less of the suspension. Larvae for sheep and goat inoculations were cultured and enumerated similarly, except that much larger doses were used.

**LARVAL INOCULATION OF ANIMALS:** Nine different larval suspensions were used: One each for Tests 1 to 7 and one each for Tests 8A and 8B. For each gerbil, 0.5 ml or less was quickly removed from the continuously stirred bulk suspension with a 0.5 ml tuberculin syringe. To the syringe was attached a #18 hypodermic needle, modified by cutting off the sharp tip and adding a small globule of solder, to prevent injury to the esophagus during inoculation. In various tests, 997 to 1,500 larvae were inoculated per gerbil (Tables 2-3).

Sheep and goats were infected with single doses of larvae in small gelatin capsules by oral inoculation with a balling gun. These capsules were prepared as follows: Individual doses of 41,000 or 50,000 larvae (Table 4) were removed from the stock suspensions with a calibrated pipette and placed on a small filter paper laid over a piece of blotting paper; the excess water was allowed to blot off and the filter paper was folded with the larvae on the inner surface and inserted in the gelatin capsule.

**ANTHELMINTICS EMPLOYED, THEIR ADMINISTRATION, AND DOSAGES:** Three anthelmintics were used; these and details concerning them were supplied by the manufacturers as indicated below:

1. Phenothiazine (Atomic Basic Chemical Co.)
  - a. N.F. product; 7  $\mu$  average particle

TABLE 1. *T. axei* and *T. colubriformis* in two isolates recovered at necropsy from various experimentally infected, unmedicated animals (controls) over the 17-month period of the anthelmintic tests reported herein.

Test number <sup>1</sup>	Infection date Month/Year	Animals infected	Stock culture animals	Per cent of each species at necropsy	
				<i>T. axei</i>	<i>T. colubriformis</i>
A. Kentucky-Horse (KH) Isolate					
1	8/63	5 Gerbils	Rabbit-Lamb 85	99.6	0.4
2	10/63	4 Gerbils	Lamb 85	97.9	2.1
3	10/63	4 Gerbils	Lamb 85	97.8	2.2
4	3/64	4 Gerbils	Goat 90	97.4	2.6
5	2/64	2 Goats	Lamb 85, Goat 90	68.2	31.8
6	8/64	3 Sheep	Lamb 207 <sup>2</sup>	28.1	71.9
7	1/65	2 Sheep	Lamb 207	27.6	72.4
8A	1/65	5 Gerbils	Lamb 207	36.1	63.9
B. Beltsville-Burro (BB) Isolate					
8B	7/64	1 Lamb (#221)	Burro	95.8	4.2
	1/65	5 Gerbils	Lamb 221	96.9	3.1

<sup>1</sup> See Tables 2-4 for further details.

<sup>2</sup> Lamb 207 was infected with larvae from 2nd stock goat.

size; 95% pure; wetting agent 1% lecithin; 1960 batch.

- b. Purified product; 6.9  $\mu$  average particle size; 99.9% pure; wetting agent 1% lecithin; 1960 batch.
  - c. Purified product; 2-3  $\mu$  average particle size; 99.9% pure; wetting agent 1% lecithin; 1963 batch.
2. Thiabendazole (Merck Institute for Therapeutic Research). Pure chemical and water dispersible powder.
  3. Ruelene as "Ruelene Wormer Drench" (Dow Chemical Co.). This product was prepared as a sheep drench for investigational use only, and consisted of the following ingredients:

<i>On printed label</i>	<i>Dow Chemical Co. assay</i>
Ruelene—21%	Ruelene—21.3%
Propylene glycol—79%	Propylene glycol—78.7%

The phenothiazine and thiabendazole powders were prepared as water suspensions for use as drenches. The fluid Ruelene Wormer Drench was used as supplied by the manufacturer; the doses were calculated on the basis of the Ruelene content determined from the assay.

The drenches were administered to gerbils by the same technique used for larval inoculations. The phenothiazine and thiabendazole drenches were prepared so that not more than 0.5 ml was administered at one time. The planned anthelmintic doses were given to gerbils in two parts, one-half in the morning

and one-half in the afternoon of the same day, and to the sheep and goats as single doses with a dosing syringe equipped with a Whitlock nozzle.

The anthelmintic doses were given to gerbils on the basis that each gerbil treated weighed 100 g, rather than as mg/kg (Tables 2, 3). As the mean weight of the gerbil test groups varied from 70 to 96 g, the mg/kg doses actually given were 4-30% higher than those listed in the tables. Because of the small quantities of drugs used per gerbil, we do not believe this method of calculating doses materially affected the results. The doses were about the normal therapeutic dose, double the normal dose, and (thiabendazole only) quadruple the normal dose.

The doses were given to the sheep and goats (Table 4) as mg/kg of body weight, and all doses were within the recommended therapeutic range.

In all tests except one, the drugs were administered 24 to 36 days postinfection, when the nematodes were mature. In one test with gerbils (Test 3, Table 3) the drugs were given 12 days postinfection, when the nematodes were immature.

**NECROPSY AND WORM COUNT PROCEDURES; EFFICACY CALCULATIONS:** All animals were killed 5 days posttreatment. At necropsy, the stomachs and small intestines of the gerbils were removed; each organ was cut into small pieces and placed in 50 ml of artificial pepsin digestion fluid (0.5% scale pepsin and 0.5%

TABLE 2. Activity of three anthelmintics on experimentally induced, mixed trichostrongyle infections involving two isolates and two species (*T. axei* and *T. colubriformis*) in gerbils. (Test 8A, B; also see Table 1).

Drug and dose <sup>1</sup> (Divided dose, ½ AM, ½ PM)	No. larvae inoculated per gerbil	No. gerbils per group	Avg no. nematodes per gerbil at necropsy		Per cent efficacy	
			T. a.	T. c.	T. a.	T. c.
A. Kentucky-Horse Isolate						
None (Controls)	1,500	5	150	264	—	—
Phenothiazine Purified, 2-3 μ 1,200 mg/kg	1,500	5	99	173	34	35
Thiabendazole 100 mg/kg	1,500	5	44	5	71	98
Ruelene 400 mg/kg	1,500	5	85	14	43	95
B. Beltsville-Burro Isolate						
None (Controls)	1,400	5	279	9 <sup>2</sup>	—	—
Phenothiazine Purified, 2-3 μ 1,200 mg/kg	1,400	5	240	4	14	56 <sup>3</sup>
Thiabendazole 100 mg/kg	1,400	5	134	0	52	100 <sup>3</sup>
Ruelene 400 mg/kg	1,400	5	135	0	52	100 <sup>3</sup>

<sup>1</sup> Treatment 24 days postinfection; necropsy 5 days later.

<sup>2</sup> Only about 3% of *T. colubriformis* in BB isolate; see Table 1 for details.

<sup>3</sup> Numbers of T. c. too small for these efficacy calculations to be meaningful.

HCl) in a 100 ml wide-mouthed bottle, and the bottle was capped. The digests were incubated at 37 C for 14 hours; formalin was then added to preserve the nematodes for counting and identification. Total worm counts were made from all gerbils.

At necropsy of the sheep and goats, the abomasums were quickly tied off with string from the small intestines at the pyloric valve, and the two organs removed separately for worm recovery. Each organ was then opened and its contents were recovered; the remaining tissues were then separately subjected to artificial peptic digestion as for gerbils. Both the contents of the organs and the separate digests were made up to 2,000 ml with water and two 100 ml samples were removed while the bulk material was continuously stirred. Total worms per animal were calculated from these samples.

Efficacy of the anthelmintic treatments was calculated on the basis of postmortem worm counts by using the following formula:

$$\frac{\text{Avg worms in controls} - \text{avg worms in treated animals}}{\text{Avg worms in controls}} \times 100 = \% \text{ efficacy.}$$

RESULTS

ANTHELMINTIC STUDIES: The results ob-

tained with the gerbil-*Trichostrongylus* spp. system are summarized in Tables 2 and 3. The three phenothiazines showed very low grade or no activity against *T. axei* and *T. colubriformis* in gerbils, even when the purified product of 2-3 μ average particle size was given at about double (1,200 mg/kg) the normal therapeutic dose recommended for domestic ruminants (Tests 4 and 8). However, the purified, 2-3 μ phenothiazine at 600 mg/kg was highly efficacious against the same nematode species in sheep and goats (Table 4).

Anthelmintic activity of thiabendazole and Ruelene was readily detected in this system. In Test 1 (Table 3), as the dose of thiabendazole increased (50, 100, and 200 mg/kg) the efficacy against the predominant *T. axei* infections also increased (40, 83, and 97%, respectively). In Tests 1 to 4, 200 mg/kg of thiabendazole gave consistent results (97, 94, 93, and 89%, respectively) against *T. axei*. In Test 3, thiabendazole was about as effective against immature *T. axei* (mainly 4th and early 5th stages) as against adult *T. axei* in the other tests. In Test 4, about double the normal dose of Ruelene showed only low grade activity against *T. axei*.

TABLE 3. Activity of three anthelmintics on experimentally induced, mixed trichostrongyle infections in gerbils. Kentucky-horse isolate used, which consisted mainly of *T. axei* (T. a.) in these tests; only small numbers of *T. colubriformis* (T. c.) were present.

Test no., <sup>1</sup> Time of treatment	Drug and dose <sup>2</sup>	No. larvae inoculated per gerbil	No. gerbils per group	Avg no. nematodes per gerbil at necropsy <sup>3</sup>		Per cent efficacy
				T. a.	T. c.	T. a. only
1 36 days postinfection	None (Controls)	1,090	5	215	1	—
	Thiabendazole 50 mg/kg	1,090	4	130	1	40
	Thiabendazole 100 mg/kg	1,090	4	37	0	83
	Thiabendazole 200 mg/kg	1,090	4	6	0	97
2 35 days postinfection	None (Controls)	997	4	214	5	—
	Phenothiazine NF, 7 $\mu$ 600 mg/kg	997	3	162	0	25
	Phenothiazine Purified, 6.9 $\mu$ 600 mg/kg	997	4	253	3	0
	Phenothiazine Purified, 2-3 $\mu$ 600 mg/kg	997	4	216	4	0
	Thiabendazole 200 mg/kg	997	4	13	0	94
3 12 days postinfection	None (Controls)	1,055	4	347	8	—
	Phenothiazine NF, 7 $\mu$ 600 mg/kg	1,055	4	283	0	18
	Phenothiazine Purified, 6.9 $\mu$ 600 mg/kg	1,055	4	213	17	39
	Phenothiazine Purified, 2-3 $\mu$ 600 mg/kg	1,055	4	220	5	37
	Thiabendazole 200 mg/kg	1,055	4	26	0	93
4 32 days postinfection	None (Controls)	1,200	4	267	7	—
	Phenothiazine Purified, 2-3 $\mu$ 1,200 mg/kg	1,200	4	279	14	0
	Thiabendazole 200 mg/kg	1,200	4	29	0	89
	Ruelene 400 mg/kg	1,200	4	196	1	27

<sup>1</sup> See Table 1 for further details.

<sup>2</sup> Divided dose;  $\frac{1}{2}$  AM and  $\frac{1}{2}$  PM.

<sup>3</sup> Necropsy 5 days posttreatment.

Test 8 (Table 2) was a comparison of the effectiveness of the three anthelmintics, at about double the normal therapeutic dose, against both the KH and BB isolates of *Trichostrongylus* spp. in gerbils. Phenothiazine had only low grade efficacy against the KH isolate, which at the time of this test consisted of about 36% *T. axei* and 64% *T. colubriformis*. However, a difference in efficacy appeared with the thiabendazole and Ruelene against the two species. Both drugs were more efficacious

against *T. colubriformis* (98 and 95%) than against *T. axei* (71 and 43%); this was confirmed with the BB isolate, although the numbers of *T. colubriformis* involved were small. Thus, sensitivity of the KH and the BB isolates to these three anthelmintics seemed to be about the same.

In the potency tests with these three anthelmintics against similar experimental *Trichostrongylus* spp. infections in sheep and goats (Table 4), all the larval cultures used had

TABLE 4. Activity of three anthelmintics on experimentally induced, mixed infections of *T. axei* and *T. colubriformis* in sheep and goats.

Test no.: <sup>1</sup> Time of treatment	Drug and dose	No. larvae <sup>2</sup> inoculated per animal	No. animals per group	Avg no. nematodes per animal at necropsy		Per cent efficacy	
				T. a.	T. c.	T. a.	T. c.
5 31 days posttreatment	None (Controls)	41,000	2 Goats	7,697	3,515	—	—
	Phenothiazine Purified, 2-3 $\mu$ 600 mg/kg	41,000	2 Goats	912	5	88	99.9
	Thiabendazole 100 mg/kg	41,000	2 Goats	0	0	100	100
6 36 days postinfection	None (Controls)	50,000	3 Sheep	9,813	25,023	—	—
	Phenothiazine Purified, 2-3 $\mu$ 600 mg/kg	50,000	3 Sheep	3	50	90.9	99.9
	Thiabendazole 100 mg/kg	50,000	3 Sheep	0	0	100	100
	Ruelene 200 mg/kg	50,000	3 Sheep	0	3	100	99.9
7 31 days postinfection	None (Controls)	50,000	2 Sheep	4,330	11,545	—	—
	Thiabendazole 50 mg/kg	50,000	2 Sheep	0	5	100	100
	Ruelene 100 mg/kg	50,000	2 Sheep	1,290 <sup>3</sup>	0	70	100

<sup>1</sup> See Table 1 for further details.<sup>2</sup> Kentucky-Horse isolate used.<sup>3</sup> One sheep had almost all worms.

substantial numbers of both *T. axei* and *T. colubriformis* (Table 1). These limited tests show that our preparations of the three drugs were of normal potency against these species of nematodes in two of their normal hosts. However, 200 mg/kg of Ruelene was superior to 100 mg/kg against *T. axei*; both levels of this drug removed practically all of the *T. colubriformis*.

**OBSERVATIONS ON *Trichostrongylus* spp. ISOLATES:** Table 1 summarizes the history of our experience with the KH isolate of *Trichostrongylus* spp. Although this isolate appeared to be pure *T. axei* when received, either it became mixed with small numbers of *T. colubriformis* or some *T. colubriformis* was present in the original shipment. The necropsy data from the untreated (control) animals showed that *T. colubriformis* in this isolate gradually increased through transfer to stock sheep 85 and goat 90, increased substantially when it was transferred from goat 90 to a second stock goat, and again increased proportionately through transfer from the second stock goat to Lamb 207. Subsequent infections of 5 sheep and 5 gerbils (Tests 6, 7, 8A) with larvae cultured from the feces of Lamb 207

over a period of 5 months maintained ratios of about 1 to 3, or 1 to 2, *T. axei* to *T. colubriformis*. About the same ratios of the two species were obtained from 5 lambs as from 5 gerbils. Thus, on a proportional basis, gerbils are about as susceptible as sheep to infection with these two species. In further support of this conclusion, approximately similar percentages of the two species were recovered from 5 gerbils infected with the BB isolate from Lamb 221 as from Lamb 221 itself.

**UNIFORMITY OF INFECTIONS IN UNTREATED, CONTROL GERBILS:** In using this gerbil-trichostrongyle system to screen the anthelmintic potential of chemicals, the range of infections established is an important consideration. The infections in control gerbils in 6 tests indicate that substantial numbers of both species were established (Table 5). In Test 8A, one gerbil had only 37 *T. colubriformis* at necropsy, whereas the other four had reasonably uniform infections of this species; all five had quite uniform infection of *T. axei* (135-166). The most uniform infections of the combined species (241-333) were established in the gerbils in Test 8B. In general, the infections were



TABLE 5. Ranges in necropsy worm counts of *T. axei* and *T. colubriformis* in individual gerbils in untreated, control groups.

	<i>T. axei</i>	<i>T. colubriformis</i>	Both species
Test 1	138-298	0-5	138-298 <sup>1</sup>
Test 2	146-299	0-11	146-299
Test 3	248-463	0-30	248-493
Test 4	135-446	0-17	139-451
Test 8A	135-166	37-451	172-594
Test 8B	227-333	0-17	241-333

<sup>1</sup> The ranges of *T. a.* and *T. c.* are not simply additive, as the *T. c.* did not always occur in the gerbils which had the highest and lowest *T. a.* worm counts.

uniform enough to make this a reliable test system.

#### DISCUSSION

Our results show that the gerbil-*Trichostrongylus* spp. system is satisfactory to screen new chemicals for treating nematode infections in animals. We have confirmed and extended Leland's (1963b) preliminary observations that the gerbil-*T. axei* system showed promise as a primary anthelmintic screen. Our results for thiabendazole activity against *Trichostrongylus* spp. below the 200 mg/kg level differ somewhat from those of Leland. He stated that thiabendazole at 100 mg/kg and below showed no activity against *T. axei*; we observed 40% activity at the 50 mg/kg dose, and 52, 72, and 83% activity in different tests at the 100 mg/kg dose. However, we used divided doses of the drug, whereas Leland used single doses, and also based our drug doses on gerbil weights of 100 g each, although the mean weight of the gerbils was 77 g. In our tests at the 100 mg/kg level thiabendazole apparently removed 98% of the *T. colubriformis*. The two species-gerbil system may be more sensitive to anthelmintic activity than the one-species-gerbil system. Thus, *T. axei* in gerbils may be more sensitive to some chemicals and *T. colubriformis* to others.

Leland's (1963b) work and ours show that phenothiazine preparations have little or no activity in the gerbil-*T. axei* and the gerbil-*Trichostrongylus* spp. systems. This is probably due to the fact that the digestive tracts of gerbils and domestic herbivores, particularly ruminants, differ anatomically and physiologically. The activity of both thiabendazole and Ruelene in the gerbil-trichostrongyle

system justifies further investigation of the system as a primary anthelmintic screen.

One advantage of this host-parasite system as a primary screen is its manipulative simplicity. Gerbils are easily infected with mixed larvae of *T. axei* and *T. colubriformis* by oral inoculation of small numbers of larvae. Because of the small size of gerbils, only very small quantities of test drugs are needed, and these are easily administered to these docile animals. Furthermore, breeding colonies are easily established following the procedures of Schwentker (1963), Ringle and Dellenback (1963), Kramer (1964), and Marston and Chang (1965).

Our limited data on controlled anthelmintic tests with sheep and goats experimentally infected with *Trichostrongylus* spp. and treated with phenothiazine, thiabendazole, and Ruelene can be compared with data reported by Drudge *et al.* (1964) on these three anthelmintics for removal of natural infections of *Trichostrongylus* spp. from sheep. The following tabulation and efficacy calculations pertain only to *Trichostrongylus* spp., based on data reported by Drudge *et al.* (1964).

Drug and Dose	Nematodes at necropsy: Avg/lamb <sup>1</sup>			Per cent efficacy		
	T.a.	T.c.	T.v.	T.a.	T.c.	T.v.
None (controls)	2,564	4,520	2,048	-	-	-
Phenothiazine Purified, 2-3 $\mu$ 550 mg/kg	32	2,032	496	99	55	80
Thiabendazole 44 mg/kg	4	0	0	99.9	100	100
Ruelene 125 mg/kg	448	675	8	82	75	99.9

<sup>1</sup> Five lambs per group (T.a.—*T. axei*; T.c.—*T. colubriformis*; T.v.—*T. vitrinus*).

These results of Drudge *et al.* for thiabendazole at 44 mg/kg are similar to our results with 50 mg/kg (Table 4, Test 7). Results are also similar for phenothiazine and Ruelene, except that we obtained somewhat higher efficacies against *T. colubriformis* with 600 mg/kg of phenothiazine and 100 mg/kg of Ruelene than they obtained with 550 mg/kg and 125 mg/kg of the same drugs, respectively.

Although the cause of the quantitative species change of our KH isolate of *Trichostrongylus* spp. is obscure (Table 1), certain factors may have been involved: (a) differences in egg productivity of the two species, (b) dif-



ferences in susceptibility of the stock animals used, (c) differences in time of peak egg production of the two species in mixed infections, (d) the effect of culture conditions on the differential development of infective larvae of the two species (Giordia *et al.*, 1966). The interaction of these two closely related species in mixed infections warrants further investigation.

The BB isolate was obtained from a burro which had grazed for several months on a pasture formerly grazed by sheep. The burro probably became infected with one or both of the two trichostrongyle species as a result of sheep contamination of the pasture. We were not able to follow this isolate through several transfers in stock animals to determine if the two species would undergo the same quantitative change as in the KH isolate. However, our work indicates that when trichostrongyle isolates are obtained from equines, such isolates may contain more than one species of *Trichostrongylus*.

#### SUMMARY

Experiments were conducted to evaluate gerbils simultaneously infected with *T. axei* and *T. colubriformis* as a host-parasite system for use as a primary screen for new chemicals of possible anthelmintic activity. Three phenothiazine products, thiabendazole, and Ruelene were used in this evaluation. This system detected activity of both thiabendazole and Ruelene, but not of the three phenothiazines. *T. colubriformis* in gerbils appeared to be more sensitive to thiabendazole and Ruelene than *T. axei*. All preparations of anthelmintics used in the gerbil tests showed normal activity against similar mixed trichostrongyle infections in sheep and goats. Quantitative changes that occurred over a period of about 17 months in one mixed isolate of *T. axei* and *T. colubriformis* are described, and the possible factors influencing these changes are discussed.

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## Zoological Nomenclature in the Jet Age<sup>1</sup>

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In this age of molecular biology and space science, there are those who regard taxonomy in general, and nomenclature in particular, as horse-and-buggy biology. So here I am, asked to speak on a subject, zoological nomenclature, that would be, I am sure, at the very bottom of any Gallup poll rating in biology. I did the best I could by dressing up the title, and hence my talk is called "Zoological Nomenclature in the Jet Age." With jets, of course, you can expect a blast of hot air and a lot of noise, but the trip will be fast.

With reference to modern science, it strikes me that there is a strange phenomenon today. The wider the horizons, the narrower the viewpoint of some scientists on the older areas of knowledge. But as a taxonomist I know that all will come home to roost some day. When men land on the moon, they will collect samples. Then they will ask the elementary question in taxonomy: "What is it?" And *then* they will submit it for identification to some taxonomist!

However, I did not come here to wring my hands. I came to talk about some problems in zoological nomenclature. I shall *not* review the history of nomenclature, or Codes, or International Congresses of Zoology. That has been

done often, and *ad nauseam*, frequently by me. Rather I shall concentrate on present and future general problems. We simply cannot escape nomenclature of some kind because we must have names, or symbols, or some means of referring to objects and concepts, of recording information concerning them, and of communicating with others about them. Nomenclature may be derided as old fashioned, but like eating and sex, it is basic, and it won't go away. And it has problems, even in modern sophisticated science, and sometimes because of that sophistication. I am seriously concerned about some of these problems. I think they are matters with which the International Commission and taxonomists in general must come to grips in this jet age. I may not have pat answers, but at least I wish to discuss the problems. I want to think out loud for a while.

### WHAT IS PUBLICATION?

My first problem is an old one, but recently it has hit me in the face: What is publication? This problem transcends nomenclature and taxonomy, but it is more acute in taxonomy because we use date of publication as an objective, factual criterion of priority.

Recently, during the meeting of the American Institute of Biological Sciences on the campus of the University of Maryland, I at-

<sup>1</sup> Presented as part of an after-dinner program at the 56th anniversary meeting of the Helminthological Society of Washington, 19 October 1966.

tended a symposium on microprint—microfilm, microcards, etc. There was a pitifully small audience, for a challenging subject with a number of important speakers. I am concerned here about two different facets of the problem, one very disturbing and one not, to me at least.

The facet that does not disturb me concerns acceptance of photographic methods as "publication" in the meaning of the International Code of Zoological Nomenclature. The Code at present flatly rejects photographic methods for the finished product. Should it recognize them? Personally I think recognition is inevitable. I presented sample microcards of the first Wildlife Diseases Bulletin, kindly furnished me by Carleton Herman, to the Colloquium on Nomenclature at the International Congress of Zoology at London in 1958. The Congress declined to recognize such methods as publication, and the new Code was prepared in accordance with that decision. I think the decision was understandable, and, at that time, defensible. Such material was brand new to many of the zoologists present. They had suspicions about the permanency of such material. They felt that the method must first be improved, and above all, must *prove* itself. They considered it untried, and indeed it was, as a method of journal publication; the Bulletin was, initially, a pilot project, its fate yet to be determined. Incidentally, the Bulletin is still appearing, now on 4 by 6 inch microfiche.

But that was nearly ten years ago. Meanwhile, the Wildlife Diseases Bulletin has been thriving, and microprint in various forms has come increasingly into use. It has also been greatly improved, and more people are familiar with it. Mechanical readers are vastly better. At the same time, the information explosion has been increasing the pressure of literature. I need not elaborate on the problems of the sheer bulk of literature and all its concomitant problems of storage and retrieval, of shelf space in libraries—indeed, of space for libraries themselves! I am convinced that for much technical material some kind of microprint publication (if I may use the word for the moment in a general sense) will be used increasingly. I think of nothing more appropriate for microprint than a great deal of taxonomic work—detailed descriptions and redescriptions,

figures, synonymies—that should be (or that is!) placed on record but that need not be consulted frequently. The Commission must soon face up to modern developments in this field. That prospect does not worry me.

The other facet of the publication problem is more disturbing. A typed copy of a Ph.D. thesis is customarily (perhaps always) deposited in the library of the institution granting the degree. Is this publication? I am sure that most or all of you would promptly say, "Of course not." So do I. But at this AIBS symposium, we were told that, "Of course it *is* publication." Frankly, I was, and am, shocked. I simply cannot agree that a typed manuscript of a thesis deposited in a university library in partial fulfillment of the requirements for a degree of Doctor of Philosophy is *bona fide issue* for *permanent, public, scientific record*.

But do I use the words of the International Code when I should be using common sense? (The two are not necessarily synonymous!) There is no question that the deposited thesis does not constitute publication in the technical meaning of the present Code. But there are some ramifications, and I am afraid that this problem will become more intense and more demanding. It is not an answer to point out that commercial men with a profit motive were chiefly the ones who insisted that a deposited thesis constitutes publication. Some others also did. Right or wrong, let us consider a few of the complications.

First of all, I may note that a summary of the thesis may be printed, distributed like a reprint, or printed in a volume of summaries of theses, and these are, of course, clearly publications in their own right. So is an abstract that is printed and published in a conventional manner, e.g., "Dissertation Abstracts." These, as they stand, constitute publication for what they themselves contain. There is no question about such summaries and abstracts, nor about separately printed theses.

A deposited thesis manuscript, however, is obviously not printed, and it is not distributed. But it can be borrowed, copied, quoted, cited in bibliographies. It may be advertised, as in "Dissertation Abstracts." It can be ordered in microprint from a master copy maintained by the commercial firm. Is the whole thesis then to be considered as published by mere deposit?

The commercial firms are in the business to make a profit, naturally, from their service in making available and disseminating the theses. Are they wrong in considering the theses published? Are universities wrong in making them available for copying? Is it fundamentally any different than an interlibrary loan, in terms of releasing the student's material? Do not universities (at least some) also require formal publication, i.e., publication in a conventional manner? If they do, and some do, is it not evidence that they themselves do not really consider the deposit of the thesis or the availability of a photocopy as true publication? Is the availability of photocopies on order any different than that of conventional publications available for purchase or free distribution upon request? Or any different than rare or limited-edition publications that can be borrowed on interlibrary loans?

Copyright problems may enter here also. Commercial companies may arrange copyright for distribution of a thesis. But for copyright, there are no standards for number of copies, and no requirement about means of reproduction. Indeed, as far as I can learn, I could type out a copy and three carbons of a description of a new species, sell one to any of you for 5 cents, and send two to the Copyright Office for a copyright. Is this what we want for publication? Obviously, legally copyrightable material and acceptable scientific publication must be two different things, at least for some types of material.

To me, "publication" implies not only the making of the finished product (printed in ink, under the present Code), but also public issuance and dissemination of a number of copies. Can that be said of a deposited thesis? Can that even be said of a deposited thesis for which an abstract has been published (printed)? Can it be said of a thesis that you can borrow? Can it be said of a thesis of which you can order a microprint?

Think of other angles important in the public dissemination of information throughout the scientific community. Think of abstract journals, the Index-Catalogue of Medical and Veterinary Zoology, the Zoological Record. Do they cover theses? Do they, or could they, or should they, pick up new names for taxa of organisms, new synonymy, type designations,

etc., scattered through deposited theses but not usually mentioned in summaries or abstracts? They could, of course, order theses regularly as soon as the abstracts appeared, and then index the full texts. But I believe that they are not now so treated. There would still remain the problem of the theses that were not selected for dissemination via microprint because they were deemed unattractive and commercially unprofitable.

Theses are often stuffed with material that is pruned out when a paper is prepared for formal conventional publication. Are not theses essentially preliminary drafts submitted for a special private purpose and not yet prepared as formal and public contributions to the literature of science?

As you may have gathered from my remarks, I am exceedingly loath to recognize theses as publications. But the problem has complications—the published abstracts, the ready availability of copies in microprint, the use being made of these copies (quotations, background). Sooner or later, and I hope sooner, taxonomists and the International Commission will have to face up to this subject. Should we hew to the line of conventional publication, or should we somehow adjust our thinking and our rules? The decision may be a tough one, but the time is upon us. Theses are already yielding problems.

#### NUMERICAL TAXONOMY

A second major topic, although one that I shall mention rather briefly, is numerical taxonomy. For the most part, and in essence, this concerns taxonomy rather than nomenclature. But in their bumptious enthusiasm and fanatic crusader's zeal, some advocates of numerical taxonomy have, at various times, made striking proposals regarding scientific names, and thus they impinge on nomenclature.

One proposal held that scientific names are "old hat," and that they would be succeeded by numbers, in order to fit into the modern world of computers and retrieval systems. I consider this a wild-eyed scheme. Certainly numbers are useful and often necessary, but they need not supplant names. We have telephone numbers and bank account numbers, social security and credit card numbers, etc. But your individual binomial or trinomial is not

affected. The numbers are always associated with names. One could, if one wished, go all the way with 12-2-10 or could like 9-11-5, but I'll stick with LBJ and Ike. Fortunately, the numerical taxonomists themselves have come back to earth from some of their flights of fancy.

There does remain a recent proposal for a monomial system, presented by C. D. Michener in an article in "Systematic Zoology." His proposal does indeed have a superficial and haunting attractiveness, in terms of avoiding such problems as homonymy, change of generic and specific combinations, and change of specific endings to agree with the gender of the generic name. But I consider it a backward step. Binomial nomenclature has often been criticized as not showing classification, but it actually does, in a significant way that is perhaps the most important step in the whole hierarchy. In its simplest form, it shows that one taxon, the species, is a member of a next higher taxon, a genus. When the classification of a group is well advanced and the genera are truly natural units, the binomial can be the basis of prediction and of phylogenetic, evolutionary, and zoogeographical speculation. The monomial would destroy this. Some advantages it may have, but also some disadvantages. I would not wish to cast away the well-established and time-tested binomial system for an untested system that might profit us in certain mechanical ways but would lose for us in fundamental ways.

#### UNIFORMITY IN SPELLING

Computers, the increasing pressures of codes and retrieval systems, and numerical taxonomy, coupled with increasing impatience with some nomenclatural trivia, have led me to think that the time is ripe for a modernizing of names and greater attention to simplification and uniformity. To put it bluntly, I believe that we should meet the computers part way. *Let systematists systematize their names.* This is a Machine Age; let us streamline nomenclature as much as possible, while still retaining that which is sound.

I am thinking here especially of uniformity in spelling. Adherence to the original spelling is all very well; it is (usually) an ascertainable and unarguable fact, good or bad. Acceptance

of the fact has a certain bibliographic merit, and it avoids arguments on transliteration, on meaning, on whether an alternate spelling did or did not exist in classical times, as evidenced by an obscure scrap of papyrus. Early in nomenclature, the classicists prevailed, and they proceeded, à la Agassiz, to correct the errors of the ignorant, the careless, and the printers. The reaction against this purism, abetted by increasing ignorance of and disrespect for the classical languages, has led to a "reign of error," an adherence to original spelling no matter how bad. In turn, this has led to a glorious mixture of spellings. I think it is high time to set our house in order. I am seriously considering the proposal of uniform spelling of the roots in scientific names. A few examples will suffice: *chaeta* vs. *cheta* (as in *Oligochaeta*, *Chaetognatha*, etc.), *palaeo* vs. *paleo*, *myia* vs. *mya*, *rha* vs. *ra*. The choice might be made either on the basis of the correct form (*chaeta* over *cheta*, for example), or on the basis of the simpler reduced form, even as *paleo* has won out, in this country at least, over *palaeo* in such words as paleontology, palearctic, and Paleocene. It would be better, in my opinion, to preserve the classically correct form for the scientific names as they are used in all countries, and let the ordinary terms be simplified in the individual languages as they will. Some uniformity of language would greatly simplify matters of spelling, assist in recording and retrieval of information, and be of great aid to memory in many groups.

#### THE LAW OF PRIORITY AND THE 50-YEAR RULE

I shall say little about this controversy except to note that it is still being argued. To a great extent the argument stems from a fundamental difference in viewpoint. In groups with well-advanced classification and little or no alpha and beta taxonomy yet to be done, the desire to hold fast to well-known and familiar names is strong. This is especially true in groups that contain familiar animals such as Aves and Mammalia. At the other extreme are the entomologists, and I believe the helminthologists also, with a vast amount of alpha and beta taxonomy still ahead of them, with much reclassification and many changes of names and generic shifting inevitable, and with much

difference of opinion on synonymy and status. For us, the Law of Priority is an operable, objective principle that we can apply without running to the Commission at the slightest hint of change. The old system of Suspension of the Rules for really serious and worthwhile cases was an acceptable and workable system. The 50-year rule, on the other hand, involves us in far too much wasteful bibliographic work and time-wasting litigation before the Commission. I suspect that this is true for many invertebrate groups.

The last International Congress of Entomology, at London in 1964, adopted overwhelmingly a resolution, prepared and moved by me, proposing to the International Commission on Zoological Nomenclature that the field of entomology be exempted from the application of the 50-year rule. The resolution was presented to the Commission but has not thus far been acted upon.

I have been asked: Why limit the resolution to entomology? The answer: That is the only field for which the International Congress of Entomology can speak. Why not fight the 50-year rule in the International Congress of Zoology? It has been fought twice, and as hard as I could fight it, but the fight failed,

for various reasons, though always with a very close vote. And the obvious final question: Why not bow gracefully to the "democratic" process and accept the vote? First of all, the Zoology Congress is not truly representative of all of zoology and never has been. The fields of entomology and paleontology, with their own International Congresses (of geology, for the latter), are not proportionately represented. Most of all, frankly, I am endeavoring to prevent outright revolution and to work within the framework of international zoological nomenclature as long as possible.

Helminthologists and their associates are, in this modern world, like taxonomic entomologists. We are carrying a torch along slow and bumpy side roads while our richer brethren are whizzing by on turnpikes and in space. We still have problems with traffic rules and road maps. I have discussed a few of these problems tonight. I hope that some day we can all meet together in the sunlight on the other side of the Slough of Despond.

Meanwhile, my favorite admonition for all taxonomists, and especially for nomenclaturists, is Oliver Cromwell's plea to the bishops of England: "I beseech ye, by the bowels of Christ, consider ye may be wrong."

### New Speleognathinae from Central and South American Mammals (Acarina, Trombidiformes)

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Recent collections of small mammals made in Panama and Colombia<sup>1</sup> have made available for description two new species of Speleognathinae from rodents and bats respectively. In addition, two more speleognathid species are reported for the first time from Panama. All measurements are in millimeters.

<sup>1</sup> The author is indebted to Drs. C. E. Yunker and J. M. Brennan who kindly furnished speleognathid specimens from Panamanian rodents and to Dr. C. J. Marinkelle for specimens from Colombian bats.

#### *Paraspeleognathopsis cricetidatum* n. sp.

FEMALE (Fig. 1): Shape, elongate oval with the greatest width in the humeral region. Length, excluding gnathosoma, 0.37–0.50 (avg 0.42); width, 0.21–0.30 (avg 0.25). Gnathosoma well developed, very weakly striated and finely punctate, measuring 0.042–0.05 long (avg 0.047) by 0.05 to 0.072 wide (avg 0.067). With two pairs of hypostomal setae. Palpi three segmented, the proximal poorly

developed as a thin band on the palpal tibia. Palpal tibia large, with a single, dorsal, expanded, fan-shaped barbelled seta. Palpal tarsus ventral, with three or four barbelled setae and a single weak, nude sensory seta.

*Dorsum*: Cuticle with weak striations having fine linear punctations. Scutum present with characteristic inner cell framing two post-sensillary setae. Sensillae elongate, barbelled, 0.027 long, set in deep, circularly striated sensory pits. With a single pair of presensillary setae. Dorsal formula 2-4-4-2-2-4-2.

*Venter*: With but two pairs of sternal setae, three pairs of genital setae and two pairs of anal setae. Legs with the following setal pattern: Coxa, 2-1-1-1; trochanter, 1-1-0-0; femur, 4-3-3-1; genu, 4-4-3-2; tibia, 4-2-2-2; tarsus, 12-8-7-7. Cuticle longitudinally striated with linear punctations. Claws, strongly curving, blunt; pulvillus entire, striated and setated. Setae of femur and genu much enlarged and expanded, sexually dimorphic.

**MALE** (Fig. 2): Length, including gnathosoma, 0.33; width, 0.20. Generally like female with minor dimorphism as pseudofusion of tibia and tarsus of legs 1 and 2 to a slightly enlarged, blocklike terminal segment. Setae on femur and genu of legs 1 and 2 not markedly enlarged. Large, multilobed testes present. General sclerotization somewhat weaker than female.

**DIAGNOSIS**: *P. cricetidatum* may be distinguished from all other known species of the genus, especially *P. galliardi* Fain, 1955, by the shape of the scutum, the presence of but one pair of sternal setae, entire, striated pulvillae, and the ornamentation of the gnathosomal base and scutum.

**HOLOTYPE**: Adult female, USNM no. 3157, is deposited in the collection of the U. S. National Museum, Washington, D. C. Type material will be deposited at The British Museum (Natural History), London, England; Museum National d'Histoire Naturelle, Paris, France; Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium; Natal Museum, Pietermaritzberg, South Africa; and South Australian Museum, Adelaide, South Australia.

**TYPE HOST**: *Oryzomys talamancae* Allen, 1891,<sup>2</sup> a rice rat of the family Cricetidae which

was collected by Dr. C. E. Yunker on 29 November 1960 at Summit, C. Z. Specimens of the series examined for this description were also taken from *Zygodontomys cherriei* (Allen, 1895) collected by C. M. Keenan at France Field and Ft. Gulick, C. Z. in December of 1960 and May and June of 1961, and *Heteromys desmarestianus* Gray, 1868, a spiny pocket mouse in the family *Heteromyidae* also collected by Mr. Keenan at Ft. Gulick on 7 December 1960.

**TYPE LOCALITY**: Panama, Canal Zone.

**REMARKS**: Although no larvae were available for inspection, certain features of developing eggs within adult females indicate that these larvae may have some remarkable claw modifications and it is probable that speleognathid species from rodents will be partially classified on the basis of larval morphology when these forms are found.

Since the number of palpal segments is highly unstable within genera of the Speleognathinae, it seems probable that all speleognathids now known from rodents belong in a single genus, *Paraspeleognathopsis* (Fain, 1958) Fain, 1962 without further subgeneric splitting.

*Paraspeleognathopsis derricki*  
(Womersley, 1954)

An adult female of this species was taken from *Rattus rattus* collected by C. E. Yunker at Ft. Gulick, C. Z. on 17 April 1962.

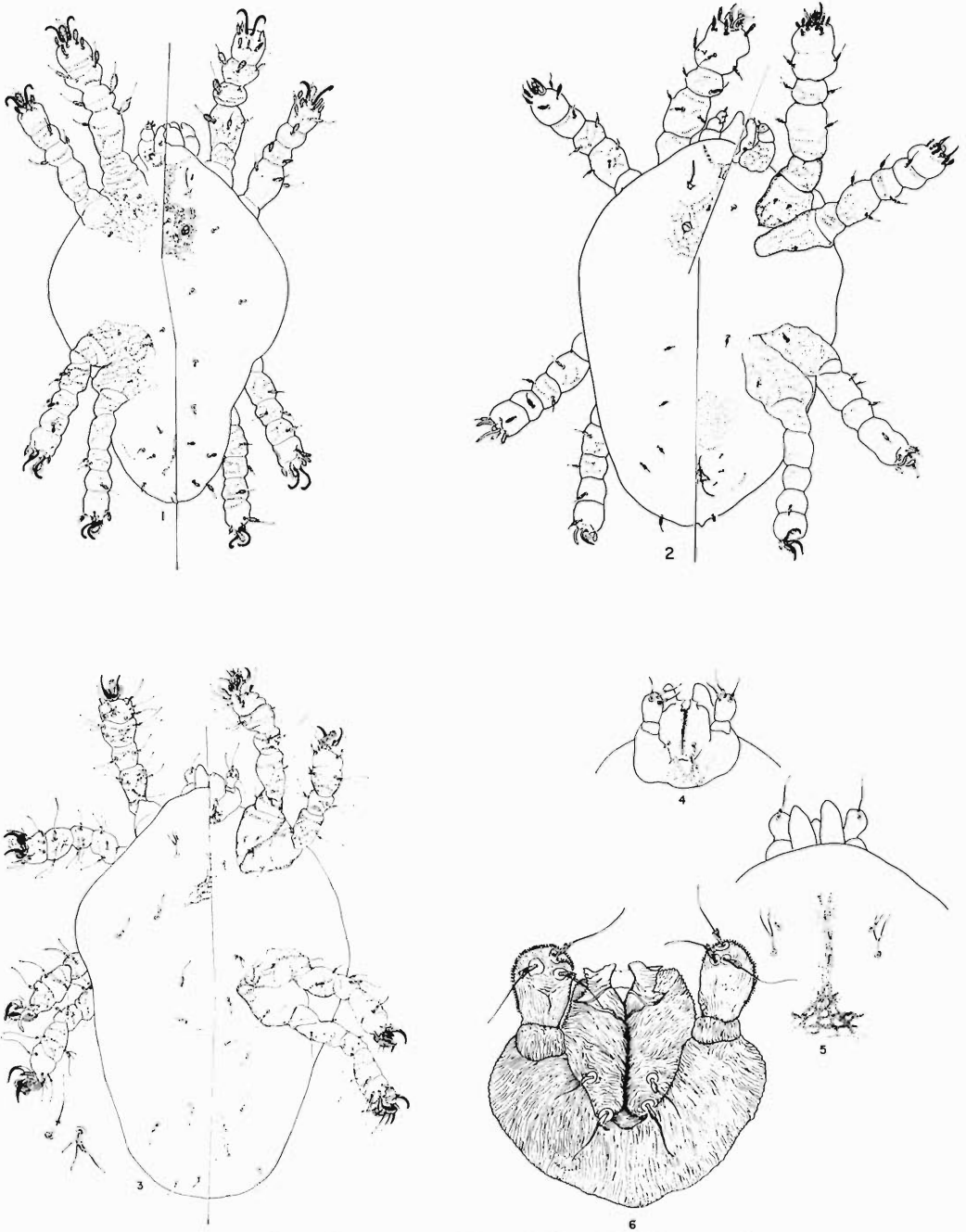
*Paraspeleognathopsis strandtmanni*  
(Fain, 1955)

A small series of adult speleognathids presently indistinguishable from *P. strandtmanni* were taken from *Sciurus granatensis* collected by C. E. Yunker at Summit, C. Z. on 18 January 1962. Proper classification of speleognathids from squirrels may also depend on larval morphology. Certainly the question of speciation in these forms must remain open until the larvae are described.

*Neospeleognathopsis phyllostomi* n. sp.

**FEMALE** (Figs. 3-6): Conforms to the characters of the genus *Neospeleognathopsis* (Fain, 1958) as recharacterized by Fain, 1963. With two palpal segments. Cuticle lightly sclerotized and characteristically striated, heaviest on the legs. Length, excluding gnathosoma

<sup>2</sup> Panamanian mammals were identified by Mr. C. M. Keenan and Dr. C. O. Handley of the U. S. National Museum.



Figures 1 and 2. *Paraspeleognathopsis cricetarum*. 1. Female: Dorsum right, venter left. 2. Male: Dorsum left, venter right.

Figures 3-6. *Neospeleognathopsis phyllostomi*. 3. Female: Dorsum left, venter right. 4. Gnathosoma. 5. Scutum and sensillae. 6. Gnathosoma showing cuticular detail.



0.50; width, 0.35. Gnathosoma, 0.080 wide, 0.079 long. Palpal tarsus with 4 setae, palpal tibia without setae.

*Dorsum*: Eyscs lacking, narrow scutum present; sensillae simple, forked 0.035 long; with one pair of presensillary setae. Dorsal formula 4-2-2-4-2. Dorsal cuticle with fine striations containing linear punctations.

*Venter*: With three pairs of sternal setae, five pairs of genital setae and two pairs of anal setae. Genital and anal areas of both female specimens are ruptured and hard to interpret. Leg setae arranged as follows: coxa, 2-1-2-1; trochanter, 1-1-0-0; femur, 6-4-3-3; genu, 4-4-3-3; tibia, 5-3-3-3; tarsus, 12, 8, 7, 7. Tarsus I bears two lanceolate, one sensory and three barbelled, flagellated setae dorsally. Claws and pulvilli unique; each claw bears basally a circular, springlike structure; pulvillus lyre-shaped and gracefully strung with fine, curving, springlike bands.

*MALE*: Shows considerable dimorphism while retaining the unique characters of the female. Has pseudofusion of tibiae and tarsi of all legs into larger blocklike segments. A trilobed testis is present.

*DIAGNOSIS*: *N. phyllostomi* may be distinguished from all other known members of the genus by the simple, bifurcate sensillae and unique characters of claws and pulvilli.

*HOLOTYPE*: Adult female USNM no. 3158 is deposited in the U. S. National Museum, Washington, D. C. The two remaining type

specimens will remain in the collection of the Rocky Mountain Laboratory.

*TYPE HOST*: *Phyllostomus hastatus* Allen, 1904, a leaf-nosed bat of the Microchiroptera.

*TYPE LOCALITY*: Colombia, near Nilo, south of Cundinamarca, 3 February 1964, by Dr. Hans Marinkelle.

*REMARKS*: The occurrence of this species with two palpal segments in the Microchiroptera, together with such linking setal features as the presence of but one pair of presensillary setae throws some doubt on the need for the subgenera *Neospeleognathopsis* and *Speleomyotis*. It seems likely that all speleognathids now known from bats will fit into a single generic concept very comfortably.

SUMMARY

Two new species of Speleognathinae are described: *Paraspeleognathopsis cricetidarium* n. sp. from *Oryzomys talmancae* Allen, a rice rat of Panama; and *Neospeleognathopsis phyllostomi* n. sp. from *Phyllostomus hastatus* Allen, a Colombian leaf-nosed bat. In addition, the speleognathid species *Paraspeleognathopsis derricki* (Wom., 1954) and *P. strandtmanni* (Fain, 1955) are reported for the first time from Panama.

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A. O. FOSTER  
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## Digenetic Trematodes of Amphibians and Reptiles from Fiji, New Hebrides and British Solomon Islands<sup>1</sup>

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ<sup>2</sup>

The trematodes of this report were collected by the junior author while serving with the U. S. Navy. Specimens of each species have been deposited in the U. S. National Museum Helminthological Collection as indicated. All measurements are in microns.

### FAMILY MESOCOELIIDAE

*Mesocoelium sociale* (Lühe, 1901) Odhner, 1911.

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

HOST: *Bufo marinus* L. (Bufonidae).

HABITAT: Small intestine.

LOCALITIES: Florida Island, British Solomon Islands; Suva, Viti Levu Island, Fiji Islands.

DATES: 8, 10 November 1944, February 1945 (Florida I.); December 1944, 13 December 1945 (Viti Levu I.).

SPECIMENS: USNM Helm. Coll. no. 61711.

MEASUREMENTS OF SEVEN SPECIMENS (six, Florida I.; one, Viti Levu I.) AND SOME PERTINENT DATA: Body 741–2,395 by 330–1,080; forebody 232–595, hindbody 392–1,585; preoral body 10–46 long; oral sucker 145–290 by 148–287, acetabulum 102–250 by 104–255, sucker length ratio 1:0.69–0.87; prepharynx (in three) 14–41 long; pharynx 58–126 by 63–145; one or both ceca terminating anterior to posterior end of vitelline fields, at same level or postvitellarian; right testis 85–230 by 75–230; left testis 85–215 by 90–206; cirrus sac 87–230 by 38–97, overlapping acetabulum

22–103; posterior chamber of seminal vesicle 31–123 by 21–90, anterior chamber 25–99 by 22–75; genital pore to oral sucker 26–126, to acetabulum 49–138, median to slightly submedian to left, at level of pharynx or esophagus; ovary 92–211 by 104–211, usually dextral but may be sinistral; 28 eggs 29–40 by 20–25.

DISCUSSION: Nine hosts from Florida I. were infected with 2, 3, 7, 8 (in two), 9, 10, 13, and 45 worms, respectively, and two hosts from Viti Levu I. with one and two, respectively. Species of *Mesocoelium* Odhner, 1911, previously reported from *Bufo marinus* are: *M. incognitum* Travassos, 1921, *M. waltoni* Pereira and Cuocolo, 1940, and *M. travassosi* Pereira and Cuocolo, 1940, from Brazil by Pereira and Cuocolo (1940); *M. travassosi* from Costa Rica by Caballero and Brenes (1958); *M. sociale* and *M. sp.* from Colombia by Ucrós (1959); *M. mesembrinum* S. J. Johnston, 1912, from Australia and *M. incognitum* from Hawaii by Yuen (1965).

Freitas (1963) declared *M. sociale* and at least 18 other species of the genus (includes those listed in the preceding paragraph) from a wide variety of amphibians and reptiles from North, Central, and South America, Africa, Asia, and Oceania synonymous with *M. monas* (Rudolphi, 1819) Freitas, 1958, described originally from Brazil. He indicated that the latter species originated on the American continent and expanded to other parts of the world through the transport of its intermediate and definitive hosts. However, at the time of Freitas' paper only the life cycle of *M. brevicacuum* Ochi in Goto and Ozaki, 1929, was known. He considered the latter distinct from *M. monas*.

According to Oliver (1949) the giant neotropical or marine toad, *Bufo marinus*, inhabited the warm subtropical and tropical areas of mainland America from Texas to Argentina. Early in the 19th century it was transported to the West Indies. In 1932 148 adult toads from Puerto Rico were introduced into Hawaii.

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Subsequently their descendants were introduced throughout the Pacific area as far as Formosa, the Philippines, New Guinea, and Australia. It is possible that some specimens of *M. monas* could have been transported to some of these localities with *B. marinus*, thus supporting Freitas' stated synonymy.

Ochi (1930) reported the land snail *Euhadra quaesita* (Deshayes) from Japan as the intermediate host for *M. brevicacum*, while Thomas (1965a, b) reported *Lamellaxis gracilis* (Hutton) from Ghana for *M. monodi* Dollfus, 1929. In personal communications Dr. Walter J. Byas, Museum Specialist, Division of Mollusks, U. S. National Museum, stated that *Euhadra* Pilsbry belongs to the family Bradybaenidae, subfamily Bradybaeninae, and *Lamellaxis* Strebler and Pfeffer to the family Subulinidae, subfamily Subulininae; both families are in the suborder Sigmurethra but are not too closely related either by shell characters or anatomy. *E. quaesita* is distributed in China, Japan and Formosa. *L. gracilis* is distributed in the tropics and subtropics of both hemispheres, including South, Central, and North America, West Indies, Africa, Madagascar, Middle East, Indo-Malayan Region, China, Japan, Philippines, Hawaii, and practically all of the island groups of Oceania; it has recently been reported from a greenhouse in Toronto, Canada. Dr. Byas noted that, ". . . some of the Indo-Pacific localities for *L. gracilis* represented in the U. S. National Museum collection are: China, Japan, Ryuku Islands, Singapore, Burma, Thailand, India, Philippines, Andaman Islands, New Caledonia, New Zealand, Loyalty Islands, Mariannas, New Hebrides, Solomon Islands, Society Islands and Cocos Keeling Atoll." He also noted that, ". . . *L. gracilis* was first described by Hutton in 1834 from Mirzapur in the Ganges Valley, India. The oldest recorded specimens in the U. S. National Museum collection do not show dates of collecting, but catalogue entries were made as follows: Singapore (1870?), New Zealand (1888), Poona, India (1870), Burma (1894). Specimens were reported under several different names over the same span of years from various localities in Florida, Central and South America and the West Indies which were proven later to be *L. gracilis*." Pilsbry and Bequaert (1927) noted that the latter species [syn. *Opeas gracile* (Hutton)], ". . . is

now so well established in the East Indies and in tropical America that it is impossible to decide whether its original home was the Old or the New World." The extensive distribution of this snail, known to serve as an intermediate host for a species of *Mesocoelium* considered synonymous with *M. monas*, tends to support Freitas' synonymy of species.

The information presented above in support of Freitas' synonymy of species with *M. monas* is essentially circumstantial. As we (1965a, b) indicated the answer to the question of species validity must await the elucidation of many more life cycles. Richard (1965) questioned Freitas' synonymy inasmuch as no experimental evidence was presented to show the degree of intraspecific variation. We cannot distinguish our present specimens from those previously identified by us (1964, 1965b) as *M. sociale* from a variety of amphibian and reptile hosts from Palawan I. (Philippines) and North Borneo (Malaysia). Balasingam (1964) reported *M. sociale* (as *M. meggitti*) from *Mabuia multifasciata* (Kuhl) (Scincidae) from Singapore, while Yuen (1965) recovered it from *Bufo melanostictus* Schneider (Bufonidae) from Malaya, Singapore, and Ceylon, and from *Bufo asper* Gravenhorst, *Rana erythraea* (Schlegel), and *R. cancrivora* Gravenhorst (Ranidae) from Malaya; none of these hosts are new but the localities are. Chatterji (1940) declared *M. meggitti* a synonym of *M. sociale*; we (1964) and Yuen (1965) concurred.

#### FAMILY ALLOCREADIIDAE

##### *Gekkonotrema postporum* n. gen., n. sp. (Figs. 1, 2)

HOSTS: Type, *Lepidodactylus lugubris* (Dum. and Bibr.); *Gymnodactylus pelagicus* (Girard) (Gekkonidae).

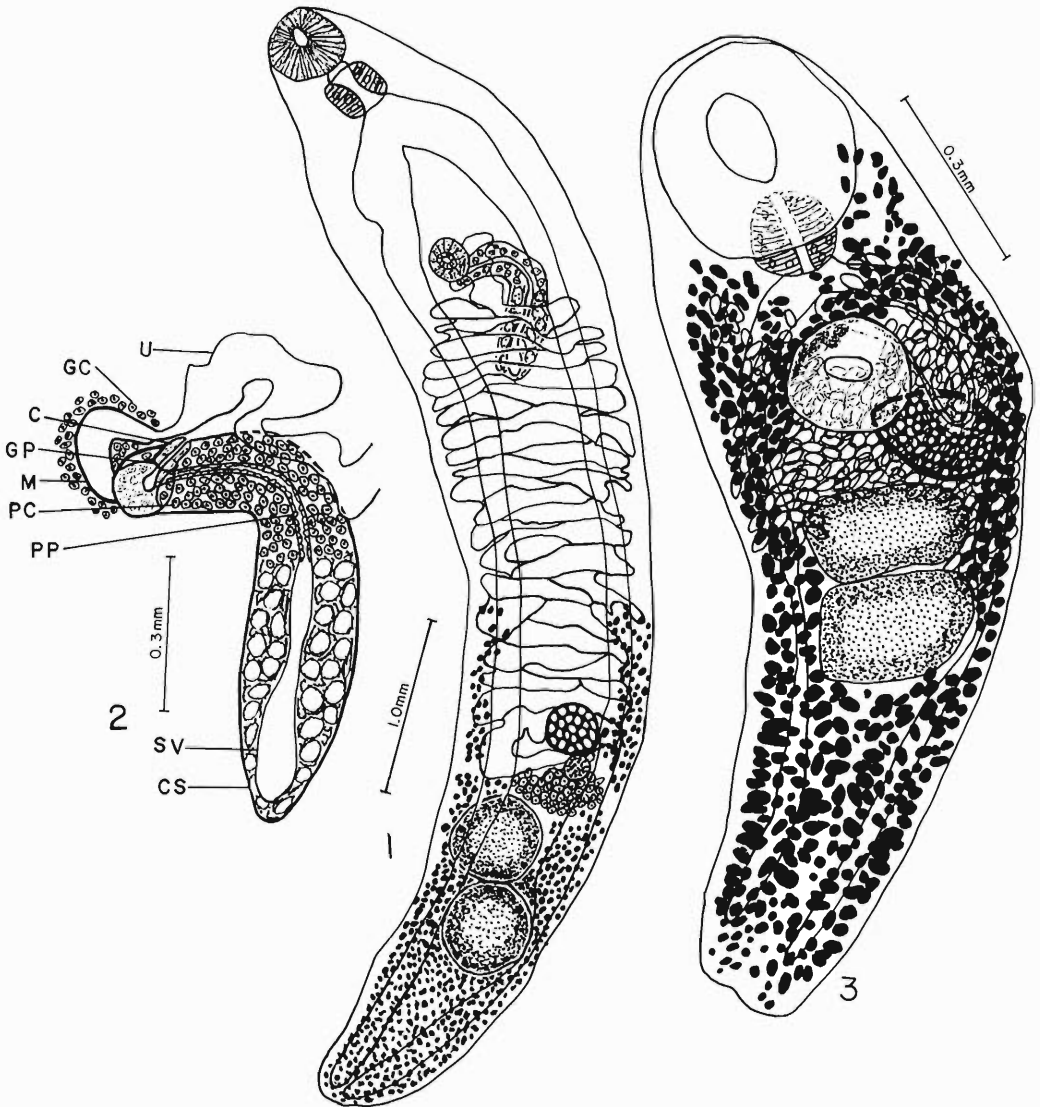
HABITATS: Small intestine, gall bladder.

LOCALITY: Espiritu Santo Island, New Hebrides Islands.

DATES: 5 March (*L. lugubris*), 8 April, August (*G. pelagicus*) 1944.

TYPES: USNM Helm. Coll. no. 61712 (holotype from *L. lugubris*); no. 61713 (paratype from *L. lugubris*); no. 61714 (paratype from *G. pelagicus*).

DIAGNOSIS (based on 13 specimens, two from *L. lugubris* and three from *G. pelagicus*



Figures 1 and 2. *Gekkonotrema postporum*. 1. Holotype, ventral view. 2. Terminal genitalia, paratype, ventral view. C, cirrus; CS, cirrus sac; GC, gland cells; GP, genital pore; M, metraterm; PC, prostate cells; PP, pars prostatica; SV, seminal vesicle; U, uterus.

Figure 3. *Dolichosaccus lygosomae*, holotype, ventral view.

measured): Body 4,234–6,779 by 744–1,212, elongate, sides nearly parallel, tapering slightly toward rounded extremities. Tegument spined almost to posterior extremity. Gland cells scattered throughout body parenchyma. Forebody 955–1,695, hindbody 3,083–5,208, pre-

oral body 19–27, posttesticular space 654–1,129, postcecal space 46–68. Oral sucker 248–335 by 237–350, longitudinally to transversely elongate, subterminal ventral. Acetabulum longitudinally to transversely elongate, 123–148 by 101–145 at ventral body surface,

182–259 by 143–257 for part lying below body surface, center at level of anterior one-fourth to three-tenths body length. Sucker length ratio 1:0.70–0.78. Prepharynx 71–201 long; pharynx 196–280 by 184–301, longitudinally to transversely elongate; esophagus 73–182 long; gland cells surrounding prepharynx, pharynx, and esophagus. Cecal bifurcation 405–795 preacetabular; ceca conspicuously cell lined, extending almost to posterior extremity. Excretory bladder elongate, wide, extending to testicular level; pore subterminal ventral.

Testes two, smooth, round to longitudinally or transversely elongate, slightly oblique, usually in contact but may be slightly separated, levels overlapping, anterior testis slightly sinistral and posterior slightly dextral but reverse condition may occur, may overlap ceca dorsally. Anterior testis 285–595 by 345–530, 1,741–3,467 postacetabular, 110–395 postovarian; posterior testis 335–610 by 402–495, 2,002–3,927 postacetabular. Cirrus sac 480–745 (longitudinal extent) by 160–230, slightly thick walled, muscular, almost entirely postacetabular, does not extend preacetabular, curving right or left in ascent, proximal part overlapping posterior, posterolateral or lateral portions of acetabulum to some extent, looping posteromedially to genital atrium lying immediately postacetabular, containing seminal vesicle, pars prostatica, prostate cells, and cirrus. Seminal vesicle straight, tubular to sacular, occasionally bipartite, slightly thick walled, muscular. Pars prostatica long, narrow, cell lined. Cirrus short, muscular, protrusible. Genital pore postacetabular, median.

Ovary 185–335 by 235–335, round to transversely elongate, smooth, usually median, pretesticular, 1,420–2,633 postacetabular. Oviduct from posterior margin of ovary. Seminal receptacle 87–165 by 94–220, round to transversely elongate, posterodorsal to ovary. Laurer's canal muscular, winding posterodorsal to dorsal body surface. Mehlis' gland dorsal, lying against tegument which is much thickened in this area, well developed, extensive, between ovary and anterior testis, may slightly overlap latter dorsally. Uterus starting ascent between anterior testis and ovary, ventral to latter, cirrus sac and ceca, coiling from one side of body to other to acetabular level, ascending to anterior margin

of latter or rarely more anteriorly before looping posteriorly. Metraterm relatively thin walled, surrounded by gland cells, descending to genital atrium ventral to descending portion of cirrus sac. Vitellaria extending from 862–2,070 postacetabular and 380–585 preovarian to posterior extremity, filling posttesticular space, usually confluent to some extent dorsal to testes, may be slightly interrupted on one side at some level between ovary and anterior testis, follicles round posttesticularly but some dendritic as for other regions. Eggs numerous, yellow, operculate, 40 measuring 42–52 by 19–25.

*Gekkonotrema* n. gen.

DIAGNOSIS: Allocreadiidae. Body elongate, tegument spined. Oral sucker larger than acetabulum, latter in anterior body third. Prepharynx, pharynx, and esophagus present. Cecal bifurcation preacetabular. Ceca extending almost to posterior extremity. Testes two, slightly oblique, in posterior body half. Cirrus sac mainly postacetabular, not extending preacetabular; seminal vesicle tubular to sacular, straight; pars prostatica long; cirrus short. Genital pore median, immediately postacetabular, opening from short genital atrium. Ovary pretesticular. Seminal receptacle and Laurer's canal posterodorsal to ovary. Uterus ascending from between ovary and anterior testis, coiling from one side of body to other, usually not extending preacetabular. Metraterm opening into genital atrium. Vitellaria extending from preovarian level to posterior extremity, filling posttesticular space. Eggs operculate. Excretory bladder elongate, extending to testicular level. Parasitic in small intestine and gall bladder of gekkonid lizards.

TYPE SPECIES: *G. postporum* n. sp.

DISCUSSION: Our collection consisted of three worms (small intestine) from one *L. lugubris*, and one (gall bladder) and nine (small intestine, gall bladder) from two *G. pelagicus*, respectively. Eight of the specimens were unsuitable for obtaining measurements.

Our species could not be placed in any existing genus, hence it was necessary to erect the new genus *Gekkonotrema* to receive it. In the keys given by Yamaguti (1958), Skrjabin and Koval (1966), and Koval (1966) our species keyed to the superfamily Allocreadio-

idea Nicoll, 1934, family Allocreadiidae Stossich, 1903, and subfamily Allocreadiinae Looss, 1902. In the key given by Koval (1966) to the genera of Allocreadiinae our form keyed to *Procaudotestis* Szidat, 1954; the latter was created for a species of trematode from a freshwater siluroid fish from Argentina. *Gekkonotrema* appears closest to *Procaudotestis* but differs significantly in having a reptilian host, the tegument spined rather than unarmed, and the genital pore post- rather than preacetabular.

#### FAMILY DICROCOELIIDAE

##### *Euparadistomum varani* Tubangui, 1931

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

HOST: *Varanus indicus* (Daudin) (Varanidae).

HABITAT: Gall bladder.

LOCALITY: Florida Island, British Solomon Islands.

DATE: February 1945.

SPECIMENS: USNM Helm. Coll. No. 61715.

MEASUREMENTS AND SOME PERTINENT DATA (based on six specimens, three measured): Body 1,902–2,085 by 1,420–1,600; forebody 665–780, hindbody 812–985, preoral body 16–27, postcecal space 330–420; oral sucker 330–395 by 355–405, acetabulum 320–435 by 330–415, sucker length ratio 1:0.86–1.32, center of acetabulum slightly preequatorial; pharynx 111–121 by 119–138; right testis 162–218 by 158–172, left testis 172–198 by 148–188; cirrus sac 148–208 by 74–75, overlapping acetabulum 12 in one, 34 and 82 preacetabular in two others; genital pore bifurcal or just postbifurcal, 106–125 postpharyngeal, 160–189 preacetabular; ovary 152–208 by 123–206, submedian to left in two, to right in one; seminal receptacle dorsal, 101–131 by 99–135; ootype complex median; Mehli's gland well developed; metraterm dextral to cirrus sac when ovary on left and sinistral when ovary on right; 18 eggs 37–56 by 21–27; Y-shaped excretory bladder bifurcating 125–162 postacetabular.

DISCUSSION: Two immature specimens were recovered in February 1945 from the gall bladder of *Lepidodactylus guppyi* Boulenger (Gekkonidae) from Florida I. They measure 750 by 413 and 1,140 by 635, respectively, and appear to be *Euparadistomum varani*. We (1964, 1965b) briefly reviewed the genus and

species, noting the presence of the latter in *Varanus salvator* (Laur.) from the Philippines, *V. rudicollis* (Gray) from North Borneo, and *Chamaeleo* spp. (Chamaeleonidae) from Madagascar. Balasingam (1964) reported this species from *V. nebulosus* (Gray) from Malaya.

*Paradistomum gregarium* Tubangui, 1929

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

HOSTS: *Gymnodactylus pelagicus* (Girard), *Gehyra oceanica* (Lesson), *Lepidodactylus guppyi* Boulenger (Gekkonidae); *Lygosoma noctua* (Lesson), *L. cyanurum* (Lesson), *L. anolis* (Boulenger), *L. solomonis* Boulenger (Scincidae).

HABITATS: Gall bladder, bile duct, small intestine.

LOCALITIES: Espiritu Santo Island, New Hebrides Islands (*G. pelagicus*, *G. oceanica*, *L. noctua*, *L. cyanurum*); Florida Island, British Solomon Islands (*L. guppyi*, *L. cyanurum*, *L. anolis*, *L. solomonis*).

DATES: 8, 12 April, 25 May, August 1944 (*G. pelagicus*); 25 August 1944 (*G. oceanica*); 11 November 1944, February 1945 (*L. guppyi*); 18 November 1944 (*L. noctua*); August 1944 (*L. cyanurum*, Espiritu Santo), November 1944 (Florida); 13 November 1944 (*L. anolis*); November 1944 (*L. solomonis*).

SPECIMENS: USNM Helm. Coll. no. 61716 (from *G. pelagicus*); no. 61717 (*G. oceanica*); no. 61718 (*L. guppyi*); no. 61719 (*L. noctua*); no. 61720 (*L. cyanurum*); no. 61721 (*L. solomonis*).

MEASUREMENTS AND SOME PERTINENT DATA (based on 13 specimens from four *G. pelagicus*, two measured; two from one *G. oceanica*, one measured; 10 from two *L. guppyi*, two measured; one from one *L. noctua*, measured; six from five *L. cyanurum*, three measured; two from one *L. anolis*, unmeasured; four from three *L. solomonis*, two measured): Body 1,271–1,960 by 495–1,290; forebody 340–500, hindbody 695–1,245; oral sucker 197–335 by 172–410, acetabulum 160–243 by 167–235, sucker length ratio 1:0.48–0.90; pharynx 59–111 by 59–145; right testis 72–218 by 71–206; left testis 65–189 by 78–182; cirrus sac 90–196 by 53–85, usually preacetabular but may overlap latter; genital pore at posterior margin of oral sucker or ventral to pharynx or

esophagus, up to 138 posterior to oral sucker, 15–178 preacetabular; ovary 85–215 by 92–265; seminal receptacle 97–150 by 63–180; 55 operculate eggs 29–44 by 18–25.

DISCUSSION: This species has been reported from *Hemidactylus frenatus* (Dum. and Bibr.) (Gekkonidae) from the Philippines, from the same host and *Gehyra mutilata* (Wiegmann) (Gekkonidae) from North Borneo, and from *H. gleodovi* Murray from Burma. Considerable variations similar to those previously noted by us (1964, 1965b) occur in the present specimens. Worms from the bile duct and especially the intestine were narrower than those from the gall bladder. This condition has been noted by Arora and Agarwal (1960) and Arora, Agarwal and Agarwal (1962) in their studies of various populations of *Paradistomum orientalis* (Narain and Das, 1929) Bhalerao, 1936. The present specimens were compared with our worms from the Philippines and North Borneo, with two specimens of *P. samoensis* Byrd, 1949, from *Emoia nigra* (Hombron and Guichenot) and *E. samoensis* (Dum.) (Scincidae) from the Samoa Islands kindly loaned to us by Dr. E. E. Byrd, University of Georgia, and with four of G. A. MacCallum's specimens of *P. trachysauri* (MacCallum, 1921) Dollfus, 1922, from *Cyclodus rugosus* (Gray) (Scincidae) from Australia (USNM Helm. Coll. No. 36473). There are considerable similarities between all but the latter species. Some of our more mature specimens showed extensive uterine coiling in the region of the acetabulum similar to that described for *P. samoensis* by Byrd (1949). In one of the latter's specimens short ascending uterine loops occurred to the right of the ovary rather than to the left; also, many of the uterine convolutions did not form transverse loops across the body but conformed to the more typical pattern for the genus; the extensive development of the uterus tended to obscure the path taken. It may be that *P. samoensis* is a synonym of *P. gregarium*, but final decision must await life cycle studies.

*Paradistomum trachysauri* (MacCallum, 1921)  
Dollfus, 1922

SYNONYMS: *Paragonimus trachysauri* MacCallum, 1921; *Cephalogonimus trachysauri* MacCallum, 1921; *Paradistomum maccallumi* T. H. Johnston, 1932.

HOST: *Cyclodus rugosus* (Gray) [syn. *Trachysaurus r.* (Gray)] (Scincidae).

HABITAT: Gall bladder.

LOCALITY: Australia.

DATE: 31 August 1918.

SPECIMENS: USNM Helm. Coll. no. 36473 (four specimens on one slide, G. A. MacCallum collection).

MEASUREMENTS AND SOME PERTINENT DATA (based on four specimens): Body 4,320–4,645 by 1,640–2,175; forebody 900–1,340, hindbody 2,805–3,045; oral sucker 410–575 by 550–635, acetabulum 455–535 by 445–610, sucker length ratio 1:0.79–1.31; pharynx 109–155 by 126–150; testes smooth to slightly lobed; right testis 205–410 by 250–465; left testis 200–505 by 230–380; cirrus sac 242–317 by 111–138, commencing 150–300 preacetabular, walls only slightly thickened, containing much convoluted, slightly thick-walled seminal vesicle in proximal one-third to one-half, a short, thick-walled, cell-lined pars prostatica, a slightly longer, thick-walled, muscular cirrus, and prostate cells anterior to seminal vesicle; genital pore to oral sucker (in two) 113–175, to acetabulum 550–555; ovary 250–315 by 255–356, lobed, ventral to seminal receptacle; latter 400–565 by 290–455, much larger than ovary; uterine coils extending postcecal; 20 operculate eggs 33–41 by 22–26.

DISCUSSION: We are briefly redescribing this species in order to supplement existing descriptions. This form was originally described as two different species by MacCallum (1921). The slide we studied is labeled *Distomum trachysauri* and appears to represent specimens described as *Paragonimus trachysauri*.

#### FAMILY PLAGIORCHIIDAE

*Dolichosaccus lygosomae* n. sp.

(Fig. 3)

HOST: *Lygosoma noctua* (Lesson) (Scincidae).

HABITAT: Small intestine.

LOCALITY: Espiritu Santo Island, New Hebrides Islands.

DATE: 19 August 1944.

HOLOTYPE: USNM Helm. Coll. no. 61722.

DIAGNOSIS (based on single specimen): Body 1,670 by 550, elongate, widest at acetabular level, tegument entirely spined, spines

more numerous and coarser anteriorly. Forebody 510, hindbody 985; preoral body 6, posttesticular space 580, postcecal space 123 (left), 143 (right). Oral sucker 336 by 310, subterminal ventral, opening longitudinally elongate. Acetabulum 175 by 188, at level of anterior body third. Sucker length ratio 1:0.52. Prepharynx short; pharynx 133 by 128, overlapping oral sucker dorsally; esophagus short, bifurcating preacetabular; ceca narrow, thick walled, extending close to posterior extremity.

Testes two, tandem, contiguous, transversely elongate, intercecal but may overlap ceca dorsally, surfaces somewhat wavy; anterior testis 140 by 265, 80 postacetabular; posterior testis 218 by 280, 210 postacetabular. Cirrus sac 280 (longitudinal extent) by 121, thick walled, muscular, curved, commencing over anteroventral part of ovary just anterior to level of posterior margin of acetabulum, partly overlapping sinistral and anterior half of latter, containing seminal vesicle, pars prostatica, prostate cells and cirrus. Seminal vesicle large, thin walled, bipartite (posterior chamber sacular and much larger than anterior), filling over half of cirrus sac; pars prostatica relatively large, cell lined; cirrus short, muscular; prostate cells surrounding pars prostatica and cirrus. Genital pore median, just preacetabular.

Ovary 140 by 215, relatively smooth, sinistral, lying 5 pretesticular, slightly overlapping left cecum and acetabulum dorsally. Ootype complex posteromedial to ovary. Seminal receptacle 68 by 130, overlapping anterior testis dorsally. Laurer's canal muscular. Uterus ventral to ovary, ootype complex, ceca, lateral margins of anterior testis, anterosinistral margin of posterior testis, and cirrus sac, extending to body margins at ovarian level, anteriormost extent to pharyngeal level. Vitelline follicles extensive, from posterolateral level of oral sucker to posterior extremity, confluent dorsally between cecal bifurcation and ovary and filling posttesticular space. Eggs yellow, operculate, 10 measuring 38–43 by 22–26.

Excretory bladder Y-shaped; stem elongate, tubular, bifurcating at anterior part of posterior testis; arms extending to level of posterior part of ovary, left arm overlapping latter dorsally; stem and arms dorsal to testes; pore terminal.

**DISCUSSION:** This is the first record of *Dolichosaccus* S. J. Johnston, 1912, from a reptile; all other species are from amphibians

from Australia, Brazil, Europe, and the Congo (Leopoldville). Manter and Pritchard (1964) noted that only four species of the eight recognized by Yamaguti (1958) have vitellaria extending to the pharynx–oral sucker level. Our species differs significantly from the four in the host being a lizard and in having the uterine coils extending to the pharyngeal level. It differs further from them, excepting *D. rastellus* (Olsson, 1875) Travassos, 1930 (from Europe and Africa), in having the vitellaria confluent anteriorly. Additionally, *D. amplivagus* Travassos, 1924 (Brazil) has the oral sucker smaller than the acetabulum; *D. ischyryus* Johnston, 1912 (Australia) has the ovary halfway between the acetabulum and anterior testis; *D. rastellus* has the ovary dextral; and *D. symmetricus* (Johnston, 1912) Yamaguti, 1958 (Australia) has the vitellaria in separate anterior and posterior masses.

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## Axenic Cultivation of *Aphelenchoides sacchari* Hooper<sup>1</sup>

RONALD F. MYERS

Axenic cultivation of plant-parasitic nematodes on oligidic, and progressively meridic and holidic media, should provide a means for research into such basic areas as (1) the dietary requirements of nematodes that are provided by the host plant, (2) the nutritional basis for resistance of plants to the parasitism by nematodes, and (3) the existence of innate nutritional requirements and biochemical differences within biological races of nematodes.<sup>2</sup>

Significant advances in the science of phytonematology will have been achieved when this information is available.

Myers (1967) recently reported the first successful cultivation of stylet-bearing nematodes on solid liver media. This paper reports further investigations on oligidic culture media, especially liver fractions, that might serve to support reproduction, growth, and development of *Aphelenchoides sacchari* Hooper.

### MATERIALS AND METHODS

*A. sacchari* was reared in liquid media consisting of extracts of various livers with dextrose added. Liver was prepared by chopping it

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<sup>2</sup> Terms relating to cultivation and media as used in this paper were defined by Dougherty (1960).

into cubes of about 3 cm subsequent to passage through a meat grinder while adding M/15 potassium phosphate buffer, pH 7.0 (1:1, w/v) at 6 C (Nicholas *et al.*, 1959). This mixture was then poured into cheesecloth, squeezed, and the resulting extract frozen at -26 C until used. This extract, after thawing, was centrifuged at 10,000 *g* for 1 hour at 4 C and, with the liquid fraction diluted to various strengths, used as the main component of media. Horse liver (4,500 g), previously stored at 10 C for 7 days, fresh calf liver (2,530 g), and lamb liver (571 g), both stored at 6 C for 48 hours, were extracted and the extracts designated HLE, CLE, and LLE, respectively. Liver extracts were heat treated at 53 C for 6 min to precipitate excess protein (Sayre, Hansen, and Yarwood, 1963). This treatment permitted larger quantities of liver extract to pass through the Seitz filter pads during sterilization of extracts and media. Media prepared with heat treated fractions were designated with an additional "H"; i.e., HHLE (heated horse liver extract). Commercial liver extract (Bacto Liver: Difco Laboratories, Detroit, Michigan) was prepared as directed by the manufacturer (DLE). HLE was autoclaved at 15 lbs pressure, 121 C for 15 min, the coagulated materials compacted by centrifugation, and the clear yellow serum used in certain experiments (AHLE).

Sterility of liver extracts, culture media, and inoculated cultures were assayed by inoculating them into thioglycolate and nutrient broths. However, microbial contamination was readily detected in media containing liver extracts after 48 hours by either visual or olfactory senses.

The strength of extracts used in several experiments ranged from 0-100%. Dextrose was added to 50% HLE at concentration from 0-2.0 g/100 ml. The final pH of extracts was as follows: HLE, pH 6.4; HHLE, pH 6.4; CLE, pH 6.8; HCLE, pH 6.8; LLE, pH 6.6; HLLE, pH 6.6; AHLE, pH 6.6; and DLE, pH 6.5. Media generally had the same pH as the extracts, but in one experiment with HLE, the pH was adjusted over a range of pH 6.0-8.0 with 1 *N* HCl or 1 *N* KOH in order to determine whether pH of the media affected reproduction.

*A. sacchari* were introduced into axenic culture media from monoxenic alfalfa callus and fungal cultures (*Pyrenochaeta terrestris*

de Not), or from other axenic cultures. Nematodes were extracted into sterile water from callus cultures or from solid axenic culture media in a test tube extraction apparatus. This apparatus, which was essentially another modification of the Baermann funnel, consisted of a wire screen covered with tissue paper with both inserted into a 20 by 100 mm test tube. The test tube contained water up to the level of the tissue paper and sterility was maintained by use of a plastic closure. Nematodes migrated from culture media which was placed onto the tissue paper into the sterile water and after 12-24 hours were pipetted into liver culture medium. Another method of obtaining sterile nematodes was to inoculate media from infested fungal or callus cultures into the center of 1% water agar plates. After 24 hours, when nematodes were evenly dispersed throughout the water agar, they were transferred into liver medium along with a disc or chip of water agar. In addition to the other methods, serial subcultivations were made by pipetting old culture medium containing nematodes into freshly prepared medium.

Preliminary results indicated that any type of tube or flask could be used in the cultivation of *A. sacchari*, just so long as sterility could be maintained. Most of these experiments utilized 20 by 100 mm test tubes covered by loose plastic closures. Tubes contained 3 ml of media and were slanted to maintain fluid depths of less than 0.5 cm. This assured sufficient oxygen content of static culture media by normal gaseous exchange with the atmosphere. All cultures were incubated at 24 C.

Total populations and linear dimensions of nematodes reared in different media were also determined. Nonheated extracts usually contained precipitate which made counting difficult at the termination of an experiment, and so the nematodes were extracted from media contained in the rearing tubes by covering the tubes with tissue paper and inverting them into 40 ml beakers. Each beaker contained a glass rod to keep the tube off of the bottom and water to a depth of 2 cm. The nematodes that migrated into the water after 24 hours were then counted. In certain experiments, media were poured into dishes and the relative numbers of eggs and nematodes were estimated. Prior to counting on a Scott eelworm counting

TABLE 1. The relationship between relative egg production of *Aphelenchoides sacchari* incubated at 24 C for 10 days and total population after 30 days. The liquid media consisted of a dilution series of horse liver extract (HLE), autoclaved horse liver extract (AHLE), heated horse liver extract (HHLE), heated calf liver extract (HCLE), heated lamb liver extract (HLLE), and commercial liver extract (DLE)<sup>1</sup> and also 0.5 g dextrose/100 ml liver extract.

Treatment	Dilution series as percentage of full strength liver extract						
	0	30	40	50	60	70	100
HLE							
egg	— <sup>2</sup>	—	+	++	++	++	+
population	—	—	++	+++	+++	+++	+++
AHLE							
egg	—	—	—	—	—	—	—
population	—	—	—	—	—	—	—
HHLE							
egg	—	+	++	++	++	+	+
population	—	++	+++	+++	+++	+++	+++
HCLE							
egg	—	—	—	—	+	+	—
population	—	+	+++	+++	++	++	++
HLLE							
egg	—	+	++	+++	++	++	++
population	—	+++	+++	+++	+++	+++	+++
DLE							
egg	—	—	—	—	—	—	—
population	—	—	—	—	—	—	—

<sup>1</sup> DLE—Bacto Liver, Difco Laboratories, Detroit, Michigan.

<sup>2</sup> Key to symbols: —, no egg production or no population increase; +, few or slight population increase; ++, many eggs or population increased over 10 times; +++, very many eggs or population increased over 100 times.

slide, the nematode suspension was diluted so that population ranged from 50–150 nematodes/ml. Linear measurements were made using a Zeiss Drawing Apparatus on nematodes that were first heat relaxed at 45 C, and then fixed in formalin–acetic acid–95% ethanol–water (6:1:20:40, v/v/v/v).

RESULTS

A. *sacchari* produced eggs when inoculated into 50% HLE containing 2% dextrose, the eggs hatched, and larval stages developed into adult females which in turn laid eggs. No males were ever observed during these cultivation experiments. Serial subcultivation was performed twice over a period of 4 months and these thriving cultures when terminated appeared to have the potential of indefinitely remaining viable. When 25 nematodes/ml were inoculated into this medium approximately 12,500/ml were harvested after 30 days.

The relationship between relative egg production on several liver media at 10 days and total population at 30 days is recorded in Table 1. Reproduction occurred at dilutions as low as 30% HHLE, HCLE, and HLLE; each of which contained 0.5% dextrose. HHLE and HLLE media were about equally suitable for

rearing nematodes. Production of eggs did not occur in AHLE and DLE media even though most of the nematodes survived for 30 days. Since larval stages predominated in AHLE and DLE media, adult development was apparently retarded.

Table 2 summarizes relative lengths, widths, vulval distances from anterior end as percentage of total lengths (per cent V), and the total number of animals resulting when 100 nematodes were inoculated into culture media. Lengths of females differed significantly at the 1% level when reared in the three types of culture media; females averaged 0.384 mm in HLE media, 0.447 mm when reared on the fungus, *P. terrestris*, and 0.514 mm when extracted from alfalfa callus cultures. In addition to differences in lengths, the total number of animals were also significantly different at the 1% level between those reared in liver extract media and in monoxenic cultures. No differences were noted in body widths or per cent V when comparing the culture media.

No decrease in egg production was observed when nematodes were reared in 50% HLE containing 2% dextrose medium adjusted to pH 6.0, pH 6.5, pH 7.0, and pH 7.5. Very few eggs were produced, however, in medium adjusted to pH 8.0. Large quantities of pre-

TABLE 2. Some mean dimensions and total numbers within populations of *Aphelenchoides sacchari* 30 days after inoculating 100 nematodes into culture media (24 C).

	Culture methods			
	50% HHLE + 0.5% dextrose	50% HLE + 0.5% dextrose	Alfalfa callus	Fungus <i>Pyrenochaeta terrestris</i>
Length female (mm) <sup>1</sup>	—	0.384 ± 0.007 <sup>2</sup>	0.514 ± 0.012	0.447 ± 0.012
Width female (mm)	—	0.018	0.017	0.018
Per cent V	—	71	70	72
Total population <sup>3</sup>	7,180 ± 900	8,450 ± 2,230	552,000 ± 105,800	617,000 ± 50,700

<sup>1</sup> All lengths significantly different at 1% level.

<sup>2</sup> ± standard error of mean (6 samples).

<sup>3</sup> Populations occurring in liver extracts significantly different at 1% level from those of alfalfa callus and fungal cultures.

precipitate, possibly protein, occurred progressively as the pH was adjusted to 6.0, but as the pH was raised by the addition of KOH, some of the precipitate returned to solution.

Reproduction occurred in all cultures containing 50% HLE whether or not dextrose was added. HLE without dextrose contained fewer nematodes after 30 days than when 0.25% dextrose was present, and between 0.5% and 2% dextrose, the total population was approximately equal.

#### DISCUSSION

Cultures of *A. sacchari* may be maintained in many types of containers. Microorganisms must be excluded since contamination usually proved fatal to the nematodes; probably through the utilization of the available oxygen supply. When the depth of liquid exceeded 5 mm, reproduction decreased and in some instances large numbers of dead animals were noted. It was not determined how fast "required nutrients" were metabolized, nor if accumulating waste products were detrimental to the nematodes.

Another detrimental factor possibly unique to liquid culture media was that the nematodes often retained portions or all of their moulted integuments. This may have increased rate of mortality since some nematodes apparently died when unable to escape from the moulted integuments. Berntzen (1965) previously mentioned this problem with respect to cultures of trichina larvae.

*A. sacchari* was subcultured twice in liquid media containing 50% HLE and 2% dextrose and as previously reported by Myers (1967), this species was subcultured four times during an 8-month period on a similar medium solidified with agar. In both types of media, the

nematodes appeared to be thriving upon termination of the experiments. It cannot be determined, however, if the successful media listed in Table 1 provided all necessary nutritional requirements until a sufficient number of generations have passed, thereby eliminating possible carry-over of some required compounds in the bodies of the inoculated populations. Since AHLE media does not support reproduction and growth, it is apparent that autoclaving destroyed certain required nutrients. One might speculate that the liver protein factor(s) required by *Caenorhabditis briggsae* which were heat labile (Nicholas *et al.*, 1959), may also be required in the diets of stylet-bearing nematodes. There may also be some basic relationship in the nutritional necessities of animal-parasitic, free-living, and plant-parasitic nematodes because all three groups showed definite growth responses in extracts of livers (Dougherty, 1960).

Egg production was extremely sensitive to modifications in the concentration of media and this may prove a useful parameter during rapid assay for the suitability of media. Reduction in body length and total numbers within a population should also be useful in formulating media (Table 2). There was no indication, however, whether these reductions were caused by the physical or the dietary properties of the culture fluid.

The ability to culture *A. sacchari* in oligidic liver media suggests that stylet-bearing nematodes may not require chemical or physical (membrane) stimuli from their hosts to trigger the feeding response. No physical or nutritional properties of successful media prevented cultivation and perhaps most stylet-bearing nematodes will eventually yield to axenic, holidic cultivation.

## SUMMARY

The stylet-bearing nematode, *A. sacchari*, was serially subcultured in liquid medium consisting of ground horse liver extracted with M/15 potassium phosphate buffer, pH 7.0, diluted to 50% strength and containing 2% dextrose. Eggs were produced, growth and development occurred, and populations increased from 25 nematodes/ml to 12,500 nematodes/ml in 30 days at 24 C. Further experiments indicated that extracts from lamb and calf livers could substitute for the extract from horse liver. Body length of females and total numbers within populations of nematodes reared in horse liver medium were reduced and differences were significant at the 1% level from those cultivated on either fungal or alfalfa callus cultures. *A. sacchari* was the first stylet-bearing nematode to be axenically cultured in liquid, oligidic media.

## ACKNOWLEDGMENTS

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Beltsville, Maryland, for the identification of *A. sacchari*.

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### *Protospirura peromysci* n. sp. (Nematoda: Spiruridea) and Other Helminths from *Peromyscus* spp. in Nevada<sup>1</sup>

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Studies of the parasites of small rodents in Nevada have not been previously undertaken and an evaluation of their existence, prevalence, and zoonotic importance cannot be properly assessed. The present paper is concerned with helminth parasites of 145 rodents, comprising four species of *Peromyscus*—*P. maniculatus* (Leconte) (87), *P. eremicus* (Baird) (42), *P. truei* (Schufeldt) (7), and *P. crinitus* (Merriam) (9)—collected from Washoe (77), Clark (57), Ormsby (10), and Lincoln (1) counties. Their distribution within the state is given by Hall (1946) and Bradley and

Deacon (1965). Although some parasites of *Peromyscus* have been previously reported in parasitological literature, along with those from certain other small rodents, few such studies have dealt with this group per se. Such investigations as have been accomplished include those by Schad (1956) in Quebec, Canada and Grundmann and Frandsen (1960) in Utah, USA.

Of the total number of *Peromyscus* examined, 26 (17.9%) were infected with helminths. Of the parasitized hosts, 1 harbored three species, 3 had two, and 22 had only one. Infection data are presented in Table 1. A brief discussion is given of each of the helminths.

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TABLE I. Collection data on helminths from 145 *Peromyscus* spp. from Nevada.

Host	Number examined	Parasites collected	Number infected	Per cent infected	Locality
<i>P. maniculatus</i>	87	<i>P. peromysci</i>	10	11.47	Washoe, Clark
		<i>R. onychomys</i>	2	2.29	Washoe
		<i>R. coloradensis</i>	1	1.15	Clark
		<i>N. dubia</i>	2	2.29	Washoe
		<i>Hymenolepis</i> sp.	1	1.15	Washoe
		<i>Moniliformis</i> sp.	1	1.15	Washoe
<i>P. eremicus</i>	42	<i>R. coloradensis</i>	1	2.38	Clark
		<i>S. peromysci</i>	5	11.90	Clark
		<i>Hymenolepis</i> sp.	1	2.38	Washoe
		<i>R. onychomys</i>	1	2.38	Washoe
		<i>N. dubia</i>	1	2.38	Clark
<i>P. crinitus</i>	9	<i>S. peromysci</i>	2	22.22	Clark
<i>P. truei</i>	7	<i>S. peromysci</i>	2	28.60	Clark
		<i>P. peromysci</i>	1	14.30	Washoe
		<i>G. peromysci</i>	2	28.60	Clark

## NEMATODA

## SPIRURIDEA DIESING, 1861

GENUS—*Protospirura* SEURAT, 1914

Spirurids were collected from the stomach and occasionally the small intestines of ten *P. maniculatus* and one *P. truei*. The range of infections was from 1 to 40 worms per host. The parasitized mice were from two counties, Clark (7) and Washoe (4). Following Yamaguti (1961), the worms were assigned to the genus *Protospirura*, although it is recognized that controversy exists concerning its congeneric relationship with *Mastophorus* Diesing, 1853 (Chitwood, 1938; Read and Millemann, 1953; Khera, 1956).

Because of certain discrepancies in morphological characters, the Nevada spirurid could not be justifiably assigned to any of the known species of the genus *Protospirura*. It is, therefore, herein described as new. All measurements are given in millimeters unless otherwise indicated.

*Protospirura peromysci* n. sp.

(Figs. 1-5)

MALE: Total length, 11.60-18.00; width, 0.39-0.50. Lips bilobed, each lobe subdivided into subventral and subdorsal pseudolabia of equal size and a larger medial pseudolabium. Each of smaller pseudolabium has two triangular denticles of about equal size, medial has four flat denticles of approximately same size. Small cervical papillae found at base of subventral and subdorsal pseudolabia. Laterally compressed buccal cavity measures 0.05-

0.07 in length. Nerve ring, 0.29-0.34 from anterior end. Excretory pore 0.39-0.41 from anterior end. Anterior glandular portion of esophagus 2.18-2.35 in length. Coiled tail with bursa, maximum width 0.63-0.72. Spicules unequal, left spicule 0.33-0.38 and right spicule 0.82-1.20 in length. Distal portion of right spicule with expanded sheath extending to blunt tip. Left spicule mostly ensheathed, containing inconspicuous barb near distal end. Six pairs of slightly pedunculated papillae of equal size present, four pairs preanal and two pairs almost immediately postanal. An unpaired papilla present on rim of cloacal aperture. Tip of tail with five pairs of small sessile papillae, four pairs linearly arranged; a single papilla situated adjacent to each member of third pair. Anus 0.28-0.32 from tip of bluntly rounded tail.

FEMALE: Total length, 24.40-30.00; maximum width 0.64-1.00. Buccal cavity 0.65-0.80. Glandular portion of esophagus 0.39-0.68 and muscular part 3.65-5.20. Nerve ring 0.34-0.43 from anterior end. Vulva near middle of body to slightly posterior to it, 13.00-17.60 from anterior end of body. Eggs 0.042-0.048 in length to 0.035-0.040 in width.

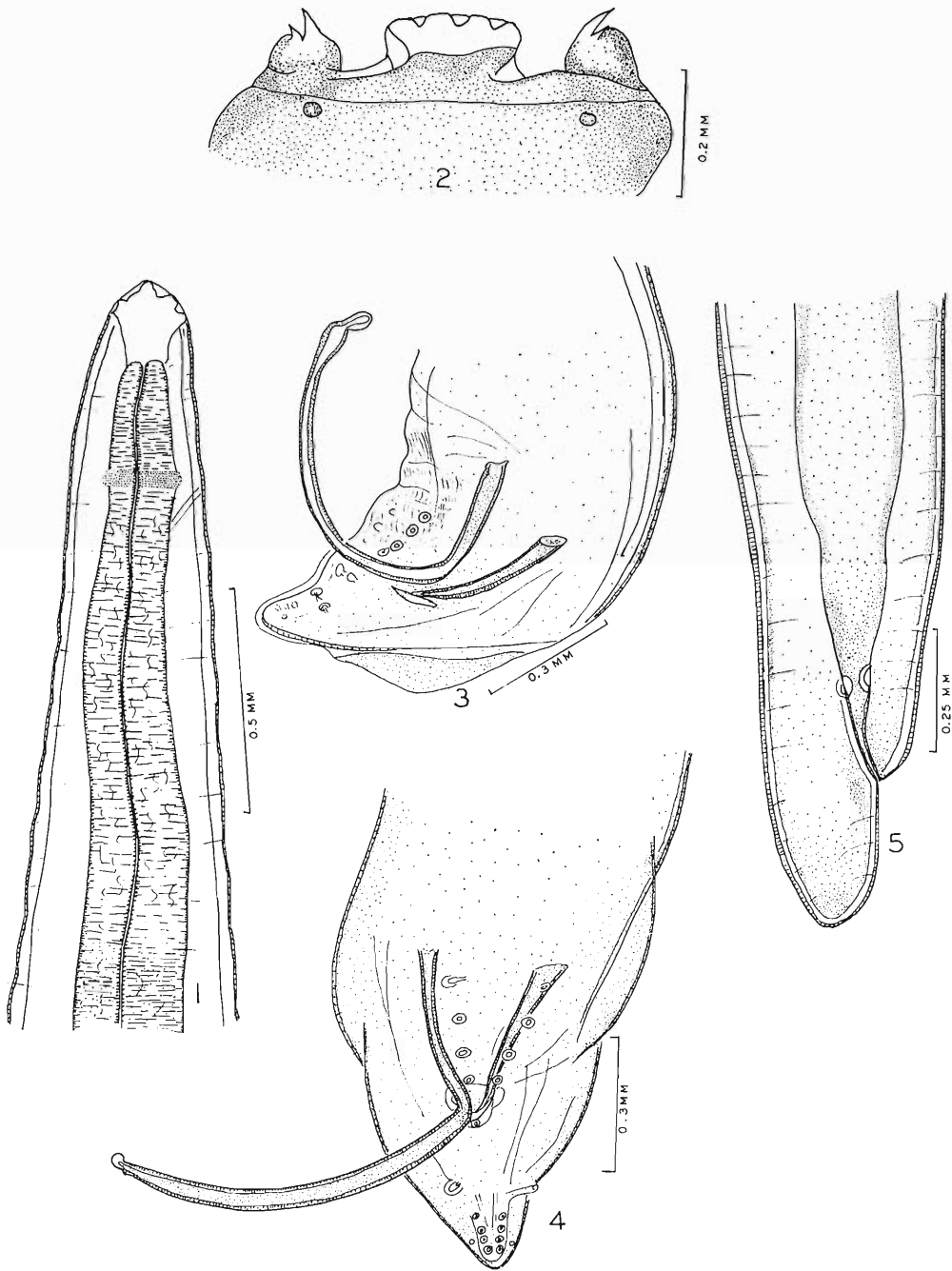
HOSTS: *Peromyscus maniculatus* and *P. truei*.

HABITAT: Small intestines and stomach.

LOCALITIES: Clark and Washoe counties, Nevada.

TYPE SPECIMENS: USNM Helm. Coll. nos. 61723 (holotype male and allotype female) and 61724 (paratypes).

Yamaguti listed 18 species of the genus *Protospirura*, *P. guianensis* Ortlepp, 1924 hav-



Figures 1-5. *Protospirura peromysci* n. sp. 1. Anterior end of worm. 2. Labial region (lateral view). 3. Posterior end of male (lateral view). 4. Posterior end of male (ventral view). 5. Posterior end of female (lateral view).



ing been rightfully transferred by Chitwood (1938) to the genus *Spirura* Blanchard, 1849. Taxonomic keys to most of these species are presented by Hall (1916), Ortlepp (1924), Cram (1926), Brumpt (1931), and Soltys (1949). Chitwood listed measurements of certain morphological structures for nine of the species listed by Yamaguti. As far as the writers are aware, only two species of the genus have been reported from *Peromyscus*, these are *Protospirura numidica* Seurat, 1914, reported from *P. crinitus* and *P. truei* by Grundmann (1957) and Grundmann and Frandsen (1960), who also found it in *P. maniculatus*, from "*Peromyscus g. gossypinus* and *P. g. megacephalus*" by Chitwood and *Protospirura labiodentata* (v. Linstow, 1899) from *P. leucopus* (Rafinesque) by Chitwood.

Of the members of the genus, *Protospirura peromysci* more closely resembles *P. numidica*, *P. muris* (Gmelin, 1790) and *P. muricola* Geddoelst, 1916.

The new species differs from *P. numidica* in the number, shape, or arrangement of denticles of the lips. According to Seurat (1914) *P. numidica* has three teeth on each lobe, whereas, *P. peromysci* has two on each subdorsal and subventral lobe and four on the middle one. Chitwood, however, stated that there were four distinct or two indistinctly divided teeth on each lobe. The number of papillae at the tip of the tail is five pairs in the new species rather than three as given. The new species also differs in the size of the spicules. Seurat gave the spicule sizes as 830  $\mu$  and 420  $\mu$ , however, Chitwood gave a size range for the right spicule as 830–1,160  $\mu$  and for the left, 340–420  $\mu$ . The egg size of the new species also is somewhat smaller than that reported for *P. numidica*.

*P. peromysci* differs from *P. muris* in its labial dentition, in the number and arrangement of sessile papillae at the tip of the tail and in the size of spicules. Egg size of *P. peromysci* also differs from those of *P. muris* (see Hall, 1916).

*Protospirura peromysci* may be readily distinguished from *P. muricola* by the presence of teeth on the mediolateral lobes of the lips and by its longer spicules and smaller egg size. In addition, the new species has seven pairs of postanal papillae, rather than six pairs.

The new species also shows some resemblance to *P. magna* (syn. *M. magnus* (Khera, 1956)), however, it differs in labial dentition, the position of the vulva, and in the sizes of eggs and spicules. *P. peromysci* is also a smaller worm than *P. magna*. The presence or absence of papillae on the tip of the tail of *P. magna* was not discussed by Khera.

#### GENUS—*Rictularia* FRÖELICH, 1802

Rictularids are geographically widely distributed, being reported in hosts throughout much of the world, particularly in carnivores, insectivores, rodents, bats, and possibly lizards. A review of the genus was given by Dollfus and Desportes (1945) and a key to species of the group was presented by Cuckler (1939). Some observations of *Rictularia* of North America and a discussion of members occurring in rodents and bats were made by Tiner (1948a). According to McPherson and Tiner (1952) the *Rictularia* in North American rodents are divisible into two groups, those with a transverse and dorsal oral opening, in which *R. citelli* McCleod, 1933 is the only representative, and those with a roughly circular and anterior opening in which the representatives are *R. onychomis* Cuckler, 1939, *R. ondatrae* Chandler, 1941, *R. dipodomis* Tiner, 1948 and the *Rictularia* spp. of the "coloradensis" group. *R. microti* McPherson and Tiner, 1952 was added to the latter group by McPherson and Tiner (1952).

From *Peromyscus* spp. only two species of *Rictularia* have been reported, *R. coloradensis* Hall 1916, type host *Eutamias quadrivittatus* (Say) and *R. onychomis* from *Onychomys leucogaster* (Wied-Neuwied). Dollfus (1960) stated, "Actuellement, les *Rictularia* des *Peromyscus* des Etats-Unis sont tous consideres comme referable a coloradensis." The morphological study of the rictularids obtained in this survey does not support Dollfus' statement. Grundmann suggested as new specimens of *Rictularia* sp. recovered from three deer mice.

#### *Rictularia onychomis* Cuckler, 1939

Seven female specimens were recovered from the small intestines of two *P. maniculatus* and one *P. eremicus* collected in Washoe County of northern Nevada. A count of the prevulvar combs and spines gave a range from



28–29. Posterior to the vulva, which was situated approximately 720–816  $\mu$  from the end of the esophagus, the spines became gradually smaller until they could no longer be discerned with accuracy; about 22–24 were counted. The maximum length of spines in the prevulvar region was about 65.5  $\mu$ , whereas those in the postvulvar area were about 22  $\mu$  long. Except for egg size, which could not be ascertained, the morphology of specimens fitted the description of *R. onychomis* as given by Cuckler; however, maximum comb-length did not agree with that given by McPherson and Tiner.

*Rictularia coloradensis* Hall, 1916

*P. eremicus* (1) and *P. maniculatus* (1), collected in Clark County of southern Nevada, each harbored a single nongravid female specimen of *Rictularia coloradensis*. The parasite obviously differed from *R. onychomis* in having larger combs and spines in the prevulvar area and very large spines in the postvulvar region of the body. The large spines were easily discernible caudad to about the level of the anus. The vulva was slightly anterior to the esophageal-intestinal junction. The worm showed some resemblance to *R. coloradensis* in the extent and conspicuousness of the cuticular armature and in having a more forward position of the vulva. Hall listed the total number of prevulvar combs as 31, whereas in the writers' specimens there were only 30. He cited the position of the vulva at the exact level of the posterior end of the esophagus, rather than being somewhat anterior to it. There was also a slight variation in the height of the combs and spines. The differences observed, however, did not seem sufficient, especially with inadequate specimens, to justify designating this material differently.

*Rictularia coloradensis* has been previously reported from *Peromyscus* spp. Harkema (1936) reported the species from *P. leucopus* in North Carolina and Tiner (1948b) examined specimens of this species collected from the same host in Maryland. Rankin (1945) reported the species from *P. maniculatus* in Washington and Tiner also reported it from this same host in Wisconsin.

Specimens of *Rictularia* collected from Nevada rodents were found free in the intestinal lumen and no pathology was observed that

could be attributed to them. On the other hand, Cuckler stated that his specimens were found deeply embedded within the intestinal wall.

Infection of rodent hosts by rictularids is affected through the ingestion of parasitized arthropods, i.e., roaches, beetles, etc.

GENUS—*Gongylonema* MOLIN, 1857  
*Gongylonema peromysci* KRUIDENIER  
AND PEEBLES, 1958

Eight females, two of which were immature and two others fragmentary, were collected from the stomachs of two animals (*P. truei*) collected in Clark County. The position of the vulva, lengths of worm and of the esophagus, and egg size fitted the description of *G. peromysci* as given by Kruidenier and Peebles (1958). The Nevada specimens are, therefore, assigned to this species. These investigators, working in Arizona, collected *G. peromysci* from the stomachs of three species of Cricetidae, including *P. maniculatus*, *P. boylii* (Baird), and *P. truei*. The parasite was not collected in the Utah survey by Grundmann and Frandsen.

OXYURIDEA WEINLAND, 1858  
GENUS—*Syphacia* SEURAT, 1916  
*Syphacia peromysci* HARKEMA, 1936

Oxyurid nematodes were collected from the cecum and in the abdominal cavity of three species of *Peromyscus*—*P. eremicus* (5), *P. truei* (2), and *P. crinitus* (2), all from Clark County. The range of infection was from 1 to 12 nematodes per host. The specimens belonged to the genus *Syphacia*. Two species of this group have been reported from *Peromyscus*—*S. peromysci*, from *P. maniculatus* collected in Arizona by Kruidenier and Peebles, by Grundmann and Frandsen in Utah, and by Schad in Quebec, and *S. samorodini* Erickson, 1938, from *P. leucopus* and *P. maniculatus* in Minnesota. Tiner (1948c) in his presentation of a key to species of the genus considered the latter parasite species to be a synonym of *S. peromysci*. The Nevada syphacids fit the description of *S. peromysci* as given by Harkema (1936) and may be readily identified by employing Tiner's key to members of the genus.

## STRONGYLIDEA DIESING, 1851

GENUS—*Nematospiroides* BAYLIS, 1926*Nematospiroides dubia* BAYLIS, 1926

This parasite was collected from the small intestines of *P. maniculatus* (2) from Washoe and *P. eremicus* (1) from Clark County, Nevada. Four adult specimens, three males and a female, were obtained. The specimens fitted the description of the species as given by Baylis (1926). The occurrence of the species in *P. maniculatus*, although cited by Yamaguti, is not reported by Schad, Grundmann, nor Grundmann and Frandsen.

## ACANTHOCEPHALA

GIGANTORHYNCHIDEA GOLVAN, 1916

GENUS—*Moniliformis* TRAVASSOS, 1915*Moniliformis* sp.

Three immature specimens were collected from the small intestines of a single deer mouse (*P. maniculatus*). Although species determination could not be ascertained, the specimens could be assigned to the genus *Moniliformis*. *M. clarki* (Ward, 1917) was reported by Grundmann and Frandsen from *P. maniculatus*.

## CESTODA

CYCLOPHYLLIDEA BEN. IN BRAUN, 1900

GENUS—*Hymenolepis* WEINLAND, 1858*Hymenolepis* sp.

Tapeworms were conspicuously absent in hosts examined during this investigation. *Hymenolepis* sp., in two hosts, *P. maniculatus* (1) and *P. eremicus* (1) constitutes the only record of Cestoda in this study. Both infected animals were collected in Washoe County of northern Nevada. About four fragmentary specimens were obtained from the small intestines. The condition along with poor staining, rendered species identification most difficult. Species of *Hymenolepis* have been previously reported from *Peromyscus*, including *H. citelli* McLeod, 1933, reported by Grundmann and Frandsen (1960) and *Hymenolepis* sp. by Kruidenier and Gallicchio (1956), both parasites having been taken from the deer mouse.

## SUMMARY

Helminth parasites of *Peromyscus* spp. from areas within Nevada have been investigated.

One hundred forty-five animals, comprising four species from four counties were examined. From these hosts, eight species of helminths of seven genera were recovered, including six of Nematoda, one of which was described herein as new, one of Cestoda, and one of Acanthocephala. Trematodes were not collected.

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### DONALD BARD McMULLEN

1903-1967

Dr. Donald B. McMullen, internationally known senior member of the staff of the Walter Reed Army Institute of Research, died of a heart attack 27 May, while participating in a scientific meeting at the Saint Clair's Hospital in New York City.

During a long and distinguished career, Dr. McMullen's principal interest was in the biology and control of schistosomiasis. Much of his effort was devoted to methods of elimination of the snails which harbor the infection and provide the source of human disease.

He was born in Tarkio, Missouri, on 20 November 1903, and received his early education at Tarkio College and Washington University (St. Louis). He became Head of the Department of Biology of Monmouth College in 1928, a position he held for ten years. During this period Dr. McMullen attended the Johns Hopkins University School of Hygiene and Public Health, receiving the Doctor of Science degree in 1935.

He was a member of the faculty of the Uni-

versity of Oklahoma School of Medicine from 1938 to 1952, rising to Professorship of Preventive Medicine and Public Health. During World War II he served as a consultant to the United States Army on the prevention of schistosomiasis in the Philippines for which he was honored with the Medal of Freedom. Dr. McMullen entered full time government service in 1952. Because of his expertise he regularly was sought as a consultant by various agencies and was loaned to the World Health Organization as a full time staff member from 1958 to 1963, serving as the leader of its schistosomiasis advisory team. During the past several years he had assumed an active role in the Army's malaria research program, in addition to serving as Scientific Advisor to the Director of the Walter Reed Army Institute of Research.

Dr. McMullen was President of the Helminthological Society of Washington in 1966, and at the time of his death was President of the American Society of Parasitologists.

## MINUTES

## Four Hundred Twenty-first—Through Four Hundred Twenty-eighth Meetings

421st Meeting: Adult Education Center, University of Maryland, College Park, Maryland, 19 October 1966. Fifty-sixth Anniversary dinner meeting. Nominations of M. Chitwood as President; W. De Witt as Vice-President, E. Buhner as Corresponding Secretary and Treasurer, A. Pipkin as Recording Secretary; Editorial Board (5-year terms): A. McIntosh and W. Hargis, were submitted to the membership of the Society and approved by acclamation. Following an excellent dinner, an interesting program on taxonomy was presented and included: Reminiscences in taxonomy, by H. Stunkard; Zoological nomenclature in the jet age, by C. Sabrosky, and Seventy-five years of morphology, by M. Chitwood. Special recognition was extended to Drs. W. W. Cort and George LaRue in the form of a presentation of the Helminthological Society Anniversary Award, the first time for this award to be shared by two recipients in the same year.

422nd Meeting: Beltsville Parasitological Laboratory, Beltsville, Maryland, 18 November 1966. Slate of officers announced at 421st meeting was elected by unanimous vote. Papers presented: The separation of kidney worm (*Stephanurus dentatus*) antigens by disc electrophoresis, by R. Romanowski; Publish or perish, by D. Segal; *Babesia caballi* in the red blood cells, by A. Johnson; Physical-chemical studies of the hemoglobin from *Syngamus trachea*, by J. Rose; Transmission of *Heterakis* by the field cricket, by A. Spindler; Cleaning and concentration of oocysts in large quantities, by J. Vetterling.

423rd Meeting: Adult Education Center, University of Maryland, College Park, Maryland, 14 December 1966. Papers presented: Studies of the micro-environment of free-living stages of nematodes, by J. Poole; Blood parasites of the wood duck, by C. Herman, Skin penetration studies on *Schistosoma mansoni* cercariae, by M. Stirewalt; Colored transparencies from Iran, by A. Eslamii.

424th Meeting: Patuxent Wildlife Research Center, Laurel, Maryland, 20 January 1967. Papers presented: Water pollution: A practical dilemma turned into an ecological opportunity, by R. Beaudoin; Notes on the biology

of *Cnephia taeniatifrons*, by B. Tarshis; *Haemoproteus* in domestic pigeons and mourning doves in Maryland, by J. Kniseley and C. Herman; A book report: About vectors, by C. Herman.

425th Meeting: Wilson Hall, National Institutes of Health, Bethesda, Maryland, 17 February 1967. Papers presented: A quantitative postmortem study of human schistosomiasis mansoni, A. Cheever; Studies on population densities of microfilariae in dogs, by G. Pacheco; Fluorescent antibody studies on malaria in hypo- and hyper-endemic areas in Malaysia, by W. Collins; Thrombocytopenia in simian malaria, by J. Sheagren; The micro-pyle, a unique structure of the sporozoa, H. Sheffield.

426th Meeting: Howard University, Washington, D. C., 10 March 1967. Papers presented: Esterases in *Schistosoma mansoni* cercariae, by G. Wells; Oxygen uptake by brain slices from trypanosome-infected rats, by J. Bruce; Growth enhancement of rats inoculated with *Trichinella spiralis*, by D. Sen; Duration of protective immunity in rats inoculated with *Trypanosoma lewisi*, by C. Lee; South American ramblings related to the 1st Latin American Congress of Parasitology, D. Lincicome.

427th Meeting: School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, 28 April 1967. A motion commemorating the 80th birthday of Dr. W. W. Cort was made by A. Foster, and was seconded and carried unanimously. A letter to Dr. Cort was read by G. Otto, who suggested that the many friends of Dr. Cort in the audience sign the letter, which would be forwarded to him as a gesture of the Society's good will. Papers presented: Effects of anti-cestode agents on P<sup>32</sup> incorporation into ATP in *Hymenolepis diminuta*, by L. Scheibel; The dietary effects of acorn on the growth and development of *Hymenolepis diminuta* in the rat, by E. Eni; Applications of the Lowry quartz fiber balance to precision dry weight measurements of parasitological tissues, by R. Lennox; Genetic aspects of susceptibility to virus hepatitis in mice, by I. Shif; Cytochrome oxidase activities in migratory larvae

of *Ascaris lumbricoides* var. *suum*, by S. Sylk; Mosquito tissue culture, by D. Gubler.

428th Meeting: New Bolton Center, University of Pennsylvania, Kennett Square, Pennsylvania, 13 May 1967. The Auditor's Report was read in summary to the Society, and approved. Papers presented: Games of chance played by worms, by G. Graham; Fine structure of the nervous system of *Echinococcus granulosus*, by D. Morseth; Demonstration of synchrony in the early development of the first asexual generation of *Eimeria tenella*, by D. Boughton; Further studies on the antigens in ascariasis, by E. Jeska; Growth and survival of *Ascaris suum* larvae in diffusion chambers in normal and immune animals, by J. Williams; Specific and nonspecific leucocyte adhesion to helminth larvae, by E. Soulsby; An illustrated talk on South America, by E. Newmeyer.

Cocktails were served in adjoining Allam House, courtesy of the School of Veterinary Medicine, followed by an excellent dinner, attended by 92 members and guests.

The following were elected to membership at the meetings indicated: **422nd**—J. W. Ward, G. M. Malakatis, J. B. Abram; **424th**—M. Warren, J. Mizelle, F. S. L. Williamson, J. J. Sullivan, R. S. Verma, and M. Shakil; **425th**—C. E. Cosgrove, C. A. Johnson, P. A. Nyberg, and D. Morseth; **426th**—N. Ballard, and L. M. Schultz; **427th**—P. Vitiello, H. D. Blankespoor, Y. Mamiya, D. Muncey, B. Premvati, M. Balasubramanian, B. G. Ngundo, C. L. Hung, R. P. Petriello, and T. W. Rowse; **428th**—G. L. Enriquez.

ALAN C. PIPKIN  
*Recording Secretary*

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