IN MEMORIAM — G. STEINER

VOLUME 29 JANUARY 1962 NUMBER 1

PROCEEDINGS

of

The Helminthological Society of Washington

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

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

Subscription $3.00 a Volume; Foreign, $3.25

Published by

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The Helminthological Society of Washington meets monthly from October to May for the presentation and discussion of papers. Persons interested in any branch of parasitology or related science are invited to attend the meetings and participate in the programs.

Any person interested in any phase of parasitology or related science, regardless of geographical location or nationality, may be elected to membership upon application and sponsorship by a member of the society. Application forms may be obtained from the Corresponding Secretary-Treasurer (see below for address). The annual dues for either resident or nonresident membership are four dollars. Members receive the Society's publication (Proceedings) and the privilege of publishing (papers approved by the Editorial Committee) therein without additional charge unless the papers are inordinately long or have excessive tabulation or illustrations.

Officers of the Society for the year 1962

Year term expires (or began) is shown for those not serving on an annual basis.

President: CARLTON M. HERMAN
Vice-President: FRANCIS G. TROMBA
Corresponding Secretary-Treasurer: EDNA M. BUHRER
Recording Secretary: VICTOR H. DROPKIN
Librarian: MILDRED A. DOSS (1945—)
Archivist: JOHN T. LUCKER (1945—)
Representative to Washington Academy of Sciences: DOYS A. SHORE
Executive Committee Members-at-Large: CHARLES G. DURBIN, 1963
LOUIS J. OLIVIER, 1962

PROCEEDINGS OF THE SOCIETY

The Proceedings of the Helminthological Society of Washington is a medium for the publication of notes and papers presented at the Society's meetings. However, it is not a prerequisite for publication in the Proceedings that a paper be presented before the Society, and papers by persons who are not members may be accepted provided the author will contribute toward the cost of publication. Each volume of the Proceedings consists of two numbers, issued in January and July.

Manuscripts may be sent to any member of the Editorial Committee. Manuscripts should be typewritten (double spaced) and submitted in finished form for transmission to the printer. Except in the case of preliminary papers to be published *in extenso* later, a manuscript is accepted with the understanding that it is not to be published, with essentially the same material, elsewhere. The Editorial Committee assumes no responsibility for statements appearing in authored articles. To appear in the January number, manuscripts should be received not later than November 15th; to appear in the July number, not later than May 15th.

Proof.—Whenever possible galley proof will be sent to authors for verification.

Reprints are furnished at cost in accordance with the schedule of prices printed below. Unless otherwise specified in the order, reprints are furnished without covers. The order for reprints should be submitted when proof is returned except in the case of authors not residing in the continental United States or Canada when the order for reprints should accompany the manuscript.

No. Pages	1–2	3–4	5–8	9–12	13–16	17–20
50 copies	5.88	6.25	10.00	13.50	15.19	21.44
100 copies	6.81	7.39	11.25	15.13	17.71	24.69
Add'l 100	1.88	2.25	2.50	3.25	4.81	6.50

Covers: 100 copies 6.00; Additional 100 2.38.

Proceedings of previous meetings.—Independent publication of the Proceedings began in 1934. Prior to this date the Society's proceedings were published in *Science* (1911-1913) and, later, in the *Journal of Parasitology* (1914-1933). A few sets of these early proceedings as well as the back volumes of the PROCEEDINGS are available. For information write the corresponding secretary-treasurer (see below).

Remittances should be made payable to The Helminthological Society of Washington and sent to the corresponding secretary-treasurer.

Correspondence concerning business items should be addressed to the corresponding secretary-treasurer, Edna M. Buhrer, Section of Nematology, Plant Industry Station, Beltsville, Md.; concerning manuscripts to the editor, Gilbert F. Otto, Parasitology Department, Research Division, Abbott Laboratories, North Chicago, Illinois.

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 29 JANUARY 1962 NUMBER 1

North American Monogenetic Trematodes. X. The Family Axinidae*

EMMETT W. PRICE

This paper represents a continuation of the series dealing with the North American monogenetic trematodes and of a general revision of the Monogenea. The purpose and organization of this installment are the same as for previous sections (Price, 1937, 1938, 1939a, 1939b, 1942, 1943a, 1943b, 1961a and 1961b**.

In the present paper the family Axinidae, proposed by Unnithan (1957), to include the genus *Axine* Abildgaard and related genera, some of which previously have been referred to the subfamily Axininae Monticelli or included in the subfamily Microcotylinae Monticelli of the family Microcotylidae Taschenberg, is recognized.

The writer is convinced that certain characters, namely, the shape of the opisthohaptor, persistence of opisthohaptoral anchors or hooks, and shape of ovary are so distinctive of the helminths in question that they comprise a family group coordinate with those of the Microcotylidae. The wing-like opisthohaptor, bearing two rows of clamps placed end to end and separated by an anchorbearing lobe or languette—in effect forming a straight angle—is distinctive. The persistence in the adult worms of anchors of a characteristic larval type (see Euzet, 1955, 1957, and Bychowsky, 1957) is also distinctive. The two characters mentioned are correlated with the shape of the ovary, which in the axinid genera is more or less U-shaped, with both proximal and distal limbs directed anteriad; in the microcotylid genera the ovary has both proximal and distal limbs directed posteriad. For these and other reasons, which will be discussed more fully in a future paper, the recognition of the family Axinidae appears justified.

AXINIDAE Unnithan, 1957

DIAGNOSIS: Body usually elongate, flattened dorsoventrally, with posterior end more or less truncate. Prohaptors a pair of aseptate suckers, usually provided with minute rounded papillae or denticles forming a more or less definite pattern, opening into oral cavity. Opisthohaptor a frill bearing microcotylid clamps along truncate posterior end of body; clamps, in two rows or forming a straight angle, rows separated by minute hook-bearing languette, representing morphological posterior end, and located roughly at a point corresponding to distal end of an imaginary line drawn through long axis of body; opisthohaptoral hooks or anchors of two dissimilar pairs—those of outer pair lunate with slender dorsal roots, those of inner pair with lunate tips articulating with long slender shafts. Genital atrium and/or cirrus

*From Jacksonville State College, Jacksonville, Alabama.
This work was supported by a grant (G13002) from the National Science Foundation.

**References to these papers, except 1961b, are given in the bibliography of Part IX.

armed or unarmed. Testes follicular, postovarial. *Ovary U-shaped or J-shaped, with proximal and distal limbs directed anteriad.* Vagina single; aperture dorsal, either lateral, submedian or median, usually provided with horn-like spine, or, rarely, with incomplete row of curved spines. Genito-intestinal canal present. Vitellaria as in other microcotyloids.

TYPE GENUS: *Axine* Abildgaard, 1794.

This family may be divided into three subfamilies, largely on the basis of the character of the opisthohaptor and terminal genitalia.

Key to subfamilies of Axinidae

1. Opisthohaptoral clamps few, in 2 equal rows separated by anchorbearing languette ..... Indocotylinae Tripathi
   Opisthohaptoral clamps numerous, in 2 unequal rows separated usually, if not always, by anchorbearing languette 2
2. Genital atrium armed with 3 groups spines; cirrus armed ..... Axininae Monticelli
   Genital atrium usually unarmed, when armed spines not in groups as above; cirrus usually armed ..... Axinoidinae n. sf.

Subfamily AXININAE Monticelli, 1903

DIAGNOSIS: Opisthohaptoral clamps numerous, in unequal rows separated by anchor bearing languette. Genital atrium armed with 3 groups of spines, 2 lateral and 1 median, situated on pad-like structures; cirrus armed with circle of relatively strong pointed spines. Vaginal aperture dorsal, near lateral margin, armed with horn-like spine. Other characters as for family.

TYPE GENUS: *Axine* Abildgaard, 1794.

Key to genera of Axininae

1. Median spines of genital atrium in single, usually incomplete, row in distal portion of atrium ..... *Axine* Abildgaard
   Median spines of genital atrium in 2 or 3 complete or incomplete rows, in anterior portion of atrium 2
2. Median spines of genital atrium in 2 or 3 horseshoe-shaped rows ..... *Heteraxinoides* Yamaguti
   Median spines of genital atrium in 2 complete rows ..... *Unnithanaxine* n.g.

GENUS *Axine* Abildgaard, 1794

SYNONYMS: *Heteracanthus* Diesing, 1836; *Cestracolpa* Meserve, 1938.

DIAGNOSIS: Spines of genital atrium in 3 groups, 1 group on each side of anterior portion of atrium and third, usually an incomplete circle, towards distal portion of atrium. Cirrus armed with circle of spines. Other characters as for subfamily.

TYPE SPECIES: *Axine belones* Abildgaard, 1794, from *Belone acus.*

INCLUDED SPECIES: *Axine cypseluri* (Meserve, 1938) Price, 1946 (syn. *Cestracolpa cypseluri* Meserve, 1938), from *Cypselurus callopterus; A. hemiramphae* Unnithan, 1957, from *Hemiramphus xanthopterus; A. japonicum* Price, 1946 (syn. *A.* (*Axine*) *cypseluri* Yamaguti, 1940, not Meserve, 1938), from *Cypselurus agoo; A. hyporamphi* n.sp., from *Hyporamphus* sp.; *A.*

triglae Beneden and Hesse, 1863 (sp. inq.)† from *Trigla hirundo; A tripathii* n.n. (syn. *A. hemirhamphae* Tripathi, 1959, not *A. hemiramphae* Unnithan, 1957), from *Hemirhamphus georgii* (sic); and *A. yamagutii* (Meserve, 1938) Price, 1946 (syn. *Cestracolpa yamagutii* Meserve, 1938), from "unidentified flying fish."

Of the above species, only *Axine hyporamphi* and *A. yamagutii* have been collected from North American localities. Since the writer has available to him specimens of *A. cypseluri* and *A. belones,* the latter donated by Hon. Miriam Rothschild, Peterborough, England, to whom thanks are hereby extended, descriptive notes and figures of these forms are included for comparison.

Axine belones Abildgaard, 1794 (Figures 1, 14, 26, 38, 50)

SYNONYMS: *Heteracanthus pedatus* Diesing, 1836; *H. sagittatus* Diesing, 1836; *Axine orphii* Beneden and Hesse, 1863; *A. platyura* Creplin, 1838.

DESCRIPTION: Body trumpet-shaped, 4.4 to 8 mm long, tapering gradually from anterior end to opisthohaptor which is about 1.7 mm wide and directed to left. Prohaptoral suckers almost circular, about 0.057 mm in diameter, provided with minute, blunt, tooth-like structures arranged as in Fig. 26. Opisthohaptor consisting of a frill along posterior end of body bearing 60 to 65 microcotyloid clamps, 0.095 to 0.115 mm wide, and a minute lobe bearing 2 pairs of anchors. Anchorbearing lobe, representing morphological posterior end, situated about 25 to 27 clamp widths from right end of opisthohaptor. Anchors typically axinoid; lateral anchors 0.040 mm long, with lunate blades and slender dorsal roots, and inner anchors about 0.050 mm long, with small lunate blades articulating with long slender shafts. Pharynx oval, 0.030 mm long by 0.020 mm wide; esophagus slender, bifurcating about midway between levels of genital and vaginal apertures; intestinal branches with short median and longer lateral diverticula, extending into opisthohaptoral area, with right limb longer than left. Genital aperture median, about 0.6 mm from anterior end. Genital atrium armed with 3 groups of spines, a posterior incomplete circle of about 20 elements, each about 0.020 mm long, and a group of about 12 spines, about 0.020 mm long, on each side at level of anterior end of cirrus. Cirrus armed with corona of about 10 spines, each about 0.015 mm long. Testes about 60 in number, postovarial. Ovary J-shaped, immediately prestesticular. Ootype fusiform, immediately anterior to cephalic end of ovary. Seminal receptacle present. Genito-intestinal canal opening into right intestinal limb immediately posterior to level of right vitelline duct. Vagina muscular, lined with heavy cuticula bearing prominent papilliform projections; vaginal aperture dorsolateral, situated on right side a short distance posterior to level of esophageal bifurcation; vaginal spine horn-like, about 0.05 mm long by 0.02 mm wide at base. Vitellaria extending from level of vaginal aperture into haptoral region. No eggs present.

HOST: *Belone acus.*

LOCATION: Gills.

DISTRIBUTION: Europe.

SPECIMENS: U.S.N.M. Helm. Coll. Nos. 37726, and 37727, collected September 30, 1932 (1 specimen) and December 10, 1933 (2 specimens), respectively, at Plymouth, England, by Hon. Miriam Rothschild.

---

†*Axine triglae* Beneden and Hesse is regarded by Dawes (1947) as a synonym of *A. belones* and it is possible that his conclusion may be correct. However, since this inadequately described species is from a fish host belonging to a different family than that of *A. belones,* it should be regarded as a *species inquirendeum* until a restudy of material from the type host is possible.

The above description agrees very closely with that given by Lorenz (1878) except that the opisthohaptoral hooks or anchors were not observed. The presence of these anchors in *Axine belones* was first reported by the writer (Price, 1946) and, independently, later in the same year by Sproston (1946). The latter author, however, appears to have overlooked the slender inner pair, since she showed only one pair in her illustration. Furthermore, it appears from the measurements given of these hooks that she failed to observe their slender dorsal roots.

Axine cypseluri (Meserve, 1938) Price 1946 (Figures 2, 15, 27, 39, 51)

SYNONYM: *Cestracolpa cypseluri* Meserve, 1938.

DESCRIPTION: The description given by Meserve (1938), except for the presence of an armed condition of the prohaptoral suckers and opisthohaptoral anchors, is adequate and need not be repeated here. The prohaptoral suckers are armed around the margin with minute tooth-like structures as shown in Fig. 27. The opisthohaptoral anchors, consisting of 2 pairs of hooks, similar to those described for *A. belones,* are located on a minute lobe or languette between the 18th and 19th clamps from the right end of the haptor. The outer anchors are 0.043 mm long and the inner 0.060 mm long.

HOST: *Cypselurus callopterus.*

LOCATION: Gills.

DISTRIBUTION: Galapagos Islands.

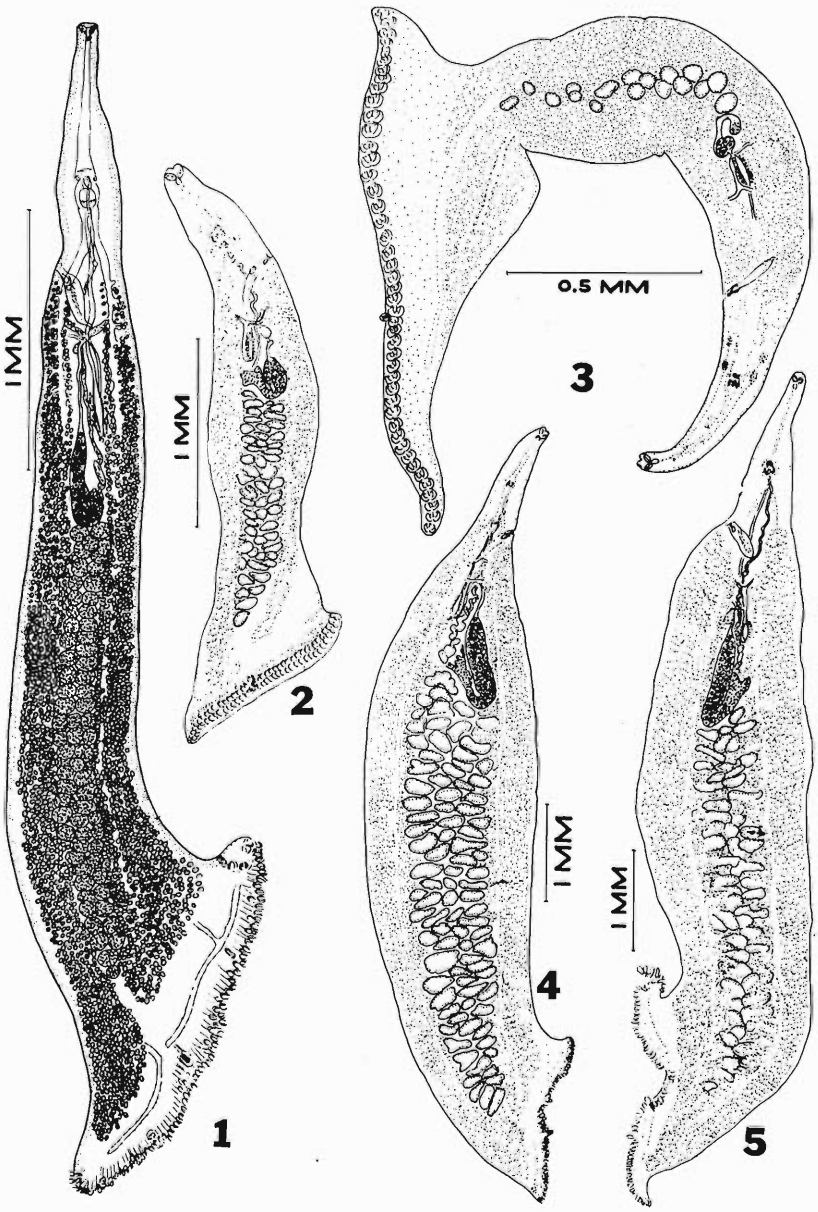
SPECIMEN: U.S.N.M. Helm. Coll. No. 9165 (holotype).

Sproston (1946) was quite emphatic in her belief that *Axine cypseluri* (Meserve) and *A. cypseluri* Yamaguti (= *A. japonicum* Price) were identical. There is a possibility that this may be true, since there are strong similarities in the genital armament and in the presence of an oval, lamellar nodule in the vaginal wall. On the other hand there are differences in the number of opisthohaptoral clamps (41 in *cypseluri* and 65-68 in *japonicum*) in the number of testes (about 60 in *cypseluri* and about 30 in *japonicum,*) and in hosts which suggest that the two species should be regarded as distinct until more evidence to the contrary is available. Yamaguti, in personal conversation, completely rejected Sproston's conclusion.

Axine hyporamphi new species (Figures 3, 16, 28, 40, 52)

DESCRIPTION: Prehaptoral portion of body lanceolate, opisthohaptoral portion wing-like; overall length 2.6 mm, width in testicular region 0.034 mm and of opisthohaptor 1.36 mm. Prohaptoral suckers about 0.040 mm in diameter, without denticles. Opisthohaptoral clamps 46 in number and about 0.050 mm wide; terminal lobe located between 21st and 22nd clamps, counting from most distal end. Anchors of typical *Axine* shape; those of outer pair 0.036 mm long, with strong lunate hooks and slender dorsal roots, and those of inner pair 0.040 mm long, with small lunate hooks articulating with slender shafts. Oral aperture subterminal; esophagus slender, bifurcating at or near level of genital atrium; remainder of digestive tract apparently similar to that of other species. Genital aperture about 0.3 mm from anterior end. Genital atrium armed with 3 groups of spines as in other species of *Axine s. str.;* lateral groups with 12 spines each, 0.010 mm long, arising from pad-like structures, distal spines 14 in number, about 0.012 mm long, in an incomplete circle. Spines of cirrus corona 12 in number, about 0.007 mm long. Testes relatively few, about 17, postovarial, not extending into haptoral area. Ovary

U-shaped, in equatorial zone. Vagina muscular, with relatively thick cuticular lining but without obvious papillae or spines; aperture dorsomarginal, about 0.5 mm from anterior end, armed with horn-like spine 0.036 mm long by 0.018 mm wide at base. Vitellaria distributed as in other species; remainder of female system not clearly distinguishable. No eggs present.



1. *Axine belones*, ventral view; 2. *A. cypseluri*, dorsal view; 3. *A. hyporamphi*, anterior portion in ventral view, haptor in dorsal view; 4. *A. yamagutii*, dorsal view; 5. *Heteraxinoides inada*, ventral view.

HOST: *Hyporamphus* sp. The label on slide gives the host as *Hyporhamphus* (sic) *brasiliensis*. However, according to Jordan, Evermann, and Clark (1930) the range of this fish does not extend as far north as Massachusetts and for that reason the accuracy of the host identification is questioned.

LOCATION: Gills.

DISTRIBUTION: United States (Woods Hole, Massachusetts).

SPECIMEN: U.S.N.M. Helm. Coll. No. 36550 (holotype), collected August 11, 1915, by G. A. MacCallum.

This species is based on a single, somewhat distorted specimen. It resembles most closely *Axine hemirhamphae* Tripathi (1959) from India. It differs, however, in number of opisthohaptoral clamps, posterior extent of testes, number and arrangement of genital spines, and host.

Axine yamagutii (Meserve, 1938) Price, 1946 (Figures 4, 17, 29, 41, 53)

SYNONYM: *Cestracolpa yamagutii* Meserve, 1938.

DESCRIPTION: Except for relatively minor details, the description given by Meserve (1938) is adequate and a detailed redescription is unnecessary. As in the preceding species, the prohaptoral suckers are armed with minute tooth-like structures (Fig. 29). The opisthohaptoral clamps differ from those of all other species in having 12 more or less vertical ribs in each clamp wall. Opisthohaptoral anchors were overlooked by Meserve. They occur on a minute languette located between the 30th and 31st clamps, counting from left or most distal point of the haptor. The outer hooks or anchors are about 0.030 mm and the inner 0.040 mm, respectively. The number of testes in the holotype specimen appears much greater than the 52 given by Meserve. It is possible that the greater number may be more apparent than real, as it is difficult to distinguish in the material available between testes and lobes of a testis.

HOST: "Unidentified flying fish."

LOCATION: Gills.

DISTRIBUTION: Mexico (Clarion Island, and open sea off coast).

SPECIMEN: U.S.N.M. Helm. Coll. No. 9164 (holotype).

This species is easily distinguished from other axines by the peculiar, ribbed opisthohaptoral clamps and in the genital spination.

GENUS *Heteraxinoides* Yamaguti, 1943

SYNONYM: *Axine* (*Heteraxinoides*) Yamaguti (1943).

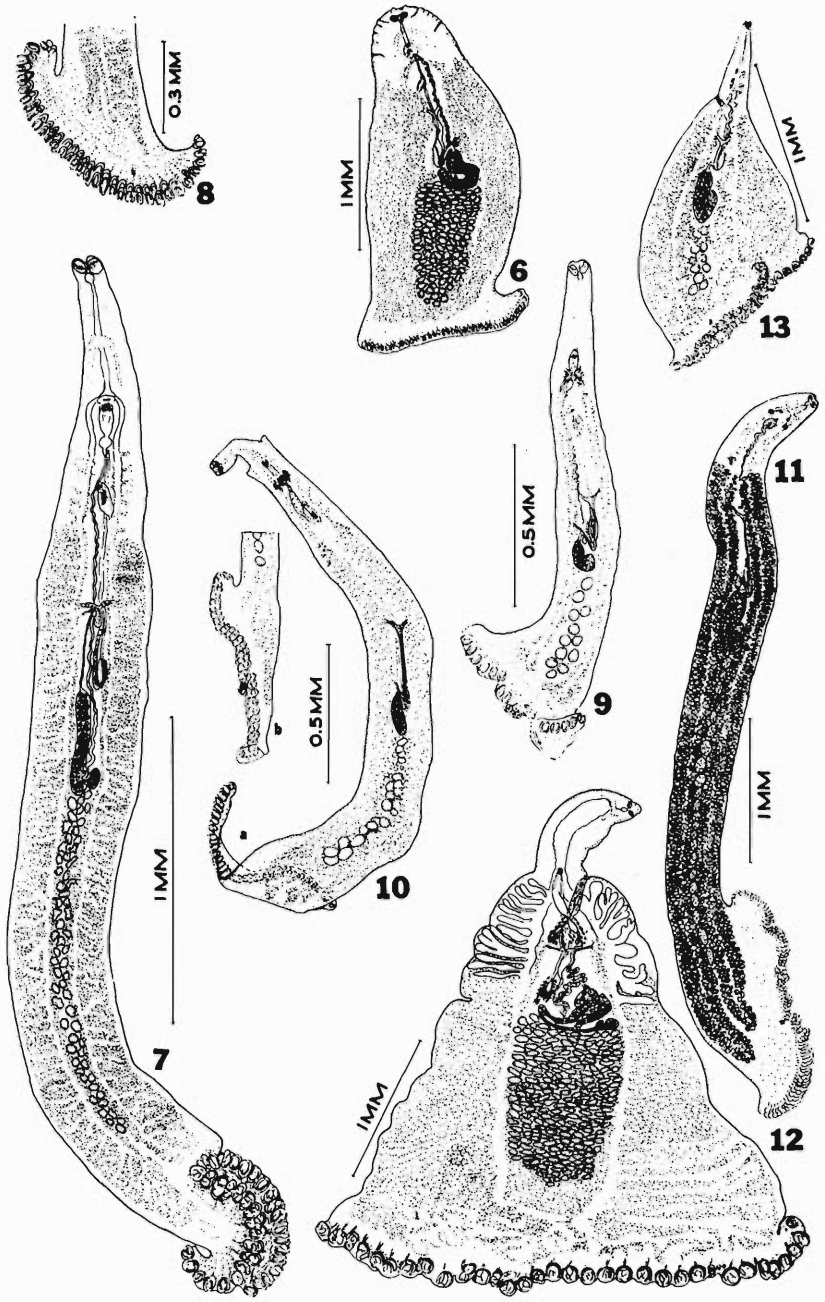
DIAGNOSIS: Spines of genital atrium consisting of 2 lateral groups, arranged in 3 indistinct rows, each on an elongate pad, and an anterior group of 3 indistinct rows on a horseshoe-shaped pad. Other characters as for subfamily.

TYPE SPECIES: *Heteraxinoides inada* (Ishii and Sawada, 1938) Yamaguti, 1943.

Heteraxinoides inada, so far the only species of the genus, is not known to occur on hosts inhabiting North American waters. However, since a specimen is available through the courtesy of Prof. T. Sawada, to whom appreciation is here expressed, and inasmuch as there are some differences between this specimen and the original account, a redescription follows:

Heteraxinoides inada (Ishii and Sawada, 1938) Yamaguti, 1943 (Figures 5, 18, 30, 42, 54)

SYNONYMS: *Axine inada* Ishii and Sawada, 1938; *Axine* (*Heteraxinoides*) *inada* (Ishii and Sawada, 1938) Yamaguti, 1943.



6. *Axnoides meservei*, dorsal view; 7. *A. oceanicum*, ventral view of holotype; 8. *A. oceanicum*, posterior end of paratype; 9. *A. raphidoma*, ventral view; 10. *A. strongylurae*, a. ventral view of holotype, b. haptor of paratype; 11. *Nudacraxine gracilis*, ventral view of holotype; 12. *Chlamydaxine resplendens*, ventral view; 13. *C. truncatus*, ventral view.

DESCRIPTION: Body lanceolate, flattened, 8 mm long by 1.7 mm wide in mid-region, expanded posteriorly to form opisthohaptor 2.5 mm wide. Prevaginal portion of body attenuated, with transversely striated cuticle. Prohaptoral suckers oval, 0.095 mm long by 0.075 mm wide, provided with minute, tooth-like prominences distributed as shown in Fig. 30. Opisthohaptor consisting of a frill along wide posterior end, bearing about 95 clamps of microcotyle-type, about 0.075 to 0.170 mm wide, and with a minute languette provided with 2 pairs of dissimilar, axine-type anchors located about 1 mm from left or most distal part of haptor; outer anchors about 0.045 mm long, inner 0.055 mm long. Pharynx piriform, 0.057 mm long by 0.035 mm wide; esophagus slender, bifurcating distal to genital atrium; remainder of digestive tract apparently similar to that of other axinids. Genital atrium about 0.9 mm from anterior end of body, provided with 3 groups of spines situated on pad-like structures; lateral groups of 30 to 33 spines, each about 0.020 mm long; anterior group of about 50 spines, each about 0.020 mm long, arranged in 3 indistinct rows on horseshoe-shaped pad. Cirrus armed with corona of 9 spines, each 0.020 mm long. Testes about 55 in number, postovarial. Ovary U-shaped, in median field, immediately pretesticular; seminal receptacle present, a short distance in front of origin of oviduct; genitointestinal canal opening into right intestinal limb near level of anterior end of seminal receptacle; ootype oval, to left of anterior pole of ovary; vitelline reservoir Y-shaped, branching about midway between anterior end of ovary and posterior end of thickened portion of vagina. Vagina muscular, lumen of distal portion lined with small spines; vaginal aperture dorsal, near right body margin and about 1.5 mm from anterior end; vaginal spine horn-shaped, about 0.065 mm long by 0.035 mm wide at base. Vitellaria almost filling body width from level of vaginal aperture to opisthohaptoral region. No eggs present.

HOST: *Seriola quinqueradiata* (= *Tylosurus quinqueradiata*).

LOCATION: Gills.

DISTRIBUTION: Japan.

SPECIMEN: U.S.N.M. Helm. Coll. No. 37728 (paratype).

The outstanding differences between the description given by Ishii and Sawada (1938) and the specimen described herein are in the position of the female organs and in the location of some of the opisthohaptoral clamps. According to the authors mentioned, also shown in their illustration of the species, the ovary and vaginal opening are on the left side of the body while in the specimen studied they are on the right side. The opisthohaptoral clamps are arranged in a single row along the free edge of the haptor and none along the right margin as stated in the original description, this arrangement apparently due to a folding over of that portion of the haptor. Furthermore, as in the case of many species, the opisthohaptoral anchors were overlooked.

Unnithanaxine new genus

DIAGNOSIS: Spines of genital atrium consisting of two lateral groups and an anterior group of 2 complete rows surrounding genital aperture. Other characters as for subfamily.

TYPE SPECIES: *Unnithanaxine parawa* (Unnithan, 1957) n. comb.

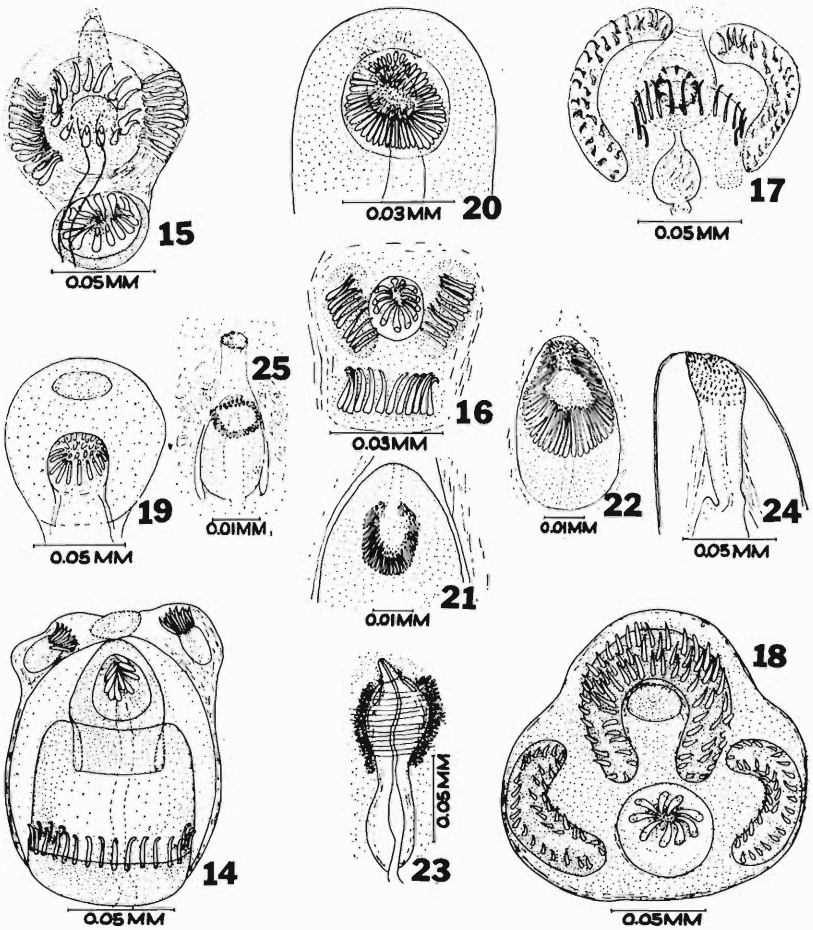
The type of this genus was described as *Axine parawa* by Unnithan (1957) from the gills of a flying fish, *Cypselurus bahiensis*, at Trivandrum, South India. The distinctive feature of this species is the arrangement of the atrial

spines, the median group of which consists of two complete rows surrounding the genital aperture. In this respect it occupies a position intermediate between the species of *Axine s. str.* and *Heteraxinoides.*

AXINOIDINAE new subfamily

DIAGNOSIS: Opisthohaptoral clamps numerous, in unequal rows separated by anchor bearing languette. Genital atrium usually unarmed; cirrus usually armed with one to several rows of spines. Vaginal aperture either dorsomedial or dorsolateral, armed with horn-like spine or, rarely, with an incomplete row of incurved spines. Other characters as for family.

TYPE GENUS: Axinoides Yamaguti, 1938.



14-24. Genital armament: 14. *A. belones;* 15. *A. cypseluri;* 16. *A. hyporamphi;* 17. *A. yamagutii;* 18. *Heteraxinoides inada;* 19. *Axinoides meservei;* 20. *A. oceanicum;* 21. *A. raphidoma;* 22. *A. strongylurae;* 23. *Nudacraxine gracilis;* 24. *Chlamydaxine resplendens;* 25. *C. truncatus.*

Key to Genera of Axinoidinae

1. Vaginal aperture dorsolateral, armed with incomplete row of incurved spines ..... *Neoaxine* Price
   Vaginal aperture usually dorsal and median, sometimes sublateral, armed with single horn-like spine 2
2. Cirrus and genital atrium unarmed ..... *Nudaciraxine* n.g.
   Cirrus armed; genital atrium usually unarmed 3
3. Vaginal aperture dorsomedian; genital atrium unarmed-*Axinoides* Yamaguti
   Vaginal aperture dorsal and sublateral, genital atrium armed or unarmed 4
4. Body triangular; genital atrium unarmed ..... *Chlamydaxine* Unnithan
   Body elongate; genital atrium armed or unarmed 5
5. Genital atrium unarmed ..... *Loxura* Unnithan
   Genital atrium armed ..... *Loxuroides* n.g.

GENUS *Axinoides* Yamaguti, 1938

SYNONYM: *Axine* (*Axinoides*) Yamaguti, 1938.

DIAGNOSIS: Genital atrium unarmed; cirrus armed. Vaginal aperture dorsal, in median field, armed with horn-like spine. Other characters as for subfamily.

TYPE SPECIES: *Axinoides tylosuri* Yamaguti, 1938.

Axinoides was proposed as a subgenus of *Axine* by Yamaguti (1938) and elevated to full generic status by Price (1946) and, independently, by Sproston later in the same year. The validity of this action, based largely on the position of the vaginal aperture, has been questioned by Hargis (1956, 1959). His objection was based for the most part on the apparent variation in the position of the vaginal aperture of *A. gracilis* (Linton) which was then included in *Axinoides*. A new genus is proposed later on in this paper to include *A. gracilis* and the validity of *Axinoides* is retained herein.

The species that may be included in *Axinoides* as presently conceived are as follows: *Axinoides aberrans* (Goto, 1864) Price, 1946, from *Tylosurus schismatorhynchus; A. kola* Unnithan, 1957, from *Ailennus hians; A. meservei* Price, 1946 (syn. *A. aberrans* Goto, of Meserve, 1938) from *Tylosurus fodiator; A. oceanicum* (Caballero, Bravo-Hollis and Grocott, 1953) n. comb., from *T. fodiator; A. raphidoma* Hargis, 1956, from *T. raphidoma; A. sebastisci* Yamaguti, 1958, from *Sebastiscus marmoratus; A. strongulurae* n. sp., from *Strongylura marina;* and *A. tylosuri* Yamaguti, 1938 (type), from *Tylosurus schismatorhynchus.*

Axinoides meservei Price, 1946 (Figures 6, 19, 31, 43, 55)

SYNONYM: *Axine aberrans* Goto 1894, of Meserve, 1938.

DESCRIPTION: Body 2 to 2.3 mm long by 1.1 mm wide in ovarian region. Prohaptoral suckers almost circular, 0.045 mm long by 0.038 mm wide, armed with minute denticles as shown in Fig. 31. Opisthohaptor 1.2 mm wide, provided with 41 clamps, about 0.040 mm wide, and armed with anchors of *Axine* type, one outer anchor between 7th and 8th clamps—counting from left—and other between 12th and 13th clamps; only one inner anchor observed, located near right outer anchor; outer anchors about 0.033 mm long and inner anchor about 0.040 mm long. Pharynx oval, about 0.036 by 0.025 mm; esophagus bifurcating posterior to genital atrium; remainder of digestive tract apparently similar to that of other axines. Genital aperture about 0.2 mm from anterior end; genital atrium unarmed. Cirrus armed with a corona of

3 or 4 alternating rows of slender spines about 0.010 mm long. Testes numerous, postovarial, occupying greater part of interintestinal field. Ovary U-shaped, equatorial in position. Seminal receptacle small, almost immediately anterior to distal pole of ovary. Genito-intestinal canal opening into right intestinal branch a short distance anterior to seminal receptacle. Vaginal aperture dorsal, median, about 0.28 mm distal to genital aperture, armed with horn-like spine; vaginal atrium provided with short lancet-like spines. Vitellaria extending from level of vaginal aperture to opisthohaptoral region. Eggs not present in specimens available.

HOST: *Tylosurus fodiator.*

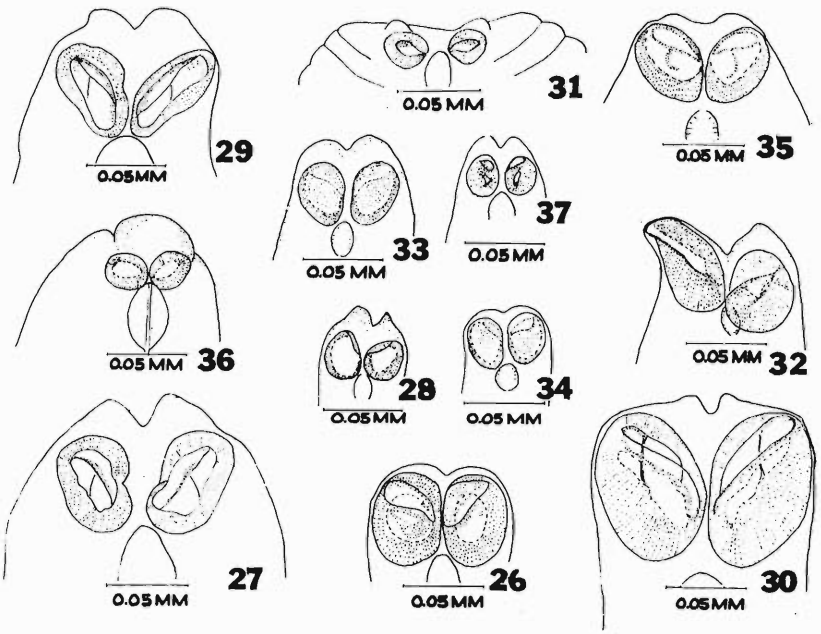
LOCATION: Gills.

DISTRIBUTION: South America (Port Utria, Columbia).

SPECIMENS: U.S.N.M. Helm. Coll. Nos. 37720 (holotype) and 37721 (paratype), donated by Dr. F. G. Meserve to whom appreciation is hereby expressed.

This species was reported as *Axine aberrans* Goto by Meserve (1938) from specimens collected by Dr. H. W. Manter while a member of the 1934 Allen Hancock Expedition to the Galapagos Islands. Beyond giving the length as 1.7 to 2.3 mm long and a reasonably good illustration, the specimens were not described. Largely on the basis of size and host differences, the writer (Price, 1946) renamed the species *Axinoides meservei.*

While there is a remote possibility that *A. meservei* may be the same as *A. aberrans* this cannot be determined with any degree of certainty until a



26-37. Prohaptoral suckers, (stippling indicates distribution of denticles): 26. *Axine belones;* 27. *A. cypselluri;* 28. *A. hyporamphi;* 29. *A. yamagutii;* 30. *Heteraxinoides inada;* 31. *Axinoides meservei;* 32. *A. occanicum;* 33. *A. raphidoma;* 34. *A. strongylurae;* 35. *Nudaciraxine gracilis;* 36. *Chlamydaxine resplendens;* 37. *C. truncatus.*

restudy of the latter species can be made. Goto's (1894) description and figure of *A. aberrans* are probably as good as might be expected from the limited number and condition of the specimens available. There is needed more detailed information on the genital and vaginal spination, number and structure of the opisthohaptoral clamps, as well as the shape and size of the opisthohaptoral anchors—so far unknown for any of the species described from *Tylosurus schismatorhynchus*. One morphological detail between Goto's and Meserve's specimens, which may be significant, is the anterior extent of the vitellaria. In *A. aberrans* these glands extend to about midway between the vaginal and genital apertures whereas in *A. meservei* they extend to about the level of the vaginal aperture.

Axinoides kola Unnithan, which was obtained from the gills of *Ailennus hians* at Trivandrum, South India, resembles *A. meservei* in body form and in most other respects. However, it is a much larger species, the cirrus spines are in a single row, and its occurrence on a host belonging to a different genus than that on which *A. meservei* was found suggest that it should be regarded as distinct until more information is available.

Axinoides raphidoma Hargis, 1956 (Figures 9, 21, 33, 45, 57)

DESCRIPTION: Except for the papillate condition of the prohaptoral suckers and the spination of the cirrus, the description as given by Hargis (1956) is accurate and need not be repeated here. The interior of the prohaptoral suckers is provided with numerous, minute papilliform projections distributed as shown in Fig. 33. The cirrus was described by Hargis (*loc. cit.*) as "muscular, eversible and protrusible, 0.070 long by 0.034 wide bearing muscular papillae in anterior end of lumen which point outwardly when cirrus is everted." Actually, the "muscular papillae" are the tips of slender spines —about 0.005 to 0.008 mm long—of which there are several rows.

HOST: *Tylosurus raphidoma*.

LOCATION: Gills.

DISTRIBUTION: United States (Alligator Harbor, Florida).

SPECIMEN: U.S.N.M. Helm. Coll. No. 38156 (holotype).

The holotype specimen is somewhat mutilated in the opisthohaptoral region and the number of clamps may have been somewhat greater than the 17 present. Furthermore, the specimen is not well stained differentially and the details of the soft structures are not too well brought out.

Axinoides strongylurae new species (Figures 10, 22, 34, 46, 58)

DESCRIPTION: Body slender, 2.4 mm long by 0.2 mm wide in ovarian region, and with shoulder-like expansions slightly anterior to level of genital aperture. Prohaptoral suckers 0.020 by 0.015 mm, armed with minute denticle-like papillae around aperture and in cavity of suckers (Fig. 34). Opisthohaptor with 34 clamps, 0.030 to 0.050 mm wide, and with anchor-bearing languette between 12th and 13th clamps, counting from right side. Anchors of typical *Axine* shape, both outer and inner pairs about 0.030 mm long. Pharynx oval, 0.020 by 0.015 mm; remainder of digestive tract apparently as in other axinids. Genital aperture about 0.2 mm from anterior end; genital atrium unarmed. Cirrus armed with several rows of straight spines about 0.010 mm long. Testes about 20 in number, postovarial, in median field. Ovary U-shaped, in equatorial zone. Vaginal aperture dorsal, median, about 0.2 mm from

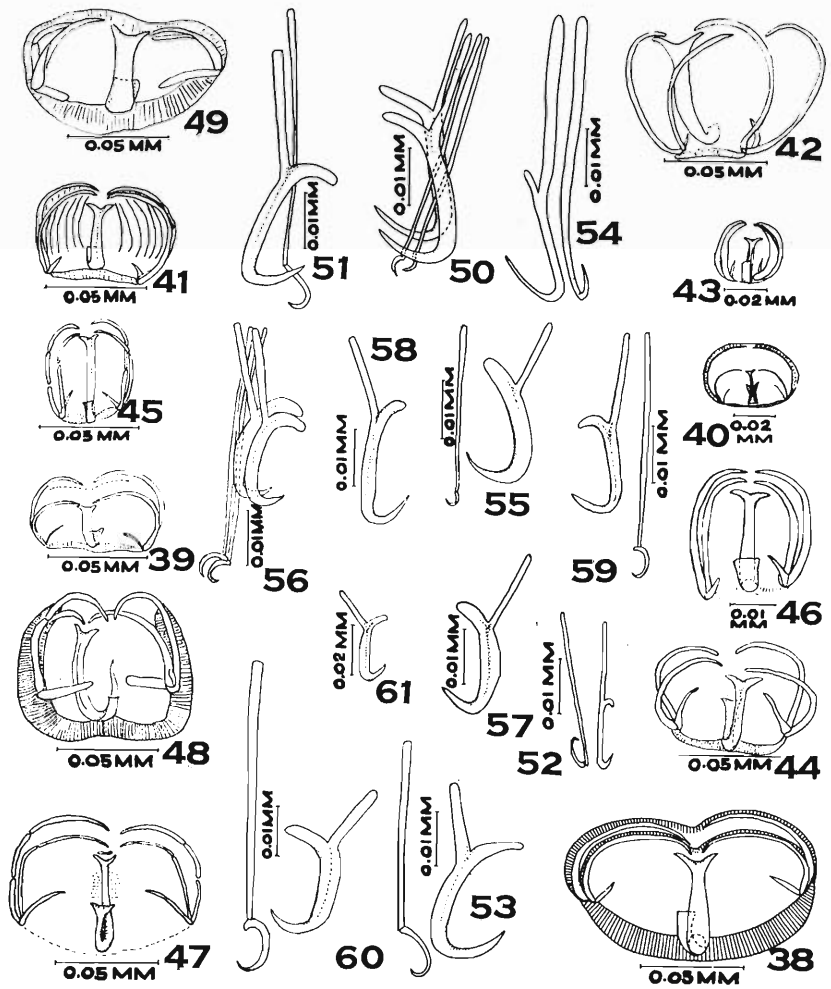
genital aperture, armed with horn-like spine; vagina armed with small conical spines. Vitellaria extending from about 0.1 mm posterior to level of vaginal aperture into haptoral region. Eggs not present in specimens available.

HOST: *Strongylura marina.*

LOCATION: Gills.

DISTRIBUTION: United States (New York Aquarium).

SPECIMENS: U.S.N.M. Helm. Coll. No. 37722 (holotype and paratype).



38-61. Opisthohaptoral clamps and anchors. 38-49. Clamps: 38. *Axine belones;* 39. *A. cypseluri;* 40. *A. hyporamphi;* 41. *A. yamagutii;* 42. *Heteraxinoides inada;* 43. *Axinoides meservei;* 44. *A. oceanicum;* 45. *A. raphidoma;* 46. *A. strongylurae;* 47. *Nudaciraxine gracilis;* 48. *Chlamydaxine resplendens;* 49. *C. truncatus;* 50-61. Anchors: 50. *Axine belones;* 51. *A. cypseluri;* 52. *A. hyporamphi;* 53. *A. yamagutii;* 54. *Heteraxinoides inada;* 55. *Axinoides meservei;* 56. *A. oceanicum;* 57. *A. raphidoma;* 58. *A. strongylurae;* 59. *Nudaciraxine gracilis;* 60. *Chlamydaxine resplendens;* 61. *C. truncatus.*

collected May 5, 1916 by G. A. MacCallum.

In many respects *A. strongylurae* resembles *A. raphidoma*. Aside from host, there are differences in the framework of the opisthohaptoral clamps as well as in their number. The most outstanding character is in the shoulder-like expansions of the forebody, assuming that they are not artifacts produced by injury during collection.

Axinoides oceanicum (Caballero, Bravo-Hollis and Grocott, 1954) new comb.
(Figures 8, 20, 32, 44, 56)

SYNONYM: *Microcotyle oceanicum* Caballero, Bravo-Hollis and Grocott, 1953.

DESCRIPTION: Body slender, 3.3 to 3.5 mm long, more or less uniform in width except in anterior portion which is gradually attenuated. Prohaptoral suckers 0.048 by 0.036 mm, armed with minute, tooth-like papillae distributed as shown in Fig. 32. Opisthohaptor of typical shape (folded in holotype) bearing 36 clamps about 0.1 mm wide, and 2 pairs of anchors located 12 to 15 clamp spaces from right side; outer anchors 0.030 to 0.032 mm long, inner 0.054 mm long. Pharynx about 0.023 by 0.016 mm; esophagus bifurcating at level of genital aperture; remainder of digestive tract similar to that of other axinids. Genital aperture about 0.490 mm from anterior end of body in holotype; genital atrium unarmed. Cirrus armed with 2 or 3 alternating rows of spines about 0.012 mm long. Testis numerous (more than 50), postovarial. Ovary somewhat J-shaped, with proximal limb twisted. Seminal vesicle, genito-intestinal canal, and vitelline duct complex similar to that in *Axine belones*. Vaginal aperture dorsal, median, about 0.290 mm from genital aperture, armed with horn-like spine; vagina provided with tubercle-like spines. Vitellaria extending from a short distance distal to level of vaginal aperture to opisthohaptoral region. Eggs not present in specimens available.

HOST: *Tylosurus fodiator*.

LOCATION: Gills.

DISTRIBUTION: Panama (Fuerte Amador, Canal Zone).

SPECIMENS: Coll. Helm. Inst. Biol. (Mexico) No. 25-13.

The description by Caballero, Bravo-Hollis and Grocott (1953) of this species, under the name of *Microcotyle oceanicum*, is very complete except for certain details. The presence of opisthohaptoral anchors was overlooked; this together with ovarian shape and presence of an armed, median vaginal aperture are sufficient to place it in the genus *Axinoides*. The above brief description was made possible through the courtesy of Dr. Eduardo Caballero y C. who graciously loaned the type specimens to the writer.

GENUS *Neoaxine* Price, 1946

SYNONYM: *Amonaxine* Unnithan, 1957.

DIAGNOSIS: Genital atrium unarmed; cirrus armed. Vaginal aperture dorsolateral, armed with incomplete circle of curved spines. Other characters as for subfamily.

TYPE SPECIES: *Neoaxine constricta* (Yamaguti, 1938) Price, 1946. (Syn. *Amonaxine constricta* (Yamaguti, 1938) Unnithan, 1957).

This genus was proposed by Price (1946) to include *Axine* (*Axine*) *constricta* which was described by Yamaguti (1938) from the gills of *Tylosurus schismatorhynchus* from Japan. This species is typically axinoid in morphology except for the apparent absence of opisthohaptoral anchors. A

careful reexamination of the original specimens would no doubt reveal their presence, since they have been found in all species of the family whenever a search has been made for them.

Nudaciraxine new genus

DIAGNOSIS: Genital atrium and cirrus unarmed. Vaginal aperture dorsal, about midway between median line and body margin, armed with horn-like spine. Other characters as for subfamily.

TYPE SPECIES: *Nudaciraxine gracilis* (Linton, 1940) n. comb.

At present, this genus contains only the type species which is redescribed as follows:

Nudaciraxine gracilis (Linton, 1940) new comb. Figures 11, 23, 35, 47, 59)

SYNONYMS: *Axine gracilis* Linton, 1940; *Axinoides gracilis* (Linton, 1940) Sproston, 1946.

DESCRIPTION: Body elongate, 1.9 to 5.1 mm long by 0.2 to 0.5 mm wide in region of ovary; anterior end bifid, attenuated, with 4 groups of cervical glands. Prohaptoral suckers oval, 0.038 to 0.047 mm long by 0.028 to 0.038 mm wide, with a band of minute denticle-like papillae around opening and with a band of similar papillae extending toward base of suckers (Fig. 35). Opisthohaptor wing-like, 0.7 to 1.6 mm wide, provided with 42 to 82 clamps, about 0.075 to 0.100 mm wide, arranged in a single row along margin; lateral sclerites of clamps slender and broken. Opisthohaptoral anchors present, typically axinid, their position indicated by indentation of haptoral border; outer anchors 0.032 to 0.038 mm long, inner 0.044 to 0.047 mm long. Pharynx oval about 0.038 mm long by 0.028 mm wide; esophagus bifurcating at or slightly anterior to genital aperture; remainder of digestive tract similar to that of other axinids. Genital aperture 0.3 to 0.6 mm from anterior end of body; genital atrium unarmed but provided with transverse ridges or striae. Cirrus unarmed. Testes oval, 20 to 22 in number, tandem, postovarial. Ovary J-shaped, about one-third of body length from anterior end; seminal receptacle present, immediately cephalad of right pole of ovary; genitointestinal canal present, opening into right intestinal limb a short distance anterior to level of seminal receptacle. Vitelline duct slender, branching about midway between level of vaginal aperture and ovary; vitellaria extending from slightly distal to vaginal aperture to opisthohaptor. Vaginal aperture dorsal, about 0.4 mm posterior to genital aperture, situated about midway between median line and body margin, armed with horn-shaped spine 0.038 to 0.043 mm long by 0.010 to 0.013 mm wide at base. Eggs not present in specimens available.

HOST: *Strongylura marina.*

LOCATION: Gills.

DISTRIBUTION: United States (Woods Hole, Massachusetts, New York Aquarium, and Alligator Harbor, Florida, vide Hargis (1956).

SPECIMENS: U.S.N.M. Helm. Coll. Nos. 8168 (holotype), coll. Edwin Linton, Woods Hole, Mass., September 10, 1912; 8167 (paratype), coll. Edwin Linton, Woods Hole, Mass., August 30, 1911; 37723, (6 specimens), coll. G. A. MacCallum, Woods Hole, Mass., Aug. 17, 1922; 37724, (5 specimens), coll. G. A. MacCallum, New York Aquarium, October 26, 1916; and 37725, (5 specimens), coll. G. A. MacCallum, New York Aquarium, October 31, 1916.

This species was inadequately described by Linton (1940) from *Strongylura*

marina collected at Woods Hole, Mass. A comparison of the data on body sizes, number of opisthohaptoral clamps, etc., as obtained from the various collections listed above, indicates that this is somewhat variable species. In spite of the variations noted, nothing consistent was observed to indicate that a mixture of species was involved.

GENUS *Chlamydaxine* Unnithan, 1957

DIAGNOSIS: Body triangular. Genital atrium unarmed; cirrus armed. Vaginal aperture dorsolateral, armed with horn-like spine. Other characters as for subfamily.

TYPE SPECIES: *Chlamydaxine truncatus* (Hargis, 1956) Unnithan, 1957.

INCLUDED SPECIES: *Chlamydaxine resplendens* (Caballero, Bravo-Hollis and Grocott, 1954) n. comb.

Chlamydaxine truncatus (Hargis, 1956) Unnithan, 1957 (Figures 13, 25, 37, 49, 61)

SYNONYM: *Axinoides truncatus* Hargis, 1956.

DESCRIPTION: See Hargis (1956). Aside from the nature of the cirrus, the original description appears to be as complete as the condition of the specimens permits. Hargis stated that the cirrus was provided with "finger-like papillae in distal portion of lumen" but a study of the holotype shows that the end of the cirrus is provided with a corona of 2 alternating rows of 0.004 mm long spines. The peculiar arrangement of the opisthohaptoral clamps is possibly an illusion due to a folding over of the right side of the haptor. Study of additional, better preserved and stained material is necessary to determine this.

HOST: *Tylosurus raphidoma.*

LOCATION: Gills.

DISTRIBUTION: United States (Alligator Harbor, Florida).

SPECIMEN: U.S.N.M. Helm. Coll. No. 38157 (holotype).

Chlamydaxine resplendens (Caballero, Bravo-Hollis and Grocott, 1954) n. comb. (Figures 21, 24, 36, 48, 60)

SYNONYM: *Axine resplendens* Caballero, Bravo-Hollis and Grocott, 1954.

DESCRIPTION: Body triangular, 3.5 mm long by 3.2 mm wide at posterior end and attenuated anterior to level of genital aperture. Prohaptoral suckers oval, 0.020 mm by 0.016 mm, without tooth-like papillae. Opisthohaptor consisting of a row of clamps, about 0.100 mm wide, along posterior end of body, and with a minute languette armed with characteristic axine-type anchors; outer anchors about 0.032 mm long and inner about 0.054 mm long. Pharynx 0.032 mm long by 0.020 mm wide; esophagus bifurcating posterior to level of genital aperture; intestinal limbs with short median and long-branched, lateral diverticula, extending to near posterior end where they approach and may be confluent. Genital aperture median, 0.064 mm from anterior end; genital atrium unarmed. Cirrus armed with about 8 alternating rows of short, 0.005 mm long, spines. Testes very numerous, postovarial. Ovary folded, with distal end directed anteriad. Vaginal aperture dorsomarginal, armed with horn-like spine about 0.050 mm long by 0.020 mm wide at base. Vitellaria extending from level of transverse vitelline ducts to opisthohaptoral region. No eggs present in specimen available.

HOST: *Tylosurus fodiator.*

LOCATION: Gills .

DISTRIBUTION: Panama (Fuerte Amador, Canal Zone).

SPECIMEN: Coll. Helm. Inst. Biol. (Mexico) No. 25-24 (holotype).

An examination of the holotype specimen was made possible through the kindness of Dr. Edwardo Caballero y C. Aside from the structure of the anterior end, the original description (Caballero *et. al.*, 1954) is adequate and as complete as possible from the single specimen on which the species is based. According to the original description, the anterior end is provided with a minute oral sucker and the illustration shows no evidence of prohaptoral suckers. According to the writer's observation, the customary prohaptoral suckers are present; the essential difference in this respect between this form and other axinids is that the anterior end is not bifid but is in the form of a rounded lobe.

This species is included in the genus *Chlamydaxine* since the body shape, unarmed genital atrium, armed cirrus, and lateral position of the vaginal aperture makes its close relationship to the genotype, *C. truncates*, a reasonable certainty.

GENUS *Loxura* Unnithan, 1957

DIAGNOSIS: Body elongate. Genital atrium unarmed; cirrus pineapple-shaped, armed with numerous spines. Vaginal aperture sublateral, armed with horn-like spine. Other characters as for subfamily.

TYPE SPECIES: *Loxura ananaphallus* Unnithan, 1957.

The type and only species occurs on the gills of *Tylosurus leiurus* in India.

Loxuroides new genus

SYNONYM: *Loxura* Unnithan, 1957, in part.

DIAGNOSIS: Genital atrium armed with incomplete rows of spines; cirrus cushion-shaped, armed. Other characters as for *Loxura*.

TYPE SPECIES: *Loxuroides sasikala* (Unnithan, 1957) n. comb.

The type and so far the only species of this genus was described as *Loxura sasikala* by Unnithan (1957) from the Indian fish *Cypselurus oligolepis*. It is separated from *Loxura* because the nature of the armament of the genital atrium appears sufficiently distinctive to warrant generic status.

Subfamily *INDOCOTYLINAE* Tripathi, 1959

DIAGNOSIS: Opisthohaptoral clamps in 2 equal rows of 4 clamps each, rows separated by anchor bearing languette. Genital atrium armed, cirrus armed or (?) unarmed. Vaginal aperture dorsal, median or sublateral, armed with horn-like spine or (?) unarmed. Other characters as for family.

TYPE GENUS: *Indocotyle* Tripathi, 1959.

This subfamily was placed by Tripathi (1959) in the family Discocotylidae. However, the opisthohaptoral clamp structure and anchors are typically axinoid and properly belong in the family Axinidae.

Key to genera of Indocotylinae

Vaginal aperture dorsal and median, (?) unarmed ..... *Indocotyle* Tripathi
Vaginal aperture dorsal and sublateral, armed ..... *Oligapta* Unnithan

GENUS *Indocotyle* Tripathi, 1959

DIAGNOSIS: Genital atrium armed; cirrus (?) unarmed. Vaginal aperture dorsal and median, (?) unarmed. Other characters as for subfamily.

TYPE SPECIES: *Indocotyle hemirhamphae* Tripathi, 1959.

The type and only species so far allocated to *Indocotyle* was described from the gills of *Hemirhamphus georgi* (sic) from India. The indicated absence of cirrus spines, or the possible confusion of cirrus spines with atrial spines, as well as the absence of a vaginal spine should be reinvestigated.

GENUS *Oligapta* Unnithan, 1957

DIAGNOSIS: Genital atrium and cirrus armed. Vaginal aperture dorsal and sublateral, armed with horn-like spine. Other characters as for subfamily.

TYPE SPECIES: *Oligapta oligapta* Unnithan, 1957.

This genus was based by Unnithan (1957) upon specimens collected from the gills of *"Hemiramphus georgeii"* from India. Its close similarity to *Indocotyle* Tripathi is evident by the nature of the opisthohaptor.

LITERATURE CITED

BYCHOWSKY, B. E. 1957. Monogenetic trematodes, their systematics and phylogeny. (In Russian), Akad. Nauk. SSR: 1-509.

CABALLERO, EDUARDO; BRAVO-HOLLIS, MARGARITA; and GROCOTT, ROBERT G. 1953. Helmintos de la Republic de Panama. VII. Descripción de algunos tremátódos de peces marinos. An. Inst. Biol., Mexico, 24: 97-136.

————. 1954. Helmintos de la Republic de Panama. XII. Descripción de dos nuevos tremátodos monogéneos, parásitos de peces marinos comestibles del Oceano Pacifico del Norte. Ciencia, 14: 81-86.

DAWES, BEN. 1947. The Trematoda of British fishes. 364 pp. London.

EUZET, LOUIS. 1955. Larves gyrodactyloïdes nageantes de quelques trématodes monogénétiques de poissons marins. Bull. Soc. Neuchâtel. Sc. Nat., 3 s., 78: 71-79.

————. 1957. Larves gyrodactyloïdes neagantes de quelques Microcotylidae. Idem., 80: 186-194.

GOTO, SEITARO. 1894. Studies on the ectoparasitic trematodes of Japan. J. Coll. Sc. Imp. Univ., Japan, Tokyo, 8: 1-173.

HARGIS, WILLIAM J., JR. 1956. Monogenetic trematodes of Gulf of Mexico fishes. Part XI. The family Microcotylidae Taschenberg, 1879 (continued). Proc. Helminth. Soc. Wash., 23: 153-162.

————. 1959. Systematic notes on the monogenetic trematodes. Idem., 26: 14-31.

ISHII, NOBUTARO and SAWADA, TOSHISADA. 1938. Studies on the ectoparasitic trematodes. (III). Jap. Jour. Expr. Med., 16: 239-249.

JORDAN, DAVID STARR; EVERMANN, BARTON WARREN; and CLARK, HOWARD WALTON. 1930. Check list of fishes and fishlike vertebrates of North and Middle America, etc. 670 pp. Washington.

LINTON, EDWIN. 1940. Trematodes from fishes mainly from the Woods Hole region of Massachusetts. Proc. U. S. Nat. Mus. (3078), 88: 1-172.

LORENZ, LUDWIG. 1878. Ueber die Organisation der Gattungen Axine and Microcotyle. Arb. Zool. Inst. Univ. Wien, 1: 405-436.

MESERVE, FRANK G. 1938. Some monogenetic trematodes from the Galapagos Islands and the neighboring Pacific. Allen Hancock Pacific Expeditions, 2: 29-88.

PRICE, EMMETT W. 1946. A note on the genus *Axine* Abildgaard (Trematoda: Monogenea). Jour. Parasitol., 31(suppl.): 22 (1945).

————. 1961. North American monogenetic trematodes. IX. The families Mazocraeidae and Plectanocotylidae. Proc. Biol. Soc. Wash., 74: 127-156.

SPROSTON, NORA G. 1946. A synopsis of the monogenetic trematodes. Tr. Zool. Soc. London, 25: 185-600.

TRIPATHI, YOGENDRA R. 1959. Monogenetic trematodes from fishes of India. Ind. Jour. Helminth., 9: 1-149 (1957).

UNNITHAN, R. VISWANATHAN. 1957. On the functional morphology of a new fauna of Monogenea on fishes from Trivandrum and environs. Part I. Axinidae fam. nov. Bull. Centr. Research Inst. Univ. Kerala, 5: 27-122.

YAMAGUTI, SATYU. 1938. Studies on the helminth fauna of Japan. Part 24. Trematodes of fishes, V. Jap. Jour. Zool., 8: 15-74.

————. 1943. Verzeichnis der ektoparasitischen Trematoden der Japanischen Fische. Privately published. 3 pp.

The Description, Feeding Habits, and Life History of *Neotylenchus linfordi* n. sp.; A Mycophagous Nematode*

HELEN CAROL HECHLER**

DESCRIPTION

This nematode is named *Neotylenchus linfordi* n. sp. in recognition of the many contributions of Dr. Maurice B. Linford to the science of nematology and also of the work he did on the feeding habits of this nematode just prior to his death.

All specimens described were from active cultures with many gravid females. Measurements were made on specimens relaxed 11 to 14 minutes in small Syracuse dishes placed in an oven at 54° C. Some were fixed in FAA or 5% formalin, and some were mounted in glycerine after fixation. Many anatomical details were observed on live and crushed nematodes.

FEMALES: (78) L = 0.70 mm. (s ± 10.465 mm.) (0.49-0.91; a = 30(25-35); b = 5.1 (3.6-6.7); c = 8.0 (5.7-12.8); V = $^{39\text{-}75}$ 79 (74-90) $^{2.4\text{-}3.7}$.

MALES: (18) L = 0.52 mm. (s ± 1.010 mm.) (0.45-0.60); a = 28 (24-33); b = 4.3 (3.3-5.5); c = 15 (13-20); T = 59 (47-69).

HOLOTYPE FEMALE: L = 0.61 mm.; a = 27; b = 4.7; c = 7.6; V = 47/ 79 3.2/.

ALLOTYPE MALE: L = 0.55 mm.; a = 27; b = 5.5; c = 16; T = 65.

FEMALES: (Fig. 1) Body slender, tapering at both ends. Cuticle 1 micron thick near middle of body, 0.6 microns at extremities. Striations 0.7 microns apart opposite stylet, 1.4 microns apart near middle of body. Lateral field 13% of body width with four incisures, the outer two slightly crenate. Incisures reduced to three that are 1 to 2 body widths in front of deirid, then to two, then to one, disappearing one stylet length behind stylet. Incisures reduced to three at anus, disappearing one half tail length behind anus. Deirids at latitude of hemizonid. No phasmids seen.

Head rounded, symmetrical, not offset. No annules on lips. Head framework eight parted, papillae on four submedial lips, amphids on lateral lips. Lateral lips reduced, located on periphery of head (Fig. 1-C).

Stylet 9-10 microns long, tylenchoid. Knob group 2 microns wide, subventral knobs slightly notched. Excretory pore prominent, located opposite anterior basal bulb. Highly refractive excretory tube often visible 2 or 3 body widths behind beginning of intestine. Hemizonid 3 to 4 striations wide, 2 to 5 striations anterior to excretory pore. Nerve ring 1 to 2 body widths anterior to excretory pore.

Corpus of esophagus elongate, fusiform, without a true median bulb. Dorsal duct opening 1 micron behind stylet. Esophageal lumen sinuous in live specimens, straight in fixed ones. Walls of lumen comparatively thick and refractive in anterior 35% of esophagus, thinner posterior to opening of ventral gland duct. Basal bulb elongate-pyriform, terminating in a more or less elongate extension similar to that of the Paurodontinae. It differs from this group in that the cuticularized lumen does not reach the end of the extension, and the tissue is organized differently in the extension than in the bulb. Lumen off center, usually to the ventral side.

*Professor Gerald Thorne of Madison, Wisconsin gave helpful advice about both the taxonomic position of this nematode and the preparation of the drawings. His kindness is gratefully acknowledged.

**Research Assistant, Department of Plant Pathology, University of Illinois, Urbana, Illinois.

Anterior end of intestine characterized by thin-walled chamber overlapping base of bulb. Behind chamber intestinal lumen narrows gradually to a sinuous tube passing between alternate triangular cells which give the nematode a tesselated appearance (Fig. 2D). Tesselation and chamber obscured by dark granules in well fed specimens. Lumen widens slightly just in front of rectum. Rectum 9 microns long, curved. Anus usually visible but inconspicuous in fixed specimens.

Vulva a transverse slit 38-41% of body width. Vagina one fourth to one third of body diameter, walls about 1 micron thick. Ovary single, anterior, outstretched, oocytes in single file, rarely in double rows through part of its length. Ovary usually extends to about three body widths behind basal bulb, although a few seen at level of nerve ring. Post vulval uterine branch round, convoluted, length one half distance from vulva to anus. Uterus elongated, walls thick and convoluted. Sperm in uterus and post vulval branch.

MALES: (Fig. 1-F, G) Male similar to female in anterior part. Lateral field reduced to 2 striations at level of spicules, continued along bursa to near tip of tail. Stylet 8 to 9 microns long. Testis outstretched, occasionally reflexed. Spermatocytes in one to three rows. Vas deferens one fourth length of testis. Sperm round, 0.4 microns in diameter. Spicules cephelated, curved, 20 microns long. Gubernaculum curved, one fourth length of spicules. Anus oval, lips protruding. Tail narrow with rounded terminus. Bursa extends from head of spicules to beyond tip of tail. Edges slightly crenate, occasionally with a distinct lobe near terminus.

DIAGNOSIS: *N. linfordi* differs from all previously described *Neotylenchus* by the long extension of the base of the esophagus. Three others, *N. latus* Thorne, 1935, *N. thornei* Meyl, 1955, and *N. italicus* Meyl, 1955, have a post vulvar sac. *N. linfordi* differs from *N. latus* by the bursa extending over the tip of the tail, the longer spear with notched knobs, and oocytes in a single row. It differs from *N. italicus* in absence of a tail peg, and from *N. thornei* by the pointed terminus of the female.

TYPE LOCALITY: Niles, Illinois.

TYPE HABITAT: Soil from around roots of a greenhouse-grown *Clematis* sp.

EXPERIMENTAL METHODS

Stock cultures were maintained in Petri dishes of 1/5 Difco potato dextrose agar and 4/5 2% water agar. The fungus used was *Pyrenochaeta terrestris* (Hansen) Gorenz, J. C. Walker, and Larson isolated from spelt roots. Its advantages for nematode culture are the thin walled, closely spaced hyphae, sparse spore production, and the growth rate is slow enough that it does not completely cover the dish, yet rapid enough for the nematodes to complete several generations before the fungus is killed. One disadvantage is a red pigment which obscures observations except at the margin of the colony. The nematodes developed better on one-fifth strength PDA than on full strength, possibly because a lower concentration of metabolic products of the fungus was present.

FEEDING STUDIES: Observations and photographs of feeding were made using nematodes which had been transferred with a sterilized needle to four-day-old fungus cultures on 2% water agar. A sterilized cover slip was placed over them and observations were begun immediately. Nematodes feeding directly against the coverslip could be watched with a 60X oil immersion lens.

LIFE HISTORY STUDIES: All life history studies were made at 22-25° C. Egg development was observed in small dishes of sterilized water by aid of a

40X water immersion lens. The number of larval stages was determined by sorting molting nematodes into size groups and examining them and intermediate sized larvae for stylet size and genital-primordium variations. Colonies from a single female were examined at least twice each day for presence of eggs, newly hatched larvae, and molting nematodes to establish the period between molts and the length of the life cycle.

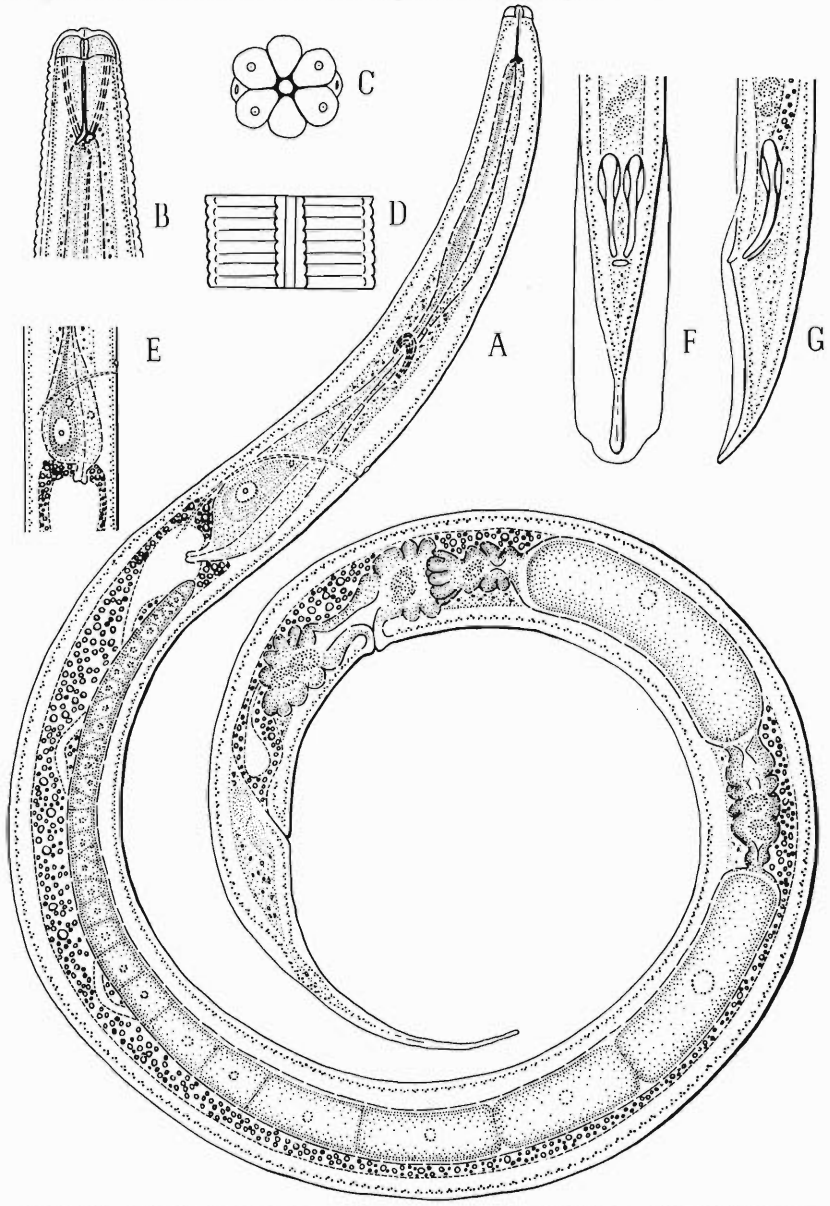


Figure 1. *Neotylenchus linfordi* n. sp. A. Female 700×; B. Head 1500×; C. Face view 2500×; D. Lateral field 1000×; E. Variation of basal bulb 700×; F. Male tail, ventral view 750×; G. Male tail, lateral view 750×.

FEEDING

PRELIMINARY ACTIVITIES: This nematode moves sinuously through the agar, thrusting the head from side to side until the lips touch a hyphal strand. Immediately it begins rhythmic probing with the stylet while moving sidewise along the hypha until penetration is achieved, usually near a septum. On coenocytic mycelium penetration is usually extremely difficult. It is probably aided by the greater structural rigidity of the cells of the septate hyphae. If penetration fails the nematode finally moves away, often still moving the stylet spasmodically.

ACTIVITIES OF DORSAL DUCT: In a nematode which has recently fed and in which the body reserves are not excessively depleted, the feeding process usually follows variations of this general pattern: Immediately after stylet insertion the dorsal duct gradually widens. After 1 to 2 minutes a hyaline fusiform vacuole appears just behind the juncture of duct with esophagus and enlarges rapidly until it is about one stylet length long and 1 ½ to 2 times as wide as the stylet knob group (Fig. 2-E). Granular material from the basal bulb flows forward around the vacuole and collects at the base of the stylet. Gradually the granules replace the vacuole, filling from the front posteriad. Occasionally the forward streaming of the granules stops and they circulate in front of the vacuole and in the rest of the dorsal duct. After 1 to 2 minutes regular forward flow resumes. When intestinal contraction begins after 5 to 15 minutes the remaining vacuole disappears and the dorsal duct width shrinks. A narrow stream of granules continues to flow toward the stylet while pumping progresses.

ACTIVITIES OF INTESTINE: Ingestion of food is apparently accomplished solely by rhythmic contractions of the thin-walled chamber in the anterior end of the intestine (Fig. 2-A). The anterior half of the intestine moves forward to compress the chamber, then moves back to the resting position. This motion proceeds at a uniform rate of 6 to 8 strokes per minute and continues 35 minutes to 3 hours. It is most pronounced immediately against the chamber and becomes progressively less posteriorly until it is very slight at about the mid point. During compression accordion folds develop in the wall of the chamber, which disappear as the chamber is relaxed (Fig. 2-C). These activities show best in starved specimens with little intestinal content since as feeding continues dark granules in the chamber conceal the folding.

ACTIVITIES OF ESOPHAGUS: Forward motion of the intestine is accompanied by a backward diagonal motion of the esophagus by which the lumen is pushed or pulled from a ventral to a central position in the chamber (Fig. 2-B). The base of the lumen appears softer and less refractive than the anterior part, and may act as a valve. Presumably as the intestine moves forward the lumen is squeezed together and chamber content is prevented from moving into the esophagus, but instead is forced back into the intestine proper. As the intestine relaxes the lumen opens and material is pulled into the chamber.

Immediately after the final contraction of the chamber the nematode retracts its stylet and moves away. Once a nematode is established in a favorable feeding location, it rarely ranges far over the agar, but withdraws the stylet from one feeding site and inserts it again as soon as it touches another strand.

EFFECTS OF GLANDULAR MATERIAL: Although no granules can be seen flowing from the stylet tip into the hyphal cell at any time during the feeding process, the hyphal cytoplasm is modified (Fig. 3-A-D). It becomes more granular

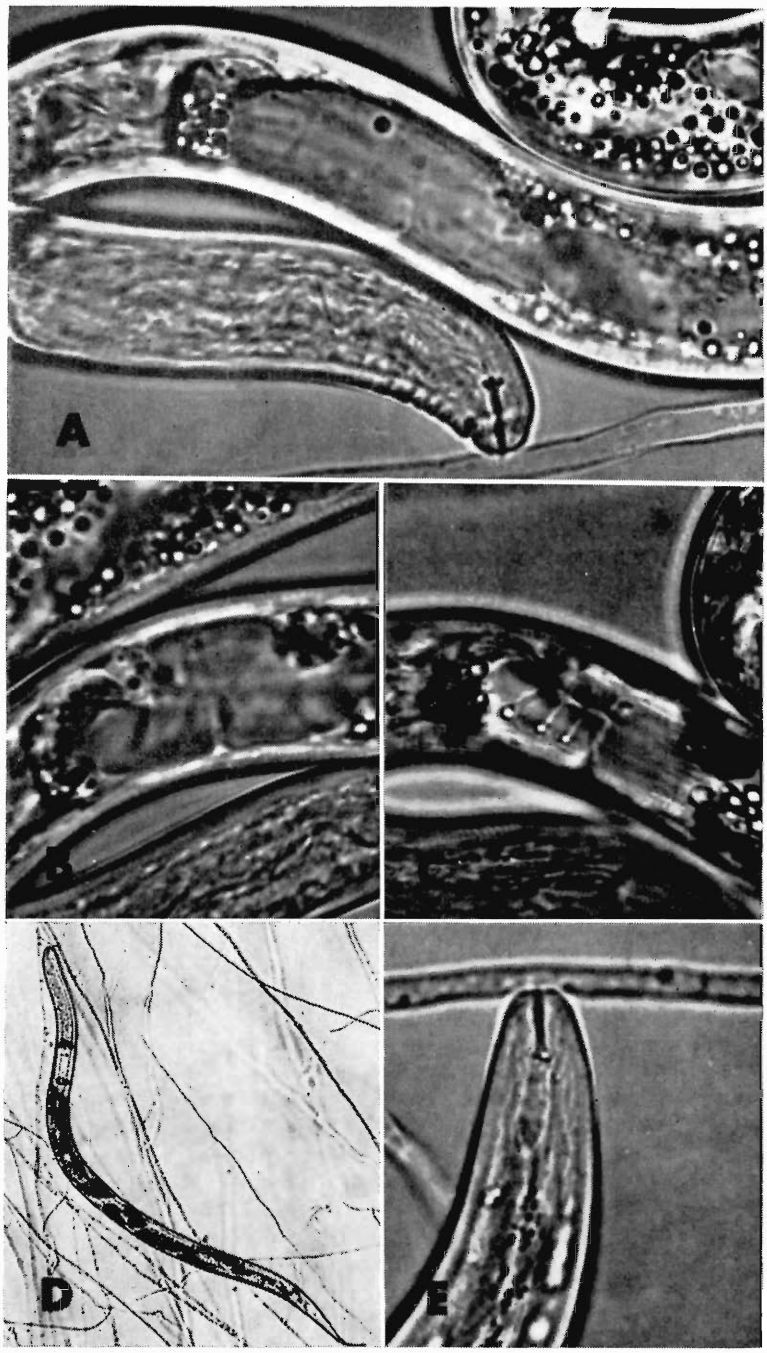


Figure 2. A. Nematode feeding, chamber relaxed; B. Chamber contracted, focus on lumen; C. Chamber contracted, focus on wall; D. Nematode feeding, showing intestinal tesselation; E. Head showing dorsal duct vacuole.

and less vacuolate, even before intestinal pumping begins. Later it becomes more hyaline. These changes progress from cell to cell during the feeding period, often as far as six cells away from the stylet. It seems reasonable to assume that the nematode is injecting an extra-oral digestive enzyme. The sudden shrinkage of the dorsal duct at the beginning of intestinal contraction suggests that an especially large injection occurs at this time. It is not clear whether the hyphal septa are completely digested away or only made more highly permeable. Ingestion lasts so long at one site, however, that it seems probable that the nematode receives food from three to six cells on either side of the stylet.

In a nematode which has been starved for several days with very few reserves in the intestine, the flow of granular material in the dorsal duct is slight. Intestinal contraction begins within 1 to 2 minutes after stylet insertion and continues only a few minutes. It is possible that such a nematode ingests food from only one hyphal cell, or even from only a part of the cell, the supply of enzyme being insufficient to modify additional cytoplasm.

In young fungus colonies containing only a few nematodes, the effects of feeding show 2 or 3 days after the nematodes are added as small dark patches visible to the unaided eye.

The most detailed observations were made on *Pyrenochaeta terrestris* as described above. Similar nematode activities and hyphal modifications also occur with the following fungi: *Epicoccum nigrum* Link, *Fusarium oxysporum* Schlechtendahl, *F. moniliforme* Sheldon, *Gliocladium roseum* (Link) Thom, *Helminthosporium sativum* Pammel, King, and Bakke, *Lentinus lepideus* Fries, *Poria monticola* Murrill, and *Sclerotium bataticola* Taubenhaus.

On *Pythium arrhenomenes* Drechsler, a coenocytic fungus, five or six pumping strokes occur only during pauses in protoplasmic streaming and the nematode immediately moves away. There is no pumping during protoplasmic streaming and no extended pumping as on septate hyphae. Probably the digestive enzyme and modified cytoplasm are carried away in the stream and the nematode can ingest only the modified material which accumulates around the stylet during the short pause.

DISCUSSION: In Petri dishes with the fungus as the only source of food, the progeny of single gravid females reached 40,000 to 70,000 in thirty days. Further population growth seems limited by various factors, including death and senescence of the fungus, senescence of the nematodes, and even invasion of the nematodes by the fungus. The nematodes feed effectively only on the young hyphae at the periphery of the colony, and as the cultures age many dead nematodes, presumably killed either by metabolic products of both fungus and nematodes or by starvation, are found in the center with their bodies filled with hyphae.

The nematodes often spend 1 or 2 hours at a single feeding site while they presumably withdraw food from only 6 to 10 cells. This plus the comparatively sparse distribution of this group in nature might suggest that the feeding process is inefficient. However, the rate of population increase, which is similar to that of other mycophagous nematodes studied under the same conditions, indicates the mechanism is quite adequate.

LIFE HISTORY

EGG PRODUCTION: A well-fed female produces 7 to 8 eggs a day. The oocytes move slowly along the oviduct. As they pass into the uterus they separate from the row of cells and both ends become rounded, fertilization

occurs, and the egg shell is laid down. As the moment of deposition nears the egg moves spasmodically toward the vagina. A small portion of the end of the egg squeezes into the vagina and expands again outside the vulva. This bubble-like expansion slowly grows as the egg is pushed along until, when about ¼ of the egg is out the remainder is expelled all at once. Nematodes lay eggs without interrupting the rhythm of the feeding process.

Eggs are 4.8-5.9 × 1.7-1.9 microns, straight to slightly curved, finely rugose, unsegmented when laid.

EMBRYO DEVELOPMENT: The protoplasm rounds up and pulls away from the egg shell about 15 minutes after the egg is laid. The first division occurs in 2 ½ to 3 hours. As in *Radopholus similus* this division is perpendicular to the long axis of the egg and results in two cells, one larger than the other (Van Weerdt, 1960). About 1 ½ hours later first the larger, then the smaller cell divides, resulting in four cells arranged in single file. In all eggs examined no cells shifted out of line. All the cells then divide parallel to the

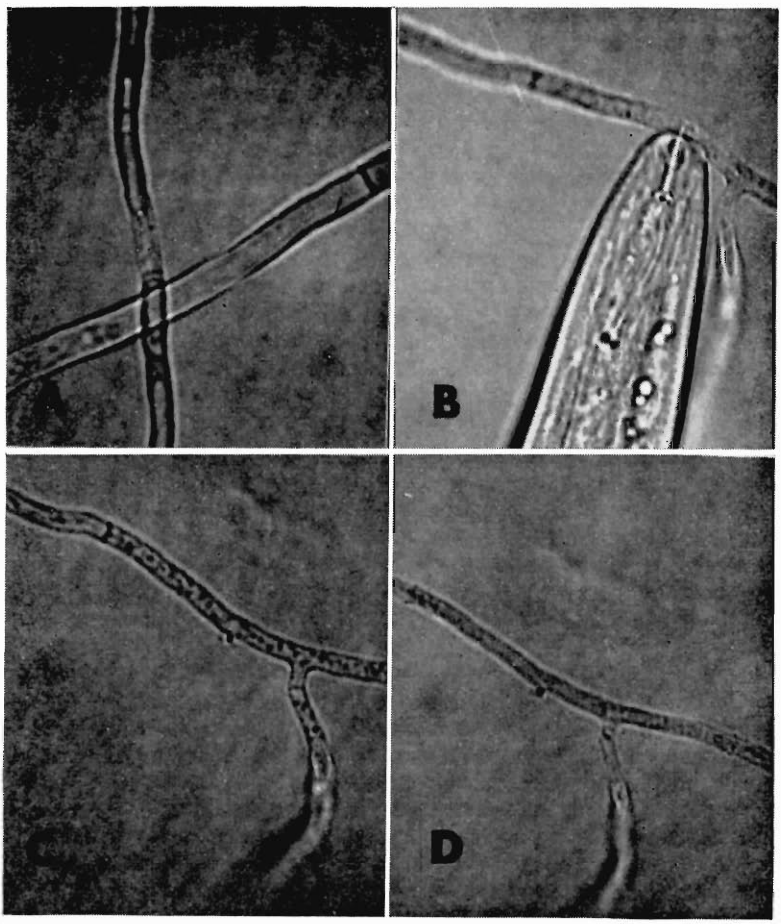


Figure 3. A. *Pyrenochaeta terrestris*, no nematodes; B. *P. terrestris*, nematode has fed 15 minutes; C. *P. terrestris*, nematode has fed 30 minutes and just moved away; D. *P. terrestris*, 3 hours after nematode moved away.

long axis of the egg, starting with the middle cells and ending with the smaller end cell. Eight cells are present after about one hour. Cell divisions continue until, about 22 to 24 hours after the egg was laid, the embryo has assumed a vermiform shape with the wider head end more hyaline than the tail.

FIRST STAGE LARVAE: Motion of the head end, and a little later a twisting of the whole embryo begins when it is about 1 ½ times as long as the egg. When twice egg length it begins to move back and forth. The various movements may be necessary for proper muscular development. One first-stage larva from a crushed egg was 240 microns long, 11 microns wide, extremely translucent, with no stylet, esophageal modifications, or intestinal granules visible. No stylet remains were seen in any cast cuticle during the first molt, which occurs 43 to 48 hours after the egg is laid.

SECOND STAGE LARVAE: When activity resumes, the larva moves back and forth and with a twisting motion, stretching the egg shell as hatching time approaches, and its fully developed stylet probes spasmodically. The stylet was never seen to penetrate the shell. The bursting of the shell seems to be an effect of the nematode trying to straighten itself out. Hatching occurs 50 to 60 hours after the egg is laid. The larvae always emerge from the end of the egg, never from one of the long sides.

L = 0.19-0.25 mm.; a = 21-25; b = 2.2-2.6; c = 4.8-6.4 G = $^{2.4\text{-}4.8}$ 55-60 $G = \frac{\text{Distance from head to posterior of genital primordium}}{\text{Total body length}} \times 100$; Stylet = 7.0-7.5 microns. Cells of the genital primordium increase from four to about eight. The second molt occurs about 24 hours after hatching.

THIRD STAGE LARVAE: L = 0.35-0.42 mm.; a = 25-30; b = 3.1-3.7; c = 5.8-7.1; G = $^{8.5\text{-}22.0}$ 68-80; Stylet = 7.5-8.0 microns. The genital primordium increases to about thirty cells. The third molt occurs in 1 ½ to 2 days.

FOURTH STAGE LARVAE: L = 0.50-0.54 mm.; a = 31-35; b = 4.1-4.9; c = 6.2-7.2; G = $^{16\text{-}42}$ 75-80 $^{1.9\text{-}3.8}$; Stylet = 8.5-9.5 microns. A hyaline area in the genital primordium marks development of the future vagina. The fourth molt occurs about 2 days after the third.

FEMALES: Adult females feed 12 to 14 hours before egg laying begins. Individual females have been followed up to ten days after the fourth molt, when they were still producing eggs. They undoubtedly produce for a longer time. Some cultures contain swollen "dowager" types, with several eggs lying separately in the reproductive tract and translucent material separating the cuticle, intestine, and ovary.

MALES: Petri dishes contained 0.0-2.5% males. Although copulation has been seen, isolated newly-molted females produced young, showing that males are not necessary for reproduction.

SUMMARY: The life cycle occupies about eight days under the conditions of these studies with four molts, one in the egg. The first stage has no stylet. All other larvae, males, and females have a stylet and feed. Increase in the a, b, and c values from stage to stage suggests that most rapid growth between molts is between the anus and esophagus.

SUMMARY

Neotylenchus linfordi n. sp. is distinguished by a post vulvar branch, stylet with notched knobs, oocytes in a single row, pointed female tail, and the bursa extending over the tip of the tail. Feeding is accomplished by alternate compression and expansion of the thin walled chamber in the anterior end of

the intestine. When feeding on *Pyrenochaeta terrestris* on PDA at 22-25° C., the life cycle is completed in eight days with four molts, one in the egg. The first stage has no stylet, all other stages have a stylet and feed.

LITERATURE CITED

MEYL, A. H. 1955. Die bisher in Italien gefunden freilebenden Erd und Süss-wasser Nematoden. Archivio Zoologico Italiano 39: 161-264.

THORNE, G. 1941. Some nematodes of the family Tylenchida which do not possess a valvular median esophageal bulb. The Great Basin Naturalist 2: 37-85

VAN WEERDT, L. B. 1960. Studies on the biology of *Radopholus similis* (Cobb 1893) Thorne, 1949. Nematologica 5: 43-51.

A New Gasterostome, *Bucephaloides megacirrus*, from the Redfish, *Sciaenops ocellata**

G. T. RIGGIN, JR.** and ALBERT K. SPARKS***

During examination of fishes from the Gulf of Mexico, many specimens of a gasterostome fluke were recovered from the redfish (*Sciaenops ocellata*). Seven hosts, which harbored twelve to several hundred flukes each, were collected at Bayou Rigaud, Grand Isle, Louisiana from December 1951 to August 1956. A single redfish collected from Alligator Harbor, Franklin County, Florida in November 1955 also harbored several hundred worms of the same species. The flukes belong to the genus *Bucephaloides* Hopkins, 1954 and are herein described as a new species.

The Florida specimens, approximately half of which were flattened by coverslip pressure, were fixed in alcohol-formal acetic acid solution and stored in 70% alcohol until stained and mounted. Frontal, sagittal and cross sections of unflattened worms were cut at 10 microns and stained with Delafield's hematoxylin and eosin. Whole mounts of flattened specimens were stained with Delafield's hematoxylin, Ehrlich's acid hematoxylin, Semichon's aceto-carmine and Borax carmine.

The Louisiana specimens were fixed in Gilson's fluid under coverslip pressure and stored in fifty percent alcohol. They were stained in Delafield's hematoxylin and alum cochineal.

Measurements of the Florida specimens are given first, followed by the measurements of those from Louisiana. All measurements are of flattened specimens and, unless stated otherwise, are in millimeters. Drawings were made with the aid of a microprojector. The following description is based on eleven specimens from Florida and three from Louisiana.

Bucephaloides megacirrus, n. sp.

DESCRIPTION: WITH CHARACTERS OF GENUS: Body linguiform, rounded at anterior extremity; round in cross section. Length .944-1.23, .910-.940, width .425-.464, .310-.440 at level of ovary. Body covered with rows of spines becoming smaller posteriorly and lacking at posterior extremity. Anterior sucker sub-terminal, rounded, .154-.201, .161-.182, in diameter. Pharynx oval, slightly postequatorial, variable in dimensions, length .075-.105, .075-.090,

*Contribution No. 91 from the Oceanographic Institute, Florida State University.

**Present address: Department of Biology, Furman University, Greenville, South Carolina; in Department of Biological Sciences, Florida State University during these studies. The author wishes to thank Dr. R. B. Short for his help during this study.

***Present address: College of Fisheries, University of Washington, Seattle, Washington; at Texas A. & M. Research Foundation, Thibodeaux, Louisiana during these studies.

width .090-.116, .090-.105. Esophagus short, extending anteriorly and opening into short, sac-like intestine. Excretory bladder elongated, sac-like, extending anteriorly to level of ovary. Excretory pore posterior, terminal. Testes rounded, .139-.165, .097 in diameter, situated on left side, tandem, usually slightly overlapping, anterior testis at about level of pharynx. Cirrus sac conspicuous, extending anteriorly to level of ovary, containing many prostate cells. Seminal vesicle large, in anterior portion of cirrus sac. Cirrus eversible, with two conspicuous genital lobes extending into genital atrium. Genital pore terminal, slightly dorsal to excretory pore.

Ovary immediately anterior to testes, rounded .079-.115, .091 in diameter. Oviduct extending dorsally and posteriorly from ovary; forming conspicuous suction chamber and entering oötype dorso-lateral to anterior testis. Oötype surrounded by Mehlis' gland. Uterus long, turning anterio-medially from oötype, forming many convolutions between pharynx and haptor before enlarging into metraterm opening into left side of genital atrium. Seminal receptacle absent. Laurer's canal present, extending from oviduct near suction chamber posterio-medially, opening dorsally in region of posterior testis. Vitelline follicles, round to oval, in two lateral groups of ten to twelve, anterior to ovary. Right and left vitelline ducts join posterior to ovary, forming common duct on left side of body entering oviduct shortly before oötype. Eggs numerous, yellow, length 31-42 microns, 25-33 microns; width 17-24 microns, 15-21 microns.

HOST: *Sciaenops ocellata,* the redfish.

LOCATION: Intestine

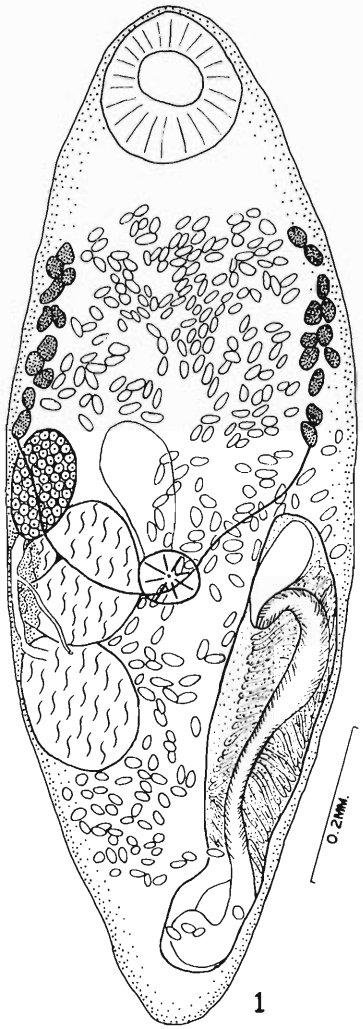
LOCALITY: Alligator Harbor, Franklin Co., Florida.

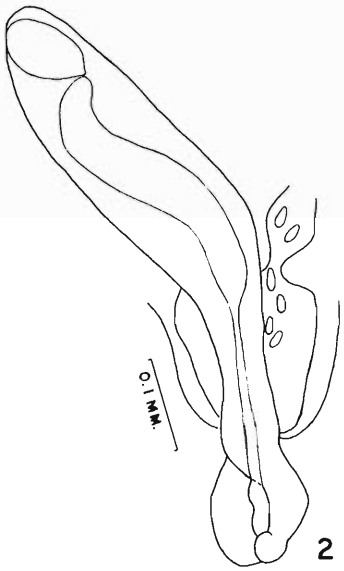
TYPE: Holotype deposited in the Helminthological Collection of the U.S. National Museum, No. 59516. Paratypes in authors' collections.

DISCUSSION

The name *Bucephaloides* has been proposed by Hopkins (1954) for the genus of gasterostomes which possess an anterior sucker but lack a hood, papillae or other accessory structures. According to Hopkins, this name should replace the generic name *Bucephalopsis* Nicoll, 1914, since use of the name *Bucephalopsis* for adult worms was based on the unproved assumption that the gasterostomes with only a plain muscular sucker developed from the cercaria *Bucephalopsis haimeanus* (Lacaze-Duthiers, 1854). The genus *Bucephaloides* contains approximately 34 species.

Bucephaloides megacirrus differs from all of the described species below in the possession of a pair of genital lobes and the position and size of the cirrus sac. Dayal (1948) described *Bucephaloides sinhai; Bucephaloides thapari;* and *Bucephaloides macronius,* each of which possesses a single genital lobe extending into the genital atrium. In addition to differences in the structure of the genital lobes, *B. megacirrus* differs from *B. sinhai* in body size and the lack of a vesicula seminalis externa; from *B. thapari* and *B. macronius* in the position of the testes and ovary. Bhalerao (1937) described *Bucephaloides karvei,* which was redescribed by Gupta (1956), as possessing a dorsal genital tongue and a pair of genital lobes immediately ventral which extend into the genital atrium. Gupta (1956) substantiated the presence of these structures in placing *B. belonea* Srivastaia, 1938 in synonymy with *B. karvei.* The present species possesses a pair of genital lobes but lacks the genital tongue as described by Bhalerao. *B. megacirrus* also differs from *B. karvei* in the size and position of the cirrus sac and in body shape.





Bucephaloides megacirrus, n. sp.
Fig. 1. Dorsal view of holotype, drawn from whole mount.
Fig. 2. Posterior end of paratype, showing cirrus, with lobes extended.

LITERATURE CITED

BHALERAO, G. D. 1937. Studies on the helminths of India. Trematoda IV. J. Helminth. 15: 97-124.

DAYAL, J. 1948. Trematoda parasites of Indian fishes. Part I. New trematodes of the family Bucephalidae. Indian J. Helminth. 1: 47-62.

GUPTA, S. P. 1956. A redescription of *Bucephalopsis magnum* (Verma 1936) Srivastaia 1938 and *Bucephalopsis karvei* Bhalerao, 1937. Ind. Jour. Helm. 8: 112-121.

HOPKINS, S. H. 1954. The American species of Trematode confused with *Bucephalus* (*Bucephalopsis*) *haimeanus*. Parasit. 44: 353-70.

The Life Cycle of *Ditylenchus triformis* (Nematoda: Tylenchida) With Emphasis on Post-Embryonic Development*

HEDWIG HIRSCHMANN

In a previous paper (Hirschmann and Sasser, 1955), the occurrence of an intersexual form was reported in the tylenchoid nematode *Ditylenchus triformis* Hirschmann and Sasser, 1955. At that time, only small numbers of nematodes were available for study due to the difficulties in colonizing the species. Later, a fungus** was found that was very suitable for growth and reproduction of this nematode. By propagating *D. triformis* in cultures of this fungus, it was possible to extend the investigations on the life cycle and the role of intersexual individuals in reproduction. Certain phases of these studies have been published (Hirschmann, 1957).

This paper deals with a full account of the life cycle and the reproductive habits of *D. triformis*. Emphasis is given to the anatomical development of the reproductive system during post-embryonic development of the nematode.

MATERIALS AND METHODS

Stock cultures of the dioecious and intersexual lines of *D. triformis* were maintained on fungus cultures (Fig. 1A) in the laboratory. Twenty females or intersexes and 20 males were added to 8-day-old Petri dish cultures of the fungus growing on potato dextrose agar. No special precautions were taken to prevent contamination during nematode inoculation, since the fungus showed a high degree of antibiotic activity. Within 1½ to 2 months each Petri dish culture yielded large numbers of nematodes in all stages of development. The nematodes for various studies were extracted by the Baermann funnel method. All tests were conducted at 24 to 26°C, the optimal temperature range for development and reproduction of this nematode species.

A number of procedures were employed in preparing nematodes for morphological and anatomical investigations. Measurements were taken from specimens killed by gentle heat and mounted in 2% formalin. Measurements of molting specimens apply to larvae in the beginning phase of molting. Observations on the process of molting and the anatomical investigations on post-embryonic development were made on specimens mounted in water or 2% formalin, or specimens stained in hot acid fuchsin lactophenol (Goodey, 1957). For a detailed study of the development of the reproductive system, the specimens were killed by gentle heat, fixed for 10 to 20 minutes in Carnoy, stained in 1% acetic orcein, and mounted in 45% acetic acid.

The following technique was employed in chronological development studies. Small blocks (5 x 5 x 2 mm) of fungus culture were cut from the margin of a fungus colony and placed in the center of a large coverslip (22 x 50 mm) which was then inverted and sealed, with vaseline, to a van Tieghem chamber (22 mm diam., 10 mm high) (Fig. 1B). Each of 100 such fungus cultures was inoculated with 10 freshly hatched second stage larvae. Fifty nematodes were examined daily for 13 days and their stage of development was recorded. To provide optimal conditions for development, the larvae were transferred to new cultures on the seventh day. Pairs of young, freshly molted females and males were transferred to new fungus cultures in van Tieghem

*Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 1304 of the Journal Series.

**Identification of the fungus has not been possible, since all attempts to induce sporulation have failed. It is suspected, however, that this fungus is a species of *Helminthosporium* or *Fusarium*.

chambers. The time required for the deposition of the first eggs, the embryonic development to first stage larvae, and the hatching of second stage larvae was recorded.

The same culturing method was employed to investigate longevity, mode of reproduction of the intersexes, and reproductive habits. In these studies, however, the nematodes were transferred to new fungus cultures every five days.

EXPERIMENTAL RESULTS

1. Post-embryonic Development

The eggs of *Ditylenchus triformis* are laid singly before undergoing cleavage. The average dimensions of 100 eggs in different stages of embryonic development (Fig. 2A) were 66.2 microns ± 0.7 (range: 59.8-74.4 microns) by 25.3 microns ± 0.4 (range: 21.4-30.6 microns).

Four molts occur throughout the life cycle. The first molt takes place within the egg (Fig. 2B) and it is the second stage larva that hatches. The differentiation of various organ systems is not completed in first stage larvae (Fig. 2B). The esophagus is not fully formed; only the anterior insertable part of the stylet is developed. The genital primordium consists of 3 nuclei- one large central nucleus which is bordered anteriorly and posteriorly by 2 smaller nuclei. The large nucleus is the germinal nucleus, whereas the 2 small nuclei are somatic nuclei. The germinal nucleus is distinguishable even in earlier embryonic stages, before the embryo has assumed the vermiform shape. When first stage larvae or molting first stage larvae are artificially released into water from eggs, they move about normally for a short period, but their bodies soon become vacuolized and the larvae die.

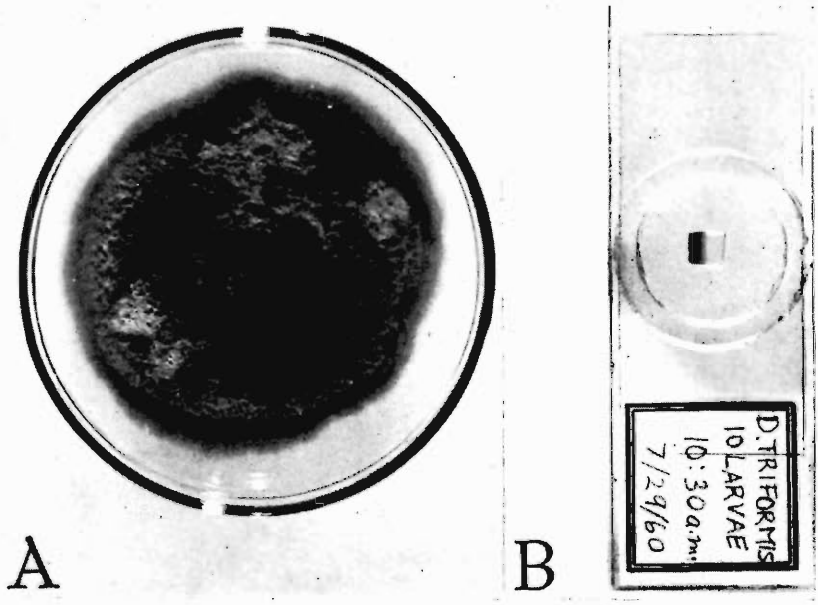


Fig. 1. A—culture of an unidentified fungus used in propagating *Ditylenchus triformis*; B—Van Tieghem chamber with small block of fungus culture inoculated with larvae of *D. triformis*.

SECOND STAGE: The second stage larvae at hatching are fully developed and resemble the adults except for body size, reproductive organs, and accessory reproductive structures (Fig. 2C). The oval-shaped genital primordium is located at approximately 63% of the body length. With the onset of feeding, the larvae continue their development. The genital primordium enlarges slightly without any division of its nuclei. The ventral- and lateral-chord nuclei, as well as the nuclei in the rectal region undergo mitotic divisions. In some fully grown second stage larvae, 2 spindle-shaped, moderately heavy staining nuclei in the ventral chord can be distinguished from the rest of rounded, darkly staining ventral-chord nuclei in the vicinity of the genital primordium. As will be shown later, these nuclei are present in female and intersex larvae, but are lacking in larvae that will develop into males. One nucleus is located slightly anterior to the anterior end and the other slightly posterior to the posterior end of the genital primordium. Both nuclei appear to have been derived from the ventral chord. Each of them divides once during the second stage, thus resulting in a group of 4 nuclei whose position varies somewhat in relation to the genital primordium (at same level with primordium or slightly posteriad).

SECOND MOLT: Larvae approaching the second molt become more and more sluggish and finally lie motionless during molting. The first indication of molting is a retraction of the protoplasm in the cephalic region away from the cuticle. The anterior insertable part of the stylet and the cephalic framework remain in this clear-appearing area, whereas the posterior part (shaft and knobs) is reduced to a thin refractive line that is still connected with the anterior part. The cuticular linings of the amphidial ducts and amphids are also shed. The protoplasm then reorganizes to form a new cephalic region with framework and faintly visible stylet (insertable part formed first, then shaft and knobs). Simultaneously with these processes in the anterior region, the cuticular lining of the rectum is molting as indicated by a clear area in the rectal region (Fig. 2D). With the completion of the new stylet the new cuticle is formed and soon the larva of the next stage appears within the molted cuticle. The process of molting is essentially similar in all stages.

During the second molt, the first nuclear divisions occur in the genital primordium (Fig. 2D). The 2 somatic nuclei divide and, with the completion of this molt, the resulting nuclei arrange in patterns that are typical for the sexes. In male larvae, the anterior somatic nucleus divides repeatedly to form several nuclei that later give rise to the male gonoduct. The division of the posterior somatic nucleus results in 2 nuclei. One of them remains as cap cell nucleus in the posterior tip of the gonad throughout the life of the nematode. The other nucleus migrates anteriad the germinal nucleus and later gives rise to somatic nuclei that will form the epithelium of the testis. In female and intersex larvae (Fig. 2D), it is the posterior somatic nucleus that divides

Abbreviations for Figures

c n—cap cell nucleus; div ger n—dividing germinal nucleus; ep n—epithelial nuclei; ep t—epithelium of testis; es—esophagus; es n—nuclei in esophageal region; gd—gonoduct (developing); gen prm—genital primordium; ger n—germinal nucleus; gub—gubernaculum (developing); int—intestine; mlt r—molting rectum; od—oviduct; pb—polar body; p ut s—postvulvar uterine sac; qc—quadricolumella; r s—receptaculum seminis; s ch n—specialized ventral-chord nuclei which later form vagina (drawn heavier for better differentiation); so n—somatic nuclei of genital primordium; sp—spermatogonial cell; spi—spicule (developing); spi prm—spicule primordia; t—testis; ut—uterus proper; va—vagina (developing); v ch n—ventral-chord nuclei; v d—vas deferens; vit m—vitelline membrane.

several times resulting in nuclei that later form the female gonoduct. The division of the anterior nucleus gives rise to the cap cell nucleus and epithelial nuclei of the ovary. The group of 4 specialized ventral-chord nuclei mentioned earlier is located posteriorly to the primordium.

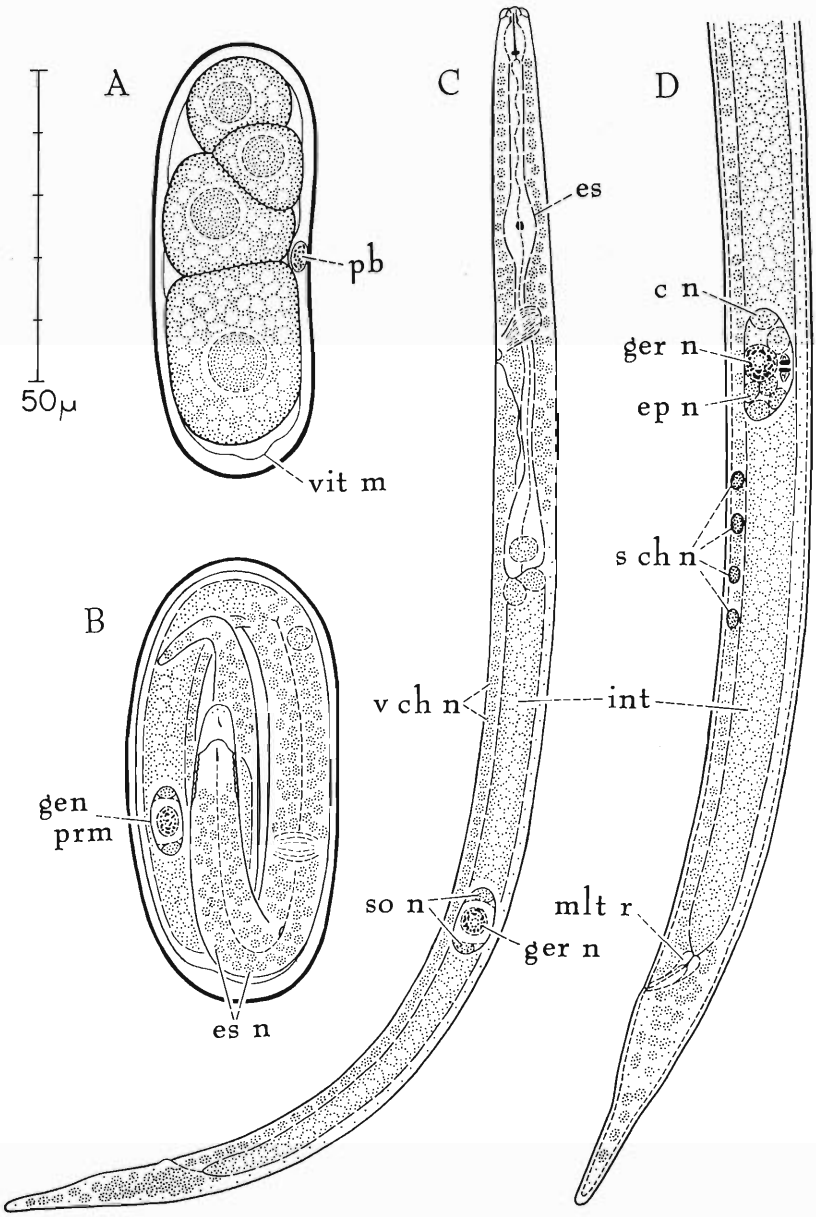


Fig. 2. Development of *Ditylenchus triformis*. A—egg with 4 blastomeres; B—molting first stage larva in egg; C—second stage larva, freshly hatched; D—second molt, posterior part of female larva.

The derivatives of the various nuclei can be traced separately throughout the development of the gonad, since they stain differentially with acetic orcein. The large germinal nucleus stains heavily in a coarse granular pattern with one or 2 dark staining areas. The cap cell nucleus and the large epithelial nuclei which form the ovary or testis stain faintly and have distinct nucleoli. The epithelial nuclei of the gonoducts are small and stain very dark.

THIRD STAGE: Early in the third stage the morphological differences between larvae of different sexes become more pronounced. In male larvae (Fig. 3A), the anterior part of the gonad is oval-shaped consisting of a group of 9 to 12 small cells and is slightly set off from the posterior rod-shaped part which comprises cap cell nucleus, germinal nucleus, and 2 large epithelial nuclei. In the rectal region a large group of small nuclei appears which later give rise to spicules and gubernaculum. In contrast, female larvae (Fig. 3B) have the group of small cells at the posterior end of the gonad, and only a small group of nuclei in the rectal area. The distance from the posterior end of the gonad to the anus is much shorter in female than in male larvae. This is due to the posteriad growth as well as backward movement of the entire gonad in female larvae. Also, the 4 specialized ventral-chord nuclei have enlarged and are now located within the extent of the gonad in female larvae, but are lacking in male larvae. Third stage intersex larvae combine characters of the 2 sexes. They resemble female larvae with respect to gonad morphology and location, but exhibit a large group of spicule primordial nuclei in the rectal region as do male larvae.

THIRD MOLT: With the beginning of the third molt, the gonad has extended in length. In male larvae (Fig. 3C), the anterior oval-shaped group of cells differentiates into 2 double rows of small spherical cells which, instead of proceeding anteriad, turn backward and grow posteriad along the ventral side. The remaining gonad with germinal nucleus, cap cell nucleus and 4 large epithelial nuclei is pushed dorsally and gradually shifts anteriad. Finally, only the end with the germinal nucleus is slightly bent dorsally and is straightened at the time the fourth stage larva emerges from the old cuticle. The nuclei in the rectal area are also dividing resulting in enlargement of the spicule primordia. In female larvae (Fig. 3D), the posterior cell group of the gonad increases in size. The gonad grows out anteriad by forming 2 single rows of cells between this posterior cell group and the 2 anterior large epithelial nuclei. The 4 specialized ventral-chord nuclei undergo further divisions, and by completion of the third molt, they are arranged in 2 groups of 8 nuclei each in the vicinity of the posterior region of the gonad. These nuclei later take part in the formation of the vagina. Their arrangement in 2 groups already indicates the future position of the vagina which will be located ventrally between them. The distance from gonad end to anus in female larvae is about one-half of that in male larvae (Fig. 3C,D; Table I). Intersex larvae resemble female larvae, but have, in addition, large spicule primordia as male larvae. The distance between posterior gonad end and anus tends to be slightly shorter than in female larvae.

FOURTH STAGE: During the fourth stage, the gonads of all 3 types of larvae continue to increase in length (Fig. 4A,B). The anterior testicular and ovarial parts are clearly distinguishable from the posterior somatic parts that will form the gonoducts. In male larvae (Fig. 4A), the germinal nucleus divides and advanced fourth stage larvae may have 4 germinal nuclei. Epithelial nuclei begin to migrate between these germinal nuclei. The posterior end of the testicular part consists of several large, irregularly arranged

epithelial nuclei. A slight constriction marks the end of the testicular region and the beginning of the posterior, more narrow epithelial part which consists of 2 double rows of small spherical cells. This part later gives rise to the vas deferens. The first 2 pairs of these cells are slightly larger than the rest and are somewhat oval-shaped. They will form a sphincter-like structure between testis and vas deferens in the mature male. In larvae approaching

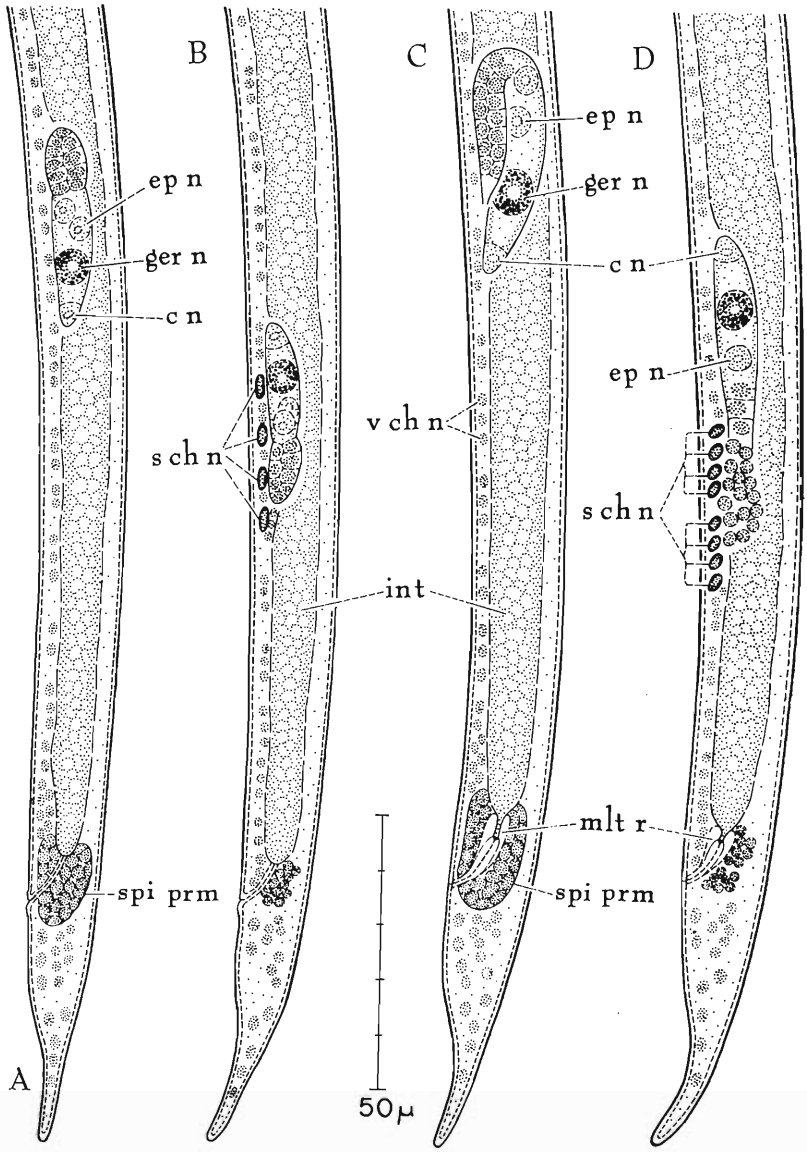


Fig. 3. Post-embryonic development of *Ditylenchus triformis*. A—early third stage, posterior portion of male larva; B—early third stage, posterior portion of female larva; C—third molt, posterior portion of male larva; D—third molt, posterior portion of female larva.

the fourth molt, the posterior end of the gonad is in the vicinity of the prominent spicule primordia. In fourth stage female larvae (Fig. 4B), the germinal nucleus does not divide. The 2 single rows of small epithelial cells extend in length by repeated divisions. They will eventually differentiate into the various parts of the female gonoduct. As development progresses, 2 double rows can be distinguished in some parts. Anteriorly, one or 2 pairs of epithelial nuclei appear to migrate into the ovarial part of the gonad. They are distinguishable from the rest of epithelial nuclei of the ovary, since they are smaller and stain similar to other nuclei of the gonoduct. Posteriorly, the formation of uterus proper and postvulvar uterine sac is indicated. The dorsal epithelium of these gonad regions develops as a continuation of the 2 single rows of small epithelial cells; the ventral epithelium differentiates from another 2 single rows of cells located ventrad. The individual nuclei of the 2 groups of 8 specialized ventral-chord nuclei begin to migrate one by one toward the center of the nematode body providing a clear space between them, in which the vagina will be formed. The development of the intersex gonad progresses in the same manner as that of female larvae and the spicule primordia are as prominent as in male larvae.

FOURTH MOLT: During the fourth molt, the gonad of male larvae clearly differentiates into testis and vas deferens (Fig. 4C). The germinal nuclei undergo further divisions (as many as 8 nuclei in advanced molting larvae) and are organized into distinct cells with well-defined membranes, particularly in the posterior end of the testis. Wedge-shaped epithelial cells between spermatogonial cells and pairs of large rounded epithelial cells posterior to the last spermatogonial cell form the epithelium of the testis. The vas deferens increases in length and its posterior end comes to lie close to the rectum. With the completion of the development, the vas deferens opens into the rectum. The cells of the spicule primordia are distinctly arranged in 2 subdorsal lobes early in this molt. As molting progresses, spicules and gubernaculum and caudal alae complete their differentiation. Freshly molted males may have as many as 13 spermatogonial cells. The most mature ones are preparing for the first maturation division. In female larvae, the germinal nucleus begins to divide for the first time (Fig. 4D). Due to this delay in division, freshly molted females have 4 or less germinal nuclei. The posterior ovarial part comprises numerous small epithelial nuclei whose further function was not determined. A slight constriction marks the end of the ovarial part and the beginning of the gonoduct. The anterior narrow part of the gonoduct, the oviduct, consists of 2 single rows of 6 columnar epithelial cells with long spindle-shaped nuclei. The oviduct is followed by a region of 6 to 10 larger cells in double rows with oval-shaped nuclei which form the spermatheca in the adult. The spermatheca constitutes the modified anterior end of the uterus and is followed by a characteristically widened part consisting of 16 large, coarsely granulated cells with spherical nuclei, the quadricolumella. Behind the quadricolumella, the uterus narrows to form another short, constricted region with 2 single rows of 3 narrow epithelial cells each. This region is followed in sequence by a few other epithelial cells, the uterus proper, and the postvulvar uterine sac. The latter 2 structures are formed by a layer of flattened epithelial cells. Vagina and vulva complete their development during the fourth molt. The 2 groups of 8 specialized ventral-chord nuclei have migrated inside creating the lumen of the vagina between them. Four nuclei lie posteriad, 4 anteriad in the median plane, and 4 take a dorso-lateral position on each side of the vagina.

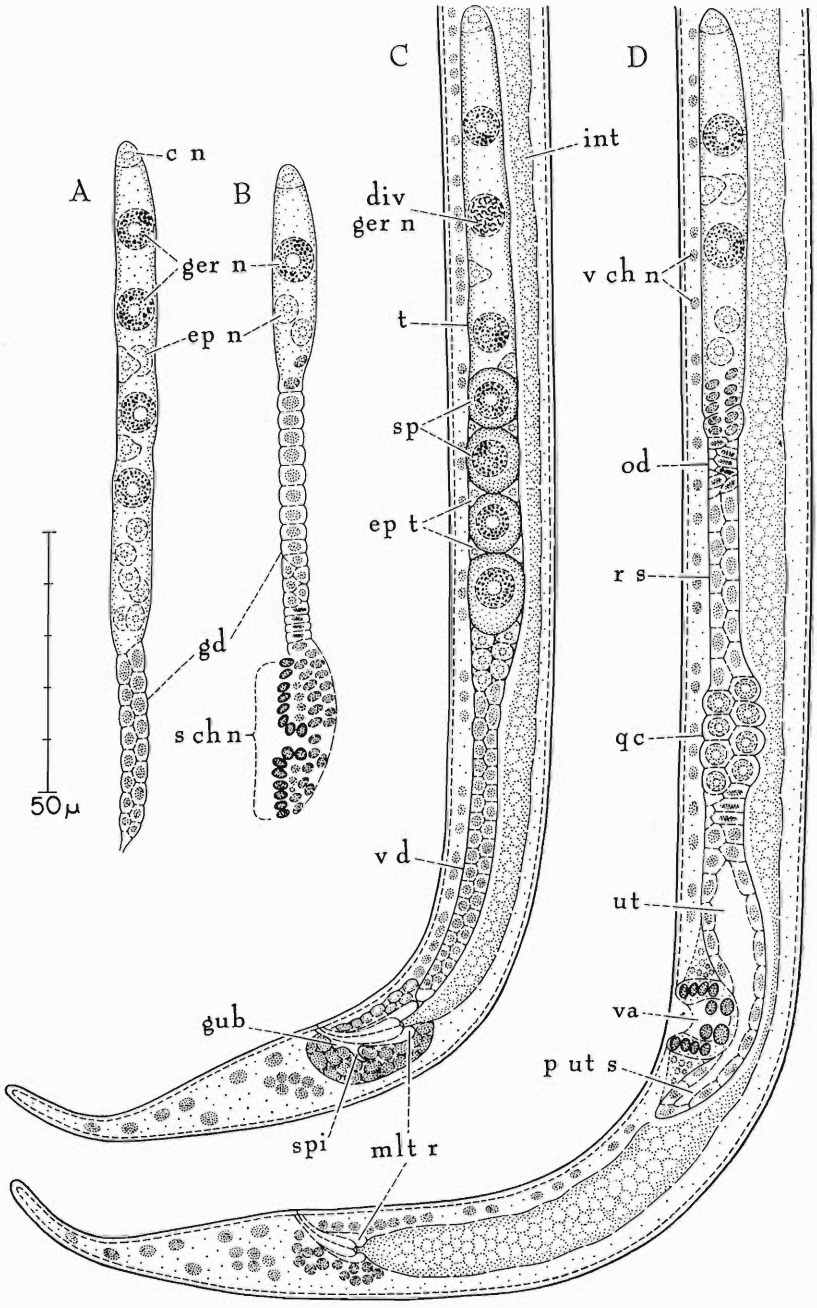


Fig. 4. Post-embryonic development of *Ditylenchus triformis*. A—fourth stage, gonad of male larva; B—fourth stage, gonad of female larva; C—fourth molt, posterior portion of male larva; D—fourth molt, posterior portion of female larva.

With the completion of this last molt, the cuticular lining of the vagina is formed. In young females the oögonial nuclei soon increase in number and cell membranes become distinct. As in previous developmental stages, intersex larvae combine the characters of female and male larvae. They have the same developmental pattern of the gonad as female larvae and, in addition, spicules, gubernaculum and caudal alae are formed in the same manner as in male larvae.

2. Comparison of molting larvae and amendation of measurements of adults.

Each molt is clearly defined by body and gonad length (Table 1). Under the conditions of these experiments, there was no overlap in measurements of these characters between the various molts.

Adult nematodes continue to grow to some extent after the last molt. An amendation of the original description of *D. triformis* with respect to certain measurements of females, males and intersexes is presented in the following:

50 females: body length = 867.5 microns ± 25.1 (691.5-1046.6 microns);
length of stylet = 7.85 microns ± 0.07 (7.34-8.51 microns);
a = 23.6-39.9; b = 52.5-73.0; c = 9.8-13.7;
$V = {}^{28.7-63.3}\,76.2 - 81.2\,{}^{2.3-4.3}$;
vulva-anus distance (%) = 11.5-15.9;
excretory pore (%) = 10.9-14.3

50 intersexes: body length = 852.2 microns ± 23.8 (712.0-1122.3 microns);
length of stylet = 7.98 microns ± 0.07 (7.40-8.42 microns);
length of spicules = 12.9 microns ± 0.6 (12.1-14.9 microns);
length of gubernaculum = 5.7 microns ± 0.3 (4.9-6.4 microns);
a = 26.4-48.8; b = 49.5-72.8; c = 10.2-13.3;
$V = {}^{24.3-64.6}\,71.8 - 82.7\,{}^{2.0-3.8}$;
vulva-anus distance (%) = 11.2-20.1;
excretory pore (%) = 11.9-14.2.

50 males: body length = 707·9 microns ± 19.5 (614.1-875.8 microns);
length of stylet = 7.63 microns ± 0.07 (7.22-8.23 microns);
length of spicules = 14.9 microns ± 0.2 (13.7-15.7 microns);
length of gubernaculum = 6.1 microns ± 0.1 (5.2-6.8 microns);
a = 32.4-43.6; b = 45.5-62.2; c = 9.2-12.4;
T = 28.8-60.0;
excretory pore (%) = 12.8-15.9.

Table 1. Measurements of various developmental stages of *Ditylenchus triformis*[1]

		Body length (μ)		Gonad length (μ)		A[3]
		Range	Mean[2]	Range	Mean[2]	Range
2nd stage larvae freshly hatched		198 — 282	242.6 ± 4.4	8.3 — 13.0	10.1 ± 0.3	16.9 — 27.4
2nd molt		317 — 376	346.6 ± 4.2	14.3 — 20.4	16.8 ± 0.4	22.1 — 28.2
3rd molt	male larvae	406 — 526	470.8 ± 6.7	40.8 — 91.8	58.9 ± 3.9	15.1 — 31.2
	female larvae	430 — 556	487.7 ± 7.1	47.9 — 83.6	64.8 ± 2.3	9.5 — 12.2
	intersex larvae	428 — 548	483.4 ± 6.9	46.4 — 86.6	68.0 ± 2.7	8.7 — 12.1
4th molt	male larvae	563 — 720	642.6 ± 15.1	155.0 — 295.8	230.5 ± 6.7	—
	female larvae	614 — 801	724.4 ± 11.4	183.6 — 265.2	220.9 ± 5.1	—
	intersex larvae	599 — 783	688.8 ± 11.7	163.2 — 230.5	196.3 ± 4.8	—

[1] Data based on measurement of 50 larvae in each case.
[2] 95% confidence interval.
[3] A = Distance from posterior gonad end to anus expressed in per cent of body length.

3. Time required for completion of life cycle in fungus cultures.

The time required for the development of freshly hatched larvae to adults in fungus cultures is indicated in Table 2. Some of the second stage larvae underwent the second molt three days after inoculation. On the fourth day, most of them were in the second molt and few had developed into third stage female or male larvae. On the fifth day, the majority of the larvae had reached the third stage and a few were in the third molt. Although few fourth stage male and female larvae appeared 6 days after inoculation, most of the larvae reached the fourth stage on the eighth day. The fourth molt began on the seventh day. On the ninth day, the larvae undergoing the fourth molt had increased in number and the first adult males appeared, whereas the first adult females were found one day later. All nematodes had developed into adults on the thirteenth day.

With a chance of immediate mating, 2-day-old adult females began to lay eggs. The embryonic development was completed within 3 to 5 days with the appearance in the eggs of fully grown first stage larvae or larvae in the first molt. Most second stage larvae hatched from the eggs within 4 to 6 days following egg deposition. Thus, the life cycle of *D. triformis,* under the conditions of these experiments, was completed within 16 to 21 days.

As evidenced from Table 2, there was a considerable variation in the rate of development of individual larvae. On the seventh day, for example, a range of different stages from larvae undergoing the second molt to larvae in the fourth molt was found. In general, the fourth stage and fourth molt were somewhat prolonged as compared to the other developmental stages.

4. Longevity, mode of reproduction of intersexes and reproductive habits.

The life span of 35 adult females averaged 63 days (range: 31 to 124) in fungus cultures at 24 to 26° C. Thirty males had a slightly longer average life period of 74 days (range: 33 to 145). The intersexes were intermediate in longevity.

It was previously suspected that intersexes might function as normal females (Hirschmann and Sasser, 1955). At that time, however, no egg formation or deposition was observed in intersexes. In order to investigate this further, each of 100 fungus cultures in van Tieghem chambers was inoculated with one fourth stage intersex larva. A few days later, the larvae had developed to adult intersexes which remained active in the cultures, but

Table 2. Development of *Ditylenchus triformis* in fungus cultures at 24 to 26°C[1]

Age—days after inoculation	2nd stage larvae (%)	2nd molt (%)	3rd stage larvae (%)		3rd molt (%)		4th stage larvae (%)		4th molt (%)		adults (%)	
			female	male	female	male	female	male	female	male	female	male
2	100	....	....	....	....	....	....	....	....	....	....	....
3	74	26	....	....	....	....	....	....	....	....	....	....
4	38	44	12	6	....	....	....	....	....	....	....	....
5	14	20	30	26	8	2	....	....	....	....	....	....
6	6	6	12	16	18	22	8	12	....	....	....	....
7	....	4	8	10	20	26	14	12	2	4	....	....
8	....	....	2	4	14	8	32	22	8	10	....	....
9	....	....	....	....	10	8	24	18	14	20	....	6
10	....	....	....	....	....	....	18	10	24	18	12	18
11	....	....	....	....	....	....	8	4	16	8	34	30
12	....	....	....	....	....	....	....	....	10	6	46	38
13	....	....	....	....	....	....	....	....	....	....	48	52

[1] Data based on examination of 50 larvae per day.

did not lay any eggs within the following 25 days. Microscopical examination of several specimens revealed, in each case, many well-developed oöcytes in the ovaries and no sperm in the spermathecae. On the 25th day, 2 young males were added to each of 50 intersexes. Two days later, the first eggs appeared in these cultures and, when examined microscopically, abundant sperm was found in the spermathecae of the intersexes. No eggs were found in the cultures containing only intersex adults. This demonstrates that the intersexes function as females and are fertile only when impregnated by males.

Females and intersexes need to be inseminated repeatedly to continue egg production. When impregnated females or intersexes were kept singly in fungus cultures, they soon exhausted their sperm reserve and egg-laying ceased, although the specimens remained active and lived as long as females in association with males. When new males were added to such single females or intersexes, egg-laying resumed shortly after copulation. This could be repeated 3 to 4 times during the life of a female or intersex.

The average number of eggs laid by a single female or intersex was 79 (maximum 168). The uterus usually contained only one well-formed egg at a time. The eggs were deposited before undergoing cleavage.

In general, adults remained sexually functional for ¾ of their life time. The last period of their life was characterized by a cessation of the reproductive ability, retardation of movement and anatomical changes in certain organ systems.

5. Anabiosis.

D. triformis has the ability to survive unfavorable conditions under dormancy, a common phenomenon in the genus *Ditylenchus*. In heavily populated fungus cultures, nematodes of all stages tended to swarm together and form clumps. In older cultures these clumps or aggregates of motionless, tightly packed nematodes were easily visible to the naked eye as white patches in the agar medium. Ten heavily populated fungus cultures were allowed to dry out slowly at room temperature. The thin sheets of dried agar medium, containing the nematodes, were then stored for 2½ years at 24 to 26° C. At the end of this period, most of the specimens revived when the desiccated medium was soaked in water for 48 hours. Larvae of all stages and adults were capable of anabiosis. When transferred to new fungus cultures, they resumed feeding, and reproduction was normal.

DISCUSSION

In recent literature (Chitwood and Chitwood, 1950; van Weerdt, 1960), it has been stated that the genital primordium of all nematodes has 2 germinal nuclei regardless of the number of gonads of the adult. Genital primordia, however, with one germinal nucleus have been described earlier in some animal-parasitic nematodes (zur Strassen, 1892; Wülker, 1923). The present studies confirm that genital primordia with one germinal nucleus do exist in nematodes. This is the case in males, females and intersexes of *Ditylenchus triformis* and also in *Aphelenchus avenae* Bastian, 1865. It appears, however, that there is no correlation between the number of gonads in the adults and the number of germinal nuclei in the genital primordium. Thus, *D. triformis* and *A. avenae* with one gonad in the adult have genital primordia with one germinal nucleus, whereas *Rhabditis lambdiensis* Maupas, 1919 and *Turbatrix aceti* (Müller, 1783) Peters, 1927 also with one gonad in the adult have genital primordia with 2 germinal nuclei.

Pai, 1928, investigating *Turbatrix aceti,* was the first to distinguish clearly between germinal and somatic nuclei in the genital primordium and to trace the derivatives of each nucleus throughout the development of the gonad. Van Weerdt, 1960 was able to distinguish between germinal and somatic cells up to the third molt in *Radopholus similis* (Cobb, 1893) Thorne, 1949. In the present studies, the derivatives of the germinal nucleus and the 2 somatic nuclei of the genital primordium of *D. triformis* could be traced separately and their differentiation into the various parts of the gonad of the adult could be followed due to the differential staining properties of the various nuclei.

The cap cell nucleus in *D. triformis* was derived from the first division of one of the 2 somatic nuclei (posterior in male, anterior in female larvae) of the genital primordium. It did not divide later, but remained part of the epithelium of testis or ovary. This supports the viewpoint of other investigators (Maupas, 1899; Pai, 1928; Chitwood and Chitwood, 1950) for other nematode species and casts doubt on Musso's, 1930 opinion that the cap cell, in general, can be considered as a germinal stem cell that gives rise to germinal nuclei as well as epithelial nuclei.

The general developmental process of the gonad of *D. triformis* resembles that of *Turbatrix aceti,* except for the different number of germinal nuclei in the genital primordium and the developmental stage at which the germinal nucleus or nuclei start to divide. Such similarity in the development of the gonad of 2 unrelated monodelphic nematode species may indicate that a similar developmental pattern probably exists in various other monodelphic nematodes.

The various parts of the female gonoduct of *D. triformis* are similar to those described for *D. destructor* Thorne, 1945 (Wu, 1958). Wu suggested that the number of cells forming the quadricolumella may have some taxonomic value in differentiating *D. destructor* from *D. askenasyi* (Bütschli, 1873) Goodey, 1951. The present investigations showed that, as in *D. destructor,* the quadricolumella of *D. triformis* and *D. dipsaci* (Kühn, 1857) Filipjev, 1936 is composed of 16 cells and can therefore not be used as a taxonomic character to separate these species.

With the exception of *Tylenchulus semipenetrans* Cobb, 1913 (van Gundy, 1958) and *Meloidogyne incognita* Chitwood, 1949 (Triantaphyllou and Hirschmann, 1960), *D. triformis* is the only other nematode species in the order Tylenchida in which sex can be distinguished in the second larval stage. Sex distinction in *D. triformis* in this stage is based on the presence of 2 to 4 specialized ventral-chord nuclei which later form the vagina in the female. During the second molt, sex can be easier distinguished by the differential development of the genital primordium itself. This feature has been used by various investigators to recognize the sex of other nematodes beginning with the third larval stage (zur Strassen, 1892; Raski, 1950; van Weerdt, 1960; Yuksel, 1961).

Steiner, 1923 observed that female intersexes of *Agamermis decaudata* Cobb, Steiner and Christie, 1923 copulated with males and most of them had normal eggs in their uteri, but further development of these eggs to larvae and adults was not studied. The present investigation showed that *D. triformis* intersexes function as females and that insemination is necessary for egg production.

SUMMARY

Four molts occurred during the life cycle of *Ditylenchus triformis*. The first molt took place in the egg and the second stage larva hatched. The genital primordium of young second stage larvae consisted of one large germinal nucleus and 2 small somatic nuclei. In females, nuclei derived from the anterior somatic nucleus gave rise to the epithelium of the ovary, whereas nuclei originating from the posterior somatic nucleus formed the gonoduct. In males, the situation was reversed, but a turn of the male gonad through 180° brought the male gonoduct into its final posteriad position. The cap cell nucleus was derived from the first division of one of the somatic nuclei (posterior nucleus in male, anterior nucleus in female) and did not divide again. The germinal nucleus could be traced to late embryonic stages and did not divide before the fourth larval stage in males and the fourth molt in females. Specialized nuclei of the ventral chord formed the vagina by invagination. Two large spicule primordia derived from the rectal epithelium gave rise to spicules and gubernaculum. Intersexes showed the same developmental pattern of the gonad as female larvae and, in addition, spicules, gubernaculum and caudal alae were formed in the same manner as in male larvae. The first indication of sex differentiation occurred in the second larval stage when 2 to 4 specialized ventral-chord nuclei appeared in female and intersex larvae. Sex of the larvae was easier recognizable during the second molt and early third stage by anatomical differences of the reproductive system and location of the gonad. The various larval stages could be clearly distinguished by differences in the anatomy of the reproductive system. Each of the 3 molts outside the egg was clearly defined on the basis of body and gonad length.

The life cycle from egg to egg was completed within 16 to 21 days at 24 to 26° C. when the nematodes fed on cultures of an unidentified fungus. A considerable variation in the rate of development of individual larvae was observed. The longevity of females averaged 63 days, that of males 74 days and that of intersexes was intermediate. The intersexes functioned as females. The average number of eggs laid by a single female or intersex was 79. Females and intersexes had to be inseminated repeatedly to continue egg production. Adults remained sexually functional for ¾ of their life time. All developmental stages including adults were capable of anabiosis.

An amendation of certain measurements of females, males and intersexes is presented.

LITERATURE CITED

CHITWOOD, B. G., and M. B. Chitwood. 1950. An introduction to nematology. Section I, Anatomy. Chapter X, pp. 136-159; chapter VIII, pp. 116-125. Baltimore, Monumental Printing Company.

GOODEY, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Ministry Agr. and Fish. Tech. Bull. 2: 1-47.

GUNDY, S. D. VAN. 1958. The Life history of the citrus nematode, *Tylenchulus semipenetrans* Cobb. Nematologica 3: 283-294.

HIRSCHMANN, HEDWIG. 1957. The life cycle and role of interex in *Ditylenchus triformis*. (Abstr.) Phytopathology 47: 524.

HIRSCHMANN, HEDWIG and J. N. SASSER. 1955. On the occurrence of an intersexual form in *Ditylenchus triformis*, n. sp. (Nematoda, Tylenchida). Proc. Helminthol. Soc. Wash. D. C. 22: 115-123.

MAUPAS, E. 1899. La mue et l'enkystement chez les nématodes. Arch. Zool. Expér. et Gén. 3s. 7: 563-628.

PAI, S. 1928. Die Phasen des Lebenscyclus der *Anguillula aceti* Ehrbg. und ihre experimentell-morphologische Beeinflussung. Z. wiss. Zool. 131: 293-344.

RASKI, D. J. 1950. The life history and morphology of the sugarbeet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40: 135-152.
STEINER, G. 1923. Intersexes in nematodes. J. Heredity 14: 147-158.
STRASSEN, O. ZUR. 1892. *Bradynema rigidum* v. Sieb. Z. wiss. Zool. 54: 655-747.
TRIANTAPHYLLOU, A. C. and HEDWIG HIRSCHMANN. 1960. Post-infection development of *Meloidogyne incognita* Chitwood, 1949 (Nematoda: Heteroderidae). Ann. Inst. Phytopath. Benaki. N. S. 3: 1-11.
WEERDT, L. G. VAN. 1960. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. Part III. Embryology and post-embryonic development. Nematologica 5: 43-51.
WU, LIANG-YU. 1958. Morphology of *Ditylenchus destructor* Thorne, 1945 (Nematoda: Tylenchidae), from a pure culture, with special reference to reproductive systems and esophageal glands. Canad. J. Zool. 36: 569-576.
WÜLKER, G. 1923. Über Fortpflanzung und Entwicklung von *Allantonema* und verwandten Nematoden. Ergebn. u. Fortschr. Zool. 5: 389-507.
YUKSEL, H. S. 1960. Observations on the life cycle of *Ditylenchus dipsaci* on onion seedlings. Nematologica 5: 289-296.

Studies on the Life-history and Habits of the Burrowing Nematode, *Radopholus similis*, the Cause of Black-head Disease of Banana

CLIVE A. LOOS*

The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, is an important pest of cultivated bananas in Central America and the West Indies. The pest invades the root system and causes lesions which may girdle and destroy roots up to one-half inch thick. Heavily infected plants are stunted, carry small fruit and topple over easily because of inadequate root anchorage (Loos & Loos, 1960 a). Large numbers of *R. similis* in all stages of development may be found in lesions in both root and rhizome cortical tissue. Since bananas are propagated by rhizomes, planting material is mainly responsible for wide-scale dissemination of the pest (Loos & Loos, 1960 b). Individual specimens, used in these studies, were obtained from rhizome lesions in Jamaica.

MORPHOLOGICAL AND POPULATION STUDIES

Lacatan, Robusta and Gros Michel are three of the chief banana varieties grown for export purposes. No significant morphological differences were observed in burrowing nematode populations collected on these three varieties at various times and places. Size range was so considerable that it left no doubt that to attempt separation of populations between numerous banana varieties, on a size factor, was not possible. Van Weerdt (1958) made similar observations for *R. similis* on citrus, corn and banana in Florida.

Burrowing nematodes, on extraction from banana tissues, are sluggish but may be stimulated to activity by passing air through the nematode suspension. On the other hand, individuals recovered from soil are active; this is understandable since those individuals were not from sedentary feeding positions but in active search for a host root. Ratios of males to females in lesions vary considerably; in large lesions, where extensive colonies had been built up, this ratio is generally in the proportion of 1.5 females to a male.

*Formerly Nematologist to the Banana Board of Jamaica, Kingston Gardens, Kingston, Jamaica, W. I. and now Plant Pathologist to the Los Angeles County Agricultural Commissioner, 808 No. Spring Street, Los Angeles 12, California.

Table 1. Egg-laying of gravid and large selected females of *Radopholus similis* in spots of distilled water.

Type	No. of Females	Eggs laid in water in 0-24 hours	24-48 hours	2-10 days
Gravid females	16	42	3	0
Large non-gravid females	34	6	0	0

EXPERIMENTS IN VITRO

EGG-LAYING: Females can be maintained from two to seven weeks in spots of distilled water on glass slides, provided they are transferred to fresh water every four to six days. An active specimen becomes sluggish a few hours after transfer but may again be induced to activity by raising it out of the water on the end of a needle for a few seconds until the film of water around its body evaporates, before replacement in the water spot.

A female may carry two eggs, one in each uterus; these eggs are generally laid in an unsegmented condition in a few hours though, due possibly to disturbance, change of environment or damage to the mother, it is not unusual for development to proceed to the four-cell stage before being deposited. A few cases occurred where eggs were not deposited but developed to the final pre-hatching stage in the uterus. In another instance a second egg formed in the anterior ovary developed up to the final pre-hatching stage but did not emerge. In all probability, in those cases, females were damaged but survived for many days. There were many instances too, where a gravid female died before laying and the egg was destroyed by bacteria. Females removed from banana lesions ceased egg production in under 24 hours though an occasional specimen laid at a much reduced rate on the second day (Table 1).

In another experiment 90 gravid females were transferred, in groups of 5 worms, to drops of fresh distilled water on glass slides and placed in damp chambers to avoid drying. During the first 24 hours mean egg-laying rate was 2.6 eggs per female. This is in close agreement with egg-laying of gravid females expressed in Table 1. None of the 90 females laid any more eggs over a 7 week observation period indicating that nutritional factors stimulated egg-laying.

At room temperature (75-90°F) larvae emerged from eggs, laid in water, from the fifth day onwards and all viable eggs were hatched in seven days. The majority of eggs hatched between the fifth and sixth days (Table 2).

EXPERIMENTS WITH LIVING ROOTS

Thick, fleshy banana roots are not suitable for accurate examination for endoparasitic nematodes. *Tephrosia candida,* a legume, was selected for these life-history studies since the seedling root is readily infected under laboratory

Table 2. Hatching of newly laid eggs of *Radopholus similis* in distilled water. Results based on viable eggs in eight batches.

	1	2	3	4	5	6	7	8	Total	% hatched
5 days	23	34	22	42	12	3	2	7	145	71.7
6 days	30	40	25	54	18	12	6	13	198	98.0
7 days	30	40	25	54	18	15	7	13	202	100.0

conditions, seedlings at a suitable stage of growth are readily available and their roots are most satisfactory for examination by methods described by Gadd & Loos (1941) and elaborated on in this paper. Temperatures in the laboratory, during the course of these experiments, ranged from 75-90°F.

INFECTION: A droplet of water was placed on a microscope slide and a solitary female, larva, or group of nematodes, depending on type of infection desired, transferred to the water. The pick-up and transfer needle was made with an eyebrow hair attached to the end of a dentist's pulp canal file with plastic cement, according to the manner described by Goodey (1957). Seedlings with straight radicles, 1½ inches in length, were obtained by planting seed in which germination had proceeded to the point where the radicle was just emerging in a pot of wet sand for a period of 24-48 hours at room temperature. The suitable seedlings were transferred to a dish of water, cleared of adhering sand particles with a soft brush and placed on a dry cloth or absorbent paper to remove excess water. Nematodes were placed in a small spot of water on a microscope slide and the seedling placed on it with the root tip resting in the water spot. If the spot of water was sufficiently small it was almost immediately drawn up around the root tip area and the nematodes brought into contact with the attractive root surface. Fine sand, which had passed through a 60 mesh sieve, was heaped lightly over the anterior one-half inch of the elongating root and dampened before the slide was placed in a damp chamber. Operations should be completed as quickly as possible, to avoid excessive drying with consequent damage to the root tip, and the slide left for 24-48 hours in a humid chamber before the infected seedling is planted to a pot of wet sand where it remains until time for examination. Addition of sand over the seedling root, though unnecessary as an aid to infection, is advisable to hold down the root tip from lifting prematurely from the slide and making twisted growth.

EXAMINATION: Females and larvae entered the root a short distance behind the tip. In under a week infection was observed as a faint discoloration which became more evident a week later. For examination purposes the seedling was

Table 3. Egg-laying and larval populations of gravid females of *Radopholus similis* in *Tephrosia candida* roots (Series 1)

	Number of females	Layers	Mean of layers	S.E. Mean
Eggs laid				
1 day	12	10	2.00	0.33
2 days	17	16	7.25	0.45
3 days	26	20	13.55	0.92
5 days	24	19	18.68	1.67
7 days	23	17	31.12	1.33
9 days	23	21	34.05	0.97
10 days	20	20	43.45	3.14
12 days	15	14	55.93	4.66
14 days	18	16	56.50	4.31
Larvae after				
7 days	23	17	0	0
9 days	23	21	4.5	0.26
10 days	20	20	9.25	0.63
12 days	15	14	15.14	1.12
14 days	18	16	18.44	1.21

carefully transferred to a dish of water, excised immediately beneath the cotyledons and the root cleared of adhering sand particles with the aid of a fine brush. Preferably at this stage, while the root is still in water, air is removed from the specimen tissues by means of a vacuum pump. Sufficient air was removed when roots sank on release of the vacuum. This air exhaustion was necessary since air bubbles in the tissues obscured accurate examination of cell contents. Roots cleared of sand and air were macerated in equal parts of 10 percent nitric and 10 percent chromic acids, maceration time depending on root thickness, though thirty minutes to one hour was generally found to be sufficient. Much of the dark orange stain of chromic acid, in the root tissues, was removed by washing macerated roots in tap water for about one hour. A root was sufficiently macerated when it flattened easily, without disintegrating, between a slide and cover slip on slight pressure to the slip. Microscopic examination was greatly facilitated when a macerated root was mounted in 10 percent caustic potash before a cover slip was applied and the specimen pressed flat. Caustic potash dissolves fat in nematode bodies and eggs but there is sufficient time for observation and counts of root contents before this occurs. The thick hypocotyl section of the seedling root was an effective holding place for transference of the root during preparation processes; this section is cut away with a sharp knife or razor blade at the final transfer immediately before placing a cover slip over the specimen.

After entry neither female nor larva travelled far. Generally a female was surrounded by her eggs; if she moved her track was recognized from the trail of eggs left behind. Consequently there was no difficulty in counting her offspring accurately during the first three weeks following infection. However, after that period eggs of the second generation became mixed with those of the original female to make accurate determination of individual efforts impossible.

Table 4. Egg-laying and larval populations of unselected females of *Radopholus similis* in *Tephrosia candida* roots (Series 2).

	Number of females	Layers	Mean of layers	S.E. Mean
Eggs laid				
1 day	32	11	2.00	1.41
2 days	28	7	2.86	0.74
3 days	28	23	7.91	0.69
4 days	24	24	11.75	1.04
7 days	26	25	29.12	1.90
9 days	19	19	36.53	3.13
10 days	26	25	36.44	3.12
11 days	28	25	34.72	3.61
12 days	17	15	45.93	5.21
14 days	17	17	50.06	3.34
17 days	13	13	53.00	6.94
Larvae after				
7 days	26	25	0	0
9 days	19	19	2.26	0.36
10 days	26	25	2.64	0.80
11 days	29	27	5.22	1.18
12 days	17	15	13.27	2.57
14 days	17	17	19.29	2.16
17 days	13	13	28.54	2.94

EGG-LAYING: Series 1.-Gravid females. In the first series of experiments only females with visible eggs (gravid) in the uterus were used for infection. Frequently, however, those eggs were deposited before worms entered a root. Though gravid females were selected to ensure that only actively laying worms would be used it did not ensure that they would continue to lay for even a week. In fact it was found that 14% of individuals causing infection did not lay any eggs and nearly as many laid very few eggs. The maximum number of eggs laid by any individual was 76 in 14 days. It was evident, therefore, that some selected worms were near the end of their egg-laying period at time of selection. A summary of examination results made between 1 and 14 days is given in Table 3.

Series 2.-Unselected females. In the second series of experiments no conscious selection of females was made though it is possible that, subconsciously, larger individuals were more frequently chosen since they were easier to pick up and less likely to be large larvae. The selections were finally viewed under a microscope to ensure that only adults were collected. At total of 258 females entered roots. Examinations made from 1 to 17 days are summarized in Table 4.

NON-LAYERS: Females referred to in Tables 3 & 4 originated from a population with a large number of males, and it is fair to assume that chances of picking unfertilized females for these experiments were, therefore, small. In series 1 (gravid females) 10 of 12 (83%) commenced laying within 24 hours and showed a mean of 2.0 eggs per layer; in series 2 (unselected, non-gravid females) in the same time period, only 11 of 32 (34%) laid and in this case too, the mean was 2.0 eggs per layer. Obviously layers of the unselected females in series 2 were in the gravid category but were picked up as unselected non-gravids since eggs had been deposited shortly before they were picked up. The great difference in numbers of non-layers in the two series (10% in gravid and 70% in unselected females) over a two-day period suggested a time lapse between adulthood and commencement of egg-laying. That this pre-egg-laying period was of short duration was demonstrated in egg-laying results of the unselected females on the third day; the percentage non-layers dropped and the mean eggs per layer rose significantly (Tables 4 and 5).

In the gravid females series there were worms which did not lay or laid only a few eggs at 3, 5 and 7 days after entry into roots. These females presumably completed egg-laying but survived for a period after reproduction ceased. There was no indication of a succession of egg-laying periods with rests between such periods.

MEAN RATE OF EGG-LAYING: The mean numbers of eggs found at each examination in Series 1 and 2 (gravid and unselected females) are indicated

Table 5. Egg-laying of gravid (G) and unselected non-gravid (NG) females in *Tephrosia candida* roots.

	Number of entries		Number of non-layers		Mean eggs Total entries		S.E. mean Total entries	
	G	NG	G	NG	G	NG	G	NG
Eggs laid.								
1 day	12	32	2	21	1.60	0.69	0.36	0.23
2 days	17	28	1	21	6.82	0.71	0.60	0.35
3 days	26	28	6	5	10.42	6.50	1.34	0.81
7 days	23	26	6	1	23.00	28.00	3.38	2.15

by a solid circle in Figs. 1 and 2. Straight lines which best fit the data are represented by equations $Y = -1.399 + 4.367x$ (gravid) and $Y = -0.8348 + 3.55x$ (unselected). Mean rates of egg-laying are 4.367 and 3.55 respectively over a two-week period. The higher rate for gravid females is attributed to higher egg production over the first three days after entry into a root. In both series it was apparent that egg-laying was proceeding unchecked beyond the observed two-week period.

Rate of hatching will be a reflection on the rate of egg-laying so long as incubation periods remain constant and all eggs hatch. Mean numbers of larvae found from 7-14 days are also shown in Figs. 1 and 2. Straight lines which best fit these observations are $Y = -19.1712 + 2.753x$ (gravid) and $Y = -21.7831 + 2.766x$ (unselected). Those mean hatching rates are in close agreement with each other (2.753 and 2.766) though somewhat less than corresponding egg-laying rates (4.367 and 3.55). Since the incubation period is constant the inference is that a fair proportion of eggs did not hatch. This inference is supported by van Weerdt (1960) who stated that a good percentage of eggs, particularly those slightly longer and narrower (length/width exceeding 2.5), more strongly bent and darker colored, failed to develop normally. Whether in the present instance a lower hatching rate is due to that factor or of non-fertilization was not clear.

RATE OF EGG-LAYING OF INDIVIDUALS: It will be realized that mean rates of

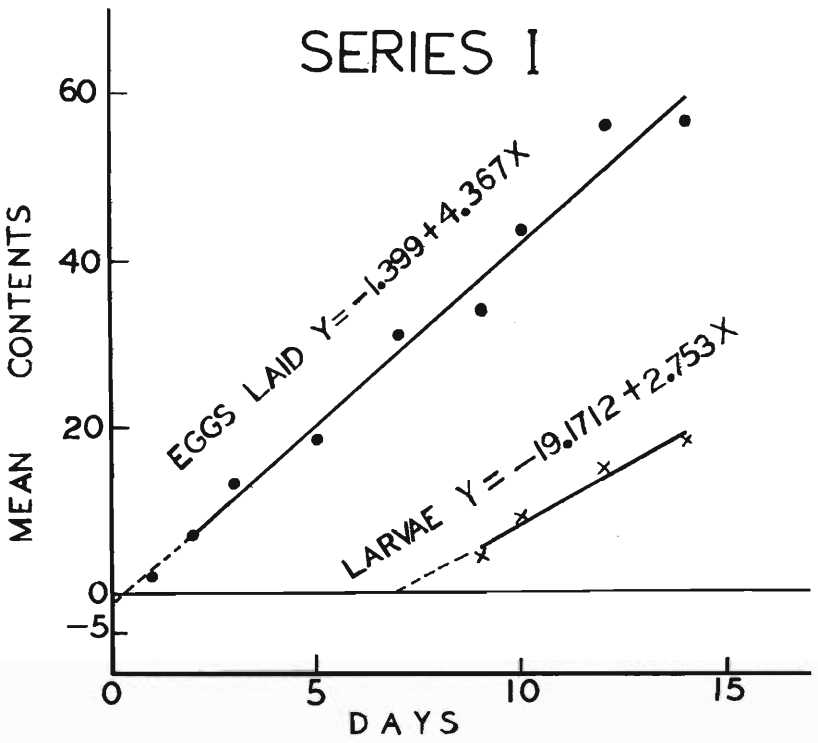


Fig. 1. Mean rate of egg-laying in *Tephrosia candida* by a population of gravid females from banana rhizome lesions and the mean rate of emergence of larvae.

egg-laying of a population give little indication of individual performances. Mean rate of a population over a period will normally be less than the mean rate of an individual unless all lay throughout the period. During 10 days in Series 1 (gravid females) 20 individuals laid 869 eggs giving an average daily rate of 4.34 eggs per individual. Of these, over that 10 day period, one laid 6, one 23 and another 31; it is possible that these individuals ceased egg-laying before the end of 10 days. The remaining 17 individuals laid from 34 to 64 eggs each with a mean daily rate of 4.76 over a 10 day period. This figure is probably fairly representative of the rate of egg-laying of individuals. Maximum rate observed was 6.4 eggs per day. Maximum rate observed in Series 2 (unselected females) was 7.7 eggs per day over a period of 9 days and maximum number laid by any one individual in 17 days, the longest period under observation, was 95 which amount, if she commenced laying on the first day, gives a mean of 5.6 eggs per day. These mean rates are in fairly close agreement to the maximum laid by a gravid female, in distilled water, over a 24 hour period.

INCUBATION PERIOD: It was previously shown that, *in vitro,* eggs may hatch in 5 days and that all hatchings were completed in 7 days. Eggs laid in roots take somewhat longer to hatch. Roots in Series 1 and 2 contained no larvae at the examination made after 7 days while larvae were observed, in some roots of both series, at the examination made after 9 days. Eggs from which these larvae emerged were probably laid shortly after females entered roots, in which case an incubation period, as observed, lies between 7 and 9 days.

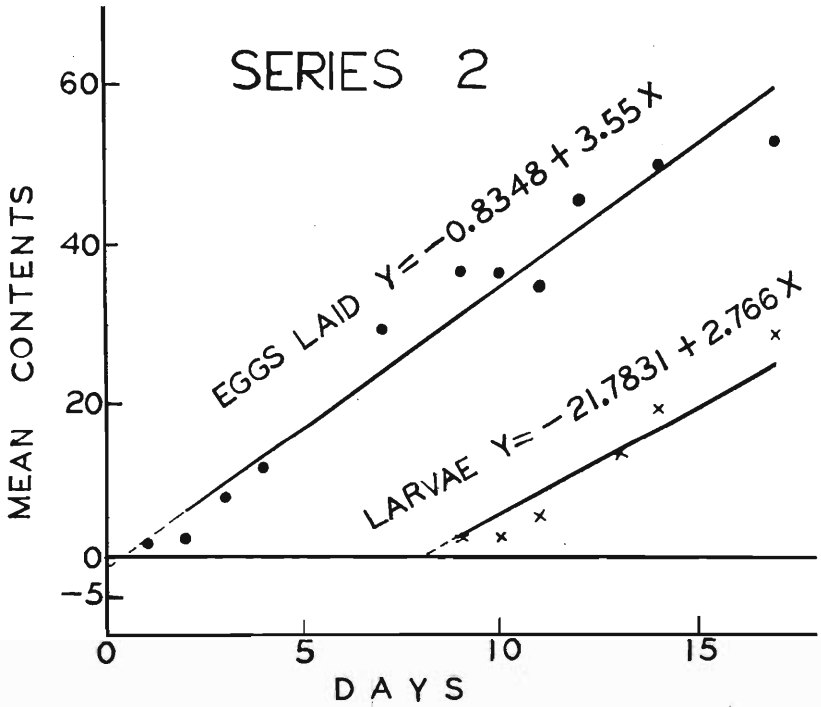


Fig. 2. Mean rate of egg-laying in *Tephrosia candida* roots by a population of unselected females from banana rhizome lesions and the mean rate of emergence of larvae.

A mean incubation period in roots may be derived from Figs. 1 and 2. If larval graphs are prolonged until they cut the X-axis, the points of intersection are 6.9 and 7.6 days for Series 1 and 2 respectively. We could conclude, therefore, that a mean incubation period for *R. similis* eggs, under the conditions of these experiments, was between 7 and 8 days.

LARVAL PERIOD: A direct estimate of the larval period was obtained by teasing roots, each of which had been inoculated with 10 recently hatched larvae, at daily intervals commencing after the ninth day. Results are set out in Table 6. Larval period was 10 to 13 days with the majority attaining adulthood in 11 days. Larval period for males was a day shorter than that for females.

PRE-EGG-LAYING PERIOD: Larvae becoming adult in 10 days did not, however, commence egg-laying until after the 12th day. Although it was not possible to determine with certainty the number of females concerned in the laying of those eggs, since the infected roots contained more than one female, the small number of eggs laid at the end of the 12th day suggested that they were laid by a single female. At the end of the 13th day egg-laying was a general feature and at the end of the 14th day 14 females had laid an average of 7.6 eggs per individual (Table 6). In comparison Gadd & Loos (1941) found that *Pratylenchus coffeae* had an adult pre-egg-laying period of 15 days.

FERTILIZATION AND EGG-LAYING: Gadd & Loos (1941) found a delay in commencement of egg-laying of unfertilized *Pratylenchus coffeae*. Two experiments were set up to ascertain if fertilization was a prerequisite to egg-laying of *R. similis* or if this species is parthenogenetic. In the first experiment 34 newly hatched larvae were inoculated, one to a root, and after 48 hours in a damp chamber the seedlings were planted to pots of wet sand. Six weeks later each of 20 positive infections contained a female without progeny. Under conditions of mass infection and colony existence egg-laying should have commenced in under 12 days and, assuming a continuous period of egg-laying, each female should have laid over 120 eggs. In a second experiment large juveniles were introduced, one to each seedling root. Sixteen days later, of 8 positive infections, 7 were females without progeny and one a male; of 10 positive infections examined 28 days after inoculation 8 were females without progeny, one a male and the other a female with 11 larvae and 67 eggs. The reason for this apparent single parthenogenetic case is not known. There is a possibility that it was a young fertilized female.

NUTRITION AND EGG-LAYING: *In vitro* studies suggested that egg-laying was governed by nutritional factors since separation of actively laying individuals from plant tissues to water caused egg-laying to cease in under 24 hours. That this cessation was due to lack of nutrition and not to disturbance caused by transfer was demonstrated in the following experiment.

Table 6. Larval and pre-egg laying periods of *R. similis* in *Tephrosia candida* roots.

Age of Days	Number nematodes	Juveniles	Females	Males	Eggs	Percentage Adults
9	21	21	0	0	0	0
10	11	7	0	4	0	57
11	15	1	10	4	0	93
12	22	2	16	4	4	91
13	16	0	13	3	52	100
14	20	0	14	6	107	100

From a collection of gravid females, recently obtained from rhizomes, 10 were inoculated to seedling roots and 60 transferred to distilled water on a glass slide. Forty-eight hours later 10 females in roots had laid 84 eggs (8.4 per female) while 60 females in water had laid 162 eggs (2.7 per female). Although the females in water were left for a further 10 days no more eggs were laid.

In a third experiment gravid females, which had ceased egg-laying over the previous 4 days of a five-day starvation period in water, were inoculated to *Tephrosia* roots. Results of examinations made 2, 3, 4 and 10 days after inoculation are expressed in Table 7. Those results are in close agreement with egg-laying of non-gravid females taken directly from banana roots (Table 4). The quick re-establishment of egg-laying of females, on transfer from starvation medium to root tissues, proved conclusively that egg-laying is correlated with nutrition.

MOLTS IN WATER: Large juveniles requiring but a single molt before adulthood completed it in water though, in many cases, they were unable to completely shed their cast cuticle. Juveniles requiring more than one molt before adulthood failed to make a second molt.

INFECTION OF ROOTS: All larval stages, from newly hatched second stage larvae to the fourth-stage pre-adult molt, are infective. Females moved in and out of roots at will but males were incapable of such movement. However, males feed in roots provided the final molt takes place in plant tissues. Fertilization occurs in the host tissues though there is no evidence that it cannot occur outside a root.

LIFE CYCLE: Life cycle, egg to egg, at temperatures ranging between 75° and 90°F, of *R. similis* in roots of *Tephrosia candida* was completed in 20-25 days; that period may be divided into 8-10 days for eggs to hatch, 10 to 13 days as larval period and 2 days as adult before egg-laying commences. The life cycle period agrees closely with Suit and DuCharm's (1957) estimate of 3-4 weeks for *R. similis* in citrus roots. Van Weerdt (1960) recorded 4 larval molts, the first occurring in the egg. The 3 molts outside the egg, occur during 10-13 days. The fourth larval or pre-adult stage is the shortest of the larval periods and there is no apparent increase in body length at that time.

Table 7. Egg-laying of single gravid *Radopholus similis* females introduced to *Tephrosia candida* seedling roots after a 5 day starvation period in water during which time egg-laying ceased.

	Egg Contents in Root After—			
	2 days	3 days	4 days	10 days
Degrees of freedom	6	7	7	6
Mean per root	2.5	8.1	12.1	31.0
Mean per root averaged per day	1.25	2.72	3.02	3.10
SEM	0.16	0.56	0.58	0.79

SUMMARY

No significant morphological differences were observed between populations of *Radopholus similis* from Lacatan, Robusta and Gros Michel banana varieties. The nematode, on extraction from banana tissues, is sluggish but may be stimulated to activity by passing air through the nematode suspension.

Gravid females when transferred from roots to water cease laying in under 24 hours. Egg-laying in water averaged 2.6 per female with a best individual performance of 6 eggs. Eggs laid in water hatch normally. A rapid method for infection and later determination of population in a seedling root is described. The value of this method in the study of the life cycle of *R. similis* is demonstrated. Females lay on an average of 3.5 to 4.6 eggs per day and continued laying beyond 2 weeks. Eggs hatch in from 5 to 7 days in water and 7 to 8 days in a root. The larval period is 10 to 13 days with the majority becoming adult in 11 days. There is a pre-egg-laying period of 2 days. Unfertilized females do not lay; there was evidence of a possible parthenogenetic case from over a large number of observations. Egg-laying of fertilized females is governed by nutritional factors. Removal of actively laying females from host tissues to water caused egg-laying to cease in under 24 hours. Reintroduction of those females to a host induced egg-laying to recommence. All larval stages and females are infective. Males are incapable of entering roots. Life cycle, egg to egg, is completed in 20 to 25 days which period may be divided to 8 to 10 days for eggs to hatch, 10 to 13 days for larval period and 2 days as adult before laying commences.

LITERATURE CITED

GADD, C. H. and C. A. LOOS. 1941. Observations on the life history of *Anguillulina pratensis*. Ann. App. Biol. 28: 39-51.

GOODEY, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture Fisheries and Food. Tech. Bull. No. 2: 47 pp.

LOOS, C. A. and SARAH B. LOOS. 1960 a. The black-head disease of bananas (*Musa acuminata*). Proc. Helm. Soc. Washington, D. C. 27: 189-193.

————. 1960 b. Preparing nematode-free banana "seed". Phytopathology 50: 383-386.

SUIT, R. F. and E. P. DUCHARME. 1957. Spreading decline of citrus. Bull. Fla. Plant Board 2 No. 11: 24 pp.

WEERDT, L. G. VAN. 1958. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. Part 2. Morphological variation within and between progenies of single females. Nematologica 3: 184-196.

————. 1960. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. Part 3. Embryology and post-embryonic development. Nematologica 5: 43-51.

Effect of Temperature on Hatching of *Aphelenchus avenae* Eggs*

DONALD P. TAYLOR**

Aphelenchus avenae Bastian, 1865, is a common soil-inhabiting nematode. Although frequently associated with plant parts, it is not considered to be an obligate parasite of higher plants, according to Steiner (1936). Since Christie and Arndt (1936) reported that this species feeds like plant-parasites and since it can be rapidly increased in the laboratory, *A. avenae* may become important in the study of nematode physiology, ecology, and genetics. This nematode may also be useful in screening chemicals for nematocidal activity because of these characteristics.

Little has been published on the hatching of eggs in the Tylenchida. Dropkin, et al. (1958) reported that the stylet of *Meloidogyne arenaria*

*Paper No. 4654, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.

**Assistant Professor, Department of Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota.

(Neal, 1889) Chitwood, 1949, is extruded repeatedly before hatching. In studies on hatching in *Paratylenchus projectus* Jenkins, 1956, Rhoades and Linford (1961) noted vigorous movement of the ovic larva and distortion of the egg membrane as hatching neared. They also stated that the stylet was thrust at the end of the egg until the membrane was broken.

This paper reports the hatching process in *A. avenae* and the effect of temperature on the time necessary for hatching.

MATERIALS AND METHODS

The population of *A. avenae* used in these experiments was increased from four females isolated from soil collected at a commercial nursery in Newport, Minnesota. These individuals were selected from a mixed nematode population, rinsed in sterile water, and placed in a petri dish culture of *Fusarium oxysporum* f. *lini* (Bolley) Snyder and Hanson on acidified potato dextrose agar. As the nematode population increased, transfers containing agar, mycelium, and nematodes were made to other plates. *A. avenae* has been maintained in the laboratory in this way for over a year.

Eggs of *A. avenae* were obtained by transferring 30-50 gravid females collected from cultures 10 to 14 days old to distilled water in a watch glass. Females were removed from the dish after 15-30 minutes and placed in another watch glass. Dishes containing eggs were placed immediately in refrigerators or incubators at constant temperatures. Dishes were examined at intervals for the presence of hatched larvae. The time elapsed from when the eggs were laid until they hatched was recorded.

Ovic larval development was observed by placing freshly-laid eggs in hanging drops of sterile distilled water as described by Taylor (1961).

EFFECT OF TEMPERATURE ON HATCHING

The results of tests to determine the effect of temperature on the time between egg-laying and hatching are given in Table 1. No eggs hatched at 5 and 42 °C. Eggs hatched in the shortest time at 36°C. At 38°C most of the eggs were killed, but those that survived required a slightly longer time to hatch than those at 36°C.

OBSERVATIONS ON HATCHING

Earliest observations of ovic larvae disclosed two distinct movements: a forward or backward churning of the larva and a rapid pulsation of the valve in the metacorpus. No outward flow of fluid from the oral opening or stylet was detected at any time. Prior to the time when metacorpal pulsations were noted, larval movements had no effect on the contour of the egg.

Table 1. Minimum time necessary for hatching of eggs of *Aphelenchus avenae* in sterile distilled water at nine temperatures.

Temperature (°C)	Hours from egg-laying to hatching
5	No embryologic development after 720 hours
10	275-282
15	120-125
20	71-74
25	46-48
30	30-33
36	28-30
38	34-45*
42	Death occurred within 24 hours

*Most eggs at this temperature were killed. The time reported is for the few that hatched

However, after pulsations had been observed, the egg membrane became more and more flexible, and conspicuous bulges occurred at the points of maximum pressure of the larva's body. When the anterior end of the larva was located at either end of the egg, the stylet was exserted and withdrawn rapidly back and forth through the oral opening and was seen projecting through the membrane into the surrounding water. Almost immediately the egg lost some of its turgidity and the end opposite the punctures collapsed except when supported by the larva. Additional pulsations were noted and many more punctures were made at both ends of the egg. The anterior end of the larva broke through the end of the egg in which most punctures had been seen. In most eggs studied, the larva slowly moved forward into the surrounding water; however, one larva emerged to about one-third its total length, backed into the egg, and then shot out of the egg tail-first.

DISCUSSION

Eggs of *A. avenae* hatched over a wide range of temperatures, but not at 5 or 42°C. Between the extremes the time necessary for hatching decreased as the temperature increased, except at 38°C. At that temperature a high percentage of eggs were killed, indicating that 38°C is near the lethal temperatures for eggs of this species.

Hatching appears to consist of three processes. After the ovic larva became active, the shell became more flexible but only after metacorpol pulsation occurred. In other nematodes such pulsations are often accompanied by an outward flow of secretions from esophageal glands. Thus, a deposition of fluids within the egg membrane by the larva at this time is likely, although not observed in this study. The initial step in hatching probably involves a chemical change in the membrane caused by activity of esophageal gland fluids as evidenced by the association of increased membrane flexibility and metacorpal pulsations. The second step in hatching is the repeated puncture of the egg by the stylet of the larva. The final step involves a rupture of the membrane by the application of pressure by the larva against a weakened part of the membrane.

SUMMARY

Eggs of *A. avenae* hatched between 10 and 38°C. The time between egg-laying and hatching decreased from 275-282 hours at 10°C to 28-30 hours at 36°C. Most eggs died at 38°C and the few eggs that hatched required a longer time than did those at 36°C. The ovic larva moved actively in a rigid egg membrane. After repeated pulsations of the metacorpal valve, the egg membrane became more flexible as hatching approached. The stylet punctured the membrane many times and the larva emerged through one of the weakened ends of the egg.

LITERATURE CITED

CHRISTIE, J. R., and C. H. ARNDT. 1936. Feeding habits of the nematodes Aphelenchoides parietinus and Aphelenchus avenae. Phytopathology 26: 698-701.

DROPKIN, V. H., G. C. MARTIN, and R. H. JOHNSON. 1958. Effect of osmotic concentration on hatching of some plant parasitic nematodes. Nematologica 3: 115-126.

RHOADES, H. L., and M. B. LINFORD. 1961. Biological studies on some members of the genus *Paratylenchus*. Proc. Helminth. Soc. Wash. 28: 51-59.

STEINER, G. 1936. The status of the nematode Aphelenchus avenae Bastian, 1865, as a plant parasite. Phytopathology 26: 294-295.

TAYLOR, D. P. 1961. Biology and host-parasite relationships of the spiral nematode, *Helicotylenchus microlobus*. Proc. Helminth. Soc. Wash. 28: 60-66.

Occurrence of *Trypanosoma cruzi* in Maryland

C. M. HERMAN* and J. I. BRUCE, JR.**

Chagas disease or South American trypanosomiasis, caused by *Trypanosoma cruzi,* is a widespread disease of man in the western hemisphere. Most human cases have been reported from South and Central America. Only two human cases have been reported from the United States and both of these were from Texas in 1955 (Woody and Woody, 1955: USPHS, 1955). In southern United States, as in the endemic areas of South America, the infection is found in a number of wild animals. Reduviids, Triatominae, are the only known insect vectors.

The first evidence that *T. cruzi* occurred in the United States was discovered in 1916 when Kofoid and McCulloch (1916) reported a new species of trypanosome isolated from reduviid bugs collected from a wood-rat nest in southern California. Fae Wood (1934) repeated and extended these studies and demonstrated that the trypanosome was *T. cruzi.* Subsequently, Sherwin Wood continued and extended his wife's studies by examining many species of cone-nose bugs and rodents from southwestern United States and Mexico. *T. cruzi* was reported from both wood rats (*Neotoma*) and deer mice (*Peromyscus*) as well as from reduviids (Wood, 1938, 1941, 1949, 1952; Wood and Wood, 1941). Packchanian (1941) reported *T. cruzi* from a number of small mammals collected in Texas, including armadillos, mice, wood rats and opossums.

In 1955, while examining blood smears from mammals trapped at the Patuxent Wildlife Research Center in Laurel, Maryland, Walton was the first to observe a trypanosome in a raccoon. This observation subsequently was reported as *T. cruzi* (Walton, *et al,* 1956); earlier findings were reported in a paper by Walton, *et al* (1958). In subsequent studies Diamond and Rubin (1956, 1958) demonstrated the susceptibility of day-old pigs, lambs and kids to this strain of *T. cruzi.*

Trypanosomes were reported from raccoons in Georgia by Brooke, *et al* (1957). A group of scientists working at the Communicable Disease Center in Atlanta reported trypanosomes from several species of mammals (McKeever, *et al,* 1958). The trypanosomes were discovered by accident in cultures made for isolation of leptospirae. The blood samples were taken from wild mammals trapped from November 1954 to April 1957 on a number of areas in southwestern Georgia and northwestern Florida. Trypanosomes were detected in primary cultures for leptospirae from 98 of 1,977 animals of 4 species. Host species and prevalences of infection were as follows: 88 of 552 opossums; 8 of 608 raccoons; and 2 of 306 striped skunks were infected. In addition, subcultures showed that one opossum, one raccoon, one striped skunk, and two gray foxes were infected. A few triatomids were collected but no infection was found in them.

---

*Section of Wildlife Diseases and Parasite Studies, Patuxent Wildlife Research Center, Laurel, Maryland.

**Department of Medical Zoology, Walter Reed Army Institute of Research, Washington, D. C.

The authors are indebted to a number of colleagues for assistance in this survey. We are extremely grateful to employees of the Patuxent Wildlife Research Center, particularly to Leonard M. Llewellyn and Fred Schmid, and to several enlisted personnel from the Walter Reed Army Institute of Research, for assistance in procuring the animals. At various times many of our colleagues on the staff at the Patuxent Wildlife Research Center, as well as personnel of the Walter Reed Army Institute of Research assigned to the Patuxent Laboratory, provided valuable assistance in handling the animals and examining some of the material.

Yeager and D'Allessandro Bacigalupo (1960) reported *T. cruzi* from raccoons and opossums in Louisiana.

RESULTS

Since 1954, we have taken blood samples from many mammals at the Patuxent Wildlife Research Center. Our routine procedure usually involved examination of heart blood in wet and in stained preparations, in concentrations by a modified Knott's technique (Herman and Price, 1955) and in culture in artificial media at room temperature (Diamond's SNB 9, Diamond and Rubin, 1958). Samples from each animal usually were examined by all four methods.

During the years 1954-1960 samples were taken from 2005 animals from the Patuxent Wildlife Research Center, including 7 chipmunks (*Tamias striatus*); 8 gray foxes (*Urocyon cinereoargenteus*); 37 (1)* red foxes (*Vulpes fulva*); 126 (1) white-footed mice (*Peromyscus leucopus*); 18 muskrats (*Ondatra zibethicus*); 219 (47) opossums (*Didelphis marsupialis*); 424 (52) cottontail rabbits (*Sylvilagus floridanus*); 472 (80) raccoons (*Procyon lotor*); 74 (7) house rats (*Rattus norvegicus*); 5 flying squirrels (*Glaucomys volans*); 79 (11) striped skunks (*Mephitis nigra*); 305 (42) gray squirrels (*Sciurus carolinensis*); 77 meadow voles (*Microtus pennsylvanicus*); 95 woodchucks (*Marmota monax*); 9 weasels (*Mustela frenata*). In addition 50 bats from neighboring areas were examined, 43 big brown bats (*Eptesicus fuscus*) and 7 evening bats (*Nycticeius humeralis*).

Specimens were collected throughout the year, but the greatest trapping effort was during the months of March and November, in connection with other studies at the Center. Special trapping was done in the other months to obtain data on incidence of blood parasites through the year. Most of the animals were released after a sample of blood was taken from the heart.

Because our data suggest a seasonal distribution of *T. cruzi* at the Patuxent Center, the monthly distribution of samples is shown in Table 1. Approximately 17% of the raccoons and 11% of all species were trapped in November. Trypanosomes were observed in the samples from ten raccoons; nine of these raccoons were trapped in November and one on October 31, 1960. The monthly distribution of the raccoon samples is given in Table 2. In 1954, samples were taken from 12 raccoons and none showed infection; in 1955, 3 of 9 (2 on November 14 and 1 on November 18) were infected; in 1956, 2 of 19 (1 on November 27, 1 on November 30); in 1957, 2 of 24 (1 on November 18, 1 on November 22); in 1958, 0 of 2; in 1959, 2 of 4 (both on November 20); in 1960, 0 of 10. In total, 9, or 11.3 percent of the 80 samples examined during November showed infection. The percentage of infected raccoons sampled throughout the year was slightly over 2 percent. All the

Table 1. Number of Animals Examined by Months

Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1954	90	52	89	95	102	92	20	23	12	61	51	17	704
1955	49	32	34	27	47	14	11	31	38	27	22	8	340
1956	7	19	77	3	14	11	10	37	46	18	51	57	350
1957	....	....	95	33	14	....	12	27	13	23	58	3	278
1958	....	....	32	31	15	4	4	7	13	13	30	19	168
1959	....	....	25	7	6	12	....	....	....	....	5	9	64
1960	2	1	3	1	37	4	3	1	....	35	14	....	101
Totals	148	104	355	197	235	137	60	126	122	177	231	113	2005

*The number in parenthesis indicates the number collected in November.

infections were classified as *T. cruzi*.

Although *T. cruzi* has been reported from rodents in other areas and a variety of trypanosomes have been reported from rodents as a result of many surveys in various parts of the world, we were unable to demonstrate any trypanosomes in the rodents collected at the Patuxent Wildlife Research Center.

We found no evidence of trypanosomes in 219 opossums, although *T. cruzi* has been reported from this host in many areas of the southern United States. McKeever, *et al* (1958) observed about the same rate of occurrence of *T. cruzi* in raccoons in Georgia (1.5%) as we did in Maryland but they also found infection in 16% of their samples from opossums. In earlier studies Walton, *et al* (1958) inoculated one of our raccoon isolates into three opossums. One opossum died 15 days later without showing any evidence of infection, one exhibited only a very low parasitemia on the 24th day after exposure and the third, which never exhibited a parasitemia, yielded a positive culture from heart blood taken on the 30th day after exposure. Brooke, *et al* (1957) studied trypanosomes isolated from 5 opossums, 1 raccoon and 1 striped skunk collected in Georgia and compared their forms with *T. cruzi* and *T. rangeli*. They concluded that isolates from the striped skunk and opossum had characteristics very similar to *T. cruzi* and that the isolate from the raccoon was more like *T. cruzi* than *T. rangeli*, although in culture it appeared different from either.

DISCUSSION

In our earlier studies with raccoons (Walton, *et al*, 1958) the three types of inocula (blood, culture and triatomids) produced nonfatal infection with eventual disappearance of organisms from peripheral blood and considerable variation in parasitemia. However, one raccoon (inoculated with cultural forms) exhibited a parasitemia up to 14 weeks after exposure and 3 weeks later yielded a positive culture. Another experimental raccoon was still exhibiting parasitemia 8 weeks after exposure. In natural infections in the wild raccoons, this continuing parasitemia was not evident.

All the *T. cruzi* we found in Maryland raccoons were found within a month's span.

It seems logical to assume that this seasonal incidence is directly related to the habits of the raccoon and the local vector of *T. cruzi*. Seasonal variations in incidence of infection also may account for our negative findings in other species. There is no evidence to date that arthropods other than triatomids can transmit *T. cruzi*. Although in earlier studies (Walton, *et al*, 1958) it was possible to transmit the Patuxent raccoon infection experimentally with laboratory reared tratomids, no cone-nose bugs were found in the field during a check for natural infection. Several local entomologists who do extensive collecting informed us that triatomids do occur in this area but are extremely rare; none were reported from the Patuxent Center. More

Table 2. Number of raccoons examined at Patuxent Wildlife Research Center, 1954-1960

Month	Number	No. Positive	Month	Number	No. Positive	Month	Number	No. Positive
Jan.	12	..	May	38	..	Sept.	35	..
Feb.	28	..	June	27	..	Oct.	24	1
Mar.	98	..	July	16	..	Nov.	80	9
Apr.	64	..	Aug.	22	..	Dec.	28	..

recently, during October 1960, one of our colleagues, Dr. Gordon M. Clark, discovered a few triatomids in a rotting log at the Patuxent Center.

SUMMARY

During 1954-1960, 2005 mammals of 18 species collected at the Patuxent Wildlife Research Center, Maryland, were examined for trypanosomes. *T. cruzi* was found in 10 raccoons between October 31 and November 30. Infection occurred in 2 percent of all raccoons sampled, and in 11.3 percent of the 80 raccoons sampled in November. Examination was by direct smears, stained smears and cultures of heart blood. Although, in previous studies, at least two experimentally infected raccoons exhibited extended parasitemia (14 and 8 weeks), no such continuing parasitemia was observed in the natural infections. No trypanosomes were found in any of the other mammals examined.

LITERATURE CITED

BROOKE, M. M., L. NORMAN, D. ALLAIN, and G. W. GORMAN. 1957. Isolation of *Trypanosoma cruzi*-like organisms from wild animals collected in Georgia. J. Parasitol. 43 (suppl.): 15.

DIAMOND, L. S. and R. RUBIN. 1956. Susceptibility of domestic animals to infection with *Trypanosoma cruzi* from the raccoon. J. Parasitol. 42 (suppl.): 21.

———— and ————. 1958. Experimental infection of certain farm mammals with a North American strain of *Trypanosoma cruzi* from the raccoon. Exp. Parasitol. 7: 383-390.

HERMAN, C. M. and D. L. PRICE. 1955. The occurrence of *Hepatozoon* in the gray squirrel (*Sciurus carolinensis*). J. Protozool. 2: 48-51.

KOFOID, C. A. and I. MCCULLOCH. 1916. On *Trypanosoma triatomae*, a new flagellate from a hemipteran bug from the nests of the wood rat *Neotoma fuscipes*. Univ. Calif. Publ. Zool. 16: 113-126.

MCKEEVER, S., G. W. GORMAN and L. NORMAN. 1958. Occurrence of a *Trypanosoma cruzi*-like organism in some mammals from southwestern Georgia and northeastern Florida. J. Parasitol. 44: 583-587.

PACKCHANIAN, A. 1941. Reservoir hosts of Chagas' disease in the State of Texas. J. Parasitol. 27 (suppl.): 30.

USPHS. 1955. Dept. Health, Education & Welfare, National Office of Vital Statistics. Communicable Diseases Summary for week ending Dec. 10, 1955.

WALTON, B. C., P. M. BAUMAN, L. S. DIAMOND and C. M. HERMAN. 1956. *Trypanosoma cruzi* in raccoons from Maryland. J. Parasitol. 42 (suppl.): 20.

WALTON, B. C., P. M. BAUMAN, L. S. DIAMOND and C. M. HERMAN. 1958. The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. Am. J. Trop. Med. and Hyg. 7: 603-610.

WOOD, F. D. 1934. Natural and experimental infection of *Triatoma protracta* Uhler and mammals in California with American trypanosomiasis. Am. J. Trop. Med. 14: 497-517.

WOOD, F. D. and S. F. WOOD. 1941. Present knowledge of the distribution of *Trypanosoma cruzi* in reservoir animals and vectors. Am. J. Trop. Med. 21: 335-345.

WOOD, S. F. 1938. A new locality for *Trypanosoma cruzi* Chagas in California. Science 87: 366-367.

————. 1941. New localities for *Trypanosoma cruzi* Chagas in southwestern United States. Am. J. Hyg. 34: 1-13.

————. 1949. Additional observations on *Trypanosoma cruzi* Chagas, from Arizona in insects, rodents and experimentally infected animals. Am. J. Trop. Med. 29: 43-55.

————. 1952. Mammal blood parasite records from Southwestern United States and Mexico. J. Parasitol. 38: 85-86.

WOODY, N. C. and H. B. WOODY. 1955. American trypanosomiasis (Chagas' disease—first indigenous case in the United States. J. Am. Med. Assoc. 159: 676-677.

YEAGER, R. G. and D'ALESSANDRO BACIGALUPO. 1960. Further studies on *Trypanosoma cruzi* in Louisiana. J. Parasitol. 46 (suppl.): 7.

The Effect of a Concurrent Infection with *Trypanosoma lewisi* on the Development and Maintenance of Acquired Immunity to *Nippostrongylus brasiliensis* in Rats*

WILLIE ASHLEY, JR.

In recent years some attention has been focused upon concurrent infections of parasitic organisms in laboratory mice and rats, especially in regard to the effect upon immunity. Brumpt (1933), Larsh and Donaldson (1944), Holmes (1947), and Cox (1952) are a few who have reported on the effects of concurrent infections on immunity in experimental animals. These investigations primarily involved nematodes and cestodes.

The literature reviewed did not reveal any reported observations on the effect of protozoan parasites on immunity to an intestinal helminth following a simultaneous entry into a host. The present report is concerned with the effect of a concurrent infection with the blood protozoan, *Trypanosoma lewisi*, on the development and maintenance of acquired immunity in rats to the intestinal nematode, *Nippostrongylus brasiliensis*.

MATERIALS AND METHODS

White laboratory rats between seven and eight weeks old were used as experimental hosts. The infective larvae of the test agent, *N. brasiliensis*, were obtained by a culture method essentially the same as that described by Yokogawa (1922), and injected into the hosts subcutaneously.

The inocula of the concurrent agent, *T. lewisi*, were prepared by taking blood from a heavily infected rat by cardiac puncture and placing it in a 2% citrate solution. Blood was used from donor rats inoculated not more than six days previously since antibody formation is probably minimal at that time. Quantitative standardization of the inocula was accomplished by use of the standard hemocytometer and blood pipette. The trypanosomes were injected intraperitoneally, and a check of the degree of parasitemia in the hosts was made daily by examining a drop of blood from the rat's tail under the microscope.

OBSERVATIONS AND RESULTS

A set of three experiments was conducted to test the effect of a concurrent infection of *T. lewisi* on the development of acquired immunity to *N. brasiliensis*, and another set of two experiments to test the effect of the concurrent infection on the action of previously acquired immunity to *N. brasiliensis*. The results are summarized in Tables 1 and 2, respectively.

Experiment I of the first set, was a preliminary attempt to observe the development of immunity to *N. brasiliensis* when relatively small numbers of *T. lewisi* are injected simultaneously with *N. brasiliensis* into rats. The concurrently infected and control rats became infected with *N. brasiliensis* and rapidly threw off the infection similarly, as was indicated by the typical daily egg count rise and gradual disappearance of eggs in the rats' feces by the 15th day after infection. Additional data were not recorded for this experiment. In experiments II and III, consisting of simultaneous injections with 50 and 150 million *T. lewisi*, respectively, and 1,000 *N. brasiliensis* larvae, the course of the *N. brasiliensis* infections was typical and similar to that in experiment I, eggs appearing in the feces of the control and concurrently infected rats on the 5th or 6th day indicating no difference in

*From the Department of Biology, The Catholic University of America, Washington, D. C.

prepatent periods. The peak of egg production generally occurred on the 8th or 9th day after infection. The lungs of necropsied control and concurrently infected rats appeared normal with no residual inflammatory reactions resulting from delayed migrating larvae. The adult worms recovered were normal and fully developed.

In experiments IV and V of the second set, all animals were rendered moderately and heavily immune to *N. brasiliensis* with 1800 and 2500 larvae, respectively, prior to the concurrent test infections. Data from the concurrent test infections, consisting of doses up to 250 million *T. lewisi*, revealed the pattern of a typical low grade infection seen when rats previously immune to *N. brasiliensis* are challenged (Table 2). Lungs of all necropsied rats in the two experiments exhibited several dark areas representing hemorrhages in the alveoli due to inflammatory reaction. Several worms were found surrounded by a nodular reaction and filled with intestinal precipitate. All worms recovered from the intestines of the control and concurrently infected rats were stunted and undeveloped. Daily observations indicated that the concurrently infected rats generally progressed slower in gaining weight than the controls, although in two out of four experiments, III and V, concurrently infected rats, as a group, showed a percentage weight gain as large as the controls at the termination of the experiments.

The trypanosome parasitemia followed a normal course in all experiments of both sets, generally reaching its peak about the 5th day and gradually subsiding until no trypansomes could be found in the rats' blood by the 15th day after infection.

Table 1. Data on primary *N. brasiliensis* infections in rats with or without concurrent infection with *T. lewisi*.

Experiment No.	Rat No.	Inoculation: No. of *N. brasiliensis*	Inoculation: Millions of *T. lewisi*	Total no. of eggs (x10⁴) passed before necropsy: Per Rat	Total no. of eggs (x10⁴) passed before necropsy: Per Group	Worms at necropsy 9th day after infection	Percent weight gain per group
I	1	650	1	58	192	----	----
	2	650	1	75		----	----
	3	650	1	58		----	----
	4	650	0	51	176	----	----
	5	650	0	54		----	----
	6	650	0	69		----	----
II	7	1000	50	79	280	----	10
	8	1000	50	70		559	
	9	1000	50	129		----	
	10	1000	0	175	289	----	
	11	1000	0	71		523	24
	12	1000	0	142		----	
III	13	1000	150	113	450	----	
	14	1000	150	172		----	24
	15	1000	150	81		543	
	16	1000	150	83		483	
	17	1000	0	98	371	----	
	18	1000	0	120		----	24
	19	1000	0	80		537	
	20	1000	0	71		499	

DISCUSSION

An evaluation of the egg count data, as well as the close similarity in the numbers of worms recovered from the rats of the two groups in each experiment, is evidence that development of acquired immunity and the action of previously acquired immunity to *N. brasiliensis* were not influenced by the presence of a concurrent infection with *T. lewisi*.

Taliaferro (1940) suggested that both inflammation and tissue sensitivity are possible factors in acquired immunity to animal parasites. In experiments IV and V, inflammation in the lungs of immune control and concurrently infected rats had undoubtedly subsided by the time of necropsy, but there was no evidence that greater cellular reaction occurred in the lungs of the concurrently infected rats than in the controls.

The presence of *T. lewisi* in these *N. brasiliensis* infected rats appeared to adversely effect the host. The rats with *T. lewisi* infections ate less, weight gains were frequently less, and they appeared to be in poorer physical condition than those not infected with this trypanosome.

SUMMARY

Egg count and worm recovery data of 5 experiments showed that in rats concurrently infected with moderate to heavy doses (1 million to 250 million) of *T. lewisi* and 1,000 *N. brasiliensis* larvae, the presence of *T. lewisi* did not influence the normal course of development of acquired immunity or the maintenance of previously acquired immunity to *N. brasiliensis*.

LITERATURE CITED

BRUMPT, E. 1933. Evolution de l'*Hymenolepis nana* var. *fraterna*. Arch. Zool. Exper. et Generale. Second Vol. Julilaire. 75: 235-246.

COX, H. W. 1952. The effect of a concurrent infection with the dog hookworm, *Ancylostoma caninum*, on the natural and acquired resistance of mice to *Trichinella spiralis*. Jour. Elisha Mitchell Scientific Soc. 68: 222-234.

Table 2. Data on rats immunized with varying doses of *N. brasiliensis* and thereafter with 1000 larvae, with and without concurrent *T. lewisi* infection.

Experiment No.	Rat No.	No. of larvae in immunizing infection	Millions of *T. lewisi* inoculated	Total eggs ($\times 10^2$) passed from test infection to necropsy		Worms at necropsy day		Percent weight gain per group
				Per Rat	Per Group	9	15	
IV	21	1800	250	495	2650	279	----	36
	22	1800	200	1280		----	139	
	23	1800	175	875		----	126	
	24	1800	0	1010	4875	245	----	
	25	1800	0	2070		----	268	31
	26	1800	0	1795		----	165	
V	27	2500	50	5	33	37	----	
	28	2500	50	12		----	9	2
	29	2500	50	11		----	----	
	30	2500	50	5		----	----	
	31	2500	0	10	41	56	----	
	32	2500	0	11		----	3	9
	33	2500	0	11		----	----	
	34	2500	0	9		----	----	

HOLMES, J. E., JR. 1957. The effects of concurrent infections with the spiny-headed worms, *Moniliformis dubius*, on the rat tapeworm, *Hymenolepis diminuta*. Jour. Parasitol. (Oct. Suppl.) 43: 20.

LARSH, J. E., JR. and A. W. DONALDSON. 1944. The effect of a concurrent infection with *Nippostrongylus muris* on the development of *Hymenolepis* in mice. Jour. Parasitol. 30: 18-20.

TALIAFERRO, W. H. 1940. Mechanisms of acquired immunity in infections with parasitic worms. Physiol. Rev. 20: 469-492.

YOKOGAWA, S. 1922. The development of *Helognosomum muris* Yokogawa, a nematode from the intestine of the wild rat. Parasitol. 14: 127-166.

Observations on the Structure of the Cysticercus of *Taenia hydatigena* Pallas, 1766*

MARIETTA VOGE

The present report is the first of a series of studies on the structural organization of cysticerci. The purpose of these studies is two-fold: first, to determine whether all larvae of the "cysticercus" type possess a comparable basic structure and tissue layers, regardless of specific scolex morphology; secondly, if the basic organization is similar regardless of the species, are there aside from the scolex sufficiently distinct differences between species so that specific identification can be made on the basis of sections not showing the diagnostic features of scolex morphology.

The material on which this report is based was collected from *Alces alces* at Fort Richardson, Alaska, in December, 1959.

MATERIALS AND METHODS

Formalin-fixed cysticerci of *Taenia hydatigena* were embedded in paraffin and sections cut at 7 microns. Stains used were Heidenhain's iron hematoxylin, Mallory's aniline blue stain, Gomori's trichrome, Mayer's hemalum and Davenport's protargol stain. All these were employed as described previously (Voge, 1960). In addition Rinehart's stain (Rinehart and Abul-Haj, 1951) was used for the demonstration of acid mucopolysaccharide and collagen.

OBSERVATIONS

The cysticercus of *Taenia hydatigena* (Fig. 1), the overall morphology of which was described by Moniez (1880), consists of a relatively thick anterior part or body which contains the scolex, and a more slender and delicate part commonly referred to as the bladder or tail. Between the body and tail there is an area of loosely packed, irregularly arranged fibers and cells. Histologi-

*From the Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, California.

Aided by a research grant (E-1583) from the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

Appreciation is expressed to Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska, for making available specimens of *T. hydatigena;* to Mrs. Hermine Bayard for making the drawings included here; and to Mrs. Nora Liu and Mr. Allen K. Berntzen, Department of Infectious Diseases, University of California, Los Angeles, for expert assistance in the staining procedures.

Abbreviations: c, cuticle; cc, calcareous corpuscles; ch, cuticular hairs; cl, cuticular layer; cm, circular muscles; fc, flame cells; fl, fibrous layer; h, hooks; lfc, loose fibers and cells; lm, longitudinal muscles; m, muscles; n, nuclei; pa, proliferative area; pc, peripheral cells; s, sucker; scc, space occupied by calcareous corpuscle; t, tail; tf, tissue fold.

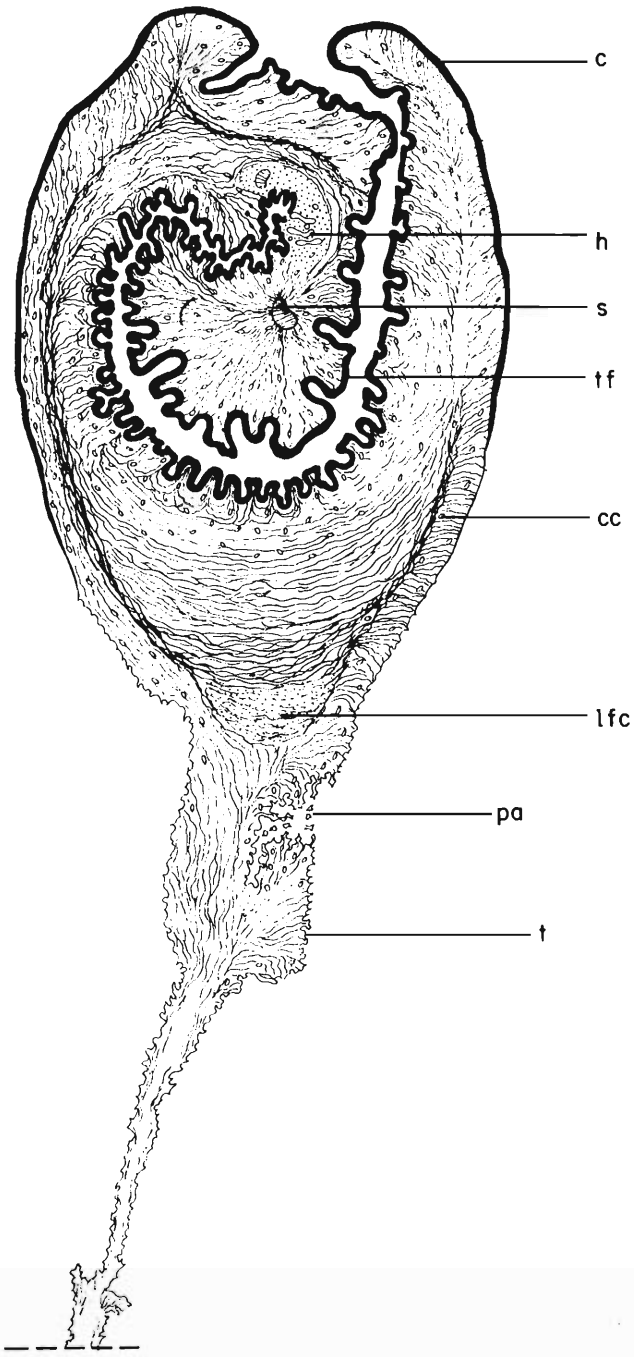


Fig. 1. Free-hand drawing of longitudinal section through a cysticercus of *Taenia hydatigena*. Tail portion much longer than shown here.

cally the two parts differ considerably. The anterior part consists of the scolex which lies more or less in the center, surrounded by much-folded tissue. The folds are lined by cuticle which extends to the outside to about the posterior third of the body and gradually becomes thinner until it cannot be seen under low magnification. The tail area shows numerous lace-like infoldings and varies greatly in size or length in different specimens. Throughout the cysticercus there are many calcareous corpuscles, situated within the delicate fibrous tissue which surrounds the scolex and makes up the major part of the larva. A few calcareous corpuscles are occasionally seen within the scolex proper. A layer of fibers staining blue with Mallory's stain is present just external to the hooks. An account of the detailed organization in the different areas of the cysticercus will now be presented.

Figure 2 shows one of the tissue folds within the anterior portion of the cysticercus near the scolex. The cuticle is a thick, homogeneous appearing structure which stains a greyish-blue in Mallory's stain and blue with Rinehart's stain. In some sections one observes a few fine hair-like structures (not shown in Fig. 1) protruding from the cuticle to the outside. Similar hairs have been described for *Cysticercus fasciolaris* by Crusz (1948). Beneath the cuticle is a narrow layer of fine fibers which stain deep blue with Mallory's and bright red with Rinehart's stain. The latter reaction indicates the

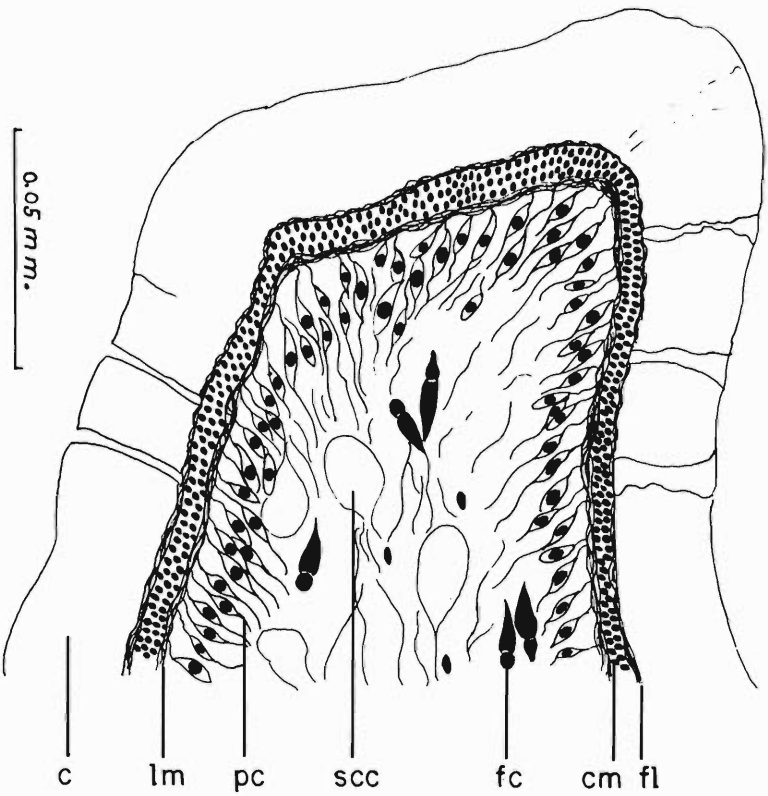


Fig. 2. Detail of tissue fold near scolex showing organization of different tissue layers and cells.

presence of collagen. The second, relatively prominent fibrous layer is probably a layer of circular muscles (Fig. 2); it stains red with Mallory's and blue with Rinehart's stain; the orientation of these fibers is at a right angle to the first layer. The fibers of the third layer lie parallel with those of the first, but in staining reactions are like the second layer. This is probably a layer of longitudinal muscles. Beneath this layer are numerous spindle-shaped cells (Fig. 2, peripheral cell layer) with long fiber-like extensions at each end, all with similar orientation. Internal to this cell layer lies a loose tissue consisting of a network with a few nuclei, many clacareous bodies and prominent densely staining, elongate structures the function of which is uncertain. Figure 4 shows these structures in greater detail. They measure 13 to 18 microns in total length and 2 to 4 microns in width. They consist of two small cone-shaped or triangular portions, one of which contains a nucleus, and of a third, elongate, portion which in preparation stained with Meyer's hemalum appears to contain fine fibers. The elongate part leads into a narrow duct of variable extent. The whole structure is enclosed by a thin membrane which thickens (Fig. 4) near the point of contact with the elongate portion. The usual orientation is with the cone toward the cuticle. These elongate structures are most numerous in the tissue folds around the scolex, but are present in other areas as well.

It was thought that silver-stained preparations would help to establish if cilia were contained within the elongate portions, and thus support the idea that this was part of the excretory system, probably the flame cells. Unfortunately neither the silver nor iron hematoxylin stain clearly demonstrated the presence of cilia, and living material was not available. As will be shown in subsequent reports, similar structures are present in cysticerci of other taeniid species. For the sake of convenience and until proven otherwise, these structures will henceforth be called flame cells. It should be noted that throughout the fibrous tissue in the body of the cysticercus one observes in

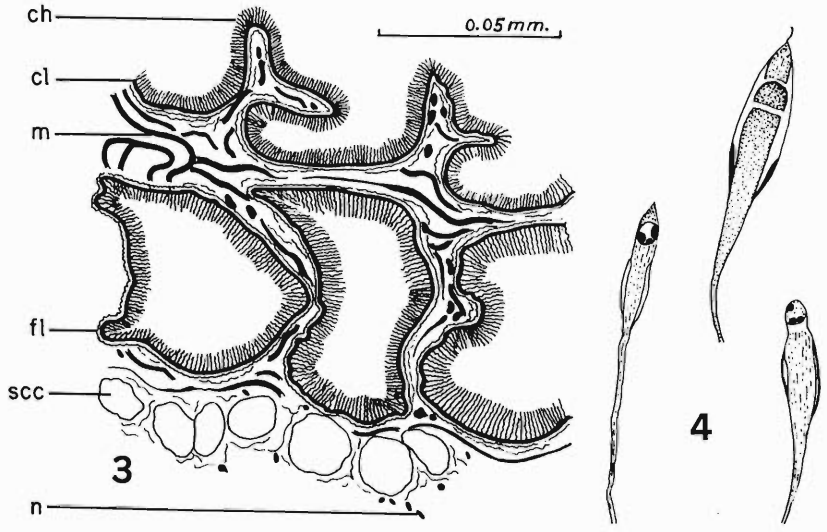


Fig. 3. Detailed structure of peripheral portion of tail, or proliferative area (see Fig. 1).
Fig. 4. Free-hand drawing of flame cells (¶).

sections parts of a duct system, perhaps excretory, which lies external to the scolex, in the tissue surrounding it. The ducts are relatively wide and their position is somewhat variable.

The peripheral tissue layers of the tail show numerous folds and outpocketings giving the margin of the tail a lace-like appearance. It appears that continuous proliferation of tissue may occur in this area. Figure 3 shows the structure of a few of these folds as they appear under high magnification. Beginning from the outside, there is first a thick layer of prominent cuticular hairs which protrudes from a relatively narrow cuticular layer. The latter stains orange with Mallory's stain while the fully developed cuticle in the anterior portion of the larva stains a uniform greyish-blue. Beneath the cuticular layer there is a delicate fibrous layer which stains blue with Mallory's stain and red with Rinehart's stain, indicating the presence of collagen. This layer appears to be the same as that described for the anterior portion of the cysticercus. Beneath the fibrous layer there are thick bundles of fibers which stain red with Mallory's stain. They are probably bundles of muscle. Numerous calcareous corpuscles are present throughout the delicate fibroid tissue making up the remainder of the tail.

If one traces the cuticular layer from the tail region anteriorly one observes a gradual increase in width of the cuticular layer with a concomitant decrease in the length of the cuticular hairs. The hairs apparently become incorporated into the cuticle. Perhaps their length in any one species determines at least to some extent the thickness of the cuticle in the anterior part of the cysticercus. Certain aspects of the relation of the fibrillar hairs to cuticle formation are discussed by Schiller (1960).

DISCUSSION

Although information on the histologic structure of cysticerci is at present insufficient for comparison between species, preliminary observation as well as the work by Crusz (1948) indicates that the basic tissue organization is probably similar in cysticerci belonging to different species of *Taenia*. Cuticular hairs have been seen by Crusz (1948), by Schiller (1960), and by myself in *Taenia hydatigena* as well as in other species. The orientation and number of the muscle layers beneath the cuticle is the same in *T. pisiformis* as in *T. hydatigena*. Further similarities will doubtlessly be found when more species are studied.

The finding of collagen-like material in the fibrous layer beneath the cuticle constitutes the first report of this substance for cestodes. However, further work on the structure of these fibers is necessary before any definitive statement can be made concerning the precise nature of these fibers.

LITERATURE CITED

CRUSZ, H. 1948. Further studies on the development of *Cysticercus fasciolaris* and *Cysticercus pisiformis*, with special reference to the growth and sclerotization of the rostellar hooks. J. Helminthol., 22: 179-198.

MONIEZ, R. 1880. Essay monographique sur les cysticerques. Travaux Inst. Zool. Lille Station Maritime Wimereux, Paris, 3: 1-190.

RINEHART, J. F. and ABUL-HAJ, S. K. 1951. An improved method for histologic demonstration of acid mucopolysaccharides in tissues. A.M.A. Arch. Pathol., 52: 189-194.

SCHILLER, E. L. 1960. The histogenesis of cuticle in the cestode genus *Taenia*. J. Parasit., 46: 9-10 (suppl).

VOGE, M. 1960. Studies in cysticercoid histology. I. Observations on the fully developed cysticercoid of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea). Proc. Helm. Soc. Wash., 27: 32-36.

New Species of Diplogasteridae (Nematoda) Associated with Bark Beetles in the United States

CALVIN L. MASSEY*

The family Diplogasteridae is abundantly associated with bark beetles throughout the United States. Many are known ectoparasites of the family Scolytidae; other are suspect. Thorne (1935) described *Diplogaster pinicola* as an associate of the mountain pine beetle, *Dendroctonus monticolae* Hopk. Massey (1956) determined the same species to be an ectoparasite of the Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk. Fuchs (1915, 1938); Körner (1954); and Rühm (1956) described several species associated with European bark beetles.

It is possible that the group in general exert considerable influence on the population dynamics of the Scolytidae. Many are thought to be predaceous on the egg stage; others may be hyperpredaceous on beetle predators and parasites.

The species herein described were taken from the galleries of various bark beetles from the southwestern, southeastern and the northern United States. Type specimens are deposited in the collection of the Rocky Mountain Forest and Range Experiment Station at Albuquerque, New Mexico.

GENUS *Acrostichus* Rahm, 1928

The genus is typified by the species *Acrostichus toledoi* Rahm, 1928. Goodey (1951) erred in synonymizing it with the genus *Diplogaster*. Several characters which are constant and prominent vary considerably from members of the genus *Diplogaster* and are sufficient to justify generic rank. These characters are: Stoma much deeper than wide; longitudinal striations prominent to very prominent; females with a reniform spermatheca; gubernaculum massive, variable in shape; tails of both sexes long and filiform.

DIAGNOSIS EMENDED: Cuticle with very prominent longitudinal and moderately fine transverse striations. Head usually narrowed forward from anterior end of neck. Stoma much deeper than wide, 10-15 microns in depth, 2.5-4 microns in width, consisting of a cheilostom with distinct cheilorhabdions; protostom with distinct prorhabdions. Meso-, meta- and telorhabdions at times fused, forming a glottoid apparatus armed with dorsal and subventral teeth, varying from two to four in number. Esophagus typically diplogasteroid. Females amphidelphic, the ovaries usually strongly reflexed and either meeting or crossing in the region of the vulva. Females with a large reniform spermatheca. Spicules paired, ventrally arcuate, cephalated. Gubernaculum massive, elaborate, variable in shape. Caudal papillae variable in number. Tails of both sexes filiform.

Several species previously described in the genus *Diplogaster* belong in *Acrostichus*:

Acrostichus lineatus (Fuchs, 1915) new combination
synonym *Diplogaster lineatus* Fuchs, 1915
Acrostichus consobrinus (de Man, 1920) new combination
synonym *Diplogaster consobrinus* de Man, 1920

*Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Fort Collins, Colorado.

The research reported was completed at the Station's Forest Insect Laboratory at Albuquerque, New Mexico.

The assistance of Mr. Gerald Thorne is gratefully acknowledged for his review of the manuscript.

Acrostichus consobrinus var. *austriacus* (Fuchs, 1938) new combination
synonym *Diplogaster consobrinus* var. *austriacus* Fuchs, 1938
Acrostichus nudicapitatus (Steiner, 1914) new combination
synonym *Diplogaster nudicapitatus* Steiner, 1914
Acrostichus occidentalis (Steiner, 1932) new combination
synonym *Diplogaster occidentalis* Steiner, 1932

Acrostichus ponderosus n. sp.

FEMALE: 0.6 mm., a = 16; b = 7; c = 3; v = 48%.
MALE: 0.4 mm., a = 15; b = 5; c = 5.

FEMALE: Cuticle with prominent longitudinal and fine transverse striae. Body widest near middle, tapering anteriorly towards the head, posteriorly to a long filiform tail. Fig. 1D. Head narrowly rounded. Amphid apertures minute pore like, located near apices of lateral lips. Stoma deeper than wide, 10 microns in depth, 3 microns in width; protostom slightly longer than cheilostom. The dorsal mesorhabdion armed with a large forward-pointing tooth. The ventral metarhabdion with a small subventral tooth as in Figure 1A. Esophagus with muscular pre-corpus and valvular median bulb; the combined pre-corpus and median bulb being somewhat longer than the isthmus and non-valvate terminal bulb. Nerve ring prominent, just forward of the terminal bulb. Excretory pore slightly posterior to the terminal bulb. Ovaries paired, reflexed, the terminal ends crossed. Fig. 1B. Vulva a transverse slit, slightly protuberant, opening into a prominent reniform spermatheca. Fig. 1B.

MALE: Testis single, slightly reflexed, at times nearly reaching the terminal bulb of the esophagus. Spicules paired, ventrally arcuate, cephalated. Gubernaculum massive, ⅔ the length of the spicules, shaped as in Figure 1C. Eight pairs of caudal papillae; 1 and 2 subventral preanal; 3 subventral postanal; 4 lateral postanal; a group of three, 5, 6, and 7 at the end of the body proper anterior to the initiation of the filiform tail; 8 subdorsal slightly posterior to 5, 6, and 7. Phasmids lateral, anterior to 4th pair of papillae. Tail filiform.

DIAGNOSIS: *Acrostichus* with above general description. Distinctive because of the size and shape of the gubernaculum.

HABITAT: Associated with *Ips ponderosae* Sw. in ponderosa pine, Bandelier National Monument, New Mexico.

Acrostichus taedus n. sp.

FEMALE: 0.63 mm., a = 15; b = 5.4; c = 3.6; v = 43%.
MALE: 0.55 mm., a = 18; b = 4.4; c = 5.5.

FEMALE: Cuticle with prominent longitudinal and fine transverse striae. Body widest near middle, tapering anteriorly towards the head, posteriorly to a long filiform tail. Fig. 1E. Head slightly narrowed. Amphid apertures minute, pore like, located near apices of lateral lips. Stoma 10 microns in depth, 2.5 microns in width, armed as in Figure 1E. Combined length of pre-corpus median bulb ⅓ longer than isthmus and terminal bulb. Nerve ring slightly forward of excretory pore. Ovaries paired and reflexed, the terminal portions crossing near the vulva. Vulva a transverse slit only slightly protuberant, opening into a characteristic reniform spermatheca. Fig. 1F.

MALE: Testis single, reflexed. Spicules paired ventrally arcuate, cephalated. Gubernaculum ¾ the length of spicules, shaped as in Figure 1G. Seven pairs of caudal papillae; 1 and 2 subventral preanal; 3 subventral postanal; 4, 5,

and 6 slightly anterior to the initiation of the filiform tail; 7 subdorsal and slightly posterior to 6. A pair of phasmids located laterally and anterior to the 4th pair of papillae. Tail filiform.

DIAGNOSIS: *Acrostichus* with characters as described. Differs from other species in the genus in the shape of the gubernaculum and in the number and arrangement of the stomatal teeth.

HABITAT: Taken from the galleries of the black turpentine beetle, *Dendroctonus terebrans* Oliv., loblolly pine, *Pinus taeda* L. near Lake City, Florida. Host material collected by R. H. Smith.

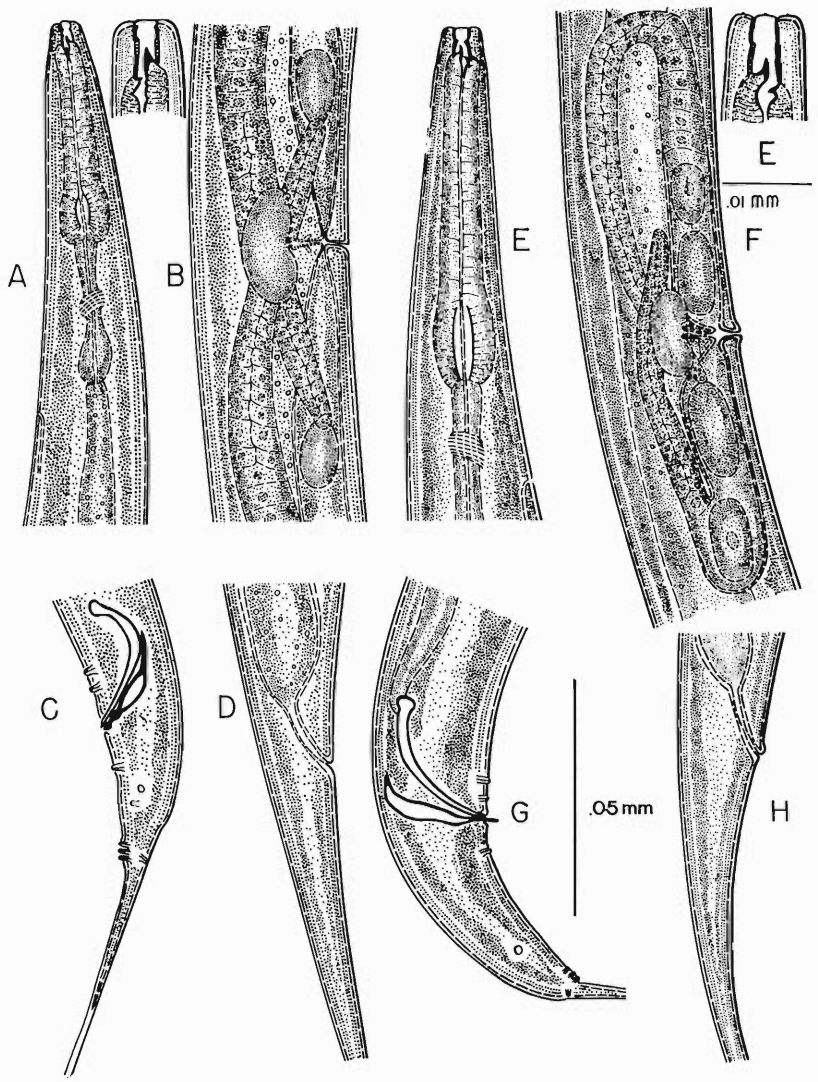


Fig. 1.—*A-D. Acrostichus ponderosus* n. sp.; *A.* Head; *B.* Female midsection; *C.* Male tail; *D.* Female tail; *E-H. Acrostichus taedus* n. sp.; *E.* Head; *F.* Female midsection; *G.* Male tail; *H.* Female tail.

Acrostichus arcuatus n. sp.

FEMALE: 0.85 mm., a = 20; b = 5.4; c = 4; v = 44%.

Cuticle with 11 very prominent longitudinal striations, striae visible at midbody. Transverse striae moderately fine. Body widest near middle, bent ventrally in a slight arc. Head strongly narrowed from middle of pharynx forward. Amphid apertures pore like, located near apices of lateral lips. Stoma 12 microns deep, 3 microns wide. Pharynx armed as in Figure 2A. Pre-corpus and median bulb of esophagus ⅓ longer than isthmus and terminal bulb. Nerve ring at middle of isthmus. Excretory pore adjacent to terminal bulb. Ovaries reflexed their entire length and meeting at midbody, as in Figure 2C. Vulva transverse, protuberant, opening into a large reniform spermatheca. Tail filiform. Fig. 2B.

MALE: Unknown.

DIAGNOSIS: *Acrostichus* with characters as described. Differs from other species in the genus in its size and in the prominence of the longitudinal striations.

HABITAT: Taken from the galleries of the black turpentine beetle, *Dendroctonus terebrans* Oliv., in loblolly pine, *Pinus taeda* L. near Lake City, Florida. Host material collected by R. H. Smith.

Acrostichus concolor n. sp.

FEMALE: 0.53 mm., a = 14; b = 5; c = 3.7; v = 47%.

MALE: 0.50 mm., a = 16; b = 4; c = 5.7.

FEMALE: Cuticle with prominent longitudinal striae. Body widest near middle, tapering only gradually towards the extremities. Head strongly narrowed from the anterior ¼ of the esophagus forward as in Figure 2D. Amphid apertures minute, pore like, located near apices of lateral lips. Stoma 12 microns in depth, 3.5 microns in width. Mesorhabdion armed with a dorsal tooth; the ventral metarhabdion with a subventral tooth, the anterior dorsal tooth the largest, the ventral metarhabdion with a small denticle. The isthmus and terminal bulb of the esophagus ⅔ the length of the pre-corpus and the median bulb. Nerve ring near middle of isthmus. Excretory pore adjacent to terminal bulb. Ovaries paired, strongly reflexed, nearly meeting at midbody. Vulva a transverse slit, opening into a reniform spermatheca. Tail filiform. Fig. 2E.

MALE: Testis single, reflexed. Spicules paired, ventrally arcuate, cephalated. Gubernaculum massive, as long as spicules, shaped as in Figure 2F. Six pairs of caudal papillae; 1 preanal subventral; 2, 3, 4, and 5 postanal subventral, located at the base of the body as it narrows into the tail proper; 6 subdorsal and adjacent to number 5. Phasmids not discernible. Tail filiform.

DIAGNOSIS: *Acrostichus* with characters as stated in description. Differs from other species of the genus in its strongly constricted head, number of male caudal papillae, and shape and size of gubernaculum.

HABITAT: Associated with *Scolytus ventralis* Lec. in white fir, *Abies concolor* (Gord. & Glend.) Lindl., Sandia Mountains, Cibola National Forest near Albuquerque, New Mexico.

GENUS *Diplogasteroides* de Man, 1912

The genus is typified by the species *Diplogasteroides spengelli*, and includes several species: *D. africanus* Micoletzky (1915); *D. bidentatus* de Cillis (1917); *D. longicauda* (Rahm, 1928); *D. stigmatus* Steiner (1930); *D.*

variabilis Micoletzky (1922); *D. crassus* Körner (1954); and *D. picicola* Rühm (1956). Both Körner (1954) and Rühm (1956) placed in Diplogasteroides several species described by Fuchs in the genus *Rhabdontalaimus* and *Neodiplogasteroides*.

Rühm (1954) erred in synonymizing the genus *Rhabditolaimus* Fuchs (1915) with *Diplogasteroides*.

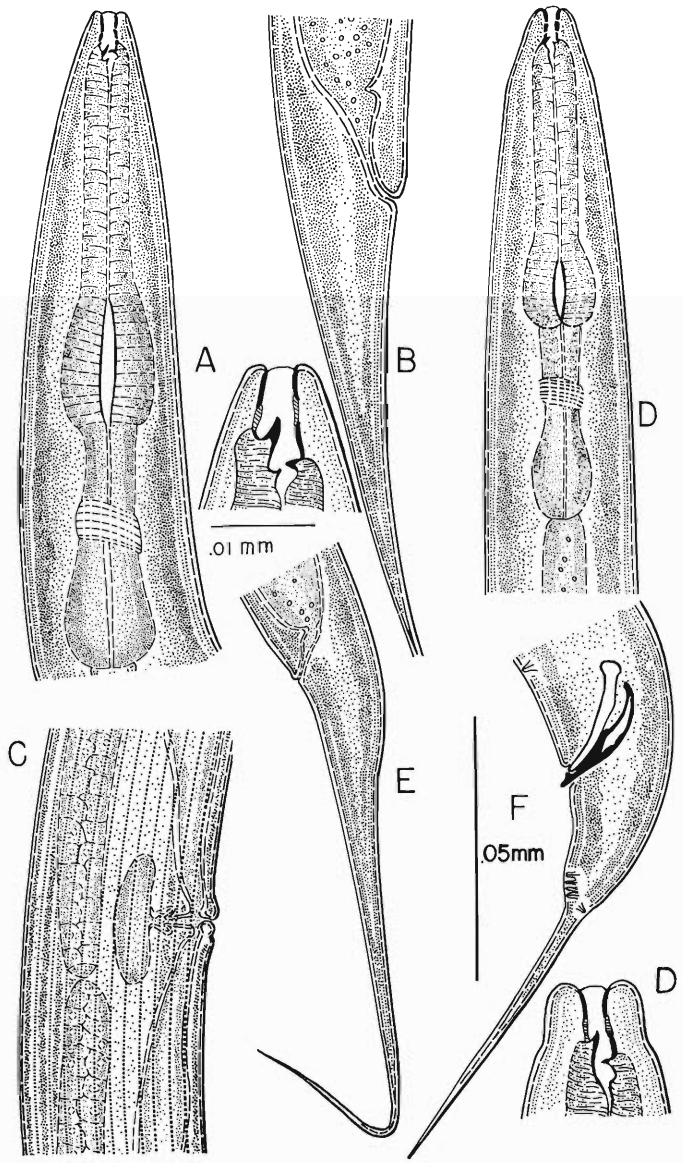


Fig. 2—*A-C. Acrostichus arcuatus* n. sp.; *A.* Head; *B.* Tail; *C.* Midsection; *D-F. Acrostichus concolor* n. sp.; *D.* Head; *E.* Female tail; *F.* Male tail.

The genus is characterized by long filiform tails of both sexes, head bearing 6 papillate lips. Stoma cylindrical, with a short cheilostom, a protostom composed of fused pro- and meso- rhabdions, dorsal metarhabdion bearing a small tooth. Telorhabdions forming a small chamber at the anterior opening of the esophagus. Amphidelphic or prodelphic. Spicules paired, ventrally arcuate. Varying numbers of caudal papillae present.

Diplogasteroides marshalli n. sp.

FEMALE: 0.58-0.75 mm., a = 16; b = 5.8; c = 5.4; v = 67%.
MALE: 0.58-0.85 mm., a = 16; b = 5.2; c = 7.4.

FEMALE: Cuticle with very fine longitudinal and transverse striae. Body tapering anteriorly towards the head, posteriorly to a moderately long filiform tail. Fig. 3D. Head rounded, slightly narrowed neck. Fig. 3A. Stoma *Rhabditis*-like, made up of a short cheilostom, the inner surface of the cheilorhabdions convex in a lateral view. Protostom with straight sides, the prohabdions approximately 3 times the length of the cheilorhabdions, dorsal mesorhabdion bearing a slender tooth. Ventral telorhabdion a knot-like structure at entrance to esophagus. Amphids not discernible. Pre-corpus of the esophagus, muscular, widening into an ovoid median bulb. Isthmus moderately slender, somewhat narrower than pre-corpus. Terminal bulb ovoid, valveless. Pre-corpus and median bulb slightly shorter than isthmus and terminal bulb. Nerve ring a broad band slightly anterior to median bulb. Excretory pore adjacent to terminal bulb. Ovary single, reflexed as much as ¾ of its length. Post-uterine branch less than half as long as body width. Vulva transverse, slightly protuberant.

MALE: Testis single, reflexed. Spicules paired, cephalated, slender, ventrally arcuate. Gubernaculum keel-like, considerably longer than wide. Fig. 3B. Nine pairs of caudal papillae; 1 and 2 preanal and subventral; 3 lateral preanal; 4 postanal and subventral; 5 postanal and lateral; a group of three, 6, 7, and 8 subventral at that portion of the tail at the end of the body; 9 postanal subdorsal and adjacent to number 6.

DIAGNOSIS: *Diplogasteroides* with characters as described. Differs from other species in the shape of the gubernaculum and the slender more arcuate spicules.

HABITAT: Recovered from the galleries of *Ips ponderosae* Sw. in ponderosa pine, *Pinus ponderosa Laws.*, Bandelier National Monument, New Mexico, and from ponderosa pine infested with *Ips lecontei* Sw. near Prescott, Arizona.

Diplogasteroides janae n. sp.

FEMALE: 0.9-1.1 mm., a = 20; b = 4; c = 8; v = 46%.
MALE: 0.73 mm., a = 21; b = 5; c = 11.

FEMALE: Cuticle with fine transverse striations. Body widest near the middle, tapering anteriorly and posteriorly. Head rather broadly rounded, made up of 6 papillate lips with prominent papillae. Fig. 4A. Stoma much deeper than wide, composed of a short cheilostom and protostom. Dorsal metarhabdion bearing a small tooth located near entrance to esophagus. Amphids located as in Figure 4A. Esophagus consisting of a muscular pre-corpus, widening into an ovoid valvate median bulb. Isthmus moderately slender, its length combined with the terminal bulb being shorter than the pre-corpus and median bulb. Terminal bulb valveless. Nerve ring near middle of isthmus. Excretory pore adjacent to median bulb. Ovaries at times reflexed their entire length. Vulva a protuberant transverse slit near mid-

body. Tail fine, filiform. Fig 4D.

MALE: Testis single, reflexed, at times nearly reaching the terminal bulb. Spicules paired, ventrally arcuate, cephalated. Gubernaculum considerably longer than wide. Fig. 4C. Seven pairs of caudal papillae; 1 and 2 preanal subventral; 3 postanal subventral; 4, 5, and 6 in a group at the posterior end

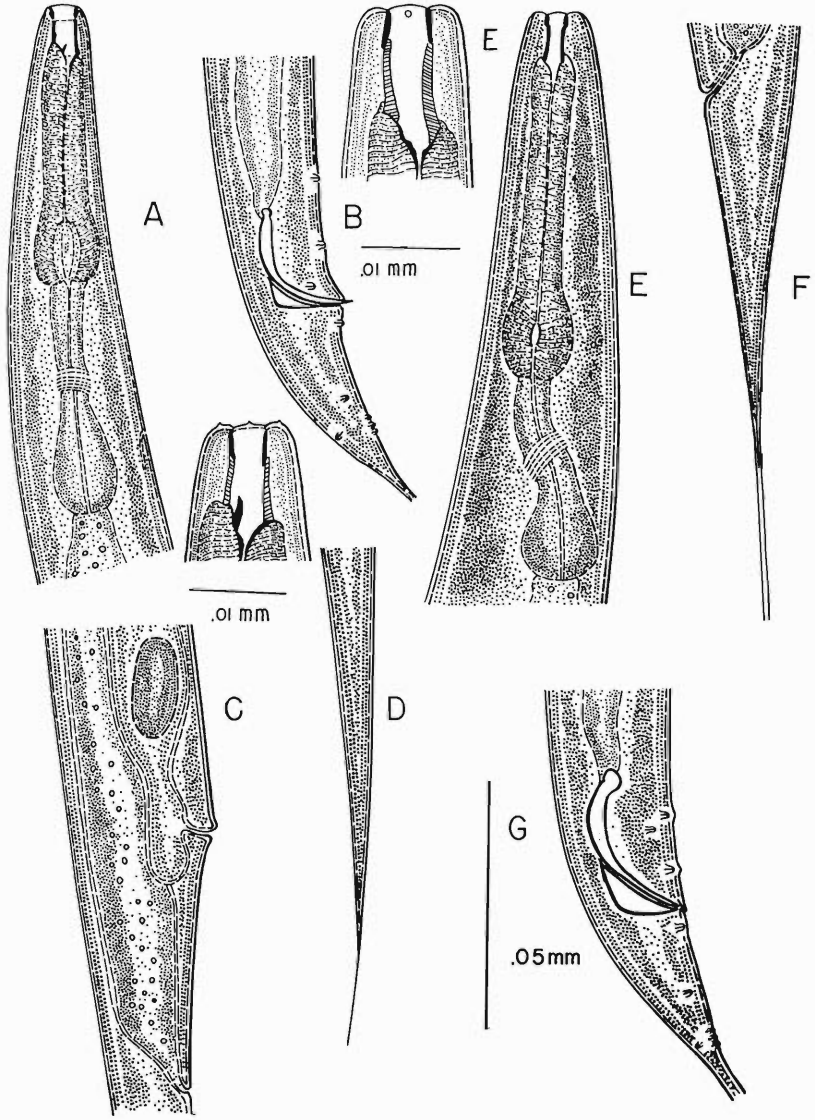


Fig. 3.—*A-D. Diplogasteroides marshalli* n. sp.; *A.* Head; *B.* Male tail; *C.* Body section, Female; *D.* Female tail; *E-G. Rhabditolaimus wesleyi* n. sp.; *E.* Head; *F.* Female tail; *G.* Male tail.

of the body proper; 7 adjacent to 6, subdorsal. Phasmids located laterally and on either side, slightly anterior to the 4th pair of papillae. Tail fine, spicate. Fig. 4C.

DIAGNOSIS: *Diplogasteroides* as described. Distinguished from other species in the shape of the gubernaculum, number and arrangement of caudal papillae, and in the tail characteristics of the male.

HABITAT: Recovered from the galleries of *Ips calligraphus* (Germ.) in longleaf pine, *Pinus palustris* Mill. at Olustee, Florida. Host material collected by E. P. Merkel.

GENUS *Rhabditolaimus* Fuchs, 1915

The genus is typified by the species *Rhabditolaimus halleri*. It is closely related to *Diplogasteroides*, but differs in that the pharynx is unarmed and the male tail possesses a small bursal structure.

Rhabditolaimus wesleyi n. sp.

FEMALE: 0.65 mm., a = 18; b = 4.6; c = 6; v = 72%.

MALE: 0.5 mm., a = 16; b = 5.3 c = 6.4.

FEMALE: Cuticle thick with very fine longitudinal and transverse striations which sometimes are scarcely discernible. Body tapering anteriorly towards the head, posteriorly to a moderately long filiform tail. Fig. 3F. Head rather broadly rounded. Fig. 3E. Amphids located as in Figure 3E. Stoma *Rhabditis*-like, made up of a short cheilostom with relatively straight cheilorhabdions. Protostom approximately 3 times the length of the cheilostom. Telorhabdions appear to be fused with the prorhabdions. Anterior end of the esophagus overlapping the posterior portion of the stoma. Pre-corpus of the esophagus muscular, terminating in an ovoid median bulb; the isthmus and terminal bulb shorter than the pre-corpus and median bulb. Nerve ring a broad band midway of the isthmus. Excretory pore not discernible. Ovary prodelphic, reflexed for about ¾ of its length. Post-uterine branch 1 ½ times body width. Vulva a transverse slit with slightly protuberant lips. Anus prominent.

MALE: Testis single, reflexed. Spicules paired, ventrally arcuate, cephalated. Gubernaculum as in Figure 3G, only slightly longer than wide. Prominent anal flap in male. Nine pairs of caudal papillae; 1 and 3 subventral preanal; 2 lateral preanal; 4 subventral postanal; 5 lateral postanal; a group of three, 6, 7, and 8 at that portion of the tail near the end of the body; 9 subdorsal and adjacent to number 7. Bursal structure not visible. Tail relatively long and filiform.

DIAGNOSIS: *Rhabditolaimus* with characters as described. Differs from other species of the genus in the anal flap of the male, the size and shape of the gubernaculum, and the number and arrangement of caudal papillae.

HABITAT: From galleries of *Dendroctonus borealis* infesting white spruce, *Picea glauca* (Moench) Voss, Anchorage, Alaska. Nematode-infested material collected by W. F. McCambridge.

LITERATURE CITED

FUCHS, G. 1915. Die Naturgeschichte der Nematoden und einiger anderer Parasiten. 1. des *Ips typographus* L. 2. des *Hylobius abietis* L. Zool. Jahrb. Abt. Syst. 38 (3/4), 109-222.

————. 1931. Die Genera: 1. *Rhabditolaimus* Fuchs, 2. *Neodiplogaster* Cobb, 3. *Tylenchodon* Fuchs. Zbl. ges. Forstw. 57 (5/6), 177-94.

————. 1938. Neue Parasiten und Halbparasiten bei Borkenkäfern und einige andere Nematoden. Zool. Jahrb. Abt. Syst. 71 (1/2), 123-90.

GOODEY, T. 1951. Soil and Freshwater Nematodes. London: Methuen and Co. Ltd., New York: John Wiley and Sons, Inc.
KÖRNER, H. 1954. Die Nematodenfauna des vergehenden Holzes und ihre Beziehungen zu den Insekten. Zool. Jahrb. Abt. für Syst., Okologie und Geographie der Tiere. Band 82, Heft (3/4).
MAN, J. G. DE. 1912. Helminthologische Beitrage. Zool. Jahrb. Suppl. 15, 1, 439-64.
————. 1920. Description D'une espece du genre *Diplogaster* Max Schultze: *Diplogaster consobrinus* nov. sp. Ann. Soc. Roy. Zool. et Malacol. Belg. 51: 47-54.
MASSEY, C. L. 1956. Nematode Parasites and Associates of the Engelmann Spruce Beetle (*Dendroctonus engelmanni* Hopk.) Proc. Helminth. Soc. of Wash. Vol. 23, No. 1, 14-24.
MICOLETZKY, H. 1915. Susswasser-Nematoden aus Südafrika. Ergebnisse einer botanischen Forschungsreise nach Deutsch-Ostafrika und Südafrika (Kapland, Natal und Rhodesien), in Denkschr. Kais. Akad. Wissensch. Wien, Mathem.-Naturw. Kl. 92, Bd. 1915.
————. 1922. Die freilebende Erd-Nematoden. Arch. Naturg. Abt. A. 87 (8/9), 1-650.
RAHM, O. 1928. Alguns nematodes parasites e semi-parasites das plantas cultureas do Brasil. Arch. Inst. Biol. Defesa Agric. e Anim. 1, 239-52.
RÜHM, W. 1956. Die Nematoden der Ipiden. Parasit. Schrift. Heft. 6.
STEINER, G. 1914. Freilebende Nematoden aus der Schweiz. Arch. Hydrobiol. 9, 426-7.
————. 1930. Nemic fauna of the slime flux of Carolina Poplar. Jour. Agric. Res. Vol. 41.
THORNE, G. 1935. Nemic Parasites and Associates of the Mountain Pine Beetle (*Dendroctonus monticolae*) in Utah. Jour. Agric. Res. Vol. 51, No. 2, 131-144.

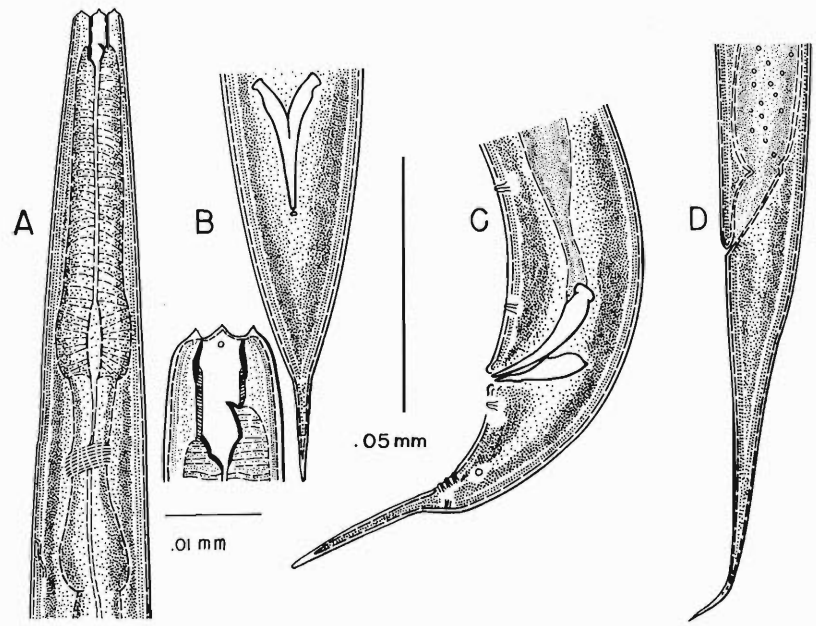


Fig. 4.—*A-D. Diplogasteroides janae* n. sp.; *A.* Head; *B.* Ventral view, male tail; *C.* Lateral view, male tail; *D. Female tail.*

The Parasites of Four Eastern Ground Doves

ROBERT M. STABLER and PORTIA A. HOLT
Colorado College, Colorado Springs

Members of the genus *Columbigallina* are widely scattered over the southern temperate and tropical regions of the Western Hemisphere. Its various species are reported as harboring *Plasmodium*, microfilariae, *Toxoplasma*, and *Haemoproteus* (Levine and Kantor, 1959). The form in the eastern United States, *C. passerina passerina* (eastern ground dove) has been reported as positive for microfilariae only, however. No reports have appeared for the western race (*pallescens*).

The authors are very grateful to Dr. O. E. Frye, Jr., of the Florida Game and Fresh Water Fish Commission, who kindly forwarded from that state four specimens of the eastern ground dove for examination.

OBSERVATIONS

The birds arrived in Colorado Springs in excellent condition on 2 February 61. Their feces, blood, bone marrow, and mouths were examined for parasites, with the following results.

NEMATODES. The feces of three of the doves contained worm eggs which ranged from 64 to 72 microns in length by 40 microns in width. They were typical of the eggs of *Ornithostrongylus quadriradiatus*. The fourth showed no eggs.

COCCIDIA: Oocysts were present in two birds only. They ranged from 17.6 to 20.8 microns in length by 15.2 to 17.6 microns in width. They were identified as *Eimeria labbeana*.

HAEMOPROTEIDS: The blood of all four birds was positive for *Haemoproteus columbae*.

TRICHOMONADS: *Trichomonas gallinae* was recovered from the mouths of three of the four doves. Subinoculations from two of them were made into the mouths of clean domestic pigeons. One pigeon showed no trichomonads at any time; the other was still positive when sacrificed six weeks later. The strain appeared to be completely avirulent.

No other parasites were detected.

DISCUSSION

So far as the authors are aware, none of the four parasites recorded herein (*Ornithostrongylus quadriradiatus, Eimeria labbeana, Haemoproteus columbae,* and *Trichomonas gallinae*) has ever previously been reported from the eastern ground dove. The nematode (Biester and Schwarte, 1959) and the coccidian (Levi, 1957) are both known to cause serious disease in the common pigeon. The trichomonad is noteworthy particularly for the lethal effects of its virulent strains on various members of the Columbiformes (Stabler, 1954). Each new type of host for this pathogenic flagellate is, therefore, of considerable interest.

LITERATURE CITED

BIESTER, H. E. and L. H. SCHWARTE. 1959. Diseases of poultry. Iowa State College Press, Ames, 4th ed.

LEVI, W. M. 1957. The Pigeon. Levi Pub. Co., Sumter, S. C., 2nd ed.

LEVINE, N. D. and S. KANTOR. 1959. Check-list of blood parasites of birds of the order Columbiformes. Wildl. Dis., No. 1

STABLER, R. M. 1954. *Trichomonas gallinae*: A Review. Exper. Parasit. 3: 368-402.

Description of *Dipetalonema interstitium* n. sp. from the Grey Squirrel and *Dipetalonema llewellyni** n. sp. from the Raccoon.

DONALD L. PRICE

Walter Reed Army Institute of Research, Washington, D.C. and
Department of Zoology, University of Maryland, College Park, Maryland

During studies on the filarioid parasites in small mammals conducted at the Patuxent Wildlife Research Center** microfilariae and adult filarioid parasites representing two new species were collected, one species from the grey squirrel, *Sciurus carolinensis* Gmelin and the other species from the raccoon, *Procyon lotor* (Linnaeus). Both filarioid parasites were placed in the genus *Dipetalonema* following the key proposed by Chabaud and Anderson (1959). No previous report on either of these parasites could be found. Measurements of both sexes of each species are expressed as an average using three males and five females followed by corresponding measurements of types.

Dipetalonema interstitium n. sp.

DESCRIPTION: Onchocercidae (Leiper, 1911); Onchocercinae Leiper, 1911; genus *Dipetalonema* Diesing, 1861. Head slightly enlarged and rounded with outer circle of eight cephalic papillae and two small interlateral papillae just inside of amphids. Amphid opening slit-like. Cuticle finely striated. Esophagus not divided, junction between esophagus and intestine indistinct. Microfilaria unsheathed and found in blood of host.

MALE (holotype): 29.80 (29.50) mm long. Lateral width: at nerve ring 0.040 (0.038) mm, of head 0.051 (0.045) mm, at excretory pore 0.048 (0.042) mm, and at anus 0.037 (0.038) mm. Nerve ring 0.090 (0.080) mm, excretory pore 0.269 (0.262) mm, and junction between esophagus and intestine 0.269 (0.295) mm from anterior end. Anus 0.110 (0.090) mm from posterior end. Posterior end coiled in about two turns. Caudal alae extremely narrow. Nine pairs of caudal papillae: 1 pair anterior to anus, 4 pairs adanal, 2 pairs just posterior to anus, 1 pair midway between anus and tip of tail, and 1 pair near end of tail. Right spicule 0.160 (0.170) mm long, left spicule 0.410 (0.380 mm long. Gubernaculum absent. Tip of tail flattened with longitudinal markings giving superficial suggestion of a bursa.

FEMALE (allotype): 66.40 (64.00) mm long. Lateral width: at nerve ring 0.071 (0.070) mm, of head 0.078 (0.075) mm, at excretory pore 0.081 (0.078) mm, at vulva 0.078 (0.076) mm, and at anus 0.048 (0.049) mm. Nerve ring 0.138 (0.140) mm, excretory pore 0.308 (0.310) mm, vulva 0.534 (0.540) mm, and junction between esophagus and intestine 0.284 (0.280) mm from anterior end. Anus 0.221 (0.210) mm from posterior end. Posterior end of body turns ventrally and ends in three or four lobes, with two lateral, and a central lobe which may be completely or only partially divided.

MICROFILARAE: 250 $\pm$ 5 microns long, 3.5 microns wide. Head rounded, cephalic space 3 microns long. Distance of anatomical points from anterior end expressed as percentage of entire length of microfilaria: nerve ring 20.0; excretory pore 24.8; excretory cell 28.9; rectal cells, G_1 60.5, G_2 62.8, G_3 67.2, G_4 69.5; anal pore 70.9. Tail ending in S-shaped curve, tapering to a fine point ,tip of last curve free of nuclei. Present in peripheral and heart blood.

---

*This dipetalonematid has been named for the late Leonard Llewellyn of the Patuxent Wildlife Research Center without whose able assistance this paper would never have been possible.

**The laboratories of the Fish and Wildlife Service, Department of the Interior at Laurel, Maryland.

HOST: *Sciurus carolinensis* Gmelin.
LOCATION: Subcutaneous connective tissue and intermuscular fascia.
TYPE LOCALITY: Le Conte State Game Refuge, Vienna, Maryland.
HOLOTYPE AND ALLOTYPE: U.S.N.M. Helm. Coll. No. 39482.
PARATYPES: (2 males and 3 females) U.S.N.M. Helm. Coll. No. 39483.

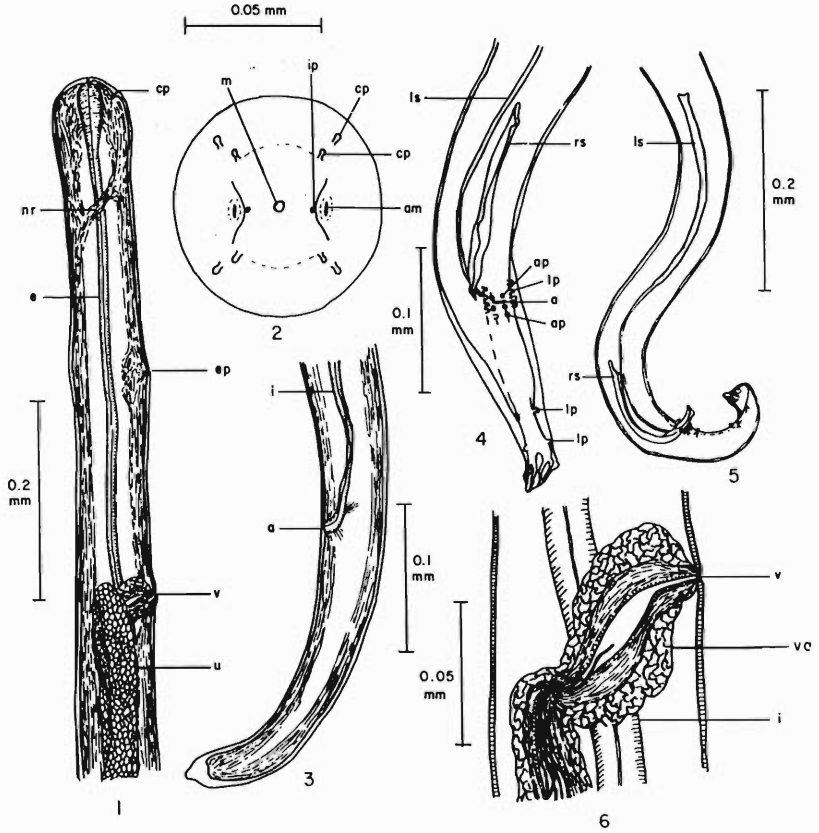


Plate I

Dipetalonema interstitium n. sp. Diagrams of adult parasites:
Fig. 1. Right lateral view of anterior end of female.
Fig. 2. *En face* view of female.
Fig. 3. Left lateral view of posterior end of female.
Fig. 4. Off ventral view of posterior end of male.
Fig. 5. Right lateral view of posterior end of male.
Fig. 6. Right lateral view of vulva and vagina of female.

Abbreviations: a, anus; am, amphid; ap, anal papilla; cp, cephalic papilla; e, esophagus; ep, excretory pore; i, intestine; ip, interlateral papilla; lp, lateral papilla; ls, left spicule; m, mouth; nr, nerve ring; o, ovary; rs, right spicule; u, uterus; v, vulva; va, vagina.
(Figures drawn with the aid of a microviewscope)

DISTRIBUTION: Le Conte State Game Refuge, Vienna, Maryland and possibly National Zoological Gardens, Washington, D.C.

DIAGNOSIS: A *Dipetalonema* having microfilariae about 250 microns long, tail ending in S-shaped curve, tapering to fine point, last curve free of nuclei. Male extremely small, having four pairs of papillae lateral to anus and longitudinal markings in tip of tail. Female averaging about 66 mm long with caudal tip of female ending in three or four lobes. This combination of characters is diagnostic for *Dipetalonema interstitium*.

Dipetalonema llewellyni n. sp.

DESCRIPTION: Onchocercidae (Leiper, 1911); Onchocercinae Leiper, 1911; genus *Dipetalonema* Diesing, 1861. Head slightly enlarged and rounded with outer circle of eight cephalic papillae and two small interlateral papillae just inside of amphids. Amphid opening slit-like. Cuticle finely striated. Esophagus not divided, junction between esophagus and intestine indistinct. Microfilaria unsheathed and found in blood of host.

MALE (holotype): 34.66 (42.00) mm long. Lateral width: at nerve ring 0.039 (0.042) mm, of head 0.045 (0.048) mm, at excretory pore 0.053 (0.065) mm, and at anus 0.033 (0.038) mm. Nerve ring 0.098 (0.110) mm. excretory pore 0.263 (0.290) mm, and junction between esophagus and intestine 0.295 (0.310) mm from anterior end. Anus 0.123 (0.140) mm from posterior end. Posterior end coiled in about two turns. Caudal alae extremely narrow. Ten pairs of caudal papillae: 1 pair anterior to anus, 5 pairs adanal, 2 pairs just posterior to anus, 1 pair about midway between anus and posterior end of tail, and 1 pair near end of tail. Right spicule 0.145 (0.158) mm long, left spicule 0.430 (0.440) mm long. Gubernaculum absent. Tip of tail flattened with longitudinal markings giving a superficial suggestion of a bursa.

FEMALE (allotype): 90.00 (92.00) mm long. Lateral width: at nerve ring 0.079 (0.079) mm, of head 0.084 (0.084) mm, at excretory pore 0.087 (0.090) mm, at vulva 0.092 (0.120) mm, and at anus 0.045 (0.046) mm. Nerve ring 0.174 (0.190) mm, excretory pore 0.343 (0.340) mm, vulva 0.750 (0.740) mm, and junction between esophagus and intestine 0.308 (0.310) mm from anterior end. Anus 0.229 (0.235) mm from posterior end. Posterior end of body turns ventrally and tip of tail ends in four lobes.

MICROFILARIA: 290 ± 5 microns long, 2.5 microns in diameter. Distance of anatomical points from anterior end is expressed as percentage of the entire length of microfilaria: nerve ring 17.9; excretory pore 23.3; excretory cell 25.0; rectal cells, G_1 69.2, G_2 77.1, G_3 78.3, G_4 80.0, anal pore 81.6. Tail ending in curve resembling delicate button-hook without nuclei. Present in peripheral and heart blood.

HOST: *Procyon lotor* (Linnaeus).

LOCATION: Subcutaneous connective tissue and intermuscular fascia.

TYPE LOCALITY: Patuxent Wildlife Research Center, Laurel, Maryland.

HOLOTYPE AND ALLOTYPE: U.S.N.M. Helm. Coll. No. 39484.

PARATYPES: (1 male and 3 females) U.S.N.M. Helm. Coll. No. 39485.

DISTRIBUTION: Maryland.

DIAGNOSIS: A *Dipetalonema* with microfilariae about 290 microns long having tail ending in delicate button-hook curve without nuclei. Male extremely small having five pairs of papillae lateral to anus; tip of tail flattened and having longitudinal marking. Female averaging about 90 mm long with caudal tip of female ending in four small lobes. This combination of characters is diagnostic for *Dipetalonema llewellyni*.

DISCUSSION

The adults of these two species of *Dipetalonema* are very similar in structure. In the males the spicules and arrangement of caudal papillae are almost the same, the only obvious difference other than size being the number of pairs of papillae lateral to the anus, *D. interstitium* having four

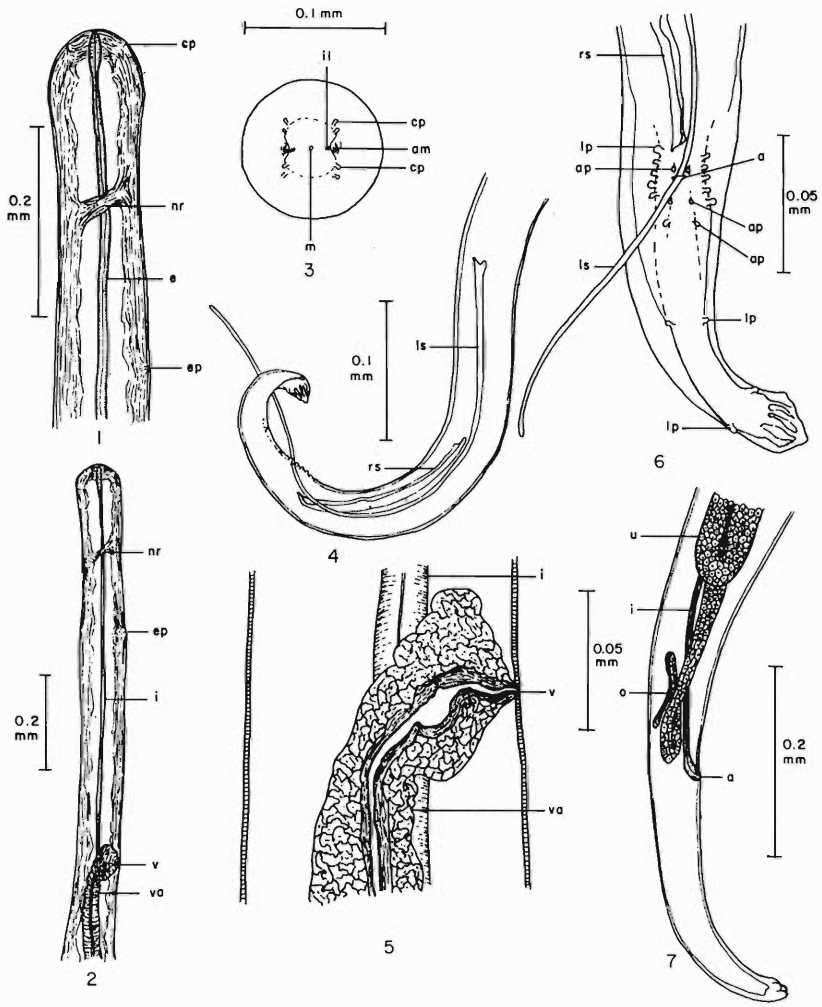


Plate III

Dipetalonema llewellyni n. sp. Diagrams of adult parasites:
Fig. 1. Right lateral view of anterior end of female.
Fig. 2. Right lateral view of anterior end of female showing vulva and vagina.
Fig. 3. *En face* view of female.
Fig. 4. Left lateral view of posterior end of male.
Fig. 5. Right lateral view of vulva and vagina of female.
Fig. 6. Off ventral view of posterior end of male.
Fig. 7. Right lateral view of posterior end of female.

and *D. llewellyni* having five. The only obvious difference between the females of these two species is size although the microfilariae removed from the uterus of one species of female can be differentiated from those of the other species. Sandground (1933), referring to the differentiation of adults of the genus *Dirofilaria,* remarks, "size relationships are cited as the chief criterion upon which most species are presumptively distinguished." Price (1959) used size along with the microfilaria to differentiate the females of *D. magnilarvatum* from those of another species found in *Macaca irus.* In the present paper there is also a difference in size which is more apparent in the females.

The morphology of the microfilaria of *D. llewellyni* from all of the infected raccoons examined was the same. Microfilariae were removed from several living females of this species to make certain that microfilariae from the adult females were the same as those seen in the blood of the host and that no other species of filaroids were involved. A similar procedure was followed in the case of *D. interstitium* in the squirrel. Since the microfilariae from the two hosts were constantly different in morphology, one from another, they could not have arisen from the same species of female *Dipetalonema.* The morphological differences were best seen in a modified Knott's concentration as described by Herman and Price (1955).

Another parasite of the same genus, *D. procyonis,* was reported by Price (1955) from the raccoon. This species is sufficiently different in structure so that it would not be confused with the presently described species.

SUMMARY

Dipetalonema interstitium n. sp., infecting the subcutaneous connective tissue and intermuscular fascia of *Sciurus carolinensis,* and *Dipetalonema llewellyni* n. sp., infecting the subcutaneous connective tissue and intermuscular fascia of *Procyon lotor,* are described from Maryland. The similarity of these two species and the necessity of using the microfilaria in diagnosis are discussed.

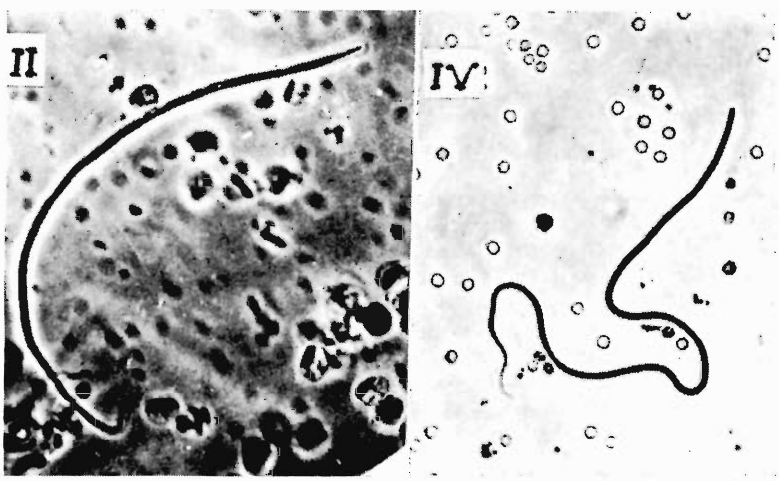


Plate II *Dipetalonema interstitium* n. sp. Microfilaria.
Plate IV *Dipetalonema llewellyni* n. sp. Microfilaria.

LITERATURE CITED

CHABAUD, A. G. and ANDERSON, R. C. 1959. Nouvel essai de classification des filaires (Superfamille des Filarioidea) II. Ann. Parasit. 34: 64-87.

HERMAN, C. M. and PRICE, D. L. 1955. The occurrence of Hepatozoon in the gray squirrel (*Sciurus carolinensis*). J. Protozool. 2: 48-51.

PRICE, D. L. 1955. *Dipetalonema procyonis* n. sp. from *Procyon lotor lotor* (Linnaeus). Proc. Helminth. Soc. Washington. 22: 38-41.

————. 1959. *Dirofilaria magnilarvatum* n. sp. (Nematoda: Filarioidea) from *Macaca irus* Cuvier. I. Description of the adult filarial worms. J. Parasitol. 45: 499-504.

SANDGROUND, J. H. 1933. Report on the nematode parasites collected by the Kelly-Roosevelt expedition to Indo-China with descriptions of several new species. Part 2. Parasites of mammals. Ztschr. Parasitenk., Berlin 5: 575-578.

On the Identification of the Cercaria of *Cotylurus brevis* Dubois and Rausch, 1950, (Trematoda: Strigeida) and Genitalia of the Adult

P. NASIR*

In a previous publication (see Nasir, 1960) a furcocercaria found in *Lymnaea stagnalis* from Edgbaston Pool, near the University of Birmingham, England, was proved to be identical with *Cercaria helvetica XXXIV* Dubois, 1934 occurring in *L. stagnalis* in Neuchâtel, and experimentally connected with the already known adult *Cotylurus brevis* Dubois & Raesch, 1950. A comparative account of related species of cercariae and details of the genitalia of adult were omitted which are presented in this paper.

DISCUSSION OF RELATED CERCARIAE

From the works of Mathias (1925) Szidat (1924; 1929A) Van Haitsma (1931) and Harper (1931) it may be deduced that the cercarial stages of species belonging to the genus *Cotylurus,* Szidat, 1928, are characterized by the presence of two pairs of penetration glands anterolateral to the ventral sucker, esophagus bifurcating either immediately anterior to the ventral sucker or far in front of it, the ventral sucker with an armature of spines, an excretory commissure anterior to the ventral sucker, and the number of flame cells, seven or ten pairs. However, an exception is met with in the cercaria of *Cotylurus communis* (Hughes, 1928) LaRue, 1932, synonyms *Strigea michiganensis* La Rue (1927) in Van Haitsma, 1930 and *Cotylurus michiganensis* (La Rue) in Van Haitsma, 1930, part of the life cycle of which has been described by Van Haitsma (1930). He describes the cercaria as possessing three pairs of penetration glands posterior to the ventral sucker and eight pairs of flame cells, six in body and two in tail stem. Van Haitsma's conclusions have been challenged by Szidat (1931) who asserted that the position of the penetration glands is of generic importance, and their location i.e., behind the ventral sucker is characteristic of cercariae belonging to the adult genus *Diplostomum.* Dubois (1938) also agrees with Szidat while Olivier and Cort (1942), as a result of experimental reinvestigation of the life cycle of *C. communis,* concluded that the cercaria which Van Haitsma assumed to be the larva of *C. communis* develops into a *diplostomulum* stage in the eyes of fish and therefore "it can have no relationship to the life cycle of *C. communis* since the metacercaria of this species is a tetracotyle found around the hearts of fishes."

*Department of Zoology and comparative Physiology, the University, Birmingham, England.

Present address: Parasitology Laboratory, Wayne State University, Detroit, Michigan, U. S. A.

The above cited characters have been regarded as of generic importance. Certain other characters have been used for the specific determination: the location of penetration gland cells and anterior excretory commisure in relation to ventral sucker, the total number of flame cells, the presence or absence of forward pointing spines, the number of rows of acetabular spines and the position of esophageal bifurcation. In addition body size has been shown conclusively by Olivier and Cort (1941) to be of significance in separating the cercaria of *Cotylurus flabelliformis* from *Cercaria douglasi*. Dubois (1934) has already emphasized the importance of esophageal length and the same author has used the number of rows of acetabular spines for the identification of *Cercaria helvetica XXXIV* Dubois, 1934 (= cercaria of *Cotylurus brevis*). Rees (1957) has not only made use of the rows and of the number of spines in each row on the ventral sucker but also of the arrangement of body spines for characterization of the cercaria of *Diplostomum phoxini* (Faust). My observations show that it is not only the length of esophagus that matters but also whether or not the course of intestinal ceca is tortuous. It seems most likely that the "unpigmented eye spots" are characteristic of all cercariae belonging to the adult genus *Cotylurus* and in those forms where their presence has not been recorded it is possible that they might have been overlooked.

There are six other related cercariae about which sufficient structural or developmental details are known to render a detailed comparison with cercaria of *Cotylurus brevis* worthwhile: *Cercaria douglasi* (Cort, 1917) Olivier and Cort (1941) from *Physa spp;* cercaria of *Cotylurus flabelliformis* (Faust, 1917) Cort and Brooks (1928) Olivier and Cort (1941) from varieties of *L. stagnalis; Cercaria A* Szidat, 1924, Dubois (1929), Wesenberg-Lund (1934) = the larvae of *Cotylurus cornutus* (Rudolphi, 1808) in Szidat (1928; 1929B) from *L. stagnalis* and *L. palastris; Cercaria sanjuanensis* Miller, H. M. Jr., 1927 from *L. stagnalis; Cercaria berghei* Fain, 1953 from *Biomphalaria alexandrina tanganyicensis* and *B. A. pfeifferi;* and *Cercaria tetraglandis* Iles, 1959 from *Planorbis corneus*.

The cercaria of *Cotylurus brevis* is distinguished from *Cercaria douglasi* by its larger size and by the fact that the tail stem in the former is always longer than the body while in the latter it is shorter than the body. Besides there are "several irregular rows of thin elongated spines" on the ventral sucker in *Cercaria douglasi* while in the cercaria of *Cotylurus brevis* there are only three rows of acetabular spines; in the former the excretory commissure lies immediately anterior to the ventral sucker whereas in the latter it lies at a considerable distance anterior to the ventral sucker at about the level of unpigmented eye spots; moreover, although the esophagus in *Cercaria douglasi* bifurcates about halfway between pharynx and ventral sucker, is quite long and intestinal ceca show only slight indentations and these too only in the postacetabular region.

Cercaria A and the cercaria of *Cotylurus flabelliformis* are readily separated from the cercaria of *C. brevis* by the position of esophageal bifurcation; in the cercaria of *C. brevis* the esophagus divides about halfway between the oral and ventral suckers but in the cercariae of *C. cornutus* and *C. flabilliformis* it bifurcates only slightly anterior to the ventral sucker.

The penetration gland cells in *Cercaria sanjuanensis* are characteristically arranged between the esophageal bifurcation and the ventral sucker but no such arrangement ever occurs in the cercaria of *C. brevis* where one of the pairs is mostly on one side of the median segittal plane of the body or the anterior glands of both sides may partially overlap. Moreover, "the alimen-

tary canal" in *C. sanjuanensis* "is of narrow calibre throughout its entire extent" and the "long esophagus" divides just at the level of the penetration glands, although a considerable distance in front of the ventral sucker; in cercaria of *Cotylurus brevis* esophagus is short and the intestinal ceca have a peculiar sinuous course broadening posteriorly. Dubois (1929) has expressed doubt as to the validity of *C. sanjuanensis* and considers it "seemingly identical with *Cercaria A*" which develops into *Cotylurus cornutus*, a distinct species from *C. brevis*.

Cercaria berghei from the Belgian Congo has about the same number of caudal bodies, i.e. 12 (in cercaria of *C. brevis* from 12-16) but they differ substantially in other points: in *C. berghei* the digestive tract traverses a sinuous course only in the esophageal region but in the cercaria of *C. brevis* the intestinal ceca along their entire extent are characteristically sinuous; moreover *C. berghei* is a smaller species, especially the furcal rami.

In 1958 the author had an opportunity to examine the living specimens of *Cercaria tetraglandis* but could not make out any other differences excepting the biological ones (see Nasir, 1960) from the cercaria of *Cotylurus brevis*. However, from the published account *C. tetraglandis* is very definitely a distinct species from cercaria of *C. brevis*: *C. tetraglandis* is considerably a smaller form and its prepharynx and esophagus are twice as long; moreover it has eight penetration spines in contrast with twenty spines in cercaria of *C. brevis*.

GENITALIA OF *Cotylurus brevis*

Dubois and Rausch (1950) gave a sufficient detail of various organ systems of adult *Cotylurus brevis*, however, they did not include the genital system which is described in this paper.

MALE GENITAL SYSTEM: Testes (fig. 1-2) median, tandem and occupy position in second and third quarters of hind body; however, this disposition is not a constant feature, depending on state of contraction of hind body. Posterior testis bulkier than anterior testis of same individual. Both testes deeply cleft into three posteriorly directed lobes. Because of great variations in shape of testes due to folding of lobes representative measurement rather unreliable.

Origin of vasa efferentia and their ultimate union to form seminal reservoir (from which common vas deferens emerges) was satisfactorily established only from a number of sections passing through different planes of body. Part of vas efferens overlying corresponding testis from which it arises usually free from sperms and therefore difficult to observe.

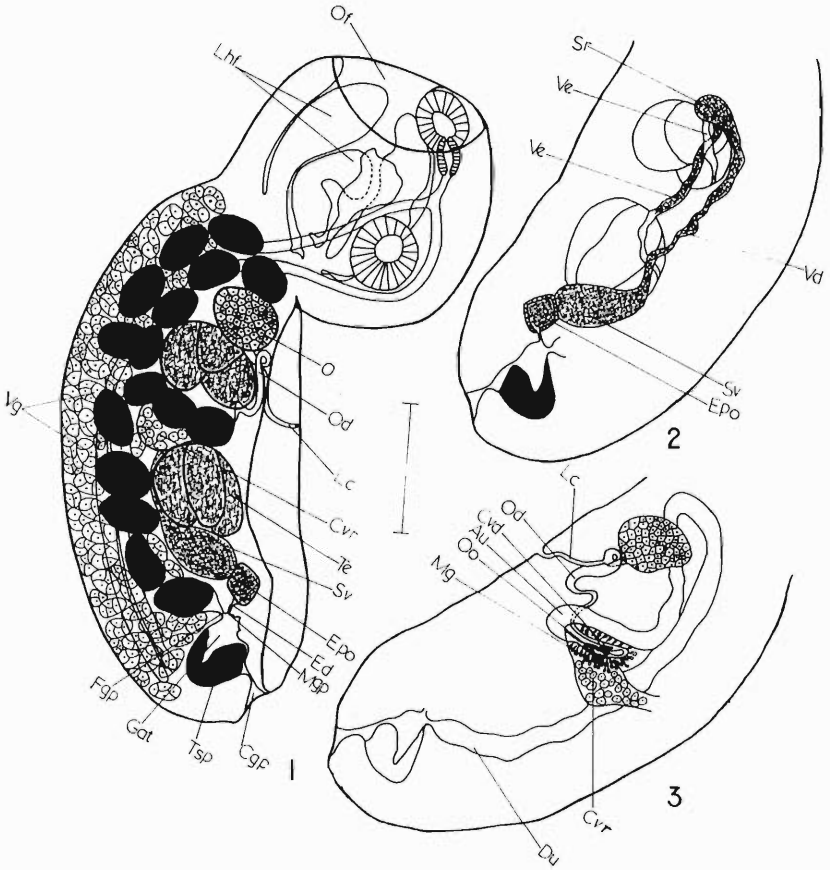
Anterior vas efferens (fig. 2) arises from the mid-ventral surface of anterior testis and runs obliquely anteriorly as far as middle of anterior border of testis; just anterior to testis uniting with vas efferens from posterior testis to form "seminal reservoir" markedly dilated with contained sperms.

Common vas deferens, on leaving seminal reservoir, curves around ventral surface of anterior testis, running nearly parallel with vas efferens from posterior testis. At antero-ventral border of posterior testis vas deferens passes between lobes of this organ. Along postero-ventral border of posterior testis dilating into large seminal vesicle, greater part is posterior to posterior testis; seminal vesicle at its distal end bends towards dorsal surface of body where it narrows rather abruptly and enters globular ejaculatory pouch.

Ejaculatory pouch furnished with thick muscular walls. Narrow slightly coiled ejaculatory duct arises ventrally from near posterior region of ejaculatory pouch and opens into genital atrium dorsal to uterine orifice.

FEMALE GENITAL SYSTEM: Transversely elongate ovary (fig. 1, 3) median and lies dorsal to and anterior to anterior testis; posterior part of ovary partly overlaps anterior part of testis. Oviduct on leaving posterior side of ovary immediately thrown into a vertical fold and at this point Laurer's canal leaves it. Laurer's canal for some distance runs parallel and dorsal to oviduct, then turns obliquely dorsally and eventually opens exteriorly on dorsal surface of hind body at a point level with posterior border of anterior testis.

Oviduct passes ventrally between testes and receives common vitelline duct



Scale equals 0.2 mm.

Fig. 1. General anatomy of adult *Cotylurus brevis.*

Fig. 2. Reconstruction of male genitalia, seen from right side.

Fig. 3. Reconstruction of female genitalia, seen from right side.

Au—ascending limb of uterus; Cgp—common genital pore; Cvd—common vitelline duct; Cvr—common vitelline reservoir; Du—descending limb of uterus; Ed—ejaculatory duct; Epo—ejaculatory pouch; Fgp—female genital pore; Gat—genital atrium; Lc—Laurer's canal; Lhf—lips of holdfast organ; Mg—Mehlis gland; Mgp—male genital pore; O—ovary; Od—oviduct; Of—opening of forebody; Oo—Ootype; Sr—seminal reservoir; Sv—seminal vesicle; Te—testis; Tsp—tongue-shaped process; Tvd—transverse vitelline duct; Vd—vas deferens; Ve—vas efferens; Vg—vitelline glands.

before bending dorsally again to enter spindle-shaped ootype. In transverse sections ootype wall strongly corrugated and receives numerous ducts from surrounding Mehlis gland.

Uterus arises from dorsal end of ootype and after running dorsally for some distance bends ventrally again as far as postero-ventral border of anterior testis; from this point it continues ventral to anterior testis as ascending limb of uterus. Anterior to ovary uterus curves ventrally and runs towards posterior end of body as descending limb of uterus until it opens in genital atrium.

Vitelline follicles occupy ventral half of hind body excepting extreme posterior region occupied by bursa copulatrix. Follicles confined entirely to hind body and never project into forebody. Dubois and Rausch (1950) observed presence of "crown of follicles" in forebody of *Cotylurus brevis* but they considered this presence of vitelline glands in forebody as an "aberrant disposition." Common vitelline reservoir generally larger than ovary and greater part of it left of Mehlis' gland. Transverse vitelline ducts pass one on either side of descending limb of uterus and enter common vitelline reservoir on ventral side. Common vitelline duct on leaving dorsal end of vitelline reservoir descends ventrally entering oviduct.

SUMMARY

A detailed comparative study of the cercaria of *Cotylurus brevis* has been made with six other related cercariae and genital system of the adult is described.

LITERATURE CITED

CORT, W. W. 1917. Homologies of the excretory system of the fork-tailed cercariae. J. Parasitol. 4: 48-57.

————; and BROOKS, S. T. 1928. Studies on the holostome cercariae from Douglas Lake, Michigan. Trans. Amer. Micr. Soc. 47: 179-221.

DUBOIS, G. 1929. Les cercaires de la région de Neuchâtel. Bull. Soc. Neuchâtel Sci. Nat. 53: 1-177.

————. 1934. Contribution á l'etude des cercaires de la region de Neuchâtel, suivie d'une note sur les cercaires du Lac Noir (Zermatt). Rev. suisse Zoologie 41 (2): 73-84.

————. 1938. Monographie des Strigeida (Trematoda) Mem. Soc. Neuchâtel Sci. Nat. 6: 1-535.

————; and RAUSCH, R. 1950. A contribution to the study of North American Strigeids (Trematoda). Amer. Midl. Nat. 43: 1-31.

FAIN, A. 1953. Contribution a l'etude des formes larvaires des trematodes au congo belge et specialement de la larve de *Schistosoma mansoni*. Mém. Inst. Roy. Colon. belge. Sci. Nat. 22: 1-312.

FAUST, E. C. 1917. Notes on the cercariae of Bitter Root Valley, Montana. J. Parasitol. 3: 105-123.

HARPER, W. F. 1931. On the structure and life history of British freshwater furcocercariae. Parasitology, 23: 310-324.

HUGHES, R. C. 1928. Studies on the trematode family Strigeidae (Holostomidae) No. XIII. Three new species of Tetracotyle. Trans. Amer. Micr. Soc. 47: 414-433.

ILES, C. 1959. The larval trematodes of certain freshwater molluses. I. The Furcocercariae. Parasitology 49: 478-504.

LARUE, G. R. 1927. A new species of Strigea from the Herring Gull *Larus argentatus* (Pont) with remarks on the function of the Holdfast organ. J. Parasitol. 13: 226.

————. 1932. Morphology of *Cotylurus communis* Hughes (Trematoda-Strigeidae). Trans. Amer. Micr. Soc. 51: 28-47.

MATHIAS, P. 1925. Recherches experimentales sur le cycle évolutif de quelques trematodes. Bull. Biol. France et Belgique 59: 1-123.

MILLER, H. M., JR. 1927. Furcocercous larval trematodes from San Juan Island. Washington. Parasitology, 19: 61-82.

NASIR, P. 1960. Trematode parasites of snails from Edgbaston Pool; the life history of the strigeid *Cotylurus brevis* Dubois and Rausch, 1950. Parasitology, 50: 551-575.

OLIVIER, L. J.; and CORT, W. W. 1941. *Cercaria douglasi* Cort, 1917, and its relation to the cercaria of *Cotylurus flabelliformis* Faust, 1917. J. Parasitol. 27: 343-346.

————. 1942. An experimental test for the life cycle described for *Cotylurus communis* (Hughes). J. Parasitol. 28: 75-81.

REES, G. 1957. *Cercaria diplostomi phoxini* Faust, a furcocercaria which develops into *Diplostomulum phoxini* in the brain of the minnow. Parasitology, 47: 126-137.

SZIDAT, L. 1924. Beiträge zur entwicklung-sgeschichte der Holostomiden. I. Zool. Auz. 58: 299-314.

————. 1928. Zur Revision der Trematodengattung Strigea Abildgard. Z. Bakt. I. Abt. 105: 204-215.

————. 1929A. Beitrage zur Kenntuis der Gattung *Strigea* (Abild). I. Allgemeiner Teil-Untersuchungen uber die Morphologie, Physiologie and Entwicklungsgeschichte der Holostomiden nebst Bemerkungen uber die Metamorphose der Trematoden und die Plylogenie derselben Z. Parasitenk 1, (4-5): 612-687.

————. 1929B. Beitrage zur Kenntniss der Gattung *Strigea* (Abild.) II. Spezieller Teil: Revision der Gattung *Strigea nebst* Beschneibung einer Anzahl neuer Gattung und Arten. Z. Parasitenk. 1, (4-5): 688-764.

————. 1931. Beitrate zur Entwicklungsgeschichte der Holostomiden. IV. Die cercariae des entenparasiten *Apatemon* (*Strigea*) *gracilis* Rud. und ihre entwicklung im Blutgefässystem des zwischenwirtes (*Herpobdella atomaria* Car.) Z. Parasitenk 3 (2): 160-172.

VAN HAITSMA, J. P. 1930. Studies on the trematode family Strigeidae (Holostomidae) No. XXI. Life cycle and description of the cercaria of *Cotylurus michiganensis* (LaRue). J. Parasitol. 16: 224-230.

————. 1931. Studies on the trematode family Strigeidae (Holostomidae). No. XXII. *Cotylurus flabelliformis* (Faust) and its life history. Pah. Mich. Acad. Sci. 13: 447-482.

WESENBERG-LUND, C. 1934. Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. K. danske vidensk. Selsk. Naturu. Math. Afd. 9, 5 (3) 1: 223.

A Redescription of the Oocysts of *Eimeria ahsata* Honess, 1942, From the Domestic Sheep

NORMAN D. LEVINE, VIRGINIA IVENS, WILLARD N. SMITH and LEONARD REID DAVIS

College of Veterinary Medicine and Agricultural Experiment Station, University of Illinois, Urbana, Illinois and Regional Animal Disease Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Auburn, Alabama

Eimeria ahsata was first described by Honess (1942) from the Rocky Mountain bighorn sheep (*Ovis canadensis*) and the domestic sheep (*Ovis aries*) in Wyoming. He used the spelling *ah-sa-ta,* but the hyphens are omitted here in accordance with the International Code of Zoological Nomenclature. Rysavy (1954) reported *E. ahsata* in the domestic sheep, *Ovis musimon* and *Capra sibirica* in Czechoslovakia. Smith, Davis and Bowman (196) and Smith and Davis (1961) found it in domestic sheep in Alabama and in flocks which had been imported into Alabama from Montana and other western states. Their identification was confirmed by Honess.

Complete descriptions of the sporulated oocysts were given in none of the above reports, and the purpose of the present paper is to do so. The oocysts described herein were from experimentally infected sheep in Alabama. They

were examined with a Leitz Ortholux microscope equipped with apochromatic objectives.

Eimeria ahsata Honess, 1942

DESCRIPTION: Oocysts (Fig. 1) ellipsoidal to somewhat ovoid, slightly flattened at the micropylar end, which is almost always the smaller one. Fifty sporulated oocysts measured 36 to 44 by 22 to 29 microns with a mean of 40 by 26 microns; their length-width ratios ranged from 1.2 to 1.7 with a mean of 1.5. Oocyst wall smooth, lavender to pinkish yellow, composed of two layers 0.9 to 1.3 microns in total thickness. The intact oocysts often appeared to have colorless outer layer 0.4 microns thick, but no evidence of such a layer could be seen in crushed oocysts, and its appearance was probably an optical illusion. The true outer layer accounts for almost the whole thickness of the wall. In intact oocysts the inner layer appears simply as a dark line on the inner surface of the wall, and may sometimes be somewhat wrinkled at the micropylar end. In crushed oocysts, however, the inner layer can be seen as a distinct membrane separate from the outer wall. Micropyle present. Micropylar cap present, dome-shaped; the micropylar caps of 44 sporulated oocysts ranged from 0.4 to 4 microns high by 7 to 11 microns wide with a mean of 2 by 9 microns. One or occasionally more oocyst polar granules ordinarily present; among 50 sporulated oocysts, a single polar granule was seen in 44, two polar granules in 2, three polar granules in 1, and none in 3. Oocyst residuum absent. Sporocysts elongate ovoid, rounded at both ends, with one end somewhat broader than the other. Stieda body absent. Fifty-three sporocysts measured 18 to 20 by 7 to 10 microns with a mean of 18 by 9 microns; their length-width ratios ranged from 1.8 to 2.7 with a mean of 2.0. Sporocyst residuum present. Sporozoites elongate, lying head to tail in sporocysts. One to three clear globules present in sporozoites.

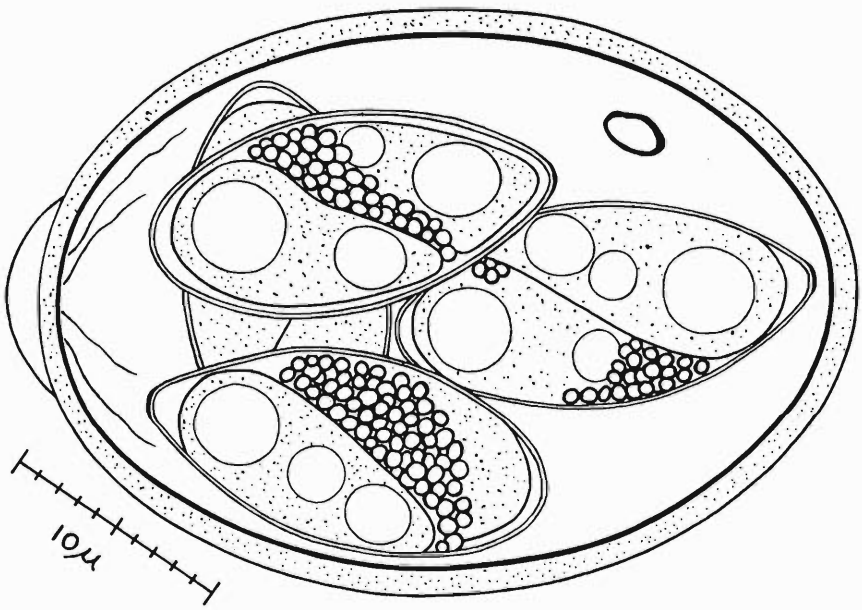


Fig. 1. Sporulated oocyst of *Eimeria ahsata* from the domestic sheep.

DISCUSSION

The oocysts described above are similar to those originally described by Honess (1942) except that they are slightly larger. He reported measurements of 29 to 37 by 17 to 28 microns with a mean of 33 by 23 microns in the domestic sheep, and 30 to 40 by 20 to 30 microns with a mean of 33 by 24 microns in the bighorn sheep. He stated that a residual mass of protoplasm half the diameter of a sporocyst was present in some oocysts; we found none such. Honess did not describe the sporocysts except to give their average dimensions (15.4 by 7.8 microns), and this only in the bighorn sheep; in his photomicrograph they appeared elongate ovoid like ours; he stated that the presence or absence of a Stieda body could not be determined.

Smith and Davis (1961) gave a photomicrograph of a sporulated oocyst of *E. ahsata* of the same provenance as ours; it did not differ markedly in appearance from that of Honess.

The oocysts of *E. ahsata* described by Rysavý (1954) from *Ovis aries*, *Ovis musimon* and *Capra sibirica* in Czechoslovakia measured 27 to 40 by 20 to 29 microns with a mean of 34 by 27 microns; their length-width ratios ranged from 1.1 to 1.5 with a mean of 1.3. They were broadly ovoid to almost spherical (illustrated as ovoid to almost broadly ellipsoidal), with a clear, thin, smooth, transparent light wall. A micropyle and micropylar cap were present, the cap being 2 to 3 microns high and 5.7 microns wide. The sporocysts were oval and measured 9 to 11 by 5 to 8 microns. There was a small sporocyst residuum. Nothing was said about an oocyst polar granule or oocyst residuum.

Rysavý's description agrees in general with that of *E. ahsata* except that the sporocysts of his form are much smaller. In addition, he did not tell whether his description was based on oocysts from one, two or all three host species. It is impossible to be certain, therefore, whether the coccidia which he called *E. ahsata* actually belonged to this species.

E. ahsata is one of a group of ovine coccidia of what might be called the arloingi type. The oocysts in this group have a cap over the micropyle, a colorless to brownish yellow wall, and are up to about 44 microns long. Included in this group are *E. crandallis* Honess, 1942, which has relatively small, ellipsodal oocysts; *E. arloingi* (Marotel, 1905) Martin, 1909, which has medium-sized, ellipsoidal oocysts; *E. granulosa* Christensen, 1938, which has medium-sized, urn-shaped oocysts; *E. punctata* Landers, 1955, which has small, subspherical, pitted oocysts; and *E. ahsata*, which has relatively large, ellipsoidal oocysts. Some workers have doubted whether the separation of all these species is justified. Lotze (1953), for instance, questioned whether there was more than one species with oocysts of the arloingi type in sheep and goats, and said that further studies were necessary to determine the status of *E. ahsata* and *E. crandallis*.

The oocyst length often given for *E. arloingi* is 17 to 42 microns; this is based on Christensen's (1938) study, which was made before the recognition of *E. ahsata* and *E. crandallis*. However, Honess (1942) reported that the oocysts of *E. crandallis* are 17 to 23 microns long, those of *E. arloingi* are 22 to 30 microns long, and those of *E. ahsata* from the domestic sheep are 29 to 37 microns long. Levine, Ivens and Fritz (unpublished) found that the oocysts of *E. arloingi* from the goat (the original host) were 22 to 31 microns long. These later findings indicate that the coccidia in the upper part of Christensen's range may have been actually *E. ahsata* and not *E. arloingi*.

Further confirmation of the validity of this separation may be drawn from

reports on the pathogenicity of *E. arloingi*. Many workers consider this species to be highly pathogenic. Horton-Smith (1958) summarized this view, stating, "*Eimeria arloingi* has been incriminated in several outbreaks of coccidiosis in Britain and it is likely that this species may be the chief cause of severe ovine coccidiosis in these islands. This view of the economic importance of *E. arloingi* is a widely held one among workers in coccidiosis outside this country, and mortality and deaths have been reported as resulting from heavy infestations."

On the other hand, Lotze (1952) found that *E. arloingi* was only moderately pathogenic in 3-month-old lambs. No visible signs were produced by infections with 1 million oocysts or less. In lambs infected with 3 or 5 million oocysts, the feces became soft on the 13th day and then returned to normal during the next 6 days; the health, general condition and weight gains of these animals were not affected. Severe diarrhea was produced with larger numbers of oocysts, but no lambs died even when given as many as 60 million, altho one was killed in extremis.

In comparison, Lotze (1954) found that as few as 50 thousand oocysts of *E. ninakohlyakimovae* caused diarrhea and as few as 500 thousand oocysts caused death in lambs, and he produced dysentery in a 2-year-old sheep by feeding as few as 1 million oocysts. Finally, Smith and Davis (1961) found that up to 3 million oocysts of *E. crandallis* produced no noticeable harmful effects, but that as few as 100,000 oocysts of *E. ahsata* killed lambs less than 15 weeks old. Under experimental conditions, *E. ahsata* is thus the most pathogenic ovine coccidium so far recognized, while *E. arloingi* is considerably less so. It is likely that most of the outbreaks of ovine coccidiosis ascribed to *E. arloingi* are actually due to *E. ahsata*. Indeed, the photomicrograph of an "*E. arloingi*" oocyst given by Horton-Smith (1958) markedly resembles that of *E. ahsata*.

SUMMARY

The oocysts of *Eimeria ahsata* from the domestic sheep are described in detail. They measure 36 to 44 by 22 to 29 microns with a mean of 40 by 26 microns, have a micropyle with a dome-shaped micropylar cap, a double-layered wall and elongate ovoid sporocysts measuring 18 to 20 by 7 to 10 microns. Under experimental conditions, *E. ahsata* is much more pathogenic than *E. arloingi*, and many of the outbreaks of coccidiosis ascribed to the latter were possibly caused by *E. ahsata*.

LITERATURE CITED

CHRISTENSEN, J. F. 1938. Species differentiation in the coccidia from the domestic sheep. J. Parasitol. 24: 453-465.

HONESS, R. F. 1942. Coccidia infesting the Rocky Mountain bighorn sheep in Wyoming. Univ. Wyoming Agric. Exper. Station Bull. 249, 28 pp.

HORTON-SMITH, C. 1958. Coccidiosis in domestic mammals. Vet. Rec. 70: 256-262.

LOTZE, J. C. 1952. The pathogenicity of the coccidian parasite, *Eimeria arloingi*, in domestic sheep. Cornell Vet. 42: 510-517.

LOTZE, J. C. 1953. The identity of *Eimeria arloingi* and *E. faurei* of sheep and goats. Proc. Helm. Soc. Wash. 20: 55-58.

LOTZE, J. C. 1954. The pathogenicity of the coccidian parasite, *Eimeria ninae-kohlyakimovi* Yakimov and Rastegaeva, 1930, in domestic sheep. Proc. Am. Vet. Med. Assoc., 19th An. Meeting, 1953: 141-146.

RYSAVY, B. 1954. Príspěvek k posnání kokcidií našich i dovezenych obratlovcu. Cesk. Parasitol. 1: 131-174.

SMITH, W. N. and L. R. DAVIS. 1961. Two species of sheep coccidia new to Alabama. Proc. Helm. Soc. Wash. 28: 95-96.

SMITH, W. N., L. R. DAVIS and G. W. BOWMAN. 1960. The pathogenicity of *Eimeria ah-sa-ta*, a coccidium of sheep. J. Protozool. 7(Suppl.):8.

Notes on the Dormancy of *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 under Constant Humidity

DONALD P. LIMBER*

The experiments reported below were undertaken in 1940. They are the outgrowth of the writer's (1938) experiments determining the weight of certain *A. tritici* larvae and the observation that the galls of this nematode are highly hygroscopic. It was thought that the length of dormancy, or anabiosis, might be greatly affected by the degree and constancy of the humidity in which the larvae were stored. Fielding (1956) has reported survival of up to 100 percent of the larvae in galls after 28 years in storage. The present experiments are at the 21 year stage with indications that the larvae in two of the test environments may duplicate the period reported by Fielding. Their value, at this time, is in the evidence which they give concerning the conditions which favor longevity in this nematode. They also cast light on the discrepancy between Fielding's report and the earlier published reports of Byars (1920), Goodey (1923), and a less well authenticated report by Needham cited by both Byars and Goodey. It should be added that the conditions under which these galls were stored would not be approached in nature except, perhaps, in some very dry climates.

The wheat galls used were supplied by Dr. G. Steiner and were received in February 1940. They were from a West Virginia collection believed by the writer to be from the 1939 crop. This sample of mixed galls and wheat kernels was kept in a 250 cc widemouth bottle. It was loosely stoppered with a cork and the unused galls were kept as a control on the experiments reported.

Galls embedded in paraffin and others sealed in nitrogen gas showed fewer viable larvae after two years than the control. The outstanding results were secured with larvae in galls sealed in glass tubes by fusing the ends of the tubes with heat. The tubes measured about 4 mm in inside diameter and averaged six inches in length. Six or more galls were placed in each tube but not such a number that any of the galls would be exposed to high heat when the open end was fused. All of the experiments shown in table 1 were started on March 12, 1940. The tubes were stored in the laboratory at Hoboken, N. J. at room temperatures.

Tubes were opened in 1961 and two galls from each were placed in about 2 cc of water in glass dishes. They were examined periodically thereafter.

Table 1. Anabiosis in Anguina tritici, second stage larvae, as influenced by constant low moisture content.

Environment All galls in glass tubes.	*Dates checked* 1952	1957	1961 Maximum Active
Ends of tubes plugged with cotton.	dead		
Ends of tubes fused, galls not dried.	alive	alive	74%
Ends of tubes fused, galls dried for 5 minutes at 70–80°C.	alive	alive	90%
Ends of tubes fused, galls dried for 15 minutes at 70–80°C.	alive	alive	0
Ends of tubes fused, galls dried for 15 minutes at 70-80°C., a few flakes of $CaCl_2$ covered by cotton plugs beneath galls.	alive	alive	8 larvae

*Plant Quarantine Division, A.R.S., U.S. Department of Agriculture, Hoboken, New Jersey.

The first evidence of activity was usually seen about 24 hours after moistening. The number of active larvae is recorded in table 1 as a percentage. However, this is not necessarily a full measure of those alive since Fielding (1951) has demonstrated that some inactive larvae may still be alive.

That the larvae which became active were in a robust physical condition is indicated by the length of time they remained active without an external food source. Of those not dried before they were sealed in the fused tube 12.9 percent were still active after 62 days in sterile water. Of those dried for 5 minutes 21.5 percent were still moving on the 53rd day in distilled water.

The larvae in the unused portion of the sample were stored in the loosely stoppered widemouth bottle at room temperature. The bottle was opened occasionally. About 30 percent of the contained larvae were viable in 1942 but all of them died sometime before 1950.

The galls which Fielding studied were stored in a laboratory at Salt Lake City, Utah, in closed boxes according to Corder (1933). They were thus subjected to a dry climate in comparison with those with which Byars and Goodey worked. The writer's stock sample in the widemouth bottle was subjected to variation in humidity. Thus the evidence seems to point to a positive correlation between longevity and a constant low humidity.

LITERATURE CITED

BYARS, L. P. 1920. The nematode disease of wheat caused by *Tylenchus tritici*. U.S.D.A. (Professional Paper), Bul. 842.

CORDER, MARGARET N. 1933. Observations on the length of dormancy in certain nematodes infesting plants. Jour. Parasit. 20: 104.

FIELDING, MAX J. 1951. Observations on the length of dormancy in certain plant infesting nematodes. Proc. Helm. Soc. Wash. 18: 110-112.

GOODEY, T. 1933. Plant parasitic nematodes. London.

LIMBER, D. P. 1938. Notes on the hot water treatment of *Anguina tritici* galls on wheat and a comparison of an Indian and a Chinese collection by use of weight criteria. Proc. Helm. Soc. Wash. 5: 20-23.

Presentation

1961 ANNIVERSARY AWARD OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

October 20, 1961

RECIPIENT: MISS MILDRED ANN DOSS—Elected a member of the Helminthological Society January, 1939. CO-PRINCIPAL INVESTIGATOR, National Institutes of Health Research Project "Revision of Stiles and Hassall Trematoda," Department of Zoology, University of Maryland; COLLABORATOR, Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture. Formerly, SENIOR PARASITOLOGIST, USDA (Retired).

ACADEMIC AND PROFESSIONAL RECORD: A. B. University of New Mexico, 1925; B. L. S. University of Illinois, 1928; additional special training in numerous languages and zoology. Parasitological bibliographer and Parasitologist In Charge of the Index—Catalogue of Medical and Veterinary Zoology, United States Department of Agriculture 1930 to 1961. Holder of Ives Scholarship, University of Mexico, 1924-1925. Elected to the Washington Academy of Science, 1950. Recipient of the Superior Service Award, USDA,

1953. One of 19,000 women honored by inclusion in "Who's Who of American Women."

AWARD CITATION: For outstanding and sustained accomplishments in parasitological bibliographic work over a period of more than thirty years, and for being largely responsible for preparing and editing for publication, with minimal assistance, a second edition of the Author Section of the Index-Catalogue of Medical and Veterinary Zoology, consisting of 29 volumes, as well as maintaining five other Sections of the Catalogue which are as yet unpublished. Futhermore, for her continuous valuable assistance and service on bibliographic problems to members of this Society and parasitologists everywhere.

CAREER HIGHLIGHTS: Miss Doss began her work with the Index-Catalogue in the USDA in 1930 under the direction of Dr. Albert Hassall. She became a member of a team consisting of Dr. Hassall, Miss Margie Potter, Miss Marion Farr and Mrs. Gertrude B. Carson. This team initiated the project of republishing the Author Section of the Index-Catalogue, but Dr. Hassall retired in 1932 and Miss Potter died in 1936. At that time Part I of the new edition of about 142 pages was published and Part II had been edited for publication, but was not published until 1938. After Miss Potter's death, Miss Doss was placed in charge of the Catalogue, a post she held for 25 years until her recent retirement from the USDA. Because of her remarkable knowledge of foreign languages and her great enthusiasm and diligence, the following record was made in the publication of the Catalogue. Parts III to XVIII, which completed the list of authors through Z, were published in the years 1939 to 1952 and consisted of 5,000 pages and 139,000 citations. Since completing the new edition of the Author Section, 11 supplements have been published, which added about 3,500 pages and 100,000 citations. In addition to her work on the Author Section, she continuously kept up-to-date the other Sections of the Catalogue, i. e., The Parasite Catalogue, The Host Catalogue, the Antiparasitics Catalogue, The Citation Catalogue of Publication, and the Checklist of Specific and Subspecific names. Although substantial contributions to this work were made by other members of the BPL staff, Miss Doss was always the main driving force behind it. By way of emphasis on the enormous and complex task that confronted Miss Doss while serving as the prime bibliographer of parasitology, it should be noted that the information in the Index Catalogue was collected from more than 22,000 different publications printed in 33 languages, and requires more than 2 million cards to record the data on about 150,000 human and animal parasites. This Catalogue enables trained persons to obtain any available published information on any known animal parasite in the world, on the diseases they cause, on their trasmission, intermediate hosts and/or vectors, on their medication, and on the methods of control and eradication.

In recent years, when much more emphasis has been given to the importance of complete and accurate bibliographic work because of the rapidly increasing volume of scientific publications, the Index-Catalogue of Medical and Veterinary Zoology has often been cited as an outstanding example of the best kind of indexing for a highly complex field of science, and the best in biology. A large proportion of the credit for the excellence of this unique catalogue of scientific information goes to Miss Doss. As further testimony of her devotion to this important work, Miss Doss has traveled widely in Russia, Europe and Central America, incidentally at her own expense, and during these travels she has devoted much time to searching out additional

material not available in this country for inclusion in the Catalogue.

Since her retirement from the USDA on June 30 this year, she has received a grant of funds for the purpose of continuing and broadening her important bibliographic work, with her immediate objective of preparing for publication a revised catalogue on the Trematoda. This work will be done under the sponsorship of the Department of Zoology, University of Maryland, with Dr. George Anastos as Co-Principal Investigator, and in cooperation with the Index-Catalogue of Medical and Veterinary Zoology, Beltsville Parasitological Laboratory, USDA. — Awards Committee: A. L. TAYLOR, P. P. WEINSTEIN, and K. C. KATES.

IN MEMORIAM

George Edward Cauthen

March 5, 1900 - November 2, 1961

Member, Helminthological Society of Washington since October 15, 1952

Gotthold Steiner

The Helminthological Society of Washington marks with deep regret the passing of Dr. Gotthold Steiner who died suddenly of a heart attack on August 21, 1961. For forty years he had been a distinguished and respected member. He was elected president in 1925 and was made a life member in 1956.

Dr. Steiner was born in Signau, Switzerland, on April 8, 1886. His higher education was at the Universities of Berne and Zurich. He received his doctor's degree from the former institution in 1910, became Privat-Docent in 1918, and served on the teaching staff until 1921. By this time his research was beginning to receive wide recognition. His publications came to the attention of Dr. N. A. Cobb who was sufficiently impressed by them to induce Dr. Steiner to continue his career in the United States and who helped secure for him an appointment as Sessel Research Fellow at Yale University. After a year at Yale, during which he became a United States citizen, he was appointed Nematologist and Technologist in the U.S. Department of Agriculture and joined the staff of Dr. Cobb's Nematology Laboratory on June 1, 1922.

After Dr. Cobb's death in 1932, Dr. Steiner succeeded him as Principal Nematologist in Charge, was promoted to the rank of Head Nematologist in 1951, and was retired on April 30, 1956. He then went to Puerto Rico where he continued his career, until the time of his death, as Nematologist for the Agricultural Experiment Station at Rio Piedras. In recognition of his service to the U. S. Department of Agriculture, which eventually spanned a period of nearly 34 years, he received the Superior Service Award in 1948 and the Distinguished Service Award in 1955.

Photograph by G. F. Weber, University of Florida

Gotthold Steiner

April 8, 1886—August 21, 1961

Nematologist, United States Department of Agriculture, 1922-56
(In Charge 1932-1956)
Member, Helminthological Society of Washington since 1922
(President, 1925-26)
Trustee, Brayton H. Ramson Memorial Trust Fund, 1936-1956
(Chairman, 1953-1956)

In breadth of training, interest, and experience in his chosen field, Dr. Steiner had few peers. The geographical broadening of his experience was begun before he came to the United States, by periods of study at the Zoological Stations in Naples, Italy; Cette, France; and Helgoland, Germany; and by identifying and describing nematodes collected by the German South Polar Expedition of 1901 to 1913. This broadening was continued by experience in many other parts of the world including a trip to Brazil in 1951 and, finally, by his recent work in Puerto Rico.

Dr. Steiner's contributions, numbering some 195 publications, deal largely, though not exclusively, with freeliving nematodes, including marine, fresh water, and soil forms, and with the nematode parasites of plants and of invertebrate animals. It is difficult to select a few for special mention because many are noteworthy. His two-part monograph wherein he described the nematodes collected by the German South Polar Expedition is a monumental work and one of several important contributions to our knowledge of the marine fauna. It is distinguished by superb drawings of the different species of Epsilonematidae. His studies on the morphology and classification of the Mermithidae, which resulted in numerous publications, were extensive and outstanding. He became one of the few who could make identifications in this taxonomically difficult group of invertebrate parasites. His publication "Plant Nematodes the Grower Should Know" is one of the more important of his many contributions in the field of phytonematology. Appearing originally in the Proceedings of the Soil Science Society of Florida, it was republished as a bulletin by the Florida Department of Agriculture and became a valuable and much consulted source of information, especially in the South.

Dr. Steiner began his work in the United States at a time when the nematode parasites of plants were receiving little attention. Dr. Cobb was engaged in a kind of crusade to convince his colleagues in the Department of Agriculture and elsewhere of the economic importance of these organisms but his efforts made little impression on a hard core of skepticism. Most leaders in agricultural research seemed convinced that, with perhaps a few exceptions, nematodes had no important effects on crop production. After Dr. Cobb's death, Dr. Steiner took up the torch. He never faltered in his convictions nor relinquished his efforts to gain proper recognition for what we now call phytonematology.

That the importance of plant parasitic nematodes would be recognized eventually was inevitable; that the efforts of Cobb and Steiner had much effect in hastening the day seems doubtful. Events that finally brought the importance of these pests into prominence had a kind of "grass-roots" origin. During the early 1940's nematicides became available that could be used on a field scale and that made it possible to demonstrate the increased yields that often result when these pests are controlled. Growers were impressed and began asking for information regarding control procedures that experiment stations could scarcely ignore. The statements and predictions of Cobb and Steiner took on new significance.

Dr. Cobb never lived to see this change but it was Dr. Steiner's good fortune to see his predictions come true and even his most extravagant claims vindicated. Prophets are not always without honor in their own country.—J. R. CHRISTIE.

MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

abama
Cairns, E. J.
Herlich, H.
Minton, N. A.
Porter, D. A
Price, E. W.
aska
Rausch, R.
izona
O'Bannon, J. H.
Reynolds, H. W.
kansas
Cross, J. H., Jr.
Slack, D. A.
lifornia
Allen, M. W.
Ayoub, S. M.
Baker, N. F.
Brown, R. L.
Chitwood, B. G.
Dougherty, E. C.
Dunn, F. L.
Hart, W. H.
Holdeman, Q. L.
Ichikawa, S. T.
Larson J. E.
Lear, B.
Lider, L. A.
Loos, C. A.
Lownsbery, B. F.
Maggenti, A. R.
Mankau, R.
McBeth, C. W.
Nigh, E. L., Jr.
Paxman, G. A.
Raski, D. J.
Sher, S. A.
Steele, A. E.
Wagner, E. D.
Van Gundy, S. D.
Viglierchio, D. R.
Voge, Marietta
Youngson, C. R.
nal Zone
Grocott, R. G.
Pipkin, A. C.
Yunker, C. E.
lorado
LaMotte, L. C., Jr.
Olsen, O. W.
Stabler, R. M.
nnecticut
Miller, P. M.
Penner, L. R.
Pistey, W. R.
laware
Fielding, M. J.
Jaquette, D. S.
Ritchie, T. M.
strict of Columbia
Ashley, W., Jr.
Ballard, Ethyl L.
Barron, C. N.
Betz, D. O., Jr.
Briscoe, M. S.
Bruce, J. I., Jr.
Buchheit, J. R.
de la Cruz, A. A.
Durbin, C. G.
Duxbury, R. E.
Kenworthy, F. T.
Lincicome, D. R.
Mollari, M.
Ostdiek, J. L.
Rajapaksa, N. K.
Robertson, Myrna J.
Rothstein, N.
Sadun, E. H.
Sarles, M. P.
Shepperson, Jacqueline R.
Schneider, C. R.
Schwartz, B.
Simaren, J. O.
Singer, I.
Slie, Rita B.
Taylor, C. R.
Tiner, J. D.
Traub, R.
Wright, W. H.
Young, Viola M.
orida
Christie, J. R.
DiEdwardo, A.
Dikmans, G.
Dieter, C. E.
Esser, R. P.
Feldmesser, J.
Hannon, C. I.
Hutton, R. F.
Kincaid. R. B.
Lefkowitz, S.
Overman, Amegda J.
Perry, V. G.
Rhoades, H. L.
Short, R. B.
Sledge, E. B.
Tarjan, A. C.
Georgia
Bogitah, B. J.
Denton, J. F.
Good, J. M., Jr.
Kagan, I. G.
Ruehle, J. L.
Smith, W. N.
Somerville, A. M., Jr.
Walton, B. C.
Hawaii
Alicata, J. E.
Goto, S.
Illinois
Bauman, P. M.
Briggs, N. T.
Ellis, H.
Garoian, G.
Hechler, Helen C.
Jaskoski, B. J.
Jeske, H. A.
Kruidenier, F. J.
Levine, N. D.
McCrae, R. C.
Otto, G. F.
Schneider, M. D.
Stewart, T. B.
Thomas, L. J.
Walton, A. C.
Indiana
Cable, R. M.
Ehrenford, F. A.
Ferris, Virginia R.
Greve, J. H.
Meade, T. G.
Seitner, P. G.
Shumard, R. F.
Thorson, R. E.
West, A. F.
Iowa
Ellis, C. J.
Engelbrecht, H.
Hsü, H. F.
Norton, D. C.
Ulmer, M. J.
Zimmermann, W. J.
Kansas
Ackert, J. E.
Ameel, D. J.
Dickerson, O. J.
Hansen, M. F.
Kentucky
Baxter, L. W., Jr.
Chapman, R. A.
Dunagan, T. T.
Louisiana
Birchfield, W.
Cordray, D. R.
Cross, H. F.
Hollis, J. P., Jr.
Martin, W. J.
Mayhew, R. L.
Newsom, L. D.
Sogandares-Bernal, F.
Standifer, Marie S.
Whitlock, L. S.
Maine
Meyer, M. C.
Maryland
Adams, F. A.
Anastos, G.
Anderson, R. I.
Andrews, J. M.
Andrews, J. S.
Bang, F. B.
Becklund, W. W.
Beye, H. K.
Borsos, T.
vonBrand, T.
Buhrer, Edna M.
Cantrell, W. F.
Chitwood, May B.
Clark, G. M.
Coatney, G. R.
Cobb, Grace S.
Colglazier, M. L.
DeWitt, W .B.
Diamond, L. S.
Doss, Mildred A.
Douvres, F. W.
Dropkin, V. H.
Elbl, Alena
Ennie, F. D.
Farr, Marion M.
Flyger, V. F.
Foster, A. O.
Freytag, R. E.
Gardiner, J. L.
Gilford, J. H.
Goldberg, A.
Golden, A. M.
Golden, Thelma S.
Grayson, Margaret A.
Green, R. E.
Habermann, R. T.
Haley, A. J.
Hasbrouck, F. E.
Herman, C. M.
Hill, C. H.
Huff, C. G.
Humphrey, Judith M.
Hwang, J. C.
Jachowski, L. A., Jr.
Jacobs, L.
Jahnes, W. G.
Jensen, Dinniemaud V.
Johnson, H. W.
Kates, K. C.
Krassner, S.
Krusberg, L. R.
LaRue, G. R.
Lawless, D. K.
Leek, R. G.
Lesser, E.
Lotze, J. C.
Lucker, J. T.
Lund, E. E.
Lunde, M. N.
Luttermoser, G. W.
Marchbank, Dorothy F.
McIntosh, A.
McLoughlin, D. K.
McMahon, Patricia A.
Morris, J. A.
Myers, R. F.
Olivier, L. J.
Parker, J. C.
Phillips, B. P.
Porter, J. B.
Reardon, Lucy V.
Reese, D. H.
Rozeboom, L. E.
Sawyer, T. K.
Schellberg, Marguerite E.
Schiller, E. L.
Schindler. A. F.
Shiroishi, Tsugiye
Shorb, D. A.
Solomon, G. B.
Spindler, L. A.
Stirewalt, Margaret A.
Taylor, A. L.
Terzian, L. A.
Tromba, F. G.
Turner, J. H.
Underwood, P. C.
Vegors, H. H.
Weathersby, A. B.
Wehr, E. E.
Weinstein, P. P.
Wilson, G. I.
Wright, S.
Young, M. D.
Massachusetts
Boyd, Elizabeth M.
Hurley, F. J.
Riser, N. W.
Rohde, R. A.
Zuckerman, B. M.
Michigan
Clark, D. T.
Cook, Alice R.
Cowan, A. B.
DeGiusti, D.
Grundbacker, F. T.
Kingston, N.
Nasir, P.
Peters, L. E., Jr.
Minnesota
Burton, P. R.
Erickson, D. G.
Taylor, D. P.
Mississippi
Knight, R. A.
Missouri
Boisvenue, R. J.
Montana
Jellison, W. L.
Senger, C. M.
Nebraska
Coil, W. H.
Kelley, G. W., Jr.
Manter, H. W.
Pritchard, Mary H.
Schuster, M. L.
New Hampshire
Bullock, W. L.
New Jersey
Coleman, E.
Cuckler, A. C.
Doscher, Mary Ehlers
Goble, F. C.
Green, D. F.
Jenkins, W. R.
Limber, D. P.
Lynch, J. E.
Pankavich, J. A.
Schroeder, P.
Shafiee, M. F.
Streu, H. T.
New Mexico
Allen, R. W.
Mayer, C. L.
Samson, R. S.
White, L. V.
New York
Berger, H.
Braun, A. J.
Fischthal, J. H.
Friedman, W.
Granek, I.
Ingalls, J. W., Jr.
Kantor, S.
Mai, W. F.
Neal, A. L.
Rohrbacher, G. H, Jr.
Stoll, N. R.
Stunkard, H. W.
Teodorovic, S.
Wilson, W. D.
Wood, I. B.
North Carolina
Cort, W. W.
Garriss, H. R.
Harkema, R.
Hirschmann, Hedwig
Hunter, Wanda S.
Miller, G. C.
Sasser, J. N.
Vargas, O.
Ohio
Elsea, J. R.
Etges, F. J.
Harwood, P. D.
Rossan, R. N.
Wharton, G. W.
Oklahoma
Lee, S. H.
McDaniel, J. S.
Self, J. T.
Smith, P. E.
Struble, F. B.
Oregon
Jensen. H. J.
Konicek, D. E.
Macy, R. W.
Murphy, D. G.
Smithson, Harriet R.
Pennsylvania
Cheng, T. C.
Chute, R. M.
Graham, G. L.
Katz, F. F.
Martin, H. M.
Meinkoth, N. A.
Ogren, R. E.
Sen, H. G.
Uricchio, W. A.
Puerto Rico
Ayala-Almodovar, A.
Oliver-Gonzales, J.
Peebles, C. R.
Roman, J.
Rhode Island
Zinn, D. J.
South Carolina
Adkins, T. R., Jr.
Dery, D. W.
Graham, T. W.
Rau, G. J.
Riggin, G. T., Jr.
South Dakota
Hugghins, E. J.
Texas
Bello, T. R.
Machmer, J. H.
Read, C. P.
Rothman, A. H
Santmyer, P. H.
Thames, W. H., Jr.
Turk, R. D.
Utah
Apt, W. J.
Frandsen, J. C.
Grundmann, A. W.
Havertz, D. S.
Jorgenson, E. C.
Virginia
Byrd, M. A.
Freund, Frances E.
Hargis, W. J., Jr.
Lautenschlager, E. W.
Sonenshine, D.
Tilton, Beverly E.
Washington
Courtney, W D.
West Virginia
Hall, J. E.
Hoffman, G. L.
Wisconsin
Epstein, A. H.
Griffin, G. D.
Noffsinger, Ella Mae
Riffle, J. W.
Saunders, J. L.
Thorne, G.
Wyoming
Honess, R. F.
Schwink, T. H.
Australia
Robinson, E. S.
Sprent, J. F. A.
Belgium
DeConinck, L.
Brazil
DeBona, Arlette
Franco, E.
Lordello, L. G. E.
Canada
Anderson, R. C.
Baker, A. D.
Bosher, J. E.
Ching, Hilda Lei
Hopper, B. E.
Mountain, W. B.
Mulvey, R. H.
Myers, Betty June
Orchard, W. R.
Sanwal, K. C.
Schad, G. A.
Welch, H. E.
Wu, Liang-Yu
Ceylon
Hutchinson, M. T.
Chile
Isaias Tagle, V.
Colombia
Gonzales-Mugaburu, L.
Costa Rica
Hunter, G. W. III
Salas, F., L.A.
Cuba
Kouri, P.
Egypt
Heyneman, D.
Oteifa, B. A.
England
Soulsby, E. J. L.
Williams, I. C.
Yeh Liang-Sheng
France
Dollfus, R. Ph.
Germany
Meyl, A. H.
Ghana
Edwards, E. E.
Thomas, J. D.
Honduras
Wehunt, E. J.
India
Basir, M. A.
Gupta, S. P.
Siddiqi, M. R.
Israel
Minz, G.
Wertheim, Guta
Ivory Coast
Luc, M.
Jamaica
Beverley-Burton, Mary
Mettrick, D.
Japan
Ichinohe, M.
Inatomi, S.
Yamaguti, S.
Yokogawa, M.
Mexico
Caballero y C., E.
Netherlands
Dorsman, W.
Nigeria
Babero, B. B.
Caveness, F. E.
Pakistan
Akhstar, S. A.
Timm, R. W.
Peru
Vargas, O. F.
Philippines
DeJesus, Z.
Marapao, B. P.
Velasques, Carmen C.
Yogore, M. G., Jr.
Poland
Bezubik, B.
Portugal
Bravo Lima, M.
South Africa
Heyns, J.
Switzerland
Baer, J. G.
Dubois. G.
McMullen, D. B.
Sudan
Satti, M. H.
Taiwan
Chiu, Jui-Kuang
Kuntz, R. E.
Turkey
Tolgay, Nermin
Uganda
Price, D. L.
Venezuela
Dao D., F.
Warren, L. G.

CONTENTS