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PROCCEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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The Structure, Synonyms and Hosts of *Physaloptera mexicana* (Nematoda; Physalopteridae)

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Caballero (1937) described the male of *Physaloptera mexicana* as having five pairs of stalked lateral genital papillae, disposed as in *P. alata,* only 3 pairs of subventral (sessile) genital papillae and moderately unequal spicules 0.334 mm. and 0.437-0.460 mm. long (a ratio of about 1:1.3-1.4). Morgan (1943), who examined the "cotypes" and other specimens borrowed from Dr. Caballero, published an original figure (1. c.; fig. 7) of the male genital region showing the spicules to be very unequal, the ratio between the length of the right and left ones being about 1:2.5; however, his figure agrees with Caballero's (1. c.; fig. 3) exactly as to the number, and rather closely as to the locations, of the genital papillae.

The agreement between these authors as to the presence of only 3 pairs of male subventral genital papillae in this species, whereas in typical *Physaloptera* 6 such pairs and an unpaired median precloacal papilla are present, and their sharp disagreement as to the ratio between the lengths of the spicules, led the writer to investigate the characteristics of the species by examining the "type" (a male and a female; borrowed from Dr. Caballero) and "cotype" (a male and a female; U.S.N.M. No. 8969) specimens. The latter were found to be the more suitable for study.

STRUCTURE OF MALE: The left spicule of the cotype male was observed to be slightly more than twice as long as the right one. Unfortunately, however, it was broken off before an actual measurement of it could be obtained. The right spicule measured about 0.40 mm. in length. From these observations and the aforementioned ratio ascertained from Morgan's figure, which probably is based on the same specimen, a calculated left spicule length of about 0.80 to 1.0 mm. is deemed acceptable for the cotype. The fifth, or posteriormost, pair of genital papillae in the series of 5 lateral pairs was found to be widely separated from the fourth pair, a pattern differing from that characteristic of *P. alata,* and there were found to be 13, rather than 6, ventral and subventral sensory organs, disposed as shown in Figure 1.

The tail of the type was found to be tightly recurved; the bursa had been damaged. The left spicule was incomplete. Only the unprotruded, proximal portion of it remained; that Caballero's description is based largely on this specimen and that his measurement (0.334 mm.) for the shorter spicule applies to this fragment seems reasonably clear. The right spicule measured about 0.43 mm. in length and has the shape shown in Fig. 2. The 5 pairs of stalked lateral papillae are disposed approximately as in the cotype. Because of the already damaged state of the specimen, excessive manipulation of it

was avoided, but enough of its subventral papillae were seen to convince the writer of its conspecificity with the cotype.

IDENTITY WITH MALES OF OTHER SPECIES. Therefore, the male of *P. mexicana* was found to agree in all essential respects with the description of the male of *P. buteonis* Morgan, 1948 (Morgan, 1948; fig. 2) and the nearly identical male-description given by Seurat (1914)* under the name *"Physaloptera subalata* Schneider." Seurat gave the spicule lengths for *P. "subalata"* as 0.84 mm. (left) and about 0.40 mm. (right); for *P. buteonis,* Morgan gave averages of 1.1 mm. and 0.38 mm. for the corresponding spicules. Morgan described all of the subventral sensory organs as papillae, whereas Seurat identified the fourth postcloacal pair in his specimens as phasmids ("Orifices des glandes caudales"). However, differentiation between sessile papillae and phasmids in totomount preparations often is practically impossible and males cannot be regarded as representing distinct species simply because one is described as having five pairs of postanal, sessile, subventral papillae and another four such pairs and a pair of phasmids.

Caballero described the precloacal portion of the bursa as greatly inflated ventrally. From examination of the aforementioned specimens, it appears that this inflation may be due partly to incipient disintegration of the worms before fixation, as indicated by the obviously abnormal separation of the cuticle from the body at and near their anterior extremities and the general disorganization of their internal structures. However, some elevation of the precloacal part of the bursa evidently is normal; it was noted by Seurat in *P. "subalata"* and the transverse precloacal arc-like line in Morgan's figure of the bursa of *P. buteonis* probably is intended to represent such an elevation.

FURTHER COMPARISON WITH THESE "SPECIES." Caballero stated that in *P. mexicana* each pseudolabium bears one internal and two external teeth; this appears to be a misinterpretation of the structures he actually observed (l. c.; fig. 2), for the writer finds the pseudolabial dentition to be as described for *P. buteonis* and *P. "subalata";* i.e., each lip bears a relatively short bluntly rounded outer tooth and an inner trifid tooth with relatively long pointed prongs. The buccal "piezas quitinosas" described as peculiar to *P. mexicana* evidently are the stomatal walls and are typical for the genus. The female reproductive system could not be made out in detail on examination of the type and cotype females. The original brief description of it lacks detail but does not conflict with the fuller female-descriptions given for *"P. subalata"* and *P. buteonis.*

The female-descriptions given under these two names admittedly do not fully agree, but the writer does not consider any of the discrepancies between them evidence of specific difference. The potentially most important disagreement concerns the mode of origin of the uteri. Seurat said that the ovijector ("trompe") expands to form a reservoir (egg chamber), resumes its original lesser diameter, and then (evidently immediately) bifurcates to join the uteri, whereas Morgan stated that a uterine "common trunk" 1.5 mm. long is present in *P. buteonis.* This, however, is not substantiated by his figure (l. c.; fig. 3), which, in near accord with Seurat's statement, illustrates a very short common trunk.** No doubt as a result of his examination of specimens, he

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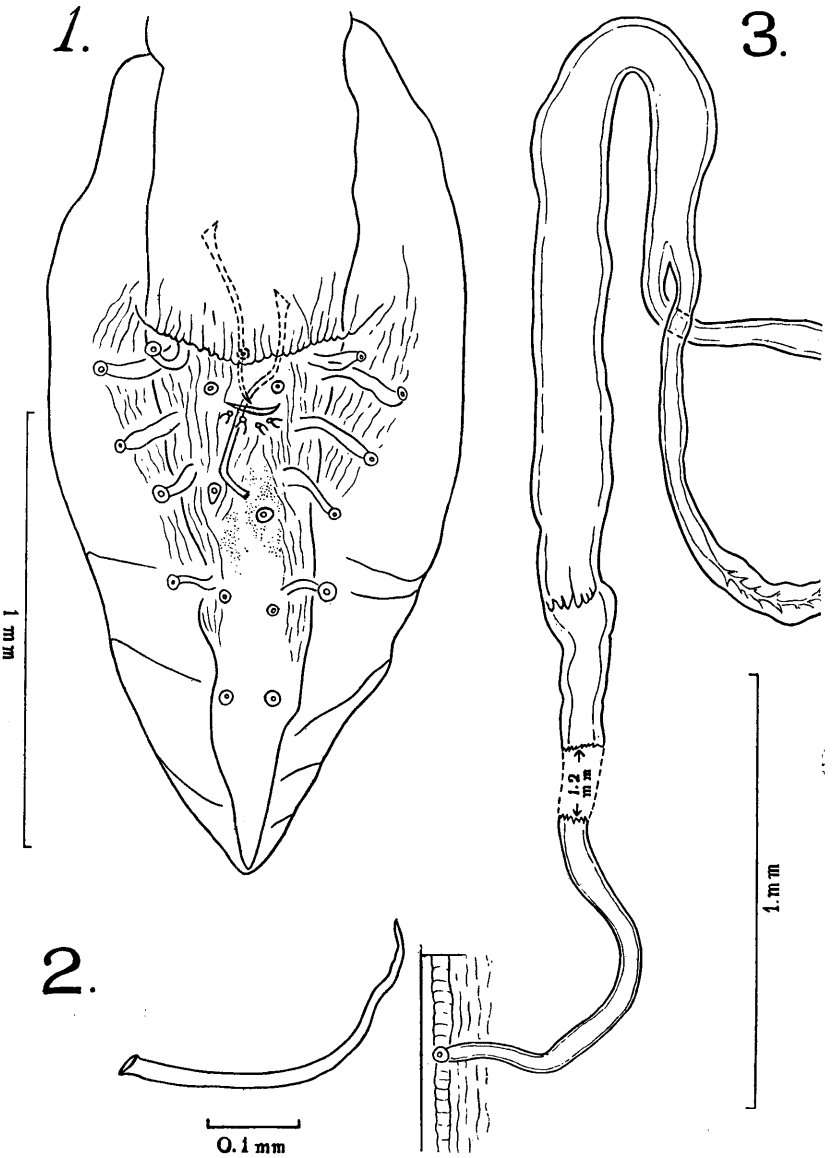
*Ortlepp's (1922) condensation of Seurat's description is better known, but is inadequate and in one respect incorrect.

**Unfortunately, efforts to locate the types and cotypes of *P. buteonis,* which Morgan (1948) stated had been deposited, respectively, in the U.S. National Museum and "parasitological collection, University of Wisconsin," have failed.



Figs. 1-3.—*Physaloptera mexicana* Caballero, 1937: 1, Bursal region, ventral aspect (cotype male); 2, right spicule, lateral aspect (type male); 3, origin of uterine bifurcation at base of egg chamber, as shown by dissection (U. S. N. M. No. 44796, female).

(1943; 1948) likewise mentioned the presence of a common trunk in *P. mexicana.*

However, in a lot of *Physaloptera* (U.S.N.M. No. 44796) from Swainson's hawk, males having the characteristics (length of left and right spicules in one specimen: 0.875 mm. and 0.40 mm., respectively) found in the cotype of *P. mexicana* are associated with females in which there is virtually no common uterine trunk, so far as is indicated by the structure (Fig. 3) observed on dissection of a suitable specimen. Hence these worms, considered identifiable as *P. mexicana,* are actually best covered by Seurat's description based on specimens from Corsica, although Swainson's hawk, like the type host of *P. buteonis,* is restricted to North America in distribution.

NOMENCLATURE; SYNONOMY; HOSTS. Seurat's tentative identification of his specimens as *P. subalata* is not acceptable because Schneider (1866) based this specific name on a male described as having 4, rather than 5, pairs of lateral genital papillae and objective evidence of descriptive error on Schneider's part is lacking.

In view of the foregoing facts and considerations and the law of priority, the writer concludes that Seurat, Caballero and Morgan (1948) had the same natural species before them and that *P. mexicana* Caballero, 1937 is its valid name, with *P. buteonis* Morgan, 1948 and *P. subalata* Schneider, 1866 of Seurat (1914) as synonyms; the known hosts and localities are: *Buteo* sp., Valley of Mexico, Mexico (Caballero, 1937); "Buse" [? *Buteo buteo*], San Martino, Corsica (Seurat, 1914); *Buteo jamaicensis borealis,* Wisconsin, U.S.A. (Morgan, 1948); *Buteo swainsoni,* Utah, U.S.A. (present paper).

DIFFERENTIATION FROM OLDER SPECIES. So far as is now evident, *P. mexicana* differs from all older species known from birds in the following combination of characters in the male: presence of 5 pairs of stalked lateral genital papillae combined with a high ratio of difference (2.0-2.5:1) between the lengths of the left and right spicules. It shares such great inequality in spicular lengths with *P. acuticauda* Molin, 1860, but only four such pairs of papillae are present in the male of this species. The genital papillary number and pattern of *P. mexicana,* including the wide separation between the fourth and fifth lateral pairs, are duplicated in *P. galinieri* Seurat, 1914, and nearly duplicated in *P. crosi* Seurat, 1914, but the spicules are short and equal, or subequal, in these species.

LITERATURE CITED

CABALLERO Y C., E. 1937. Contribution al conocimiento de los nematodes de las aves de Mexico. II. Rev. Med. Trop. y Parasitol., Habana, 3: 25-35.

MORGAN, B. B. 1943. The Physalopterinae (Nematoda) of Aves. Tr. Am. Micr. Soc., 62: 70-80.

MORGAN, B. B. 1948. *Physaloptera buteonis* n. sp., a nematode from the eastern red-tailed hawk. Tr. Am. Micr. Soc., 67: 183-186.

ORTLEPP, R. J. 1922. The nematode genus Physaloptera Rud. Proc. Zool. Soc. London, pp. 999-1107.

SCHNEIDER, A. F. 1866. Monographie der Nematoden. Berlin. 357 pp.

SEURAT, L. G. 1914. Sur les physaloptères des rapaces. Bull. Soc. Hist. Nat. Afrique Nord, 6: 244-253.

Monogenetic trematodes of Gulf of Mexico Fishes. Part VIII. The superfamily Diclidophoroidea Price, 1936. (Continued)*

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This is the eighth paper of the present series treating the monogeneids of the Gulf of Mexico. It is a continuation of the presentation of the data concerning members of the suborder Polyopisthocotylea Odhner, 1912 obtained during a recent study of these ectoparasites of fishes. Specifically treated herein are several members of the subfamily Anthocotylinae Price, 1936 which is included in the family Discocotylidae Price, 1936 *diag. emend.* The organization and purpose are the same as for preceding installments.

All measurements were made using the ocular micrometer and are cited in millimeters. In the cases of curved structures, measurements are of lines subtending the greatest arcs of those structures. In the descriptions given below the mean is given first, followed by the minima and maxima in parentheses. The number of measurements used to derive the mean is usually the same as the number of individuals measured; otherwise the actual number employed appears in parentheses before the measurements. All drawings were made with the camera lucida.

SUBORDER POLYOPISTHOCOTYLEA Odhner, 1912

SUPERFAMILY DICLIDOPHOROIDEA Price, 1936

Family Discocotylidae Price, 1936, *diag. emend.*

DIAGNOSIS: Diclidophoroidea. Body elongate, flattened dorso-ventrally, bilaterally symmetrical. Opisthaptor with eight clamps either pedunculated or sessile; usually bearing one to three pairs of anchors, each pair being dissimilar, on a terminal lappet. Discocotylid-type clamp structure as follows: ventral loop usually incomplete, dorsal loop elements fairly prominent, middle loop incomplete, may be in two or four parts, center piece usually terminally expanded, accessory sclerites usually not present (except in some groups, *e.g. Plectanocotyle,* whose discocotylid affinities are very questionable). At least one of the anchor pairs usually has shape distinctive of family with a long, delicate shaft and lunate hook. Genital atrium armed or unarmed.

TYPE GENUS: *Discocotyle* Diesing, 1850.

DISCUSSION: The above diagnostic emendation is made in order to exclude the characters of the subfamily Vallisiinae Price, 1943 which is transferred to the family Gastrocotylidae Price, 1943 where it more properly belongs by virtue of its accessory sclerites, *etc.* (These alterations will be further supported in a later installment of this series wherein a detailed discussion of Gastrocotylidae will be presented.) Plectanocotylinae Monticelli, 1903, is left in this family because the present author has not had access to the type materials which should be studied before reassignment is properly made. It may later have to be transferred to another grouping because its members have accessory sclerites in their clamps and their internal genitalia are very peculiarly arranged, with testes both pre- and postequatorial.

As presently conceived the family Discocotylidae consists of the subfamilies Discocotylinae Price, 1936, Plectanocotylinae Monticelli, 1903,

*Contribution from the Biological Laboratories of the Citadel and the Zoology Department of Florida State University, Tallahassee.
Acknowledgements and dedications of the present installment are the same as for preceding ones.

incertae sedis and Anthocotylinae, Price, 1936. The last taxon may later have to be split so that the group formed by the genera *Winkenthughesia* Price, 1943 and *Anthocotyle* van Beneden and Hesse, 1863 can be separated from the genera, *Tagia* Sproston, 1946, *Hemitagia* Sproston, 1946 and *Bicotylophora* Price, 1936, which have different anchor and body shapes. As a matter of fact *Winkenthughesia* and *Anthocotyle* may not even belong in the family Discocotylidae.

This arrangement is different from that of Palombi (1949) who included the family Discocotylidae in his family Arreptocotylidae in recognition of its microcotylid affinities.

It is highly probable that the sucker-like clamp of the Choricotylinae evolved from the slightly asymmetrical discocotylid type as exemplified by *Tagia equadori* (Meserve, 1938) Sproston, 1946 through the diclidophorinid type (see below). This same tendency toward the opening of sclerites can be seen in some gastrocotylinids and some microcotylids, *e.g. Heteromicrocotyla* Yamaguti, 1953.

Subfamily Anthocotylinae Price, 1936

GENUS *Hemitagia* Sproston, 1946. This genus was described by Sproston (1946) to accommodate *Hemitagia galapagensis* (Meserve, 1938) Sproston, 1946, the type species. Because the type specimen, USNM Helm. Coll. slide No. 9184, is torn and distorted and some important characters are obscured, the genus must be left as it is even though the type species is very similar in many characteristics to species of the genus *Tagia* Sproston, 1946.

Hemitagia galapagensis (Meserve, 1938), Sproston, 1946 (Figs. 18, 19).

SYNONYM: *Heterobothrium galapagensis* Meserve, 1938.

HOST AND LOCALITY: Gills of *Paranthias furcifer* (Cuvier and Valenciennes), from Tagus Cove, Albermarle Is., Galapagos Islands.

DISCUSSION: This fluke is not in the present collection but is included herein for taxonomic reasons.

Meserve (1938) did not mention the genito-intestinal canal which enters the right crus immediately posterior to the level of the right transverse vitelloduct.

A study of the cotylophore indicates that in addition to the 4 clamps present there are places for at least 3 and probably 4 more. This would bring the total to the usual 8. There is evidence that not only have clamps been accidentally lost but that the haptor asymmetry is due to mechanical distortion. It is, therefore, evident that the material at hand is insufficient to yield a clear picture of either the species or genus. It is certain that this form is closely related to, and perhaps congeneric with *Tagia equadori* (Meserve, 1938) Sproston, 1946 in that the clamp sclerite details, body shape and genital corona, are similar. The clamps show asymmetrical ventral and middle loops similar to those of some *Tagia* spp. The middle loop has 4 sclerites and the center piece is mostly in the wall of the middle loop.

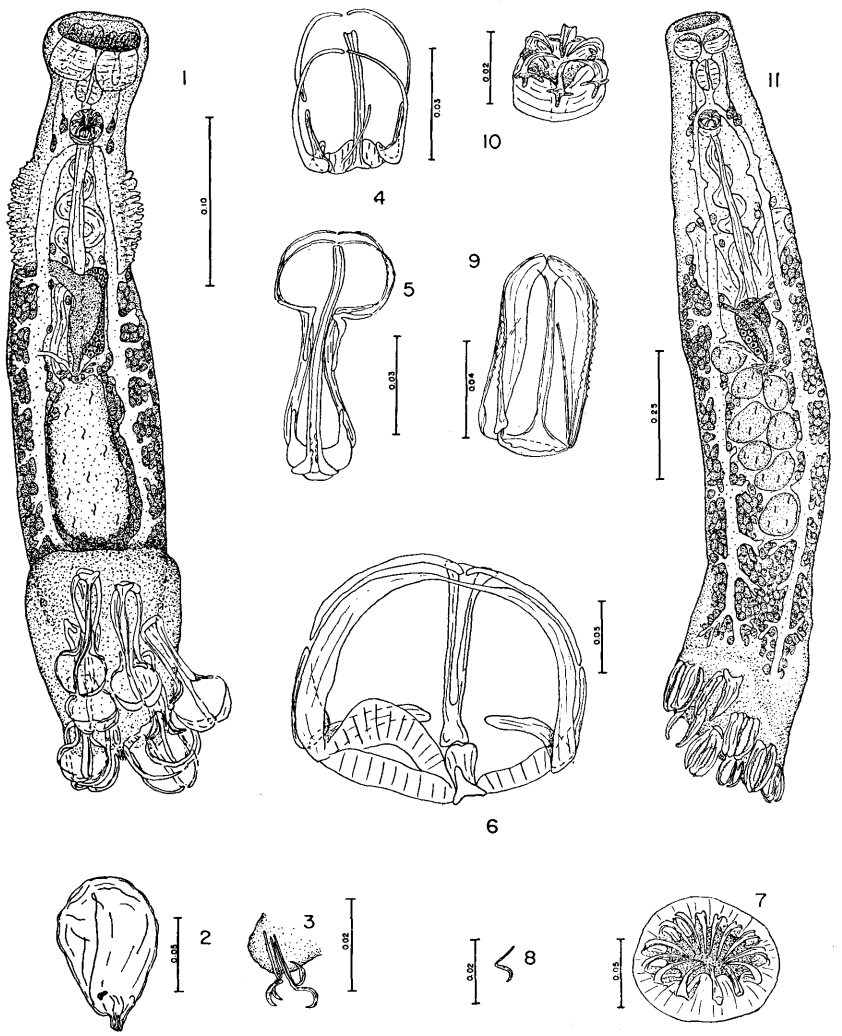
GENUS *Tagia* Sproston, 1946, *diag. emend.*

DIAGNOSIS: Anthocotylinae. Body elongate, flattened dorso-ventrally. Opisthaptor bearing four pairs of ventro-lateral clamps which may be highly modified in shape, anteriormost clamp pair reversed in position dorso-ventrally so that the ventral loop is actually dorsal. Terminal lappet with one to two pairs of discocotylid type anchors. Middle loop and ventral loop are usually incomplete and in several pieces. Gut bifurcated, ramified. Testes postequatorial. Genital atrium armed by a hemispherical, muscular piece

which is surmounted medially by incurved spines arranged radially. Vaginae usually paired, present in most species.

TYPE SPECIES: *Tagia equadori* (Meserve, 1938) Sproston, 1946.

DISCUSSION: The above emendation is made to accommodate characters of the three species, described below. Two of these are new to science. It is apparently through this genus, possibly a form similar to the type species,



Tagia bairdiella n. sp.
1. Whole mount, ventral view.
2. Egg.
3. Lappet, showing anchors.
4. Posterior clamp, ventral view.
5. Anterior clamp, ventral view.

Tagia equadori
6. Clamp, ventral view.
7. Genital corona.

Tagia cupida n. sp.
8. Anchors.
9. Clamp, ventral view.
10. Genital corona.
11. Whole mount, ventral view.

that evolution to the diclidophorid type proceeded as discussed below.

Tagia is a good example supporting the opinion of the present writer that the taxonomic conclusions regarding the various Diclidophoroidea families cannot be based on the shape of the clamps, but should rather be based on details of the clamp sclerites themselves, plus the general body shape and the shape and arrangement of the internal organs. Two members of the genus exhibit "fire-tong" clamps much like those of *Pyragraphorus* of the family Microcotylidae.

Yamaguti (1953) described *Kuhnia otolithis* from the gills of *Otolithus* sp., family Otolithidae, from Celebes. The affinities of this species with those of the present genus have been described above in the discussion of the genus *Kuhnia* (Mazocraeidae). In view of these similarities Yamaguti's species is recombined in the genus *Tagia*. Its name is now *Tagia otolithis* (Yamaguti, 1953), n. comb. with *Kuhnia otolithis* Yamaguti, 1953 as a synonym.

Tagia equadori (Meserve, 1938) Sproston, 1946 (Figs. 6 and 7).

SYNONYM: *Heterobothrium equadori* Meserve, 1938.

Host and locality: Gills of *Cheilichthys annulatus* (Jenyns) from Tagus Cove, Albemarle I., Galapagos Islands.

DISCUSSION: This fluke is not represented in the present collection but is discussed for taxonomic reasons. Both the genital corona and clamp skeleton have been figured in detail. USNM Helm. Coll. type slide No. 9183 was employed for this study.

Anchors may be present between the posteriormost clamp pair but were not positively observed. It is highly probable that a lateral vagina, much like that of *Tagia cupida* n. sp., is present in this form. At least a definite sperm-filled chamber was observed immediately beneath the cuticle on the right side and it is almost certain that several small openings to the outside were present. The genito-intestinal canal joins the right crus.

The anteriormost pair of clamps are reversed dorso-ventrally so that the ventral side is dorsal as in other *Tagia* species. In addition, and probably indicative of the phylogenetic trend in the alteration of the clamp skeleton to the diclidophorid type, there is a very slight asymmetrical development of the clamp sclerites and muscles so that the center piece is off center and noticable muscular pads are present basally on only one side. The ventral loop is complete. The dorsal loop elements are slightly unequal with one side, the muscle side, longer than the other. The middle loop is in 4 pieces, *i.e.* incomplete medially and laterally. The center piece is almost entirely situated in the middle loop capsule with a small, extra center piece sclerite basally.

As indicated above it is herein hypothesized that the diclidophorid-type clamp evolved through or from a form similar to the *Tagia*-type and that, therefore, the family Diclodophoridae is closely related to and probably derived from the family Discocotylidae or discocotylid forms. Similarities in other structures support this thesis (see below under Diclidophoridae), *i.e.* genital corona, anchors, number of clamp pairs and so forth.

Tagia bairdiella n. sp. (Figs. 1 to 5).

HOST: *Bairdiella chrysura* (Lacépède), Silver Perch, a bentho-littoral euryhaline marine sciaenid.

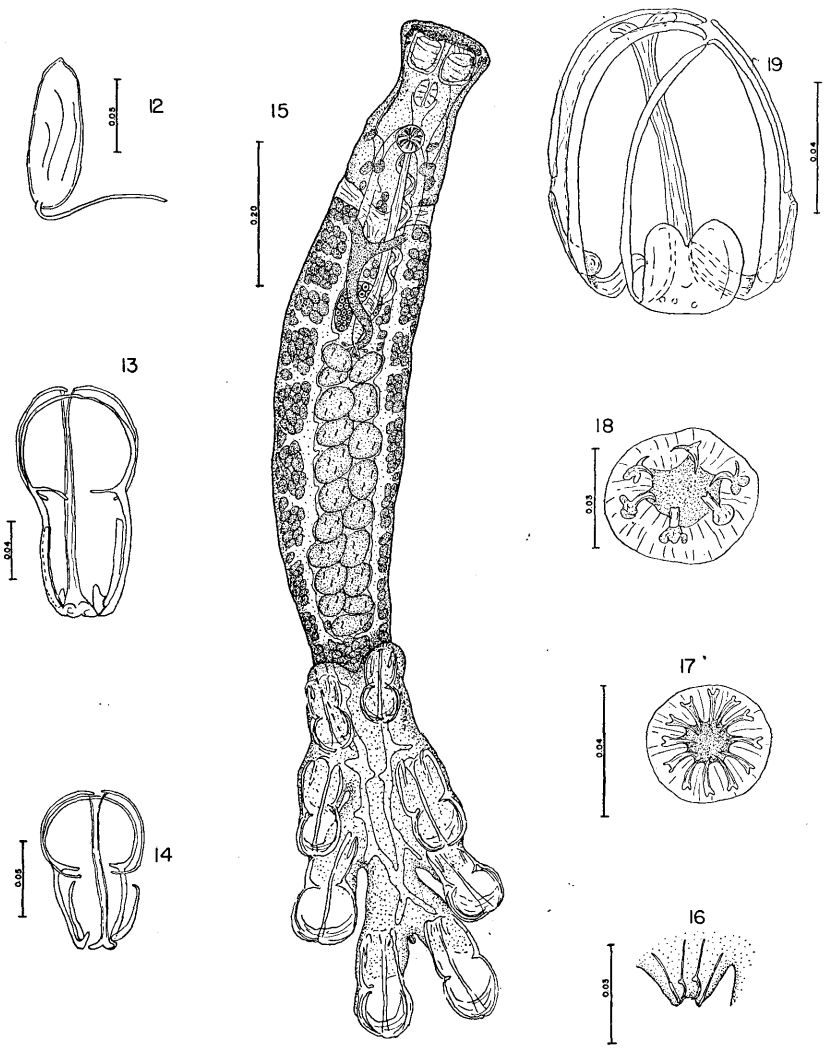
LOCATION: Gills.

LOCALITY: Alligator Harbor, Franklin Co., Florida.

Number studied: 49; Number measured: 5.

HOLOTYPE AND PARATYPE in USNM Helm. Coll. No. 47487.

DESCRIPTION: Body elongate, cylindriform, 0.479 (0.446-0.516) long by 0.106 (0.089-0.121) wide, flared anteriorly, widened posteriorly to fuse with haptor. Cuticle thin and smooth except in the antero-lateral region where it is thrown up into plicated placodes which extend dorsally but not ventrally. Prohaptor a pair of irregular, somewhat cylindrical muscular buccal suckers,



Tagia micropogoni

12. Egg.
13. Posterior clamp, ventral view.
14. Anterior clamp, ventral view.
15. Whole mount, ventral view.
16. Terminal lappets, showing anch
17. Genital corona.

Hemitagia galapagensis

18. Genital corona.
19. Clamp, ventral view.

(4) 0.035 (0.027-0.043) long, in postero-lateral walls of buccal funnel. Opisthaptor a cotylophore that is vaguely trapezoidal in outline, 0.174 (0.146-0.197) long by 0.085 (0.070-0.096) wide, armed with 4 pairs of ventro-lateral clamps, anterior clamp pair reversed dorso-ventrally, and 2 pairs of dis-cocotylid anchors on a small terminal lappet. Clamps unequal in size and shape, 3 anterior "fire-tong" clamps, 0.078 (0.073-0.086) long by 0.034 (0.031-0.038) wide, posterior rounded clamps, 0.049 (0.043-0.054) long by 0.035 (0.034-0.038) wide; "fire-tong" clamps, ventral loop apparently complete laterally, incomplete medially, dorsal loop elements short, middle loop in-complete, with 3 pairs of sclerites, 1 pair basal and 2 others making up rest of frame-work of the loop, center piece entirely in middle loop capsule, straight distally, flared proximally; rounded clamps, ventral loop incomplete medially, dorsal loop elements short, middle loop with 2 pairs of sclerites, 1 basally located, center piece in the middle loop capsule, expanded proximally. Anchors with delicate shafts and stout lunate or sickle-shaped ends, (2) 0.015 (0.014-0.017) long. Mouth terminal. Pharynx ovoid, 0.024 (0.020-0.028) long by 0.020 (0.015-0.024) wide; esophagus short. Gut bifurcated, crura ramified, rami forked, crura aparently confluent dorsal to haptor. Testis saccate, 0.105 (0.089-0.124) long by 0.046 (0.039-0.054) wide, between in-testinal crura posteriorly; vas deferens first curving to right of ovary then running sinuously in midline to genital pore. Genital pore ventral to gut bifurcation opening into armed genital atrium. Genital corona, hemispherical, muscular, 0.022 (0.020-0.026) in diameter, armed medially by 6 radially ar-ranged, curved, grooved hooks, 0.009 (0.008-0.010) long. Ovary tubular, to left of midline, bent into an inverted J-shape; oviduct running postero-medially from lateral lobe. Ootype dorsal to vitelline reservoir; uterus mid-ventral to genital atrium. Genito-intestinal canal from right crus to ootype. Pair of antero-lateral plicated placodes interpreted as ornamentation of the region of vaginal openings because vaginal pores occur in the same position in other species of the genus; openings and ducts not discernible. Mehlis' gland at base of ootype. Vitellaria follicular, extending from level posterior to vaginal region to a level dorsal to anterior haptor edge; right transverse vitelloduct (left not observed) apparently expanding to form vitelline reservoir midventrally. Egg *in utero* weakly piriform, (2) 0.103 (0.100-0.105) long by (2) 0.062 (0.061-0.063), no terminal filaments observed. Brain and excretory vesicles not seen.

DISCUSSION: *Tagia bairdiella* n. sp. differs from all other known members of the genus in the following respects: (1) details of clamp sclerites, (2) clamps of two different shapes, one a highly modified fire-tong shape, (3) vaginal placodes present, (4) testis apparently saccate and not follicular, (5) host.

T. bairdiella n. sp. and *T. micropogoni,* whose clamp modifications are most similar, are apparently closely related within the genus. This degree of relationship may reflect the relationship of their hosts which are both members of the family Sciaenidae.

Tagia cupida n. sp. (Figs. 8 to 11).

HOST: *Orthopristis chrysopterus* (Linn.), Pigfish, a bentholittoral marine haemulid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Franklin Co., Florida.

Number studied: 20; Number measured: 5.

HOLOTYPE AND PARATYPES in USNM Helm. Coll. No. 37488.

DESCRIPTION: Body elongate, somewhat cylindriform, 1.4 (1.2-1.5) long by 0.213 (0.172-0.268) wide, bluntly rounded anteriorly, widened posteriorly before joining haptor. Cuticle fairly thick and smooth. Prohaptor a pair of hemispherical buccal suckers, 0.042 (0.035-0.051) in diameter, in lateral walls of buccal funnel; several pairs of cephalic glands lateral to cirrus apparently connected by ducts to buccal suckers, probably opening into buccal funnel. Opisthaptor a cotylophore, rectangular in outline, 0.288 (0.268-0.331) long by 0.148 (0.096-0.191) wide, frontal plane of haptor forms a forty-five degree angle with the frontal plane of body; facing postero-ventrally; armed ventro-laterally by 4 pairs of clamps, anterior clamp pair reversed dorso-ventrally, *i.e.* ventral loop dorsal and middle loop ventral, rest of clamps normal in position; and a pair of anchors situated between the posterior pair of clamps. Anterior clamps significantly larger, 0.105 (0.089-0.115) long by (4) 0.054 (0.049-0.061) wide; posterior clamps smaller, 0.066 (0.055-0.077) long by (4) 0.041 (0.038-0.049) wide. Clamp skeleton difficult to study because of great modification and light sclerotization; ventral loop large, incomplete medially, dorsal loop almost nonexistant and hard to distinguish, middle loop prominent, incomplete medially, center piece entirely in wall of middle loop, expanded at both ends, edges of sclerites or cuticle of ventral and middle loops have many tooth-like serrations. Anchors, not seen clearly, probably situated on a small posterior lappet between the posterior clamps, one pair with long, delicate shafts and small lunate ends, (1) 0.015 long. Mouth terminal, oval. Pharynx ovoid, 0.047 (0.034-0.061) long by 0.037 (0.031-0.043) wide; esophagus very short. Gut bifurcated, crura ramified medially and laterally, some rami forked, posterior limits of gut not observed. Testes follicular, 7-13 in number, irregularly ovoid, between intestinal crura in posterior region of body; vas deferens a wide, sinuous running anteriorly in midline. Genital pore ventral to gut bifurcation, opening into an armed genital atrium. Genital corona hemispherical, 0.025 (0.020-0.028) in diameter, armed with a circle of 6 or 7 curved, grooved hooks, 0.009 (0.008-0.009) long. Ovary saccate, pretesticular, slightly preequatorial, looped; oviduct running posteriorly from right portion of ovary. Ootype dorsal to vitelline reservoir; uterus coursing anteriorly in midline to join genital atrium. Genito-intestinal canal not clearly seen, apparently extending from right crus to vitelline reservoir. Vaginae appearing to open dorso-laterally, often through multiple pores, at one-fourth level of body; vaginal ducts expanding immediately to form large sac-like structure, possibly seminal receptacles, remainder of vaginal ducts not visible. Mehlis' gland present. Vitellaria follicular, around intestinal crura, sparse anterior to vaginae, mostly situated between vaginae and opisthaptor; transverse vitelloducts fusing midventrally to form Y-shaped vitelline reservoir. Egg *in utero* badly distorted, may have short, stout filaments. Brain and excretory vesicles not seen.

DISCUSSION: *Tagia cupida* n. sp. is specifically different from all other members of the genus in the following respects: (1) clamp structure unusual, alligator jaw-like with tooth-like serrations, (2) vaginal pores apparently multiple, (3) host. The anchors are much like those of *Octomacrum* Mueller, 1934 and other discocotylids. This similarity is interpreted as reflecting their close phylogenetic relationship.

Tagia micropogoni Pearse, 1949 (Figs. 12 to 17).

HOST: *Micropogon undulatus* (Linn.), Atlantic Croaker, a bentho-littoral marine sciaenid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Florida.

PREVIOUSLY reported host and locality: *Micropogon undulatus* from Beaufort, N. C.

Number studied: 23; Number measured: 5.

HOMOTYPE in USNM Helm. Coll. No. 37489.

REDESCRIPTION: Body elongate, slightly fusiform, 1.6 (1.4-1.8) long by 0.208 (0.172-0.286) wide, flared slightly anteriorly, anterior end truncate, narrowed posteriorly to meet haptor. Cuticle thin and smooth. Prohaptor a pair of cylindrical buccal suckers, 0.054 (0.047-0.061) long, situated laterally in the buccal funnel; 3 or more pairs of very small head organs in rim of mouth, connected by ducts to 3 pairs of cephalic glands which are postero-lateral to genital corona. Opisthaptor a cotylophore, roughly rectangular, facing ventrally, 0.532 (0.465-0.618) long by (4) 0.129 (0.102-0.159) wide; armed ventro-laterally with 4 pairs of clamps, anterior clamp pair reversed dorso-ventrally; 2 pairs of anchors situated on a small terminal lappet. Clamps similar in structure, unequal in size, anterior clamps smallest, 0.112 (0.108-0.115) long by 0.061 (0.051-0.068) wide, posterior clamps, 0.152 (0.140-0.161) long by 0.085 (0.061-0.095) wide; clamp sclerites highly modified, appearing like those on *Pyragraphorus pyragraphorus* which resemble fire-tongs (see below); ventral loop shortest, complete, dorsal loop vestigial, middle loop longest, incomplete laterally and medially, *i.e.* in 4 pieces, 2 straight basal pieces and 2 curved terminal pieces, center piece entirely in middle loop capsule, elongate, expended at both ends. Anchors typically discocotylid. (3) 0.018 (0.015-0.022) long with long delicate shafts and stout lunate or sickle-shaped ends, situated in a row. Mouth terminal. Pharynx ovoid, (4) 0.048 (0.034-0.076) long by (4) 0.038 (0.031-0.061) wide; esophagus short, extending to over genital corona. Gut bifurcated, crura ramified, rami probably forked, crura confluent in haptor. Testis follicular, 8-24 in number, mostly oval in outline, between intestinal crura postequatorially; vas deferens sinuous, running anteriorly on left side. Genital pore ventral to gut bifurcation, opening into genital atrium. Genital corona hemispherical, 0.042 (0.036-0.050) in diameter, muscular, armed with 10-12 radially placed, curved, grooved hooks, 0.012 (0.009-0.014) long. Ovary pretesticular, saccate, looped; oviduct running medially from left lobe. Ootype slightly fusiform, dorsal to vitello-vaginal reservoir; uterus slightly to left of midline to genital pore. Genito-intestinal canal not observed, probably extending from right crus to ootype. Vaginal pores large, on both margins at one-fifth level of body, vaginal ducts apparently fusing medially to form common duct which enters vitello-vaginal reservoir anteriorly. Mehlis' gland present. Vitellaria follicular, near intestinal crura, sparse anterior to vaginae, situated mostly from level of vaginae posteriorly to haptor; transverse vitelloducts fusing in midline to form vitello-vaginal reservoir. No eggs were observed in the present collection. Egg *in utero*, from one of Pearse's specimens, spherical, about 0.109 long by 0.035 wide, with at least one terminal filament. No brain or excretory vesicles seen.

DISCUSSION: Pearse (1949) described this species from *Micropogon undulatus* taken at Beaufort, N. C. The conspecificity of the forms in Pearse's collection and these in the present collection was established by a study of the USNM Helm. Coll. slide No. 36961, the types and paratypes. The above redescription is given because the original figures and description were incomplete.

Tagia micropogoni Pearse, 1949 is apparently most closely related to *T. bairdiella* n. sp. but differs from all other species of the genus in the follow-

ing characters: (1) details of the clamp sclerites, (2) shape of clamps fire-tong-like but less highly modified than others, (3) all clamps on haptor similar in shape, (4) broad vaginal pores and ducts, (5) host.

GENUS *Bicotylophora* Price, 1936. *Bicotylophora trachinoti* (MacCallum, 1921) Price, 1936.

SYNONYMS: *Dactylocotyle trachinoti* MacCallum, 1921 *D. trachynoti* MacCallum, 1921, a misspelling, and *D. trachinoti* MacCallum, 1921 of Gallien (1937).

HOST: *Trachinotus carolinus* (Linn.), Common Pompano, a nerito-pelagic marine carangid.

LOCATION: Gills.

LOCALITY: Alligator Harbor, Florida.

Previously reported hosts and localities: *Trachinotus carolinus* and *Roccus saxatilis* (= *R. lineatus*) from the New York Aquarium (see below).

Number studied: 73.

DISCUSSION: This species needs redescription since it has never been adequately described or figured. Though there are many specimens in the present collection this task must be left until later because of a lack of time.

The taxonomic affinities of this genus are not clear. Though it is probably basically discocotylid, its clamps are more microcotylid in structure, and may later be shown to be intermediate in nature to these groups.

As in many other multiple host records reported by MacCallum for worms collected from the New York Aquarium, it is highly probable that *Roccus saxatilis* is an "unnatural" host for *B. trachinoti* and that *Trachinotus carolinus* is the natural host.

SUMMARY AND CONCLUSIONS

In this, the eighth installment of the present series, certain aspects of the systematics of the superfamily Diclidophoroidea Price, 1936 are considered. As a result of the transferral of the family Vallisiinae Price, 1943 to the family Gastrocotylidae Price, 1943 the diagnosis of the family Discocotylidae Price, 1936 is emended. The discocotylid, *Hemitagia galapagensis* (Meserve, 1938) Sproston, 1946 is partially redescribed for taxonomic reasons.

The genus *Tagia* Sproston, 1946, subfamily Anthocotylinae Price, 1936, is redefined. *Tagia equadori* (Meserve, 1938) Sproston, 1946, *T. micropogoni* Pearse, 1949, *T. bairdiella* n. sp. and *T. cupida* n. sp. are described and discussed, and *T. otolithis* (Yamaguti, 1953) n. comb. is imported from the genus *Kuhnia* to which it was originally assigned. Another anthocotylinid, *Bicotylophora trachinoti* (MacCallum, 1921) Price, 1936, is given as a new locality report.

Part IX will continue treatment of the superfamily Diclodophoroidea with the presentation of data concerning the family Diclidophoridae Fuhrmann, 1928, *sensu* Price, 1943.

LITERATURE CITED

GALLIEN, L. 1937. Recherches sur quelques trematodes m monogeses nouveaux ou peu connus. Ann. Parasitol Humaine et Comp. 15:146-154.

MESERVE, F. G. 1938. Some monogenetic trematodes from the Galapagos Islands and the neighboring Pacific. Reports of the Allen Hancock Pacific Expeditions. 2:31-89.

PALOMBI, A. 1949. I trematodi d'Italia. Parte I. Trematodi monogenetici. Archivo Zoologico Italiano. 34:203-408.

PEARSE, A. S. 1949. Observations on flatworms and nemerteans collected at Beaufort, N. C. Proc., U. S. Nat'l. Mus. 100:25-38.

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

YAMAGUTI, S. 1953. Parasitic worms mainly from Celebes. Part 2. Monogenetic trematodes of fishes. Acta Medicinae Okayama. 8:203-256.

Nematode Parasites and Associates of the Engelmann Spruce Beetle (*Dendroctonus engelmanni* Hopk.)

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The Engelmann spruce beetle (*Dendroctonus engelmanni* Hopk.) is one of the most serious pests of spruce forests in western United States. During the period 1940 to 1954 spruce, amounting to approximately 8 billion board-feet of lumber, were killed by this insect in outbreaks that occurred in Colorado and in northern Idaho and Montana.

Studies on the biology and control of the beetle were started in 1944. Artificial control was found to be expensive. As a consequence, work was started in 1950 to determine the feasibility of using biological control. Observations had indicated that the beetles and their galleries were heavily infested with nematodes.

Little was known of the effect of nematodes on the ecology of the insect. The study of nematode parasites and associates of bark beetles is comparatively new in the field of forest entomology. A few papers have been published in recent years on the relationship of the animals to bark beetle infestations. Steiner, 1932, described three species of nematodes taken from lodgepole pine infested with the mountain pine beetle, *Dendroctonus monticolae* Hopk. The paper is taxonomic in nature with a few remarks on probable habits of the nematodes described. Thorne, 1935, described nine new species taken from the mountain pine beetle and its galleries. Observations on the habits of some of the species are recorded in the paper. The nematodes in both papers were collected in Utah.

As the initial objective of the study was to determine the various species of nemas parasitic on and associated with the spruce beetle, collections were made in representative areas of the infestations. Collections were made first in Colorado and later in northwestern Montana. The associated nemas were washed from beetle-infested galleries. The internal parasites were obtained by dissecting live adult beetles, pupae, and larvae. The relative abundance of the nematode endoparasites was determined by examining 25 beetles from each of 25 trees in local areas of infestation.

NEMATODE ENDOPARASITES OF THE ENGELMANN SPRUCE BEETLE

Four endoparasitic species were taken from the collections of beetles. Three were taken from the body cavities of various stages of the beetle. The other occurred in the gut. One species, belonging to the genus *Sphaerularia*, was collected from adult beetles. *Aphelenchulus reversus* Thorne and a species belonging to the genus *Ektaphelenchus* were taken from adults, pupae, and larvae. Larvae of *Rhabditis obtusa* Fuchs were taken from the gut of larvae, pupae, and adults of the insect. Descriptions of the four endoparasites follow.

Sphaerularia dendroctoni new species

EGGS.—Deposited after segmentation, size 40u x 80u. Eggs develop in uterus outside body wall, hatching occurs immediately after deposition.

FIRST-STAGE LARVAE.—Length, 0.48 mm. to 0.67 mm.; width 20u to 25u;

[1]The writer wishes to express appreciation to Mr. Gerald Thorne whose aid made the study possible and for his review of the manuscript. Appreciation is also expressed to Mr. W. F. McCambridge for his collection of *Dendroctonus borealis* Hopk. for examination and to Mr. F. B. Knight who participated in some of the laboratory studies.

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cuticle very finely annulated; tail conoid, rounded at the terminus; spear moderately fine with basal knobs.

SECOND-STAGE LARVAE.—Similar in appearance to first-stage larvae. Length 0.64 mm. to 0.70 mm.; width 20u to 25u; genital primordia becomes apparent in this stage. Female larvae can be determined by the enlargement of the vagina (Figs. 1 G and H).

MALE.—Length 0.70 mm. to 0.78 mm.; width 20u; a = 43.3, b = 6, c = 48.7, and t = 75;[1] cuticle very finely annulated; lip region flat to slightly rounded, set off by a very slight constriction, or rather a narrowing of the neck region, a little wider than high; spear moderately fine with basal knobs, slightly longer than the width of the lip region; excretory pore slightly posterior to the nerve ring and located approximately 1/15 of the body length from the anterior end; hemizonid adjacent to excretory pore; esophagus, without a median bulb; constricted as it passes through nerve ring, ending in a cylindroid basal bulb which slightly overlaps the intestine on the dorsal side. Bulb with 3 prominent nuclei generally visible; testes outstretched or reflexed, at times almost reaching the excretory pore, the lower third distended with spermatatheca; spicula curved, ¾ as long as the tail; gubernaculum thin, trough-like, almost straight, about ¼ as long as the spicula; tail conical with a small, rounded terminus; bursa enveloping the tail extending a short distance anterior to the anus (Fig. 1 E and F).

IMMATURE FEMALES.—Length 0.80 mm.; width 32u; cuticle finely annulated in younger specimens, becoming strongly wrinkled with age, wrinkles very deep giving the appearance of segmentation, the wrinkles occur at variable regions on the cuticle; lip region similar to that of the male; ovary reflexed nearly half its length in some individuals; vulva narrow, transverse slit becoming greatly distended as the genital organs descend within the body cavity. Protrusion of the vagina takes place as in Fig. 1B; tail conical with small, rounded terminus.

MATURE FEMALES.—Vagina in this stage is the most prominent part of the individual (Fig. 1 A). As it protrudes from the body wall the cells enlarge to tremendous size, each with a prominent nucleus. The size of the vagina may reach a length of 1.6 mm. and a width of 0.25 mm. The growth is outside the body wall. Cells of the vaginal wall, while more or less globular in juvenile females, become elongate with maturity, giving the vaginal sac a smooth appearance. The size of the female changes but little. However, as the reproductive system extrudes the cuticle of the female becomes wrinkled. The spear becomes obscure and nonfunctional, anal opening disappears (Fig. 1 C and D).

As the vagina enlarges and protrudes from the body of the female, it evidently turns inside out, carrying the ovary and the uterus with it, so that the uterus remains attached to the anterior end of the extruded vagina. The anterior end of the ovary floats more or less freely in the lumen of the vagina adjacent to the posterior end of the female. The uterus remains attached to the anterior end of the vagina and the eggs are deposited through a small opening at that end.

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[1] a = Total length of nema / Greatest width of body
b = Total length / Length of esophagus
c = Total length / Length of tail
v = Percentae of length from anterior end to where vulva is located.
t = Percentage of body occupied by testes.

TYPE SPECIMENS.—Catalog numbers 18E-Z & Y, Allotype 17, collection of Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Fort Collins, Colorado.

TYPE HOST—*Dendroctonus engelmanni* Hopk.

TYPE LOCALITY—Red Table Mountain, Eagle County, Colorado.

DIAGNOSIS—Sphaerularia with protruding vagina. *Sphaerularia dendroctoni* differs from *S. bombi* Leuckart in its much smaller size, the closer spacing of the cells of the protruded vagina, and in the more conically-shaped tail.

LIFE HISTORY NOTES.—Several thousand adults, pupae, and larvae of the Engelmann spruce beetle have been examined for nematode parasites of this species. Only adults have been found infested. Information as to the mode of infection is based on observation, it may be either through the cuticle of the abdomen or by ingestion. Both male and female nemas are parasitic. As many as 50 juvenile females have been taken from the body cavity of an adult beetle. Many hundreds of larvae and eggs are present in the fluids of the body cavity of infested insects. The males are present in all infested beetles, and under the lower powers of the microscope have much the same appearance of the second-stage female.

EFFECT ON HOST.—*Sphaerularia dendroctoni* is a true parasite; it does not kill its host. The egg production of female beetles infested with the species is sharply reduced. Laboratory studies in which individual pairs of beetles were allowed to attack spruce bolts showed that in some cases egg production was prevented. The average number of eggs produced by infested females was 28.8. The average number produced by uninfested females was 76.5. Males infested with the species act as carriers of the nemas and aid in their dissemination.

To determine a trend in the status of the parasite from year to year, beetles were collected and examined in 1952 and 1953. The beetles were taken from the same areas, Red Table Mountain on the White River National Forest and Rabbit Ears Pass on the Routt National Forest. Each collection consisted of 25 beetles from each of 25 trees. The results of the examination are listed in Table 1.

TABLE I.—Percentage of Engelmann spruce beetles infected with *Sphaerularia dendroctoni* in 1952 and 1953 from two areas in Colorado.

	Percentage Infested	
Year	Red Table Mountain	Rabbit Ears Pass
1952	10.7	1.0
1953	35.4	5.6

Males and females were equally infested. *S. dendroctoni* infestation of beetles in an individual tree ran as high as 76 percent on Red Table Mountain. On Rabbit Ears Pass the highest percentage in an individual tree was 12. As the table shows, the number of beetles infested with the nematode increased tremendously on Red Table Mountain and was beginning to increase on Rabbit Ears Pass.

Beetles also were collected and examined for *Sphaerularia dendroctoni* in three relatively new infestations during the summer of 1953. Collections were made on the Lolo and Kootenai National Forests in northern Montana and on the Uncompahgre National Forest in Colorado. The percentages of beetles infested with the nema on these forests were: 11.2 on the Lolo National Forest, 0.8 on the Kootenai National Forest, and 4.2 on the Uncompahgre National Forest.

DISTRIBUTION OF *Sphaerularia dendroctoni*.—The species probably occurs

wherever *Dendroctonus* infests spruce in western North America. It has been collected from *Dendroctonus engelmanni* Hopk. from southern Colorado to northern Montana and from *D. borealis Hopk.* near Anchorage, Alaska. This species has not been observed in pine-attacking *Dendroctonus.*

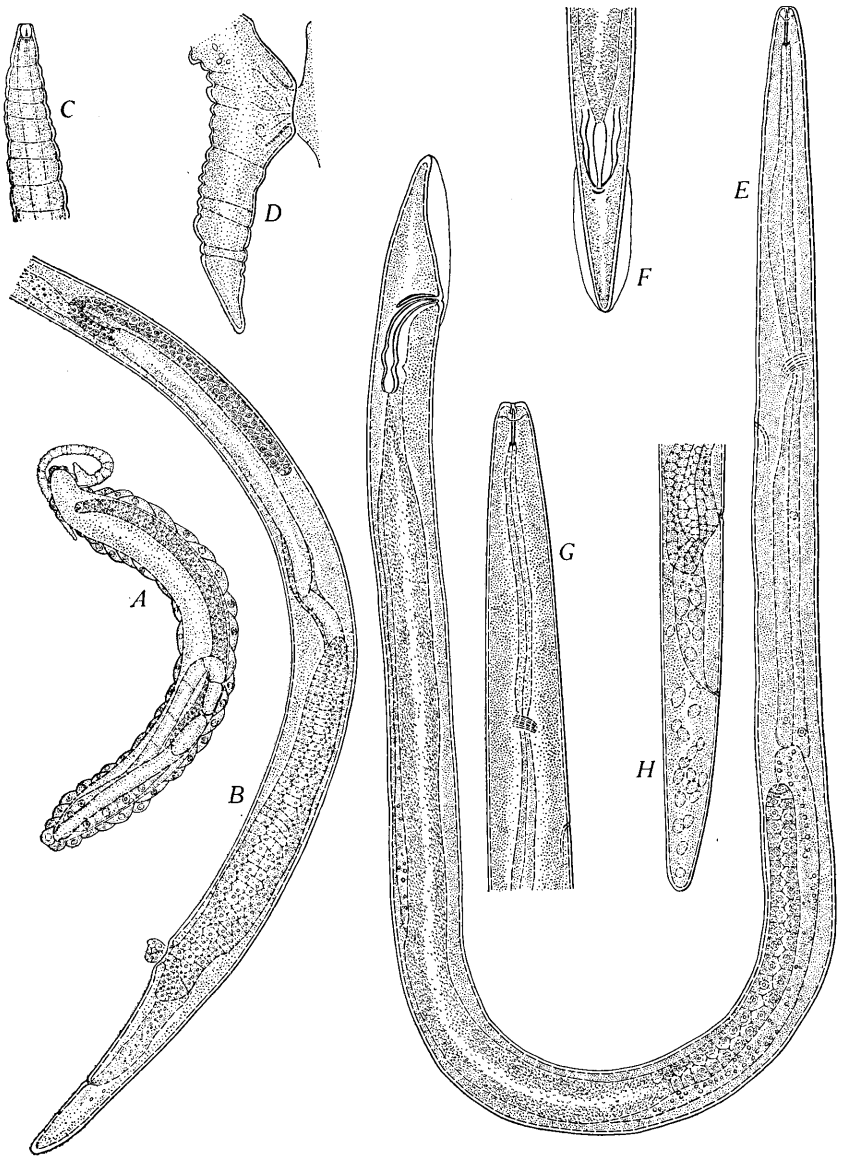

Fig. 1. *Sphaerularia dendroctoni* n. sp. A, Mature female showing evaginated vagina x 54; B, Posterior portion of immature female x 425; C, Head of mature female x 425; D, Tail of mature female x 425; E, Male x 425; F, Ventral view of male tail x 425; G, Head, second-stage larva x 425; H, Tail of second-stage larvae showing enlargement of vagina x 425.

Aphelenchulus reversus Thorne

The following description is taken from Thorne 1935:

"EGGS.—Deposited before segmentation. Size variable, 30μ by 60μ to 42μ by 90μ. Several hundred deposited by each female in the body cavity of the grub or adult beetle. Segmentation and hatching occur immediately after deposition.

"NEWLY HATCHED LARVAE.—Length 0.22 to 0.30 mm; width 12μ to 16μ. Cuticle finely striated. Lip region rounded and expanded. Tail conoid to the small blunt terminus. Spear exceedingly slender, without basal knobs. Esophagus a slender tube, narrowing as it passes through the nerve ring, then gradually expanding and merging with intestine. Excretory pore a little posterior to nerve ring. . .

"SECOND-STAGE LARVAE.—Similar in appearance to the young larvae except for the uniformly tapering anterior end and the developing gonads. Genital primordium visible at beginning of first molt. From it the single ovary develops forward until it is about half as long as body, its terminus reflexed a distance equal to 3 to 5 body widths. A prominent gland usually is visible just back of the nerve ring. During this stage little or no increase in body length but marked development in width. . . .

"FEMALES FROM GRUBS AND ADULT BEETLES.—Length 1.0 to 1.8 mm; width 50μ to 180μ. Vulva 94 to 96 percent. Body bent dorsally, more or less cylindrical throughout greater part of its length but tapering conspicuously at the very narrow lip region, which is not set off in any manner. Cuticle annulated near the head and at the terminus; on some specimens annules conspicuous, on others almost invisible. Body constricted at vulva, especially ventrally. Tail broad, bearing dorsal, hornlike, annulated terminal projection which actually is the upturned original tail of the immature nema. . . . The four labial papillae almost invisible even from a face view. The amphids lie close to oral opening. Four large glands are prominent feature of head region. Spear 12μ to 14μ long, slender, with short ventrally located aperture. Knobs of the spear vary from obscure to distinct. Lumen of esophagus can be traced only a short distance from the spear. A series of 15 to 18 pairs of conspicuous lateral structures distributed throughout the body. Vulva a broad transverse slit. Three glands lie opposite vulva, causing construction of the organs. Anus and rectum absent. Ovary extending forward about three-fourths the length of body, then reflexed a distance equal to 1 to 2 body widths. Oviparous. . . ." (Figs. 2, A, B, and C).

Thorne did not find the male of this species. Only recently have males been found, they were taken from the galleries of both the Engelmann spruce beetle and the black turpentine beetle, *Dendroctonus terebrans* Oliv.

The description of the male follows. Length, 0.47-0.53 mm.; a = 33, b = ?, c = 23, head as in Figure 3,A; lip region rounded; cuticle finely annulated, finely striated; spear very slender, knobs for the most part obscure. Esophagus a straight tube without bulb, narrowing as it goes through nerve ring which is very prominent. Excretory port slightly posterior to nerve ring. Testes outstretched almost reaching the excretory pore, at times reflexed for a very short distance, vas deferens distended with spermatozoa; spicula curved, 2/3 as long as the tail; gubernaculum thin, troughlike, almost straight. Tail with sharp mucronate tip curved as in Figure 3B, bursa enveloping the tail and extending forward to a point slightly anterior to the spicula.

TYPE SPECIMENS.—Allotype 7W,X,Y. Collection of Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Fort Collins, Colorado.

TYPE LOCALITY.—Uncompahgre National Forest, Dolores County, Colorado.

TYPE HOST.—*Dendroctonus engelmanni* Hopk.

The mature females generally burst when removed from host unless removed in a physiological salt solution.

LIFE HISTORY NOTES.—Mature females and larvae of this species have been taken from all stages of the Engelmann spruce beetle except the egg.

Eggs are laid in the body cavity of the host and hatch immediately after deposition. The infective stage of the species is evidently the first larval stage. Hundreds of nema larvae are produced in the body cavities of the various stages. The nema larvae penetrate through the wall of the gut and are passed out with the excrement. Infection of other individuals is accomplished either through the cuticle of the abdomen or through the oral cavity of the feeding beetle larvae and adults.

Males of the species have been taken from the frass of beetle galleries containing infested beetles. None has been recovered from the body cavity of the insect.

There evidently is a very high mortality of the larvae from the time they are hatched until they gain entrance to another insect. Hundreds of larvae are produced in the body cavity of a single beetle; only a few grow to maturity. A maximum of 29 juvenile and mature females was taken from the body cavity of an adult beetle. The normal is 2 to 3.

The adult nemas probably remain in the body as the beetle transforms from larva to pupa, and from pupa to adult. Adult beetles may be infested in a similar manner.

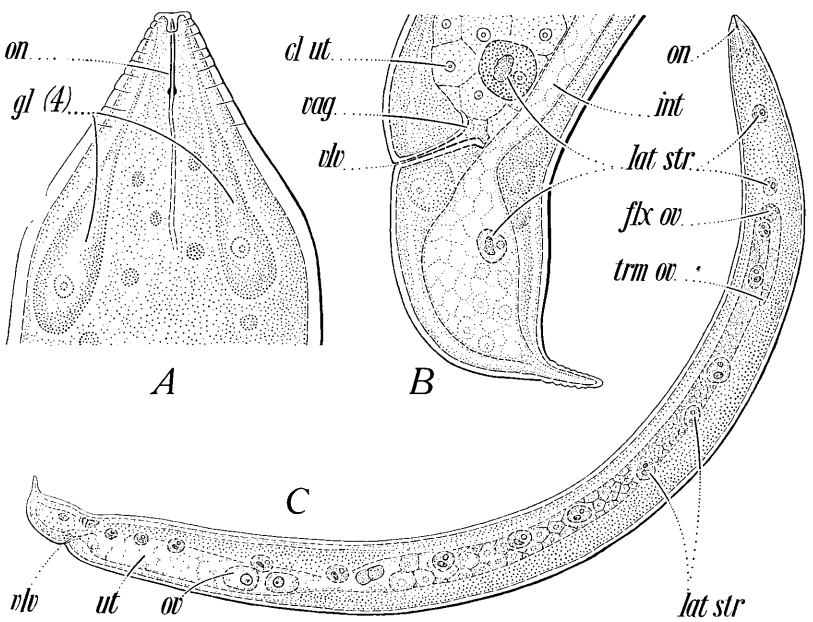

Fig. 2. *Aphelenchulus reversus* (female). A, Anterior end: *on*, Spear; *gl* (*4*), 2 or 4 glands near head x 800; B, Posterior end: *cl ut*, Cell of uterus; *vag*, vagina; *lat str*, lateral structure; *int*, intestine; *vlv*, vulva x 400; C, Female: *on*, Spear; *lat str*, lateral structure; *flx ov*, flexure of ovary; *trm ov*, terminus of ovary; *ov*, egg; *ut*, uterus; *vlv*, vulva, x 100 (from Thorne 1935).

EFFECT ON HOST.—*Aphelenchulus reversus* is a true parasite. The egg production by spruce beetle females infested with this nema is greatly reduced. The average number of eggs produced by infested females was found to be 35. The average number of eggs produced by uninfested females was 63. Infested larvae and adults, both male and female, spread the nematodes.

To determine the number of beetle larvae infested in broods where either the males or females, or both, were infested with *Aphelenchulus reversus* Thorne, larvae from 10 pairs of parent beetles were examined. A total of 90 larvae of all sizes was dissected; 20 (22 percent) were infested. There was a tendency for more of the larger larvae to be infested; few of those less than one-half grown contained the nemas.

The status of this parasite was determined for 1952 and 1953. Beetles examined for *Sphaerularia dendroctoni* were also examined for *Aphelenchulus reversus*. The results of this phase of the study are listed in Table 2.

TABLE II.—Percentage of Engelmann Spruce Beetles Infested with *Aphelenchulus reversus* in 1952 and 1953 in Two Areas in Colorado

	Percentage Infested	
Year	Red Table Mountain	Rabbit Ears Pass
1952	8.6	18.5
1953	18.4	23.6

Both males and females were equally infested. The maximum percentage of beetles infested in an individual tree was 36.

Beetles collected during 1953 from areas in southwestern Colorado and in northern Montana were infested as follows: Uncompahgre National Forest, 26 percent; Kootenai National Forest, 12.2 percent; and Lolo National Forest, 16.4 percent.

Two percent of the beetles examined from all areas were infested with both *Sphaerularia dendroctoni* and *Aphelenchulus reversus*. The percentage infestation by both species by area was as follows: Kootenai, 0 percent; Uncompahgre, 1.0 percent; Lolo, 1.2 percent; Rabbit Ears Pass, 0.8 percent; Red Table Mountain, 5.7 percent.

DISTRIBUTION AND HOSTS.—*Aphelenchulus reversus* is evidently a common parasite of the genus *Dendroctonus* and probably occurs in all species of the genus throughout North America. It has been collected from *D. borealis*, *D. ponderosae* Hopk., *D. monticolae* Hopk., *D. engelmanni*, and *D. terebrans* Oliv. In addition, it has been recovered from the body cavity of *Ips pilifrons* Sw. and *I borealis* Sw.

Ektaphelenchus obtusus new species

Female = 0.8 mm.; a = 30, b = 8, c = ?, v = *41*/78

Male = 0.7 mm.; a = 23, b = 7, c = ?, t = 34

Cuticle with moderately fine annulations; lip region flattened, definitely set off (Fig. 3E); face view reveals four prominent labial papillae and the amphids which are similar in size and shape to the papillae; spear moderately slender, three times as long as the width of lip region, without basal knobs; esophageal bulb ovate, elongate, approximately twice as long as wide; esophageal glands very prominent, elongate, extending dorsally several body widths along anterior end of intestine; nerve ring one bulb length behind bulb; ovary outstretched, posterior uterine branch very short; anus and rectum not observed; tail length undetermined; lumen of intestine broad and conspicuous throughout its length; tail convex conoid, almost blunt (Fig. 3C); male similar

in shape and conformation to that of female; testes short, not reflexed; spicula mitten-shaped; three pairs of prominent caudal papillae, one air preanal, two pairs postanal (Fig. 3D).

DIAGNOSIS: *Ektaphelenchus* with prominent esophageal bulb and glands; differs from *Ektaphelenchus hylastophilus cunicularii* Fuchs in that the spear is without basal knobs; in the more obtuse terminus and in the absence of a conspicuous anal opening. It differs from *E. ateri* Fuchs in the size of the spear and the absence of knobs on the spear. It is distinguished from *E. typographi* Fuchs, particularly by its larger size and the location of the vulva which is more anterior than that of *typographi.* Male with a mitten-shaped spicula and three pairs of caudal papillae.

TYPE SPECIMENS.—Catalog numbers 8D and 11F; allotype 21B and 22. Collection of the Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Fort Collins, Colorado.

TYPE HOST.—*Dendroctonus engelmanni.*

TYPE LOCALITY.—Red Table Mountain, Eagle County, Colorado.

The female of this species has been collected from the body cavity of all stages of the Engelmann spruce beetle. It also has been collected from the external surfaces of all stages of the insect and from its galleries. The male has been taken only from the galleries. Nothing is known of the life history of this parasite.

EFFECT ON HOST.—Its effect on the beetle is not known. As many as 10

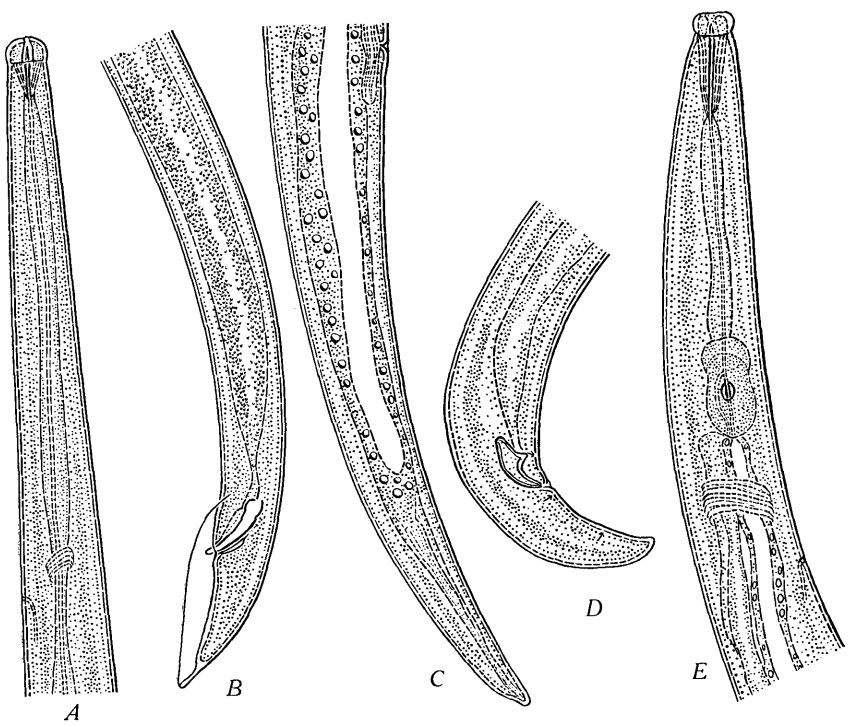

Fig. 3 A, B, *Aphelenchulus reversus* male. A, Anterior end x 725; B, Posterior portion x 725; C-E, *Ektaphelenchus obtusus.* C, Posterior portion of female x 525; D, Male tail x 525; E, Anterior portion of female x 525.

adult females have been taken from the body cavity of an adult beetle. Only the adult nemas have been found infesting the body cavity.

DISTRIBUTION AND HOSTS.—*Ektaphelenchus obtusus* has been taken from the galleries and from the body cavities of the Engelmann spruce beetle in Colorado, northern Idaho, and in the Kootenai and Lolo National Forests in northern Montana. It also has been collected from *Dedroctonus pseudotsugae* Hopk. in the vicinity of Weed, New Mexico, and from *D. borealis* at Anchorage, Alaska.

Rhabditis obtusa Fuchs 1915

FEMALE = 0.8-1.1 mm.; a= 25, b = 5, c = 5.5, v = *64*/95

MALE = 0.6-0.8 mm.; a = 30, b = 4.5, c= 2.3, t = 60

The following description is from Thorne 1935:

"Bodies of both sexes almost cylindrical between esophagus and genital opening. Neck tapering uniformly to lip region, which is about one-third as wide as base of neck. Female tail short, bluntly conoid. Vulva exceedingly far back. Striae about lu wide at mid body, slightly wider near head. Lip region almost continuous with neck contour. Six conical, forward-pointing, labial papillae were visible but other details of head were always obscured by clinging debris. Amphids minute. Pharynx about three times as deep as wide. Cheilorhabdions and protohabdions slightly convex. Telostom absent. Esophagus: Corpus cylindrical; medial bulb slightly wider than corpus; isthmus same length as corpus and half as wide; terminal bulb ovate, two-thirds as wide as neck. Nerve ring midway of isthmus. Excretory pore slightly posterior to nerve ring. Female prodelphic. Vulva elevated. Vagina extending almost straight forward. Uterus one-third as long as body. Ovary extending forward from uterus, then reflexed until the blind end reaches one-half to three-fourths the distance back to the vulva. Posterior uterine branch absent. Testis single, extending nearly to esophagus, then reflexed a short distance. Spicula and gubernaculum as shown in figure 9, C (Thorne 1935). Bursa enveloping the tail, with 2 pairs of preanal ribs, then 3 pairs grouped close together just posterior to anus, followed by 4, rarely 3 or 5, pairs; general bursal formula being 2()3, 1, 1, 2."

There is considerable tail variation in the females of this species. Figure 4 shows the tail variations of the species collected during the study. Other characters remain constant.

LIFE HISTORY NOTES.—The larvae of this species occur in the gut of all stages of the Engelmann spruce beetle.

Free living forms of the nema are found in the egg and larval galleries of the insect. The infective stage is probably the egg or first larval stage. The eggs or larva are evidently taken into the gut by ingestion. Here they hatch, or molt once or twice, and are passed out into the galleries with the excrement. Maturity is attained in the insect galleries. The larval forms from the gut of the beetle are easily reared to maturity on malt agar.

EFFECT ON HOSTS.—The nema has little or no effect on its host.

DISTRIBUTION AND HOSTS.—*Rhabditis obtusa* has been taken from all collections of Engelmann spruce beetle. In addition, it has been collected from the gut of *Dendroctonus ponderosae, D. monticolae, D. pseudotsugae, D. borealis, Ips pilifrons,* and *I. borealis.* It is probably cosmopolitan in distribution.

NEMATODES ECTOPARASITES OF THE ENGELMANN SPRUCE BEETLE

Diplogaster pinicola Thorne was the only external parasite of the beetle collected.

Diplogaster pinicola Thorne 1935

FEMALE = 1.0 mm.; a = 25, b = 7, c = 15, v = *20*/51/*22*

MALE = 1.0 mm.; a = 30, b = 6.2, c = 15, t = 52

The following description is in part from Thorne 1935:

Cuticle finely annulated, finely striated; body moderately slender, tapering gradually toward the anterior end; pharynx bearing six visible teeth; isthmus with terminal bulb slightly longer than corpus with median bulb; nerve ring midway of the isthmus; excretory pore slightly posterior to the nerve ring; vulva with protuberant lips; female tail convex, conoid with an acute terminus; male tail curved with a spicate terminus; spicula arcuate; gubernaculum with a trough-like distal extension in which the spicula glides; testes reflexed, single; 8 pair of caudal papillae.

The larvae of this species occur in large cottony masses beneath the wing covers of adult beetles. They also occur in the folds of the abdomen of the adults and larvae. The nema larvae are readily reared to maturity on malt agar.

DISTRIBUTION AND HOSTS.—This nema has been collected from *Dendroctonus engelmanni, D. ponderosae, D. monticolae, D. borealis, D. pseudotsugae, D. brevicomis* Lec., *Ips pilifrons,* and *I. borealis.* It probably occurs throughout western United States, Canada, and Alaska.

NEMATODE ASSOCIATES OF THE ENGELMANN SPRUCE BEETLE

Following is a list of the nematodes taken from the egg and larval galleries of *Dendroctonus engelmanni* Hopk.:

Ditylenchu pinophila Thorne

Ditylenchus sp.

Laimaphelenchus brachycephalus Thorne

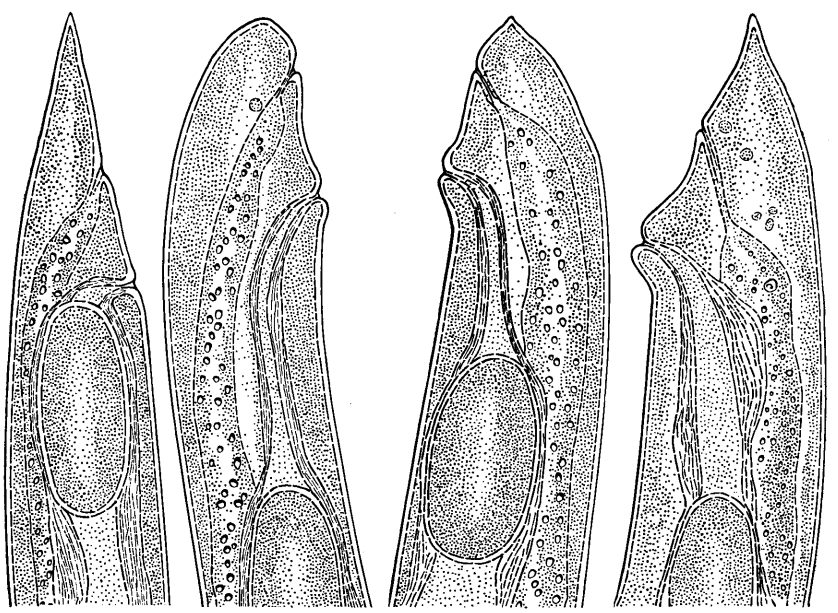

Fig. 4. *Rhabditis obtusa,* female tail variations x 475.

Bursaphelenchus talonus Thorne
Bursaphelenchus sp.
Ektaphelenchus obtusus n. sp.
Aphelenchulus reversus Thorne (males)
Aphlenchoides tenuidens Thorne
Aphlenchoides sp. Several undetermined sp. collected from egg galleries
Diplogaster pinicola Thorne
Diplogaster sp.
Rhabditis obtusa Fuchs
Panagrodontus dentatus Thorne

It is interesting to note that the nematode fauna of spruce infested with *Dendroctonus engelmanni* is fairly constant throughout the range of the insect.

SUMMARY

Four internal nematode parasites were collected from the Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk.; namely *Sphaerularia dendroctoni* n. sp., *Aphelenchulus reversus* Thorne, *Ektaphelenchus obtusus* n. sp., and *Rhabditis obtusa* Fuchs. The three former species are parasite in the body cavity; the last-named species occurs in the gut.

Studies carried on during 1952 and 1953 revealed that the egg-laying capacity of adult female beetles was reduced sharply by infestations of *Sphaerularia dendroctoni* n. sp. and *Aphelenchulus reversus* Thorne. The effect of *Ektaphelenchus obtusus* n. sp. on its host is not known.

In the areas studied, parasitism by *Sphaerularia dendroctoni* n. sp. and *Aphelenchulus reversus* Thorne increased considerably from 1952 to 1953.

LITERATURE CITED

STEINER, G. 1932. Some nemic parasites and associates of the mountain pine beetle, *Dendroctonus monticolae*. Jour. of Agr. Research 45: 437-444.

THORNE, GERALD. 1935. Nemic parasites and associates of the mountain pine beetle, *Dendroctonus monticolae*, in Utah. Jour. of Agr. Research 51: 131-144.

Some Experiments on the Effect of Low Level Phenothiazine on the Development of *Ostertagia ostertagi* Larvae in Bovine Feces

JAMES F. LANDRAM AND GEORGE E. CAUTHEN*

Larval development of hookworm was found to be inhibited by the addition of 1½ grams of phenothiazine daily in the grain concentrate by Mayhew (1948). It was reported by Mayhew (1949) that 1½ grams given daily for six days stopped egg production in nodular worm. Mayhew (1950) found that 0.4 gram of phenothiazine daily for 7 days caused abnormal eggs to be produced by nodular worm and that 0.5 gram fed for 14 days brought about stoppage of egg production. Cauthen (1951) showed that 1 gram daily prevented development of 99% of the ova in feces of a calf with a mixed infection. Mayhew (1952) demonstrated that 1½ grams given daily to calves infected with *Haemonchus contortus* resulted in abnormal eggs withn 48 hours and that egg production ceased in 5 to 14 days.

In the course of experiments in progress at the Texas Agricultural Station, Angleton, using pure infections of *Ostertagia ostertagi,* an opportunity

*From the Texas Agricultural Experiment Station, sub-station No. 3 Angleton. Technical article No. 2244. The investigation reported here is in connection with a project of the Texas Agricultural Experiment Station, and is published by the permission of the director.

was afforded to test the effect of feeding low amounts of phenothiazine on the development of the free living stages of *O. ostertagi.*

Two calves were used in the tests. The phenothiazine was administered in a commercial grain ration. Fecal samples were taken from the rectum in the forenoon. Egg-per-gram (EPG) counts were made using a modified Stoll flotation technique. Development was determined by two methods: 1) calculating percentage of hatchability of ova that had been recovered by flotation and cultured in petri dishes containing a small amount of water; 2) by spreading 3 grams of feces on cheesecloth and incubating for 6 days; larvae being recovered by use of a Baermann apparatus and percentage calculated from EPG count. In calf #19 only the latter method was used.

Calf #1 was 5½ months old and weighed approximately 150 pounds. The EPG at the beginning of the experiment was between 70 and 100. Larval development in fecal samples taken the day preceding and on the day of administration of phenothiazine was 95.5% and 96.7% as determined by observation of the ova in water cultures, and 51.7% and 10.1% as determined by fecal cultures. One gram of phenothiazine was given daily for 3 days at which time dosage level was lowered to ½ gram daily for 4 days. Within 24 hours after the first feeding of phenothiazine the larval development was 0.0% as determined by both culture methods and remained so for the next 5 days. Larval development, as determined by observation of the ova in water cultures, was 88.4% from samples taken 48 hours after phenothiazine was stopped. No fecal cultures were made on this day.

Calf #19 was 7½ months old and weighed approximately 200 pounds with an EPG count between 175 and 200 at the beginning of the experiment. Six days prior to the first administration of phenothiazine, larval development was 78.5%. The sample taken on the morning of the first feeding showed larval development of 27.0%. This animal was given 0.5 gram of phenothiazine daily for 4 days. Larval development dropped to 4.9% within 24 hours after the first feeding, 0.1% in 48 hours, 0.0% in 72 hours, and 0.0% in 96 hours. Within 48 hours after cessation of phenothiazine, larval development was 43.9%. Development from samples taken the next two days was 37.6% and 48.1%.

These experiments show that daily administration of 0.5 g. and 1.0 g. of phenothiazine to a calf 5½ months old and a calf 7½ months old effectively prevented the development of the free living stages of *O. ostertagi* to infective larvae. Effects of the drug on larval development can be seen within 24 hours after administration. Within 48 hours after the administration is stopped, the larval development is normal.

LITERATURE CITED

CAUTHEN, G. E. 1951. The effect of small amounts of phenothiazine on the development of the ova of the gastrointestinal nematode parasites of cattle. Jour. Parasit. 37 (5 part 2): 14.

MAYHEW, R. L. 1948. Phenothiazine as a larvicide against the hookworm of cattle. Ibid., 34 (6 part 2): 14.

————. 1949. The results of feeding small amounts of phenothiazine in pure infections of the nodular worm (*Oesophagostomum radiatum*) in the calf. Ibid. 35 (6 part 2): 12.

————. 1950. Results of additional experiments in which small amounts of phenothiazine was fed in pure infections of the nodular worm in calves. Ibid. 36 (6 part 2): 35.

————. 1952. Results of feeding small amounts of phenothiazine in pure infections of the large stomach worm *Haemonchus contortus* in the calf. Ibid. 38 (4 part 2): 30-31.

A New Tapeworm, *Mesocestoides carnivoricolus*, from Carnivores of the Great Salt Lake Desert Region of Utah

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During a survey of the helminths of animals from the Great Salt Lake Desert region of western Utah, an apparently new tapeworm belonging to the genus Mesocestoides was recovered from the badger, *Taxidea taxus taxus* (Schreber). Subsequently, specimens belonging to the same species were recovered from the coyote, *Canis latrans* Say, and the bobcat, *Lynx rufus pallescens* Merriam, indicating that the species is present in the common carnivores of the area. The type material from the badger consisted of 42 worms, all of which appeared to be of the same approximate age and probably due to ingesting a single host containing tetrathyridia. Subsequent lots of material included 67 worms from one specimen of bobcat and 9 from a coyote.

Tetrathyridia, which are assumed to be the second larval stage of this species were recovered from a number of specimens of *Peromyscus maniculatus sonoriensis* (Le Conte), the deer mouse, and *P. crinitus pergracilis* Goldman, the canyon mouse, during the summers of 1953 and 1954. The infection in *Peromyscus* seems to be widespread as 12 *P. maniculatus* and 3 *P. crinitus* were taken from varied localities in Tooele County, Utah, containing medium to large numbers of tetrathyridia in the liver, lungs and body cavity. The first larval stage of the tapeworm has not been located, but considering the diet of Peromyscus in this area, mites are considered to be logical hosts.

Only in the case of the badger were the specimens removed in a living state. In the other cases, the intestinal tracts had been preserved in 10% formaldehyde. The specimens from the badger were killed and fixed in warm Bouin's. Measurements were made on preserved specimens and mounted material. Whole mounts were stained with acid alum carmine, cleared in clove oil, and mounted in piccolyte. Drawings were made with the aid of a microprojector.

Mesocestoides carnivoricolus n. sp.

SPECIFIC DESCRIPTION: Small cestode with well defined scolex. Length of 20 specimens ranging between 62 and 112 mm. and 600 to 850 microns wide. Number of proglottids ranging from 210 to 318. Anterior proglottids as much as 9 times broader than long with mature segments about twice as wide as long. Gravid segments about twice as long as broad and barrel-shaped.

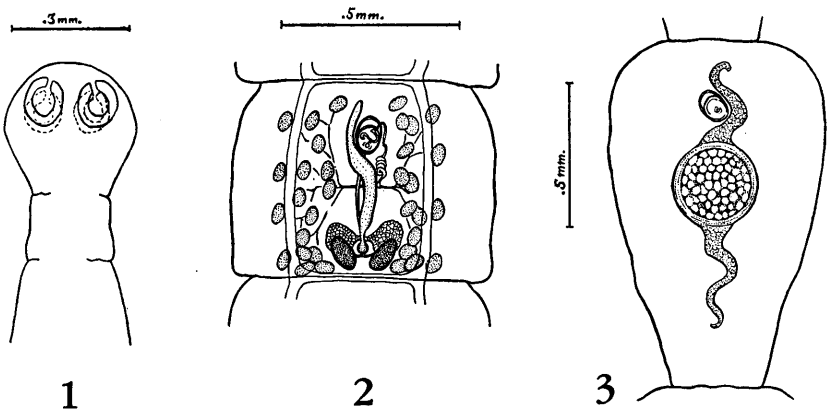
Scolex somewhat rectangular in cross-section, averaging 300 x 416 microns in preserved specimens. Measurement of ten scolices ranged from 980 to 1128 microns in length. The two long sides of scolex quite flat and each containing two suckers with rims narrowly notched anteriorly. Neck distinctly narrower than the scolex, 3 to 4 mm. in length. First segments much wider than long, averaging 49 microns long by 466 microns wide. Strobilar margins variable, from straight to serrate in the different worms examined. Measurements of the 100th segment of ten worms averaged 210 microns long by 343 microns wide.

Segments becoming mature about 100 to 120 behind neck. Mature proglottids numbering 16 to 25 in specimens examined, varying in shape and over-all dimensions between specimens. Anterior mature segments varying from 750 to 850 microns wide and 275 to 360 long. Early gravid segments becoming more square and completely gravid proglottids much longer than

wide. In mature segment, osmoregulatory ducts dividing proglottid into approximately equal thirds. Testes 25 to 35 in two more or less distinct longitudinal rows adjacent to the ducts, with 3 to 4 testes on each side being lateral to the duct. Testes more distributed in older proglottids with five or six on each side being crowded against the posterior border. Testes ovoid, 24 to 32 microns wide by 32 to 38 microns long. Cirrus pouch central to slightly anterior in less mature segments with the genital pore approximately in the center of ventral surface. In more mature segments, cirrus pouch migrates more anteriorly and comes to lie about 24 to 40 microns from the anterior edge of proglottid. Pouch ovoid, averaging about 61 x 81 microns. Ovaries near posterior border, 60 to 72 microns in length, triangular in shape; developing close together in mid-line and connected by short oviducts to the vagina. Ootype distinct, in the midline, between the ovaries. Yolk glands ovoid, 32 to 42 microns wide by 50 to 64 microns long, long axis inclined posteriorly toward the midline. Glands close together; distance between lower end averaging 28 microns, ventral and mostly posterior to the ovaries, overlapping ovaries only slightly in some cases. Uterus extending forward from ootype with several distinct bends, most conspicuous bend about the cirrus pouch. Cirrus pouch found on one side of the uterus for a distance of 3 or 4 proglottids, then reversed for several. Mature proglottid showing 21 distinct cord-like longitudinal muscle bands.

Gravid proglottids somewhat barrel-shaped in the anterior segments, becoming longer and more narrow posteriorly in the last segments of the strobila; vary from 550 to 650 microns wide to 1175 to 1340 microns long; width at anterior end 200 microns. Paruterine body ovoid, averaging 275 x 400 microns in size, with walls 32 to 35 microns thick. Position of the sac central in anterior proglottids and becoming more posterior in those at the end of the strobila. Paruterine sac developing from uterus just anterior to the oviducts. All internal structures other than a remnant of uterus and cirrus sac absent in fully gravid proglottids. Onchospheres ovoid to round, 22 x 26 microns in size.

It is considered likely that the tetrathyridia found in *Peromyscus manicu-*



Mesocestoides carnivoricolus

Fig. 1. Scolex.

Fig. 2. Mature proglottid, ventral view, showing arrangement of internal structures.

Fig. 3. Gravid proglottid from a position near the end of the strobila.

latus sonoriensis and *P. crinitus pergracilis* belong to this species. The larvae were removed with the cysts from the liver and lungs on autopsy. Following removal of the cysts, the larvae were killed and fixed in AFA, a treatment which expanded and caused the scolices to evert. Measurements of the expanded tetrathyridia showed them to be 5 to 7 mm. long by 1 to 1.3 mm. wide. Scolex 575 to 600 microns wide. The four suckers showed rims notched anteriorly 184-196 microns long by 136 to 144 microns wide. Sucker aperture slit-like. Osmoregulatory canals, distinct, lateral, branching and anastomosing posteriorly. Canals emptying in the posterior tip into a short common duct which leads to a terminal pore. The large ducts entering the common duct anterolaterally, the smaller ducts more posteriorly. Longitudinal muscle strands mostly central.

HOSTS. Adult: *Taxidea taxus taxus* (Schreber), badger
Canis latrans Say, Coyote
Lynx rufus pallescens Merriam, bobcat
Tetrathyridia: *Peromyscus maniculatus sonoriensis* (Le Conte), the deer mouse.
P. crinitus pergracilis Goldman, the canyon mouse

LOCALITY: Tooele County, Utah

COLLECTORS: F. R. Evans, J. Miles Butler, and A. W. Grundmann

TYPE SPECIMEN (From badger #2985 U. of U. coll.) Deposited in the Helminthological Collection of the University of Utah. Paratypic material deposited in the U. S. National Museum.

DISCUSSION

The two species of Mesocestoides with which this species may be confused are *M. variabilis* Mueller (1927) and *M. corti* Hoeppli (1925). Differences exist in the number, size and distribution of testes between *M. carnivoricolus* and *M. variabilis,* the former having 25 to 35 and the latter 90 to 110. The yolk glands of *M. variabilis* are lateral and separated while those of *M. carnivoricolus* are close together. The Paruterine structure in *M. variabilis* is much larger and nearer the posterior border.

M. carnivoricolus differs from *M. corti* in that the sucker rims of the latter are notched posteriorly while in the former the rims are notched anteriorly. The testes of *M. corti* are found on both sides of the osmoregulatory duct and number 36 to 60. The neck region in *M. corti* is shorter and the first segments are twice as wide as long instead of about 9 times as wide as long. Furthermore, *M. corti* is a parasite of rodents while *M. carnivoricolus* inhabits carnivores.

A Note on *Ribeiroia ondatrae* (Price, 1931) in Puerto Rico

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Collections of *Australorbis glabratus* from the streams infested with *Schistosoma mansoni* in Puerto Rico usually contain snails infected with a leptocercous cercaria which resembles those of the echinostomes. In the early studies on schistosomiasis mansoni on the island this cercaria was first

*The author wishes to express his appreciation to Dr. D. B. McMullen and Dr. H. W. Harry for their interest and assistance during the course of this study.

described by Marin (1928) as "Cercaria III." Faust and Hoffman (1934) redescribed this form and gave it the name of *Cercaria marini.* It had been observed by Harry (1954) that snails infected with this trematode were often castrated. A study of its life cycle was undertaken.

Most of the snails used came from the Rio Caguitas. Usually 3-4% of the snails were infected with *C. marini* but occasionally an incidence of 6% was obtained. In the laboratory the cercariae were shed during the night, although they could be induced to emerge during the day if the snails were placed in the dark. A study of the morphology of the redia and cercaria indicated that the species was at least very closely related to *Ribeiroia ondatrae* (Price, 1931; Price, 1942), as reported by Beaver (1939). Metacercariae were obtained in the lateral line canal of the guppy (*Lebistes reticulatus*) and *Poecelia vivipara,* and in the cloaca of tadpoles exposed to the cercariae.

Adult trematodes were obtained in the laboratory by feeding mature metacercariae to a parakeet and a pigeon. Attempts to infect chickens and ducks were unsuccessful. Natural infections were found in two of eight Green Herons (*Butorides virescens*); one from Trujillo Alto and the other from La Torrecilla Lagoon near San Juan. In all of the birds the parasite had caused deep lesions in the mucosa of the proventriculus. With the exception of the size of the adults and the number of eggs found in the uterus, the worms were identical with the descriptions given by Price (1931) and Beaver (1939) for *Ribeiroia ondatrae.* The greatest length of the adults obtained in the present study was 1.22 mm., compared to 1.6-2.0 mm. (Price) and 1.4-4.2 mm. (Beaver). In drawings these authors showed numerous eggs in the uterus. The specimens obtained in Puerto Rico contained only 6-10 eggs. In spite of these differences, a thorough comparison of the morphology of the redia, cercaria, metacercaria and adult observed in this study leaves no doubt that *C. marini* is the larval stage of *Ribeiroia ondatrae.*

SUMMARY

In an investigation of the life cycle of *Cercaria marini* Faust and Hoffman 1934, from *Australorbis glabratus* in Puerto Rico, it has been found that the metacercariae develop in the lateral line canal of *Lebistes reticulatus* and *Poecelia vivipara,* and the cloaca of tadpoles. Adult worms, identified as *Ribeiroia ondatrae* (Price 1931), were recovered from a parakeet and pigeon fed metacercariae. Natural infections were found in the Green Heron (*Butorides virescens*).

LITERATURE CITED

BEAVER, PAUL C., 1939. The morphology and life history of *Psilostomum ondatrae* Price 1931 (Trematoda: Psilostomidae). Jour. Parasit., 25:383-393.

FAUST, E. C. and HOFFMAN, W. A., 1934. Studies on schistosomiasis mansoni in Puerto Rico: III. Biological studies; the extra-mammalian phases of the life cycle. Puerto Rico Jour. Pub. Health & Trop. Med., 10:1-47.

HARRY, H. W., 1954. Host parasite relationships between *A. glabratus* and its infecting trematodes in Puerto Rico. Trop. Res. Med. Lab. Quarterly Report, April-June.

MARIN, RAFAEL A., 1928. Studies in schistosomiasis in Puerto Rico; III. Cercariae from *Planorbis guadeloupensis.* Puerto Rico Rev. Pub. Health & Trop. Med., 3:397-402.

PRICE, E. W., 1931. Four new species of trematode worms from the muskrat, *Ondatra zibethica,* with key to the trematode parasites of the muskrat. Proc. U. S. Nat. Mus., 79:1-13.

————, 1942. A new trematode of the family Psilostomidae from the scaup duck, *Marila affinis.* Proc. Helminth. Soc. Wash., 9:30-31.

Studies on the Metabolism of *Panagrellus Redivivus* (Nematoda, Cephalobidae)

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Rapid techniques for the preliminary screening of nematocides have been developed using saprophytic nematodes of the genus *Panagrellus*. *Panagrellus redivivus* (Linn., 1767) Goodey, 1945, is usually the organism of choice. This species can be easily cultured on a medium of boiled oatmeal, from which it can be separated by passage through a Baermann funnel. In some screening techniques an estimation of a quantitative response to chemical treatment is based upon a decrease in the rate of movement over specific intervals of time.

It has long been assumed that a high rate of motility is associated with a high rate of O_2 consumption. Nielsen (1949) questioned this premise when he found that several species of Rhabditis and Cephalobus showed a high movement activity but not a correspondingly high metabolic rate.

The oxygen consumption of various soil nematodes has previously been investigated by Nielsen (1949). Using the Cartesian diver technique of Halter (1943) and Linderstrom-Lang (1943), Nielsen's investigations were conducted at 16°C. on small groups of worms or in some cases on individual worms. For purposes of broad comparison our studies were made on comparatively large groups of worms over a wide temperature range.

METHODS

Respiration studies were conducted in the differential micro-respirometer described by Cunningham and Kirk (1940), later modified by Barth and Kirk (1942), and Stern and Kirk (1948). A suspension of worms was placed in a glass cup of 150 ml. capacity and the cup placed in one chamber of the differential respirometer. A small plastic cup containing 50 ml. of 50% KOH as a CO_2 absorbent was placed on top of the cup of worms; the control side of the respirometer contained a similar cup of water and cup of KOH. The Respiratory Quotients (R.Q.) were determined by repeating the experiments without the KOH.

A great deal of difficulty was experienced in developing a technique which would eliminate enough of the variable factors to obtain uniform and reproducible measurements of O_2 consumption. To maintain sufficiently uniform temperatures above 20°C. it was necessary to place the respirometer in a covered container which in turn was suspended in a thermostatically controlled water bath accurate to $\pm$ 0.5°C. Measurements made at 20°C. and lower were carried out in a thermostatically controlled refrigerator.

Worms were separated from their culture medium and prepared for experimentation by several passages through a Baermann funnel. When these worm populations were examined, they were found to contain variable and unpredictable proportions of large adult males and females and larvae of all sizes and stages of development. Since it can be assumed that the metabolic rate of small larvae would differ from the rate of adult specimens, it was necessary to develop a sedimentation process for separating worms as to size. This was accomplished by adding an aliquot of washed worms containing approximately 5000 individuals to distilled water in a 10 ml. centrifuge tube, and after shaking, the tube was allowed to settle for exactly

two minutes. At the end of two minutes, 0.5 ml. of the suspension of worms was removed from the cone of the centrifuge tube. Five to ten passages through the sedimentation cycle resulted in a selected group of 500-1000 individual worms of equal size and rate of motility which could be transferred to the respirometer by Micropipette. All respiration and R.Q. measurements were made on groups of worms containing 400-600 individuals selected by this method and suspended in 50 μ l. of distilled water.

The average weight of individual worms was determined by the camera lucida method described by Nielsen (1949). Weight determinations made on several hundred individuals, both male and female gave an average weight of 1.21 micrograms/worm.

At the conclusion of each respiration experiment the worms were removed from the respirometer, killed by gentle heat, transferred to a watchglass and counted under the low power microscope. The results of the respiration measurements were calculated on a basis of cc of O_2 per kilogram of tissue per hour. All respiration measurements were conducted for a period of several hours following a one-hour period of temperature-pressure equilibration.

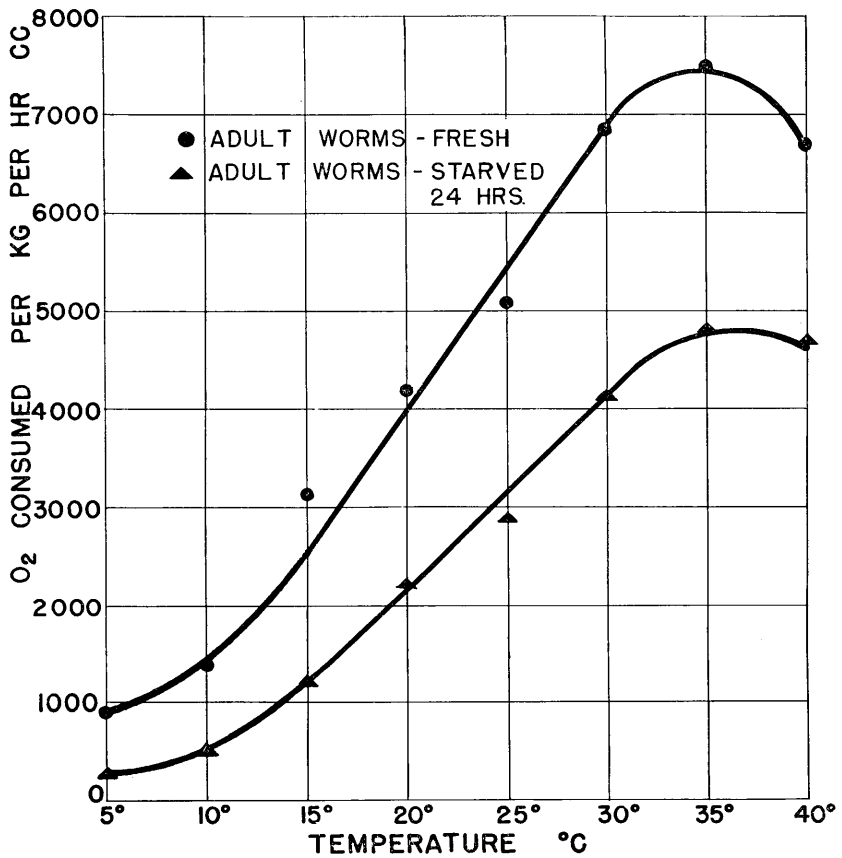

Fig. 1.—Consumption of O_2/kg/hr.

The measurements of motility were made by isolating individual worms in distilled water and counting the oscillations over a timed interval. This method, though tedious and eye-straining, gave satisfactorily uniform results with a percentage error of 15%. The average rate of oscillation of the adult worm selected by the sedimentation process was found to be 120 ± 20 cycles per minute at 25°C.

The observations on the effect of chemical treatment with respect to temperature were recorded as the LD_{50} after two hours of exposure. The LD_{50} was established as that concentration of a standard chemical toxicant (Loralkyl ethylenediamine) which resulted in the complete loss of motility of approximately 50% of the individuals under test.

All observations of respiration, motility and response to chemical treatment were carried out over the temperature range 5°C.-40°C. Observations were made with worms freshly removed from culture media and with worms that had been starved in distilled water for 24 hours and 48 hours.

RESULTS AND DISCUSSIONS

Consumption of O_2 over the temperature range 5°C-40°C. is reported graphically in Figure 1. Each point established on the curve represents an average of at least five respiration determinations made with different worm populations. The results of individual respiration determinations made on fresh worms were somewhat erratic; however, after 24 hours of starvation the variation in the rate of O_2 consumption became much less and the metabolic rate dropped to a lower level than with fresh worms. There was very little, if any, difference in O_2 consumption between worms starved 24 hours and those starved for 48 hours. Respiration measurements conducted at temperatures above 40°C. became erratic and no satisfactory results were obtained for 45°C. Previously we had demonstrated that *Panagrellus redivivus* will not tolerate a temperature of 45°C. for periods longer than two

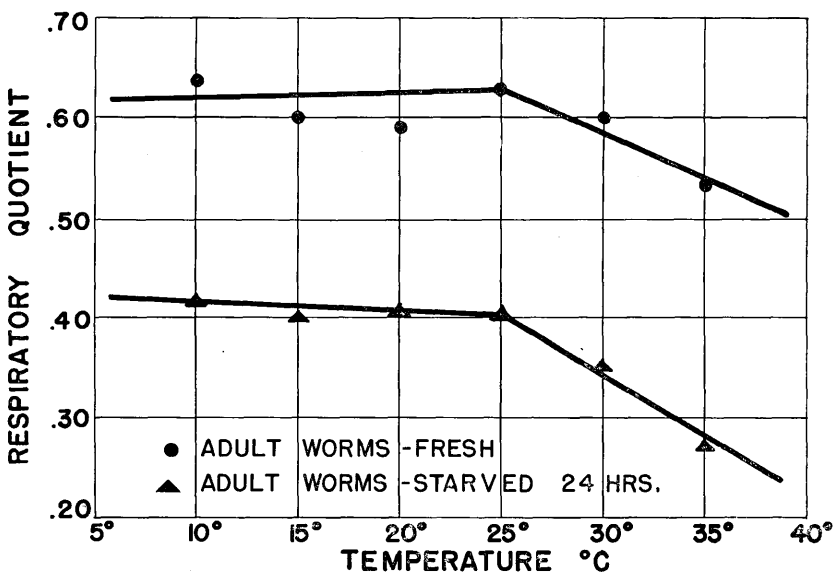

Fig. 2.—Respiratory quotient.

hours. (Santmyer 1955). The fall in O_2 consumption at 40°C. and above is probably due to thermal death of individual worms during the experiment. Determination of R.Q. (Figure 2) made on fresh worms gave significantly higher figures than for worms starved 24 and 48 hours at each temperature investigated. There was no apparent difference in the R.Q. of worms starved for 24 or 48 hours. Worms freshly removed from their culture medium have digestive tracts filled with ingested food which is still being metabolized and which may account for this difference. After periods of starvation the R.Q. values fall to lower figures which represent the metabolism of endogenous food reserves.

In the studies of the effect of temperature on motility (Figure 3), we were surprised to find that there is no apparent difference in the rate of

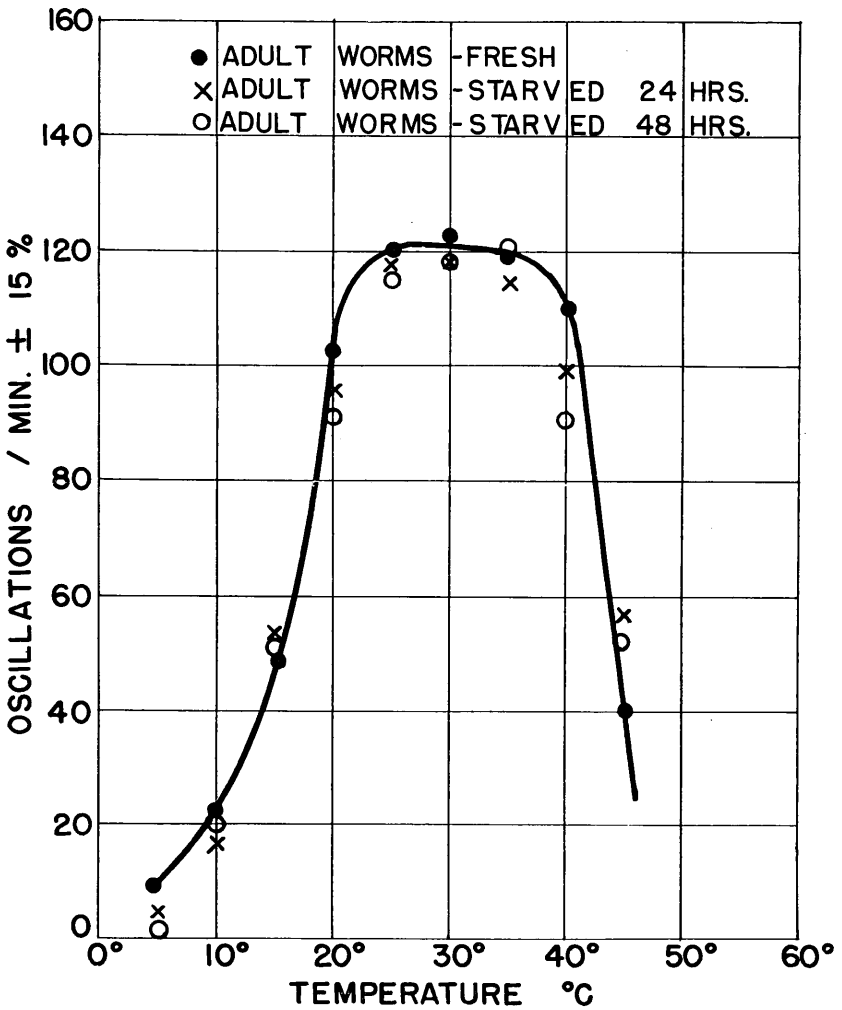

Fig. 3.—Motility rate in oscillations per minute of P. Redivivus after 1 hr. exposure at recorded temperatures.

motility between fresh worms and those starved for periods of 24 and 48 hours. There also appears to be a maximum rate of motility which is reached at a temperature of 20°C.-25°C. In fresh worms this motility is equivalent to an O_2 consumption of approximately 5000 cc O_2/kg/hour. With worms starved 24 and 48 hours the motility is equivalent to an O_2 requirement of only 2500 cc O_2/kg/hour. This difference in the energy requirement for motion in fresh and starved worms is not easily explained. It is possible that the experimental error in the measurement of the motility rate is too great to demonstrate a difference in motility, or possibly there are actual metabolic differences between fresh and starved worms which influence motility. However, it is clear that the energy compared with the total metabolic energy as measured by O_2 consumed at temperatures above 25°C. It is also probable that any nematocide screening procedure using loss of motility as the criterion of assay will not register a chemical effect until the metabolic activity falls to a level equivalent to the energy consumed in movement.

Nematode response to chemical treatment is plotted as the log of the concentration vs. temperature (Figure 4). Here again it is evident that freshly prepared worms do not respond in the same degree as starved worms. The

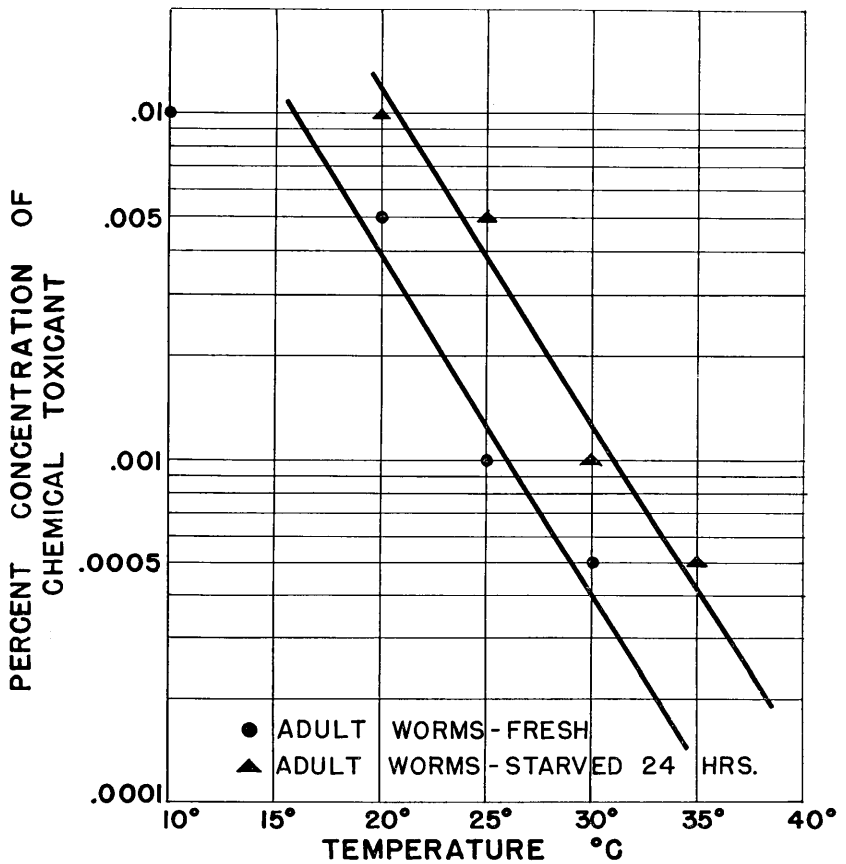

Fig. 4.—Concentration of chemical necessary to obtain LD50 in 2 hrs. at recorded temperature.

log-concentration curve is displaced approximately 5°C. up the temperature scale for starved worms. This would indicate that the starved worms are more resistant to chemical effects. The marked correlation of chemical response with metabolism is demonstrated here. As the O_2 consumption increases with increasing temperatures the resistance to chemical toxicity decreases. LD_{50} resulting from a concentration of 10 ppm of loralkyl ethylenediamine at 25°C. in fresh worms is equivalent to an O_2 consumption of approximately 5000 cc/kg/hour while 10 ppm of the toxicant will approach an LD_{50} for starved worms at 30°C. which is equivalent to an O_2 consumption of approximately 4500 cc/kg/hour.

The log of the O_2 consumed by starved worms is plotted vs. temperature in Figure 5. The plot of these data results in two lines which intersect at approximately 20°C. The increase in metabolic rate per increment of temperature is greater below 20°C. than above. It is interesting to note that this intersection at 20°C. corresponds to the maximum rate of oscillation (Figure 3) and a marked change in the values for the R.Q. (Figure 2). The exact meaning of the metabolic shift that occurs at 20°C.-25°C. is not clear, but it appears that the metabolic energy consumed in motion reaches a saturation level at that temperature and metabolic energy produced at higher temperatures becomes increasingly inefficient.

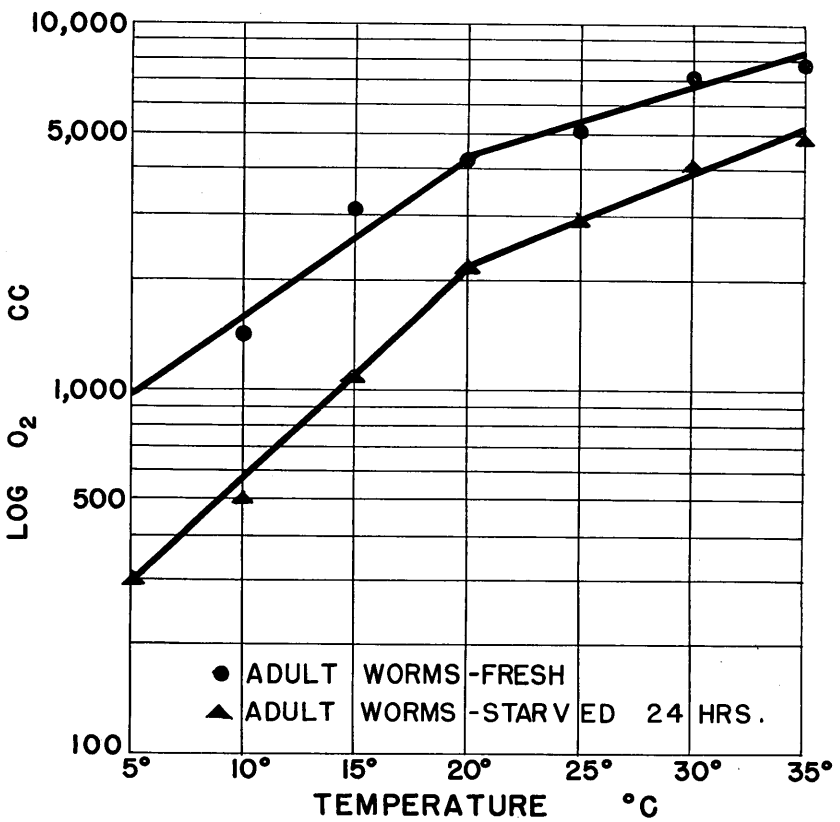

Fig. 5.—Log O_2—temperature.

The temperature-metabolic correlation with chemical treatment may necessitate a reappraisal of rapid nematocide screening methods; however, with strict adherence to a standard set of environmental conditions, uniform and reproducible results should result from *in vitro* screening.

SUMMARY

The studies reported here show that a direct correlation exists between metabolic activity and response to chemical treatment. These studies also demonstrate that a comparatively small proportion of the total metabolic energy is consumed in oscillatory movement.

LITERATURE CITED

LINDERSTROM-LANG, K. 1953. Compt. rend. trav. lab. Carlsberg, Ser. Chim. 24:334.
HOLTER, H. 1943. Compt. rend. trav. lab. Carlsberg, Ser. Chim. 24:400.
CUNNINGHAM, B., KIRK, P. L. 1940. J. Gen. Physiol. 24:135.
BARTH, L. G., KIRK, P. L. 1942. J. Gen. Physiol. 25:663.
STERN, H., KIRK, P. L. 1948. J. Gen. Physiol. 31:243.
NIELSEN, C. Overgaard 1949. Studies on the soil microfauna II. The soil inhabiting nematodes. Naturhistorish museum, Aarhus, Denmark.
SANTMYER, P. H. 1955. Proc. Helm. Soc. Wash. 22:22-25.

The Effect of Antibiotics, Phenothiazine, Sodium Fluoride, and the Combined Action of These Drugs, in the Removal of Oxyurids from Mice

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The efficacy of antibiotics in the treatment of bacterial, rickettsial, and protozoal diseases in man and animals has presented the possibility that these drugs alone or in combination with other anthelmintics might be useful in the removal of gastro-intestinal helminths. The development of an effective treatment with antibiotics for pinworms in mice might provide valuable information in the use of such drugs for *Enterobius vermicularis* infections in man, and for oxyurid infections in domestic and laboratory animals.

Wells (1951) found that terramycin and aureomycin when given orally to mice infected with *Aspicularis tetraptera,* at the rate of 500 mg. per kilogram of body weight, were effective in eliminating most of the worms and stunting the growth of the remaining worms. She reported that bacitracin at the rate of 20,000 units per kilogram of body weight, reduced the numbers of *A. tetraptera* but that it was not as effective as terramycin and aureomycin.

Chan (1952) found that terramycin, aureomycin, and bacitracin in the concentrations used by Wells against *A. tetraptera* were also effective against *Syphacia obvelata* of mice. He found, however, that when mice were offered phenothiazine at the low level of 250 mg. per kilogram of body weight for 3 days, the worm burden was only slightly reduced. Habermann (1951) reported that phenothiazine was effective in the removal of *A. tetraptera* from the colons of mice when offered 0.5 gm. of phenothiazine in molasses feed. Well (1951), Deschiens and Long (1945), and Thompson and Reinertson (1952) reported that phenothiazine eradicated *A. tetraptera* in mice. In screening a group of compounds effective against *A. tetraptera,* Hsieh (1952) found that phenothiazine at the rate of 250 mg. per kilogram of body weight for 6 days was more effective than gentian violet, tetrachlorethylene, egressin,

hexylresorcinol, and diphenan in removing pinworms from mice. Standen (1953) reported that gentian violet was rated four plus for the removal of *A. tetraptera* at the dose rate of 50 mg. per kilogram of body weight, but was ineffective at the dose rate of 25 mg. per kilogram of body weight. He also reported that piperazine hydrate gave good response at 400 and 200 mg. per kilogram of body weight unit dose.

Most oxyuridicides are effective in removing these worms from the colon of mice but are not so effective in removing them from the cecum. Since antibiotics have been reported to be effective in removing oxyurids from mice, presumably by entering through the gut wall through the blood-vascular system, this experiment was conducted to determine whether or not, by synergistic action, orally administered anthelmintics in combination with antibiotics would be more effective than either drug alone.

Previous workers offered small dosages of the antibiotics for 3 days or more, but in this experiment the antibiotics were offered in the feed in larger dosages for a 24-hour period.

MATERIAL AND METHODS

In this study a total of 400 one-month-old female, white Swiss mice weighing 10 to 12 gm. were used. Heavy worm burdens of *A. tetraptera* and *S. obvelata* were obtained in the mice by placing 45 to 55 animals ranging in weight from 10 to 12 grams in an unclean cage with 10 to 12 heavily infected mice for 14 days. Prior to exposure of the experimental mice, the infected mice were fed pellets from the floor of the cage and the cage was not cleaned for a two-week period. At the end of the exposure period, the experimental mice were divided into 4 to 5 groups (10 animals per group) and offered a molasses-medicated feed. The molasses-medicated feed for each mouse was prepared by mixing the drug with 4 to 5 cc. of molasses and then admixing the molasses-drug with 10 gm. of ground mouse feed. Unmedicated feed was prepared by mixing 4 to 5 cc. of molasses with 10 gm. of ground mouse feed.

Animals receiving aureomycin, bacitracin, and terramycin were offered 1,000 mg. of antibiotic per kilogram of body weight (10 mg. per 10 gm. molasses feed). Animals treated with phenothiazine were offered 500 mg. per 10 gm. molasses feed. Animals treated with sodium fluoride were offered 75 to 100 mg. per 10 gm. molasses feed. Mice in the experimental control groups were offered 10 gm. of molasses-ground feed.

The mice would usually eat the medicated feed within 24 hours, but if any remained, unmedicated ground feed was mixed with the remaining portion for another 24-hour period. Fecal material was collected from each group of mice for 2 days following the day of treatment. This material was screened in 20 and 60 gauge wire pans, and the worms recovered were counted. On the third day following treatment, necropsies were performed on each mouse. The cecum and colon contents were screened, and the total number of worms recovered was recorded. Gross necropsy findings were recorded, tissue was saved for histopathological examination, and the efficacy of each of the drugs was calculated.

RESULTS

Table I shows the anthelmintics tested, the number of worms passed and recovered from each of the mice, and the percent worm removal of the different anthelmintics. It will be noted in Experiment 1 that phenothiazine and bacitracin were the most effective anthelmintics in the removal of oxyurids,

TABLE I—Efficacies of Phenothiazine, Sodium Fluoride, and Antibiotics Alone and in Combinations for the Removal of Pinworms from Mice.

Exp. No.	Drug	No. Animals	No. Worms Passed in Feces	No. Worms Recovered at Necropsy			Total No. Worms	% Worms Passed in Feces
				Cecum	Colon	Total		
	Sodium Fluoride	10	61	95	117	212	273	22.3
1	Bacitracin	10	197	67	10	77	274	71.8
	Phenothiazine	10	108	12	16	28	136	79.4
	Control	10	47	269	49	318	365	12.8
	Aureo. and Sod. Fluoride	10	212	250	16	266	478	44.3
2	Terra. and Sod. Fluoride	10	190	164	14	178	368	51.6
	Sodium Fluoride	10	141	123	23	146	287	49.1
	Control	10	157	466	32	498	655	23.9
	Bacitracin	20	304	417	54	471	775	40.5
	Aureomycin	20	101	1,610	237	1,847	1,948	5.1
3	Terramycin	20	389	1,085	109	1,194	1,583	24.5
	Phenothiazine	20	744	122	79	201	945	78.7
	Control	20	167	879	156	1,035	1,202	13.8
	Aureo. and Phenothiazine	40	688	199	130	329	1,017	67.6
	Terra. and Phenothiazine	40	816	381	235	616	1,432	56.9
4	Baci. and Phenothiazine	40	1,433	303	693	996	2,429	58.1
	Phenothiazine	50	993	241	157	398	1,391	71.3
	Control	50	1,249	1,797	857	2,654	3,903	32.0
	Totals	400						

removing 79.4 per cent and 71.8 per cent of the worms, respectively.

Experiment 2 shows that sodium fluoride alone and combined with aureomycin and terramycin respectively, removed less than 55 per cent of the worms from the cecum and colon.

Experiments 3 and 4 show that phenothiazine alone removed 71.6 per cent of the oxyurids and was the only drug that removed a high percentage of *A. tetraptera* and *S. obvelata* from the mice and that the antibiotics alone, and in combination with phenothiazine, were less effective.

Gross and histopathological findings of the tissues and organs from animals offered phenothiazine, antibiotics, and phenothiazine and antibiotics respectively showed no evidence of toxicity due to the drugs. In animals offered sodium fluoride and sodium fluoride plus antibiotics, some of the animals showed hemorrhages of the colon and bloody feces.

The relatively low percent worm removal of phenothiazine for oxyurids in mice (71.6 per cent) is probably due to the fact that phenothiazine does not enter the cecum in some cases, and therefore the oxyurids in these cases are not removed. It is apparent from these experiments that phenothiazine is more effective in the removal of *A. tetraptera* from the colon than for the removal of *S. obvelata* from the cecum.

Since large single dosages of the antibiotics in the feed, alone and with other anthelmintics, were less than 70.0 per cent effective in the removal of oxyurids from mice, it appears from the experiments of Wells (1951) and Chan (1952) that longer treatments of antibiotics with other anthelmintics might be effective. Experiments to determine the efficacy of the drugs over a longer period of time are now in progress.

CONCLUSIONS

1. Phenothiazine was the most effective of the anthelmintics tested for the removal of *Aspicularis tetraptera* and *Syphacia obvelata* from mice, expelling 74 per cent of the pinworms (uncorrected average efficiency).

2. The antibiotics tested were less effective than phenothiazine in the removal of oxyurids from mice.

3. Sodium fluoride alone and in combination with other antibiotics was not effective in the removal of oxyurids.

4. Phenothiazine plus aureomycin, bacitracin, and terramycin respectively did not increase the anthelmintic efficacy of phenothiazine.

5. Phenothiazine is more effective for the removal of *Aspicularis tetraptera* of the colon than for the removal of *Syphacia obvelata* from the cecum.

LITERATURE CITED

CHAN, KAM-FAI, Chemotherapeutic Studies on *Syphacia obvelata* Infection in Mice. Am. J. Hyg., Vol. 56, 22, July 1952.

DESCHIENS, R., and LONG, L., Sur les propriétés anthelminthiques de quelques derivés de diphénylamine. Compt. rend. soc. biol., 139, 447-449, 1945.

HABERMANN, ROBERT T., Gastrointestinal Parasites of Laboratory Animals. Proc. of the 2nd Annual Meeting of Animal Care Panel, November 1951.

HSIEH, KNO-YENG NYEH, The Effect of the Standard Pinworm Chemotherapeutic Agents on the Mouse Pinworm, *Aspicularis tetraptera*. J. Hyg., Vol. 56, 3, 287, November 1952.

STANDEN, O. D., Experimental Chemotherapy of Oxyuriasis. Brit. Med. Jour., Oct. 3, 757-758, 1953.

THOMPSON, PAUL E., and REINERTSON, J. W., Chemotherapeutic Studies of Natural Pinworm Infections in Mice. Exptl. Parasitology, Vol. 1, No. 4, 384, 1952.

WELLS, HELEN S., Studies of the Effect of Antibiotics on Infections with the Mouse Pinworm, *Aspicularis tetraptera*. J. Inf. Dis., Vol. 89, 190, Sept. 1951.

Rictularia lucifugus, n. sp. (Nematoda: Thelaziidae), from the Little Brown Bat, *Myotis lucifugus lucifugus* (Le Conte, 1831)

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Two hundred and seventeen little brown bats, *Myotis lucifugus lucifugus* (LeConte, 1831) were collected from caves in Pendleton County, West Virginia during the winter months of 1952 and 1953, while the bats were in hibernation. The bats were found to harbor a member of the genus *Rictularia* Froelich, 1802, of the family Thelaziidae Railliet, 1916. One hundred and four sexually mature females, 18 immature females, and 8 males were found, with 1 to 17 females and one or no males per host. Not all bats examined harbored this nematode species. The female specimens were found in the lumen of the small intestine; whereas the males were difficult to find, being deeply imbedded in the intestinal mucosa.

Comparison with known forms indicates these specimens constitute an hitherto undescribed species for which the name *Rictularia lucifugus* is proposed. Specific identification of the genus *Rictularia* is based solely on the female, except for two species, *R. vulpis* and *R. muris* described by Galli-Valerio (1932), whose descriptions were based on the males. Two keys for identifying species of this genus, one by Cuckler (1939) and one by Dollfus and Desportes (1945) were both based on female characteristics. Conforming with the literature, the description and the identification of this new species is based on the sexually mature female. The present species was distinguished from other species of *Rictularia* when comparisons were possible, on the bases of: (1) the total number of cuticular appendages (combs and spines) and the position occupied by the vulva in relation to these appendages (Sandground, 1935; Cuckler, 1939; and Dollfus and Desportes, 1945), (2) the maximum measurements of the combs and spines (McPherson and Tiner, 1952), and (3) the distance of the last spine from the posterior end (Tiner, 1948a). Other individual characteristics were used in conjunction with those just mentioned to determine resemblances or differences.

MATERIALS AND METHODS

All measurements and camera lucida drawings were made from specimens cleared with lacto-phenol or mounted in glycerine-gelatin. *En face* preparations were made according to methods described by Buhrer (1949) and Tromba and Douvres (1953). To observe the presence or absence of an oesophageal tooth, heads of many paratype-female specimens were cut directly behind the mouth opening, mounted with adequate support and studied with a microscope, according to the technique described by Chitwood (1952).

Rictularia lucifugus, new species

DESCRIPTION: Holotype—Female. Length 22.00 mm. Widths: at base of buccal capsule 154 μ; anterior to vulva 299 μ; posterior to vulva 342 μ; maximum mid-body width 611 μ; at level of terminal end of intestine 267 μ. Cuticle provided with pronounced transverse and longitudinal striations. Mouth

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The writer expresses appreciation to F. G. Tromba, W. N. Smith, C. Yunker, L. Costello for their assistance in collecting the bats.

opening directed anterodorsally and surrounded by a circlet of denticles (Figs. 2 and 3). General arrangement of cephalic papillae as in other members of the genus. Buccal capsule well developed and heavily cuticularized. Oesophageal tooth absent. Buccal cavity maximum depth × height on inner border 76 × 52 μ. From anterior end: nerve ring 316 μ; excretory pore 383 μ; cervical papillae 534 μ; vulva 2.93 mm. Paired cervical papillae each armed with a sharply pointed spine; located posterior to the excretory pore and dorsad to the 7th pair of combs (Fig. 2). Oesophagus length 3.56 mm.; width immediately anterior to terminal end 122 μ. Genital system of opisthodelphic type. Vulva banked anteriorly and posteriorly by slightly projecting rugose-like lips (Fig. 1); located 725 μ anterior to base of oesophagus. Orifice of vagina bordered by oval or elliptical nucleated cells, presumably of glandular nature (Fig. 1). Vagina followed by a muscular ovijector, extends backwards for a distance of 464 μ, before dividing into two uteri. Maximum width of vagina 81 μ. Total number of paired cuticular appendages (combs and spines) 77: 32 pairs of combs and 3 pairs of spines anterior to vulva; 42 pairs of spines posterior to vulva. Distance from base of buccal capsule to middle of first comb 52 μ; maximum comb length × height 104 × 46 μ. Spine lengths: last prevulvar spine 59 μ; third postvulvar spine 69 μ; last postvulvar spine 26 μ. Distance from tail tip to: last postvulvar spine 203 μ; anus 211 μ. Tail is bluntly conical and appears to be spiked (Fig. 7). Uteri filled with embryonated smooth, oval, thick-shelled eggs (Fig. 6); length × width 46 × 33 μ.

HOST: *Myotis lucifugus lucifugus* (LeConte, 1831)
LOCATION: Small intestine
LOCALITY: Pendleton County, West Virginia
DATE: 1951
COLLECTOR: Frank W. Douvres
HOLOTYPE SPECIMEN: Deposited in the United States National Museum Helminthological Collection #41548.

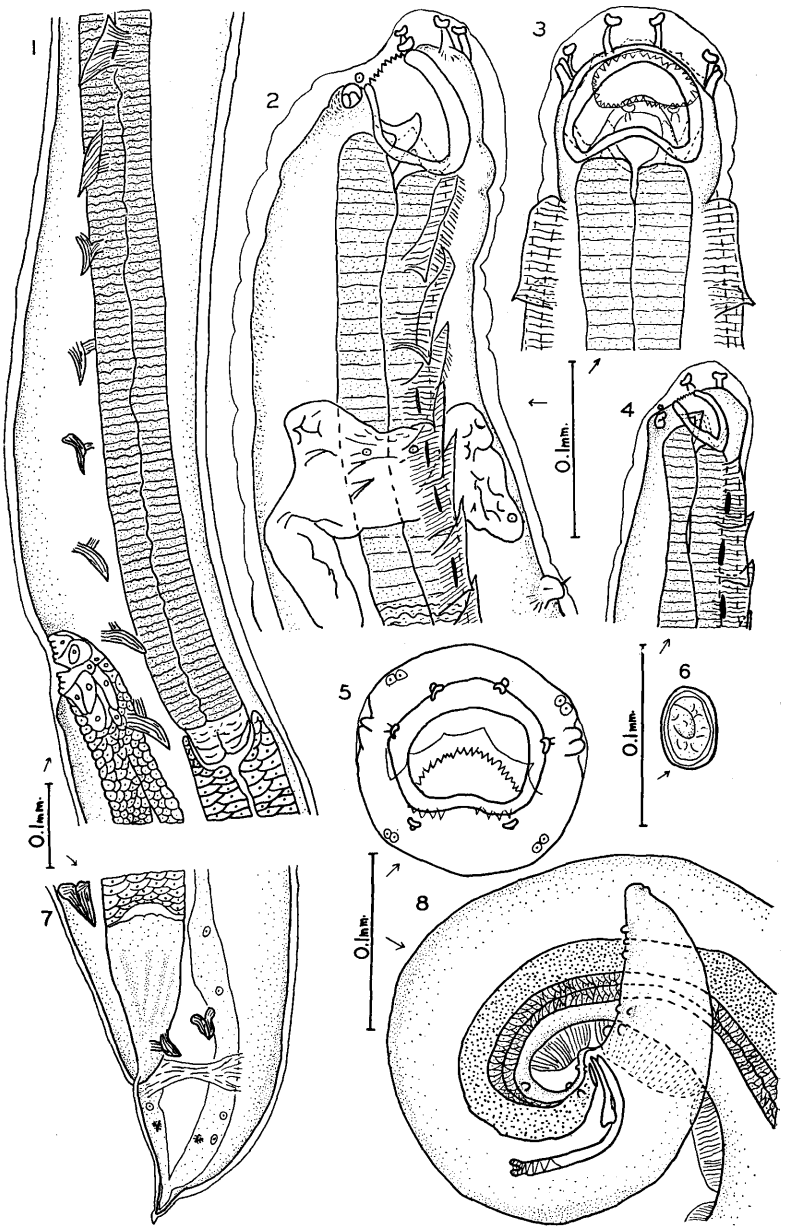
PARATYPES: Allotype—Male. Length 1.88 mm. Widths: at base of buccal capsule 47 μ; at level of 16th pair of combs 104 μ; at level of 24th pair of combs 111 μ; at level of 29th pair of combs 117 μ; at level of posterior lip of cloaca 39 μ. Mouth opening almost terminal but directed dorsad; surrounded by a circlet of denticles. General arrangement of cephalic papillae as in other members of the genus and holotype-female (Fig. 4). Buccal capsule well developed and heavily cuticularized. Buccal cavity maximum depth × height on inner border 26 × 13 μ. From anterior end: nerve ring 108 μ; excretory pore 163 μ; cervical papillae 195 μ. Paired cervical papillae each armed with a sharply pointed spine; located posterior to excretory pore and dorsal to the 7th pair of combs. Oesophagus length 334 μ; width immediately anterior to terminal end 39 μ. Total number paired combs 43, no spines present as in holotype. Distance from base of buccal capsule to middle of first comb 13 μ. Length × height: first comb 33 × 13 μ; 16th comb 52 × 23 μ; 24th comb 61 × 26 μ; 28th (maximum) comb 65 × 22 μ; 43rd (last) comb 26 × 7 μ. Distance last comb to: cloaca 169 μ; tail tip 254 μ. Last pair of combs located at an equal level with the first of four medio-ventrally located caudal fans (Fig. 8). Maximum caudal fan length × height 46 × 14 μ. Distance from cloaca to tail tip 85 μ. Spicules unequal (Fig. 8). Left spicule 112 μ long, protrudes from the cloaca, curving anteriad into body; anteromedial portion covered by a coiled, thread-like mantle. Right spicule 46 μ

long, rod-like with no mantle covering. Gubernaculum 20 μ long, rod-shaped, optically refractive; located alongside the 2 spicules at the elevated or swollen-like lips of the cloaca (Fig. 8). Cloacal papillae arranged along a median or medio-lateral plane: three precloacal pairs, the most anterior of the 3 pairs located posterior to the last caudal fan and the most posterior pair immediately anterior to the cloacal lips; and 5 postcloacal pairs. External caudal phasmids located slightly dorsolateral of the median plane of the posterior end, and appear notch-like on the tail tip (Fig. 8). Deposited in the U.S.N.M. Helminthological collection, Allotype #41549 and Paratypes #41550 (three females and one male).

Range of 103 females: 8.0-33.0 mm. long. Width: at base of buccal capsule 90-180 μ; anterior to vulva 120-398 μ; posterior to vulva 135-443 μ; maximum mid-body width 234-939 μ; at level of terminal end of intestine 110-323 μ. Buccal cavity maximum depth × height on inner border 45-120 × 26-60 μ. From anterior end: nerve ring 240-473 μ; excretory pore 270-518 μ; cervical papillae 383-601 μ; vulva 1.74-3.48 mm. Excretory pore may or may not be covered by a small protuberance. Paired cervical papillae dorsal to 6th-9th pairs of combs, usually 7th pair. Oesophagus length 1.76-4.28 mm., width immediately anterior to terminal end 75-240 μ. In 99 specimens, vulva at same level or up to 1.16 mm. anterior to terminal end of oesophagus; in 4 specimens, vulva was 30-75 μ caudad to terminal end of oesophagus. Vagina length × width: 180-443 × 30-75 μ. Total number paired combs and spines 76-79: 29-32 pairs of combs and 3-6 pairs of spines anterior to vulva; and 41-43 pairs of spines posterior to vulva. Distance from base of buccal capsule to middle of first comb 15-55 μ; maximum comb length × height 87-150 × 30-90 μ. Spine lengths: last prevulvar spine 40-75 μ; third postvulvar spine 75-180 μ; maximum postvulvar (16th-18th) spine 120-225 μ; second from last postvulvar spine 30-135 μ; last postvulvar spine 20-30 μ. Distance from tail tip to: last postvulvar spine 90-240 μ; anus 96-225 μ. Uteri filled with embryonated smooth, oval, thick-shelled eggs 34-55 × 20-37 μ.

Range of 8 males: 0.73-4.48 mm. long. Widths: at base of buccal casule 35-65 μ; at level of 16th pair of combs 62-135 μ; at level of 24th pair of combs 62-139 μ; at level of 29th pair of combs 62-165 μ; at level of posterior lip of cloaca 35-83 μ. Buccal cavity maximum depth × height on inner border 20-38 × 7-17 μ. From anterior end: nerve ring 108-255 μ; excretory pore 163-380 μ; cervical papillae 127-455 μ. Paired cervical papillae dorsal to 7th or 8th pairs of combs. Oesophagus length 0.26-1.53 mm.; width immediately anterior to terminal end 31-135 μ. Total number paired combs 41-43. Distance from base of buccal capsule to middle first comb 13-60 μ. Length × height: first comb 28-62 × 7-28 μ; 16th comb 28-110 × 20-28 μ; 24th comb 35-110 × 17-34 μ; maximum comb 35-130 × 17-35 μ; last comb 20-95 × 7-20 μ. Three to four caudal fans, range fan length × height: 35-75 × 14-35 μ. Distance from cloaca to tail tip 64-120 μ. Spicules unequal, left 110-120 μ; right 43-47 μ long. Gubernaculum observed in 4 specimens, 20 μ long. Three pairs precloacal and 5 pairs postcloacal papillae in each of the specimens examined; although caudal phasmids could only be located in two of the specimens.

Range of 18 immature females: 4.5-10.0 mm. long. Widths: at base of buccal capsule 55-120 μ; anterior to vulva 120-293 μ; posterior to vulva 126-308 μ; maximum mid-body width 150-398 μ; at level of terminal end of intestine 75-180 μ. Buccal cavity maximum depth × height on inner border 35-75 ×20-60 μ. From anterior end: nerve ring 150-293 μ; excretory pore 259-338 μ;



Drawn with the aid of the Camera Lucida

Fig. 1. *Rictularia lucifugus* n. sp. prevulvar region of paratype-female, latero-ventral view.

Fig. 2. *Rictularia lucifugus* n. sp. anterior end of paratype-female, lateral view.

Fig. 3. *Rictularia lucifugis* n. sp. anterior end of paratype-female, ventral view.

Fig. 4. *Rictularia lucifugus* n. sp. anterior end of paratype-male, lateral view.

Fig. 5. *Rictularia lucifugus* n. sp. anterior end of paratype-female, *en face*.

Fig. 6. *Rictularia lucifugus* n. sp. embryonated egg from paratype-female.

Fig. 7. *Rictularia lucifugus* n. sp. posterior end of paratype-female, latero-ventral view.

Fig. 8. *Rictularia lucifugus* n. sp. posterior of paratype-male, lateral view.

cervical papillae 326-466 μ; vulva 1.84-2.70 mm. Paired cervical papillae dorsal to 7th or 8th pairs of combs, usually the 7th pair. Oesophagus length 1.22-2.42 mm.; width immediately anterior to terminal end 60-169 μ. In 16 specimens, vulva 105-736 μ posterior to terminal end of oesophagus; in 2 specimens vulva 30-240 μ anterior to terminal end of oesophagus. Vagina length $\times$ width: 120-240 $\times$ 45-60 μ. Total number paired combs and spines 77-79: 30-32 pairs of combs and 4-6 pairs of spines anterior to vulva; and 41-42 pairs of spines posterior to vulva. Distance from base of buccal capsule to middle of first comb 14-30 μ; maximum comb length $\times$ height 75-105 $\times$ 30-45 μ. Spine lengths: last prevulvar spine 30-75 μ; third postvulvar spine 60-111 μ; maximum postvulvar (16th or 17th) spine 58-150 μ; second from last postvulvar spine 20-60 μ. In all specimens the last postvulvar spine was too rudimentary to measure. Distance from tail tip to: last postvulvar spine 90-165 μ; anus 83-150 μ. No eggs present in any of the specimens.

The allotype is included in the ranges of the paratype series. The data for the allotype and the remainder of the paratype series are: host, location, locality, collector, the same as for the holotype; date: 1951-1952.

DISCUSSION

Careful comparison of *R. lucifugus,* n. sp. with the existing descriptions of other species showed that it was unlike the known species of the genus *Rictularia.* It was found that *Rictularia macdonaldi* (Dobson, 1880), one of 6 *Rictularia* species reported parasitizing bats, resembled the present specimens. A detailed comparison of the differences and similarities (Table 1) between the females of the two speices was made because of the above resemblance and because of Tiner's (1948a) conclusions that the *Rictularia* sp. harbored by *Myotis l. lucifugus* in North America is identical with descriptions of *R. macdonaldi* (Dobson, 1880; Macdonald, 1880; and Jaegerskiold, 1909). *R. lucifugus* differs from *R. macdonaldi,* because the former species has (Table 1): (1) a greater total number of paired cuticular appendages (combs and spines), (2) a greater number of paired prevulvar cuticular appendages, (3) a much shorter last postvulvar spine, (4) a much shorter distance between the last postvulvar spine and the tail tip, (5) a smaller maximum comb height, and (6) a smaller vagina length. Although there is no difference indicated by the measurements of the tail lengths (Table 1), it should be noted that Jaegerskiold's (1909) description was based on a few sexually mature females whose body length was given as about 19.44 mm.; whereas the present description was based on 104 sexually mature females, whose body lengths ranged from 8.00-33.00 mm. The minimum tail length given for *R. lucifugus* was equal to the maximum or average measurement given for *R. macdonaldi* by Jaegerskiold (1909). Therefore, it is presumed that the tail length of *R. lucifugus* is greater than that of *R. macdonaldi.* To further support this presumption, the average tail length for the 104 specimens measured, was 166 μ and 81 percent of the specimens fell within a range of 146-186 μ. Another marked difference not cited in Table 1, was as follows: in *R. macdonaldi* the transition from combs to spines was very sharp and the first spine was situated constantly behind the vulva (Jaegerskiold 1909); whereas in *R. lucifugus,* the transition was gradual and the first spine was definitely situated anterior to the vulva (Fig. 1). On the basis of the aforementioned differences and those given in Table 1, of the sexually mature females, it is concluded that *R. lucifugus,* n. sp. and *R. macdonaldi* are two separate and distinct species.

TABLE 1. A comparison between the sexually mature females of *Rictularia macdonaldi* (Dobson, 1880) and *Rictularia lucifugus*, n. sp.

	R. macdonaldi[a]	*R. lucifugus*[b]
Total prs. cuticular appendages[c]	70-73	76-79
No. prs. prevulvar cuticular appendages	30-32 Combs	29-32 Combs & 3-6 Spines
No. prs. postvulvar cuticular appendages	42 Spines	41-43 Spines
Maximum comb length × height	120[d] μ	87-150 × 30-90 μ
Maximum postvulvar spine length	150 μ	120-225 μ
Distance last spine to tail tip	3.0 mm.	90-240 μ
Length last postvulvar spine	80 μ	20-30 μ
Length of vagina	650 μ	180-443 μ
Length of tail	96 μ	96-225 μ

[a]Based on Jaegerskiold (1909) redescription of *R. macdonaldi* (Dobson, 1880).
[b]Figures and measurements represent total ranges for 104 specimens, including the holotype-female.
[c]Cuticular appendages refer to combs and/or spines.
[d]Measurement for height only.

Although species identification of this genus is based on the females, a review of the literature revealed that the males of *R. lucifugus* were unlike any of the males reported for the genus *Rictularia*. In so far as there was a resemblance between the females of *R. macdonaldi* and *R. lucifugus*, a comparison between the males of these two species was made (Table 2). Jaegerskiold's (1909) description of the males of *R. macdonaldi* based on 2 specimens recovered from the bat, *Megaderma frons* and Seurat's (1915) description of the males of this same *Rictularia* species based on a single specimen recovered from a genette were given separately in Table 2. The major differences between these two species are the presence in *R. lucifugus* (Table 2) of: (1) a greater tail length, (2) a shorter distance between the last paired combs and cloaca, (3) a longer gubernaculum, and (4) the difference in spicule lengths. Pertaining to the last mentioned difference, in *R. macdonaldi* the measurements reported by Jagerskiold (1909) and Seurat (1915) for the left and right spicules, created a total range that could include

TABLE 2. A comparison between the males of *Rictularia macdonaldi* (Dobson, 1880) and *Rictularia lucifugus*, n. sp.

	R. macdonaldi		
	Jaegerskiold (1909)	Seurat (1915)	*R. lucifugus*[a]
Total body length	2.63 mm.	2.70 mm.	0.73-4.48 mm.
Total prs. combs	43	42	41-43
Mouth opening position	SD[b]	SD	SD
Buccal cavity depth	20 μ	----[c]	20-38 μ
Max. comb. length × height	60 × 30 μ	----	35-130 × 17-35 μ
Dist. last comb to cloaca	----	650 μ	111-303 μ
Dist. tail tip to cloaca	60 μ	----	64-120 μ
Left spicule length	134 μ	110 μ	110-121 μ
Right spicule length	42 μ	60 μ	43-47 μ
Gubernaculum length	8 μ	----	20 μ
No. caudal fans	5	6	3-4

[a]Figures and measurements represent total ranges for 8 specimens, including allotype.
[b]SD: subdorsal.
[c]Measurement not reported.

the spicule measurements for *R. lucifugus*. The present writer was able to study 8 males whose total body lengths ranged from 0.73-4.48 mm., a minimum and maximum unattained by either of the two male descriptions of *R. macdonaldi,* and still have spicule lengths that fell in the total range (Table 2). Therefore, it is believed that these measurements represent the minimum and maximum spicule lengths for *R. lucifugus.*

Although the mouth opening of *R. lucifugus* is subdorsal, it was possible to verify the generic cephalic papilla pattern described by Chitwood and Wehr (1934). The 6 cephalic papillae of the internal circle were very conspicuous and crescent-shaped (Figs. 2-5). The internoventral papillae were located ventrad to the median line; the 2 amphids were slightly more ventrad and external to the internolateral papillae; and the internodorsal papillae were located on or near the dorsal border of the denticulated mouth opening. The 8 cephalic papillae of the external circle were arranged accordingly: the ventroventral and lateroventral papillae were located ventrad to the amphids (Fig. 5); the dorsodorsal and laterodorsal papillae were located dorsad to the internodorsals (Figs. 2 and 5).

Application of the method described by Chitwood (1952) revealed that what appeared in a lateral view as an oesophageal tooth (Fig. 2), was in reality the arched dorsal wall of the buccal cavity (Fig. 3). Similar observations were reported for *R. halli* Sandground, 1935 by Chitwood (1952) and by Baylis (1928) in the description of *R. mjobergi.* Although oesophageal teeth have been reported in other species of *Rictularia,* there are none present in *R. lucifugus.*

Dollfus and Desportes (1945) in a review of the genus *Rictularia* suggested that the species of this genus could be separated into 3 groups according to the relationship of the vulva to the base of the oesophagus: group I, vulva anterior to base of oesophagus; group II, vulva at equal level with base of oesophagus; group III, vulva posterior to base of oesophagus. *R. lucifugus* females as reported in the holotype and paratype descriptions could be placed into all three of these groups. This varied relationship of the vulva and base of the oesophagus within a single *Rictularia* species has been reported by Tubangui (1931) and Baylis (1934) in the descriptions of *R. whartoni* and *R. harrisi,* respectively. Because such variability can occur within single species, the present writer does not believe it feasible to group *Rictualaria* species according to the methods described by Dollfus and Desportes (1945).

In the majority of the paratype females the last postvulvar spine was rudimentary. In many instances, the free posteriorly directed extremity of the last spine hardly projected beyond the cuticle; or as in many of the specimens this spine was represented by a striated (like the core of the other spines) thickening of the cuticle (Fig. 7). Similar observations of the last postvulvar spines were reported by LeRoux (1930) in his description of *R. aethechini.*

SUMMARY

A spirurid nematode, *Rictularia lucifugus,* n. sp. (Family Thelaziidae) from the little brown bat (*Myotis lucifugus lucifugus*) is described.

LITERATURE CITED

BAYLIS, H. A. 1928. Some further parasitic worms from Sarawak. Ann. Mag. Nat. Hist. 1: 608-611.

———. 1934. On a collection of cestodes and nematodes from small mammals in Tanganyika Territory. Ann. Mag. Nat. Hist. 13: 349, 351.

BUHRER, E. M. 1949. Technique for the beheading and *en face* examination of nematodes and similar animal types. Proc. Helm. Soc. Wash. 16: 3-6.
CHITWOOD, B. G., and E. E. WEHR. 1934. The value of cephalic structures as characters in nematode classification with special reference to the superfamily Spiruroidea. Ztsch. Parsit. 7: 273-335.
CHITWOOD, M. B. 1952. Some observations on *Rictularia halli* Sandground, 1935 (Nematoda). Proc. Helm. Soc. Wash. 19: 121-123.
CUCKLER, A. C. 1939. *Rictularia onychomis* n. sp. (Nematoda: Thelaziidae) from the Grasshopper Mouse, *Onychomys leucogaster* (Weid). J. Parasitol. 25: 432-433.
DOBSON, G. E. 1880. An annelidan entozoon. Nature. 22: 583.
DOLLFUS, R. P., and C. DESPORTES. 1945. Sur le genre *Rictularia* Froelich 1802 (Nematodes, Spiruroidea). Ann. Parasitol. Hum. et Comp. 30: 6-34.
GALLI-VALERIO, B. 1932. Notes de parasitologie et de technique parasitologique. Zentralbl. Bakteriol. I. Abt. Orig. 125: 135, 137.
JAEGERSKIOLD, L. A. 1909. Nematoden aus Aegypten und dem Sudan (eingesammelt von der Schwedischen Zoologeschen Expedition). Results of the Swedish Zoological Expedition to Egypt and the White Nile, 1901, under the direction of L. A. Jaegerskiold. Part 3, No. 25: 1-45. K. W. Appelberg, Uppsala.
LEROUX, P. L. 1930. A new nematode (*Rictularia aethechini*, sp. nov.), a *Physaloptera* and an Acanthocephala from the Hedgehog (*Aethechinus frontalis*). 16 Rep. Director Vet. Serv., Dept. Agric. Union South Africa, Aug., 217-232.
MACDONALD, J. D. 1880. On the anatomy of a new parasitic worm found in the intestine of a bat (*Megaderma frons*). Ann. Mag. Nat. Hist. 6: 400-411.
MCPHERSON, S. E., and J. D. TINER. 1952. A new nematode (*Rictularia microti*) from a Vole on St. Lawrence Island, Alaska. Nat. Hist. Misc., Chicago Academy of Sciences. 108: 1-7.
SANDGROUND, J. H. 1935. *Spirura michiganensis* n. sp. and *Rictularia halli* n. sp., two new parasitic nematodes from *Eutamias straitus lysteri* (Richardson). Trans. Am. Microsc. Soc. 54: 155-166.
SEURAT, L. G. 1915. Sur les *Rictulares* des Carnivores du Nord-Africain et les affinites du genre *Rictularia*. Compt. Rendu. Soc. Biol. 78: 320.
TINER, J. D. 1948a. *Rictularia dipodomis* n. sp. (Nematoda: Thelaziidae) from the kangaroo rat *Dipodomys* sp. J. Parasitol. 34: 1-4.
———. 1948b. Observations on the *Rictularia* (Nematoda: Thelaziidae) of North America. Trans. Am. Miscrosc. Soc. 67: 192-200.
TROMBA, F. G., and F. W. DOUVRES. 1953. A modified *en face* technique. Proc. Helm. Soc. Wash. 20: 59.
TUBANGUI, M. A. 1931. Worm parasites of the brown rat, *Mus norvegicus*, in the Philippine Islands, with special reference to those forms that may be transmitted to human beings. Phillip. J. Sc. Manila. 46: 579-581.

Tylenchorhynchus martini, A New Nematode Species Found in the Sugarcane and Rice Fields of Louisiana and Texas

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A nematode belonging to the genus *Tylenchorhynchus* has been found to be widespread in the sugarcane and rice soils of Louisiana, and in the rice soils of Texas. This nematode is herein described as *Tylenchorhynchus martini* n. sp. The host range of this nematode is unkown; in greenhouse studies, however, it has been observed to propagate readily on sugarcane hybrids (*Saccharum officinarum* L.), rice (*Oryza sativa* L.) and dallis grass (*Paspalum dilatatum* Poir.)

MEASUREMENT: Female: total length = 0.75 mm; a = 31, b = 5; c = 13.8; v = ^{26} 54 ^{21}.

DESCRIPTION: The lip region bears three annules and is set off by a

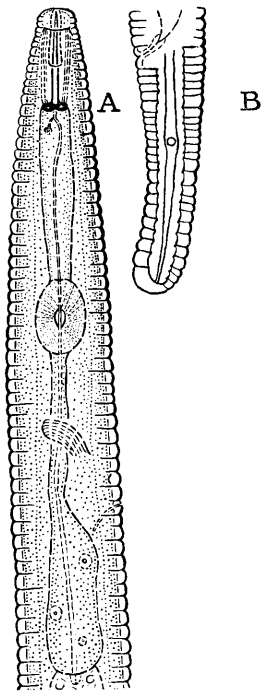

Fig. 1. *Tylenchorhynchus martini* n. sp. A. Anterior portion of female; X 675. B. Female tail; X 675.

slight construction. Cuticle marked by conspicuous annules which average 1.9 μ apart at mid-body, and are slightly smaller near the head. The annules on the tail are variable in size. Female terminus blunt, tail shape distinctive (Fig. 1-B). Phasmids prominent, located slightly anterior to middle of tail. Cervical papillae (deirids) not observed. Lateral fields marked by four lines, anteriorly beginning in region of buccal stylet knobs, and ending near caudal terminus, and are 1/4 as wide as body width at mid-body. Annulation interrupted at lateral fields as illustrated (Fig. 1-B). Buccal stylet about 19 μ long, slender, with strong basal knobs. Dorsal esophageal gland opening near base of spear. Median esophageal bulb ovoid, with a conspicuous crescent-shaped valve. Nerve ring encircling isthmus of esophagus near the middle. Basal portion of esophagus forming a distinct bulb bearing three gland nuclei. Excretory pore located near the proximal end of the basal esophageal bulb. Hemizonid located two body annules anterior to excretory pore.

Vulva a transverse slit. Vagina extending inward 1/3 the distance across the body. Ovaries paired, outstretched, oögonia forming a single line. Eggs 1/2 as wide and twice as long as body width.

Males not observed. Among 80,000 specimens examined not a single male was seen.

DIAGNOSIS: Resembled *Tylenchorhynchus claytoni* Steiner 1937, but different because of simple body annulations, shape of female tail, and presence of a slight construction setting off lip region. Males absent.

TYPE LOCALITY: Laplace, Louisiana.

TYPE HOST: Collected about the roots of sugarcane hybrid (*Saccharum officinarum* L.) by Dr. Weston J. Martin, May 28, 1953 and is named in his honor.

The Dual Antibody Response to Experimental Trichinosis*

ROBERT M. CHUTE**

The initial purpose of this investigation was the re-examination and further elaboration of the reported differential response of adult and larval *Trichinella spiralis* to immune sera. However, during the course of the present investigation, no differences which were wholly consistent with the postulate of qualitative antigenic differences between adults and larvae were demonstrated. Therefore, the study was continued on the assumption that the antigens involved in the development of protective immunity were probably present in both stages of the parasite. The investigation was then directed toward a study of the manifestation of immunity, within the body of the host, against the parasite in both the adult, or intestinal, phase and the larval, or muscle phase.

Oliver-Gonzalez (1940, 1941) reported that adult worms placed in the sera of infected rabbits first developed precipitates at the body openings or in the medium in sera taken from rabbits 15 days after infection. The percentage of adult worms developing precipitates at their body openings reached a peak in sera taken 25 to 35 days after infection; then dropped to zero on the 50th day. Precipitates around adults again occurred in sera taken after reinfection. When larvae were tested in the same sera, precipitates were first observed in sera taken 30 days after the preliminay infection. The percentage of larvae having precipitates at their body openings reached a peak in the 50 day sera and remained relatively constant in sera taken at intervals until the end of the observation period, 120 days after infection. The percentage of larvae developing precipitate did not vary appreciably during a series of reinfections. He reported that the capacity of a serum to react with larvae could be removed by adsorping the serum with powdered larvae. Changes in the precipitin titer, using a Coca's solution extract of powdered larvae, paralleled the percentage of larvae showing precipitate at the body openings. Antigen prepared in a similar manner from powdered adults showed no activity. The per cent of adult and larval worms which died when exposed to immune sera for four days appeared to be correlated with the per cent of the same stage of the parasite which showed precipitate at the body openings in the same sera.

In a study of the effect of a low protein diet on the development of immunity to trichinosis in rats, Taliaferro, Woolridge, and Benditt (1940) state that the two antibodies described by Oliver-Gonzalez were differentially affected. A low protein diet 7 days before the infection and during the infection lowered the antilarval but not the antiadult antibody. This differential response was attributed to the fact that the adults liberate their antigen earlier in the course of the infection than do the larvae. A low protein diet started 33 days before infection was reported to lower both antibodies.

Hendricks (1952) subjected mice to from 1 to 6 immunizing infections, at three day intervals, with normal or irradiated larvae, followed by a challenge infection. Mice receiving 2 or more infections with irradiated larvae

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showed an increasing degree of resistance to reinfection. Infections with normal larvae resulted in a slightly higher degree of resistance. The precipitin titer of sera drawn from mice receiving immunizing infections was correlated with the number of infections. Hendricks also reported that, when irradiated larvae were used for the immunizing infections, the antibody titer against antigen prepared from adult worms arose to a significantly higher level than the titer against antigen prepared from larval worms. When normal larvae were used for the immunizing infections this difference was not observed. The difference in resistance and titer in the two groups was attributed to the lesser degree of stimulation by larval antigen in the animals infected with irradiated larvae.

Campbell (1954) collected secretions and excretions of larvae in a nutrient fluid. Mice immunized with such secretions and excretions were reported to lose adult worms from the intestine more rapidly and fewer larvae developed than in controls. Campbell suggested that the lowered larval recovery was due to stunting.

MATERIALS AND METHODS

The strain of *T. spiralis* utilized has been maintained for over 25 years in this laboratory, chiefly in pied rats. Two strains of rats were used, pied rats from this laboratory and albino rats of the Wistar strain. Rats were 30 to 60 days old and weighed 70 to 110 grams at the beginning of an experiment. Experimental and control groups were made up by equal representation of rats from two or more litters from the same stock which had been born within 7 days of one another.

Larvae were recovered from host muscle by artificial digestion and counted by the dilution method on a Scott Hookworm counting slide (Scott, 1928). Adult worms were recovered by placing slit segments of intestine of infected rats on the screen of a modified Baermann apparatus (Cort et al., 1922) filled with 0.85 NaCl at 37° C. Adults were counted by dilution methods in a small petri dish with a cross-ruled bottom. Worms were measured by determining the length of their camera lucida image with a map-measure. Before measuring, the worms were immobilized by chilling in a refrigerator at 8° C. Worms were administered to animals *per os,* using a blunted 16 or 18 gauge hypodermic needle as a stomach tube.

Worms used for the in vitro test or for the preparation of antigen were washed repeatedly with sterile saline. Larvae were then washed in 2 changes of 20 minutes each in 1:10,000 methiolate. Adults were given two similar washings of 10 minutes each.

The antigen referred to as secretions and excretions of larvae was prepared by incubating living larvae in sterile saline solution for 6 days at room temperature. The saline contained 400 units of penicillin and 40 mgs. of streptomycin per cc. Twenty thousand larvae were used per cc. of saline. At the end of the incubation period the preparation was centrifuged and the supernatant stored in the freezing compartment of the refrigerator. The larvae to be used as whole worm antigen were washed, treated with merthiolate and stored frozen.

Rats, rabbits, and guinea pigs were bled by cardiac puncture. Serum was separated from clotted blood and stored frozen. Sufficient 1:1,000 merthiolate was added to bring the final concentration of merthiolate to 1:10,000. Serum used for in vitro tests was used unheated the day after drawing. Serum used for passive transfer was inactivated by heating at 56° C. for 30 minutes.

In vitro tests were conducted by placing a small drop of saline containing 50 to 80 larvae or 5 to 10 adults in a paraffin ring on a slide and adding three drops of serum. The preparations were sealed with a cover glass and melted paraffin. The suspension from which the worms were taken for the in vitro tests contained 400 units of penicillin and 40 mgs. of streptomycin per cc. The slides were numbered at the time of preparation. The numbers were then concealed with tape, the slides mixed and the tapes renumbered. The tests were left for 18 to 20 hours at room temperature, then read and decoded. The results were recorded as the per cent showing precipitates; not less than 50 larvae or less than 40 adults were examined.

RESULTS

PRELIMINARY OBSERVATIONS. In vitro tests with adult and larval *T. spiralis* were made in the sera of 45 rats in which stock infections were carried. The length of the infection in days varied from 2 to 300 and the infecting dose varied from 1,000 to 10,000 larvae. The results of these tests indicated that in the sera of infected rats precipitates were formed around both adult and larval worms as long as 300 days after infection and that there was no interval beyond the 30th day after infection when tests with either adult or larval worms were not positive. There was a suggestion, nevertheless, that adult worms reacted with sera more frequently than larvae, early in the course of the infection.

IN VITRO TESTS DURING INITIAL INFECTION. Since no distinct difference between the reaction of adults and larvae to immune sera were observed in the preliminary tests, an experiment was undertaken in which the conditions more closely approached those of the original investigations of Oliver-Gonzalez. Nine rabbits were divided into three groups of three, infected, and their sera tested with both adults and larvae. Three rabbits were infected with 10,000, 20,000, and 30,000 larvae respectively (group 1), three were infected with 10,000 larvae each (group 2), and three were infected with 80,000 larvae each (group 3). All the infected rabbits, together with 2 uninfected controls, were bled on the day of infection and at intervals thereafter up to 120 days.

The results shown in Table 1 are the average results of the tests of the sera of the 3 rabbits in each group and of the sera of the two uninfected rabbits. In immune sera, drawn from the 30th day after infection, oral precipitates were observed in all adult and larval tests, with only two exceptions. Negative larval tests were obtained in the sera of one rabbit in group 1 on the 100th and 120th day. In immune sera drawn up to and including the 30th day after infection, more adult than larval tests were positive and the per cent of worms which showed oral precipitate was generally higher in the adult tests. In immune sera drawn from the 50th day after infection, the per cent of worms which showed oral precipitate was higher for adults than for larvae in 13 of 19 pairs of tests. In 11 of 54 tests of normal sera (sera drawn from uninfected rabbits or drawn from rabbits at the time of infection) worms were observed with oral precipitate. The mature of the material at the oral openings of these worms was not determined, but it appeared similar to that seen in immune sera.

IN VITRO TESTS FOLLOWING REINFECTION. Six rats and 3 rabbits which had been originally infected for other purposes were utilized to study the response of the in vitro test to reinfection. Since these animals were not infected initially for this purpose, no control animals were available. The six rats received an initial infection of 1,500 larvae each, followed by re-

TABLE 1.—The Percentages of Adult and Larval *T. spiralis** showing oral Precipitate on the Indicated Days after Infection with *T. spiralis* Larvae.

Rabbit Group Number	Infecting Dose	Stage When Tested	Days After Infection													
			0	5	10	15	20	25	30	40	50	60	70	80	100	120
1	10 to 30,000	Adult	1		12		10		9	11	39	24	46		86	16
		Larvae	0		5		7		7	62	84	75	44		3	7
2	10,000	Adult	1	0			49		63	70	85	84		67		
		Larvae	1	3			4		30	29	48	13		20		
3	80,000	Adult	0	0		12		48		83		83				
		Larvae	0	0		4		43		65		14				
Uninfected Control	None	Adult	0		0		4		0	0	2	0	2		0	0
		Larvae	0		0		0		2	0	2	2	0		2	0
Uninfected Control	None	Adult	0	12			0		0	0	0	0	0			
		Larvae	0	0			0		0	0	0	0	0			

*50 larvae or 40-60 adults observed for each test.

infection with the same number of larvae on the 80th and 90th days. They were bled just before each infection and twice more after the last infection. At each bleeding a pool of equal volumes of serum from each animal was made. These five lots of pooled sera were tested after the last bleeding with the same populations of adults and larvae.

The 3 rabbits were given an initial infection of 10,000 larvae each and one reinfecting dose of 20,000 larvae 130 days later. The rabbits were bled and their sera pooled on the day of infection and on the 120th, 130th and 140th day thereafter. These four lots of sera were tested after the last bleeding with the same populations of adults and larvae.

The results of the in vitro tests of the sera of both the rats and rabbits are shown in Table 2. In the sera of both groups of animals the percentage of both adult and larval worms which showed oral precipitate increased after reinfection. The increase was greater for the adults in both cases. The greater and more rapid increase of the percentage of adults showing precipitate following reinfection is in accord with the majority of the observations made during the course of primary infections, in which generally a higher percentage of adults than larvae reacted.

IN VITRO TESTS FOLLOWING INJECTION OF ANTIGEN. Two types of larval antigen preparations were used in an attempt to stimulate, in rabbits, the antibody or antibodies involved in the in vitro test. One rabbit received whole larvae which had been killed by freezing, another received a saline solution of the secretions and excretions of larvae. A third rabbit (the uninfected control) received injections of a control solution containing saline, merthiolate, penicillin and streptomycin in the same proportions used in preparing the antigens. The fourth rabbit (the infected control) was given an infecting dose of 20,000 larvae.

Three cubic centimeters of the two types of antigens and the control solution were administered by subcutaneous injection on days 0, 3, 5, 7, 9, 14 and 104. A second lot of antigen and control solution was prepared for the final injection. Blood was drawn for testing on days 19, 104, and 109. After each bleeding, tests were carried out with both adult and larval worms. Serum from the infected control was collected 30 days after infection, stored, and

TABLE 2.—The Percentages of Adult and Larval *T. spiralis** showing Oral precipitate in the Pooled Sera of Rats and Rabbits Before and After Reinfection

Days from initial infection	Treatment	Per cent of adults showing oral precipitate	Per cent of larvae showing oral precipitate
	Pooled Rat Sera		
0	Initial infection: 1,500 larvae	0	0
80	1st reinfection 1,500 larvae	10	2
90	2nd reinfection 1,500 larvae	98	4
100	----------	100	22
120	----------	64	0
	Pooled Rabbit Sera		
0	Initial infection: 10,000 larvae	2	0
120	----------	25	8
130	reinfection 20,000 larvae	10	10
140	----------	52	28

*50 larvae or 40-60 adults observed for each test.

used as a positive control for each of the three sets of tests. Both antigen preparations stimulated the in vitro response to both adults and larvae (table 3). There was no difinite demonstration that the reaction was more pronounced against one stage of the parasite than the other. In general, however, more adults than larvae did react.

The observations reported above concerning the in vitro test during the course of infection, following reinfection, and following the injection of antigens of larval origin are distinctly different from those reported by Oliver-Gonzalez (1940, 1941). Since he carried out his in vitro tests at 37° C. rather than at room temperature and in the absence of preservatives or antibiotics, a direct comparison was set up. Duplicate tests were carried out at room temperature and at 37° C., in the presence and absence of antibiotics and merthiolate, in heated and unheated immune sera, and in the presence and absence of fresh and heat-inactivated guinea pig sera. Tests were also conducted to determine the possible effect of variations in serum dilution and the number of worms per test cell. None of these experimental conditions resulted in variations greater than were observed between duplicate tests carried out under uniform conditions.

TABLE 3.—The Percentages of Adult and Larval *T. spiralis** showing Oral Precipitate in the Sera of Rabbits following Injections of Antigens.

Rabbit Numbers	Antigen injected	Day of bleeding (from initial infection or injection)	Per cent of adults showing oral precipitate	Per cent of larvae showing oral precipitate
5	Secretions and excretions of larvae	19	96	68
		104	0	30
		109	94	92
6	Killed whole larvae	19	89	98
		104	5	40
		109	100	74
7	Uninfected control	19	6	0
		104	0	0
		109	0	0
3	Infected control	30	56	16
		30	66	36
		30	42	24

*50 larvae or 40-60 adults observed for each test.

THE IMMUNE RESPONSE AGAINST THE PARASITE IN VIVO. Since the in vitro test did not provide a definitive answer concerning the existence of separate adult and larval antigens, experiments were designed to determine the effect of immunity upon both stages of the parasite *in vivo*.

a. *Protective Immunization with Larval Antigens.* Two types of larval antigens, secretions and excretions of living larvae and killed whole larvae, were used to immunize rats. The immunized rats were given challenge infections and the adult or larval worm recoveries were compared with control groups.

In the first experiment, 15 rats were divided into 3 groups of 5 each. One group received 6 subcutaneous injections of 0.3 cc. of larval secretions and excretions on the 1st, 3rd, 5th, 7th, 10th, and 14th day. The second group received similar injections of 30,000 whole, killed larvae at the same intervals. The third group received a similar series of injections of a solution of suspending and preservative agents in physiological saline.

Five days after the last injection all 15 rats were given a challenge infection of 1,000 *T. spiralis* larvae. On the 5th day after the challenge infection all rats were killed and the number of adult worms in the small intestine of each rat was determined. The results of this experiment are shown in Table 4. Students "t" test was used to determine the significance of the difference between the means. The mean number of adults (58.2) recovered from the rats which were immunized with killed whole larvae was unquestionably significantly lower than that (196) of control rats (p less than 0.01). Thus immunization with whole killed larvae had clearly had an adverse effect upon the intestinal phase of the infection, affecting the adult worms, the developing stages preceding the adults, or both. There is also an indication that immunization with secretions and excretions of larvae had a similar effect. However, the adults (123) recovered from such immunized rats were not unquestionably fewer than in the controls (196) since p is greater than 0.05.

TABLE 4.—The Numbers of Adult *T. spiralis* Recovered from the Small Intestine of Rats which had received Subcutaneous Injections of Killed whole larvae or the Secretions and Excretions of Larvae in comparison with those from rats receiving only a Solution of the Suspending and Preservative Agents.

	The numbers of adult *T. spiralis* recovered from rats receiving:		
	Killed whole larvae	Secretions and excretions of larvae	Suspending and preservative agents
	No. adults recovered	No. adults recovered	No. adults recovered
	55	50	250
	45	96	300
	100	100	190
	66	260	110
	25	108	130
Totals	291	614	980
Means	58	123	196
SE_m	12	36	34

In the second experiment 10 rats were divided into two groups. Five rats were given a series of injections of secretions and excretions following the same procedure outlined in the previous experiment. A control group of five rats were given similar injections of physiological saline, merthiolate and antibiotics.

Ten days after the last injection a challenge infection of 650 larvae was administered to both groups. Thirty-five days after the infection all rats were killed and larvae recovered from their muscles by artificial digestion. The mean number of larvae recovered from immunized rats (table 5) was significantly lower than for the controls (p less than 0.01). The protective immunity demonstrated by the lowered larval recoveries could be a measure of the effect of the immune response on the adults from which these larvae came, the effect upon the larvae themselves, or a combination of both.

b. *The Effect of Passive Immunization on Developing Larvae.* In an attempt to determine whether or not the developing larvae were directly affected by the immune response, hyperimmune sera from rabbits was injected into infected rats after the intestinal phase of the infection was essentially complete but before the larvae had completed their development in the mus-

TABLE 5.—The Numbers of Larvae Recovered from Rats Receiving Subcutaneous Injections of the Secretions and Excretions of *T. spiralis* Larvae in comparison with those from rats receiving only a Solution of Suspending and Preservative Agents.

	Secretions and excretions of larvae	Merthiolate and antibiotic solution
	Number of larvae recovered	Number of larvae recovered
	4,500	20,000
	11,300	58,000
	9,600	20,600
	33,600	19,500
	10,800	39,700
Totals	69,800	157,800
Means	13,960	31,560
SE_m	118	157
Mean recovery ratio*	21.5	48.5

*Number of larvae recovered/number of larvae administered (650).

cles. The hyperimmune sera were obtained from rabbits 5 days after a stimulating infection with 10,000 larvae; the rabbits had previously been immunized by several infections beginning 80 to 200 days earlier. The pooled sera of six rabbits were used.

Twenty rats were given a single infection with 2000 to 2500 larvae each. Six were selected at random for sacrifice, two each on the 14th, 16th, and 20th days, as indices of the adult infections at those times. The two killed on the fourteenth day yielded 450* and 12 worms; one on the 16th day yielded 10 worms. No adult worms were found in the other rat killed on the 16th day or in either of the two killed on the 20th day. Seven of the remaining 14 rats received the hyperimmune rabbit serum and seven received normal rabbit serum. Three of each group received five doses of the respective sera on alternate days, from the 16th to the 24th day after infection; four from each group received four doses from the 20th to the 26th day after infection. All fourteen were killed 35 days after infection. The number of larvae recovered from those receiving immune sera and those receiving the normal sera was not significantly different (means of 219,000 and 178,000). Larvae recovered from the two groups did differ in size, however (table 6). A comparison of these mean measurements (7.9 cm. and 8.7 cm.) by Student "t" test gave a value for p of about 0.05.

DISCUSSION

Since Ducas (1921) first demonstrated immunity to trichinosis as a result of previous infection and concluded that the immunity was directed primarily against the adult worms, there has been increasing acceptance of this concept. McCoy (1940) found that in an immune rat a large portion of the worms of a challenge infection were lost between the fourth and eighteenth hour after infection. Culbertson and Kaplan (1938) stressed the point that the reduced number of larvae developing in an immunized rat could be explained by a reduced number of adult maturing in the intestine. Since some larvae did develop in the muscles of immunized rats, they argued that there was no evidence that the immune process directly affected the larvae develop-

*Over 100 of these appeared to be degenerating.

TABLE 6.—The Mean Length of the Camera Lucida Images* of *T. spiralis* Larvae from Rats Receiving Injections of Normal or Immune Rabbit Sera.

Immune Sera		Normal Sera	
Nos. larvae counted	Mean length of image (cm.)	Nos. larvae counted	Mean length of image (cm.)
Rats receiving 5 injections from 16th to the 24th day:			
25	7.7	25	8.4
25	7.2	25	8.1
25	9.3	25	8.9
25	7.3	25	7.9
Rats receiving 4 injections from the 20th to the 26th day:			
33	8.8	34	8.1
33	7.3	33	9.0
34	7.7	33	9.1
means	7.9		8.7
SE_m	0.09		0.05

*Scale: 1 cm. = 0.13 mm. worm length.

ing in the tissues. It should be pointed out, however, that even if all stages of the worm are equally affected by the immune response, the adults in the intestine may be lost from the host more easily than the larvae in the muscles and any response against the larvae might not be reflected in worm recovery data.

A number of workers (Anderson and Leonard, 1940; Levine and Evans, 1942; Roth, 1942; and Hendricks, 1952), using either a single sex or sterilizing the infective larvae with X-ray, were able to establish infections with adults in the intestine without production of larvae and have shown that such infections induced immunity against subsequent infection. The report of two distinct antibodies in the serum, an anti-adult antibody and an anti-larval antibody (Oliver-Gonzalez, 1941) is commonly interpreted as supporting the previous evidence that immunity is directed primarily, if not exclusively, against developing or mature adult worms in the intestine.

In the present study evidence was obtained which indicates that serum antibody has a direct effect upon the larvae. They were not reduced in number, but their growth was retarded. Since passive immunization was used and innoculations were initiated on the 16th and 20th day of infection, when some larvae may have been fully developed, it is probable that the procedure minimized rather than exaggerated the effect of immunization on the larvae. This does not deny that immunity has a powerful effect upon adults in the intestine. It does, however, suggest that there is a direct effect upon the larvae in the tissue, that hitherto this has been overlooked for the most part, and that more refined technics may show the effect to be more powerful than demonstrated here.

These studies have failed to confirm the previous indications of qualitatively different antibodies against adults and larvae. The results do not deny that the adults and larvae are antigenically different. However, the results of these studies and those of others (Oliver-Gonzalez, 1940, 1941; Taliaferro, Woodridge, and Benditt, 1940; and Hendricks, 1952) are amenable to the interpretation that the antigenic differences of the adults and larvae are quantitative. It is suggested that the rapidly developing adults in the intestine elaborate more antigen than do the migrating and developing larvae. It is of interest in this connection that Ross (1952) reported a markedly dif-

ferent serum antibody picture than previously reported by Oliver-Gonzalez. She found that during primary infections the titer against larval antigens rose more rapidly and was consistently higher than the titer against the adult antigens. She points out the difficulty in preparing pure adult antigens since mature females contain larvae in utero.

Thorson (1953) has found that the secretions of the third stage larvae of *Nippostrongylus muris* are antigenic. The present study has confirmed the antigenicity of the secretions or excretions of trichina larvae, reported by Campbell (1954, 1955). Perhaps the nature of the antigenic differences of adult and larval trichina can be clarified by a more detailed study of these excretions and secretions of the several stages of the worms.

SUMMARY

1. Excretions and secretions of trichina larvae are shown to be antigenic and capable of producing immunity.

2. Evidence is also presented which indicates that the immune response acts directly upon the larvae during migration or development in the muscles as well as upon the intestinal stages.

3. It is suggested that the "dual antibody" basis of immunity in trichinosis may be a reflection of quantitative, rather than qualitative, antigenic differences in the two stages.

LITERATURE CITED

ANDERSON, C. V. and LEONARD, A. B. 1940. Immunity produced in rats by the intestinal phase of Trichinella infections. J. Parasitol. 26 (suppl.), 42-43.

CAMPBELL, C. H. 1954. The antigenic role of excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. J. Parasitol. 40 (sec. 2), 18.

———. 1955. The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. J. Parasitol. 41: 483-491.

CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L., and PAYNE, F. K. 1922. Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil. Amer. Jour. Hyg., 2, 1-16.

CULBERTSON, J. T. and KAPLAN, S. S. 1938. A study upon passive immunity in experimental trichinosis. Parasitol. 30, 156-166.

DUCAS, ROBERT. 1921. L'Immunite dans la Trichinose. Jouve and Cie., Editerus, Paris, 47 pp.

HENDRICKS, J. R. 1952. Studies in mice on the dual antibody basis of host resistance to *Trichinella spiralis*. J. Elisha Mitchell Sci. Soc. 68, 12-26.

LEVIN, A. J., and EVANS, T. C. 1942. The use of roentgen radiation in locating an origin of host resistance to *Trichinella spiralis* infections. J. Parasitol. 28, 477-483.

MCCOY, O. R. 1940. Rapid loss of *Trichinella* larvae fed to immune rats and its bearing on the mechanism of immunity. Am. J. Hyg. 32, 105-116.

OLIVER-GONZALEZ, JOSE, 1941. The dual antibody basis of acquired immunity in trichinosis. J. Infectious Diseases 69, 254-270.

———. 1940. The in vitro action of immune serum on the larvae and adults of *Trichinella spiralis*. *Ibid.*, 67, 292-300.

ROSS, WINIFRED. 1952. A preliminary comparative study of test antigens prepared from adult and larval forms of *Trichinella spiralis*. Canadian J. Med. Sci. 20, 334-342.

ROTH, HANS. 1943. The role of the intestinal phase of trichina infection in the establishment of immunity to reinfection. Am. J. Hyg. 38, 99-111.

SCOTT, J. ALLEN, 1928. An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Am. J. Hyg. 8(2), 158-204.

TALIAFERRO, W. H., WOOLRIDGE, B. L., and BENDITT, E. P., 1949. The effect of protein depletion on acquired immunity in trichinosis. Science 109, 443.

THORSON, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode, *Nippostrongylus muris*. Am. J. Hyg. 58, 1-15.

The Attractiveness of Plants to Larvae of Root-Knot Nematodes. II. The Effect of Excised Bean, Eggplant, and Soybean Roots on *Meloidogyne hapla* Chitwood

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In a previous paper (Wieser 1955) it was shown that the excised distal portion of the tomato root consists of 3 regions with respect to its effect on larvae of *M. hapla*. The following pattern of this root portion was found: apical 2 mm (calyptra and meristematic region) repellent: following 6 mm (region of elongation) attractive; remainder, up to 16 mm behind the root apex, neutral or slightly repellent. The question arose whether the roots of other host plants show a similar pattern of repellent, attractive and neutral regions. In order to find this out a series of experiments was undertaken with plants known to be favored hosts of *M. hapla*. Only excised roots were tested and the experimental procedure employed was exactly the same as that described in the previous paper. It need only to be recalled that the experiments are based on offering nematodes a root bit in one half a sand-filled dish while the other half is devoid of any root. In the following diagrams a high percentage signifies attractiveness and a consistently low percentage repellency of the tested root bits to the nematodes.

EXPERIMENTS

1) BEAN, (*Phaseolus vulgaris* L.), VAR. PINTO. It is well known that beans are very susceptible to *M. hapla*, but to be on the safe side I planted the variety Pinto in soil infested with *M. hapla* and after 6 weeks found the plants heavily infected with this nematode. This made certain that the plants used for the following experiments were good hosts of *M. hapla* when growing normally.

The experiments with excised root ends, however, yielded quite unexpected results (Fig. 1). It appeared that root bits measuring 1 to 16 mm in length (measured from the apex of the root) were not attractive to the nematodes. That is, of all the nematodes hatched only an average of 30% was found in that half of the dish which contained the roots. Almost all samples were below the 50%-level. This shows a decidedly repellent effect of the distal portion of the roots on the nematodes. The length of the tested piece of root had no influence on the effect.

2) GARDEN EGGPLANT, (*Solanum melongena* L.) VAR. BLACK BEAUTY. This variety was shown to be very susceptible to *M. hapla* by Sasser (1954). The experiments with excised distal portions of the roots (Fig. 2) revealed an enormous variation among the individual samples. The variation is random and shows no obvious correlation between the length of the tested root and its effect on the nematodes. It is uncertain whether this great variability has its cause in the method employed or is actually based on physiological differences between the roots. If the former is true then the excised roots of the eggplant must be regarded as neutral in their effect on *M. hapla* since the axis of the range of variability is the 50% (that is, the 'neutral') level; if the latter is true then the variation in the effect on *M. hapla* would reflect an

interplay between attractive and repellent factors in the roots, of which sometimes the attractive, sometimes the repellent, factor would dominate.

3) An effect similar to that of the eggplant is displayed by the soybean (*Glycine max* (L.) Merr.) Var. Bansei. In this plant also the distal portions of the roots show an enormous variation in their effect on the larvae of *M. hapla*.

In order to find out whether this variation is purely statistical and inherent in the method employed I ran a few control tests without any roots in the dishes. It turned out that the distribution of the nematode in the two dish-halves varied considerably, but apparently not as much as in the experiments with the eggplant and soybean roots. However, I did not make a thorough statistical analysis of the case since it is irrelevant for my purpose whether the root bits are neutral to the nematodes or display an effect which varies between all degrees of attractiveness and repellency, but always in a random fashion with respect to the 50% level. This will be explained below.

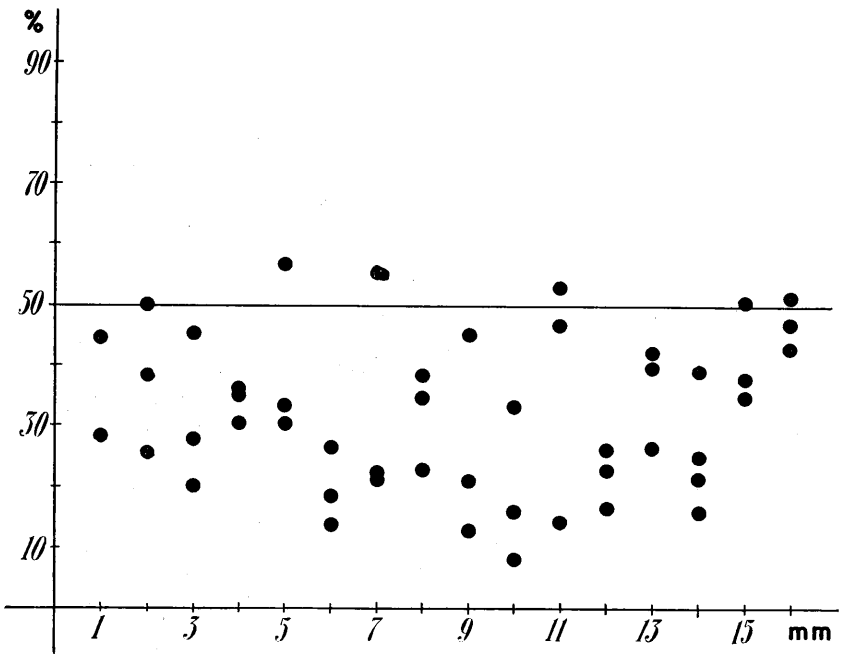

Fig. 1. Effect of excised bean root ends on larvae of *M. hapla*. Abscissa: length of excised root end. Ordinate: Percentage of nematodes found in half of dish occuppied by seedling. Each dot one sample.

DISCUSSION

If we survey the facts so far put forth it seems that in excised roots of host plants of *M. hapla* we have to consider the presence of two factors, one attractive and the other repellent. The attractive factor is present in the region of elongation of excised tomato roots for at least 24 hours after they have been severed from the plant. It also seems to be present in excised eggplant and soybean roots, though in a more or less random fashion. The repellent factor was discovered in the apical 2 mm of excised tomato roots

(Wieser 1955); it seems to be present in the eggplant and soybean roots, also in a random fashion, and it is the dominant factor in excised bean roots. It can be assumed that these two factors are of quite different origin. Since we are dealing with host plants of *M. hapla* there can hardly be any doubt that the attractive factor is the one present in the living plant. If a repellent agent is excreted by the living roots then it could be only in such small amounts that its effect is wholly superseded by that of the attractive agent. It is much more reasonable to assume that the repellent factor comes into play only after the roots have been severed from the plant, that is, with the death of some or all root cells, and with the onset of decay of the root tissues (since the experiments were not undertaken under sterile conditions).

Since the attractive factor was still apparent in tomato roots 24 hours after excision, while it was not in beans, we might assume that in the latter plant it broke down more rapidly, or was present in smaller amounts, than in the former.

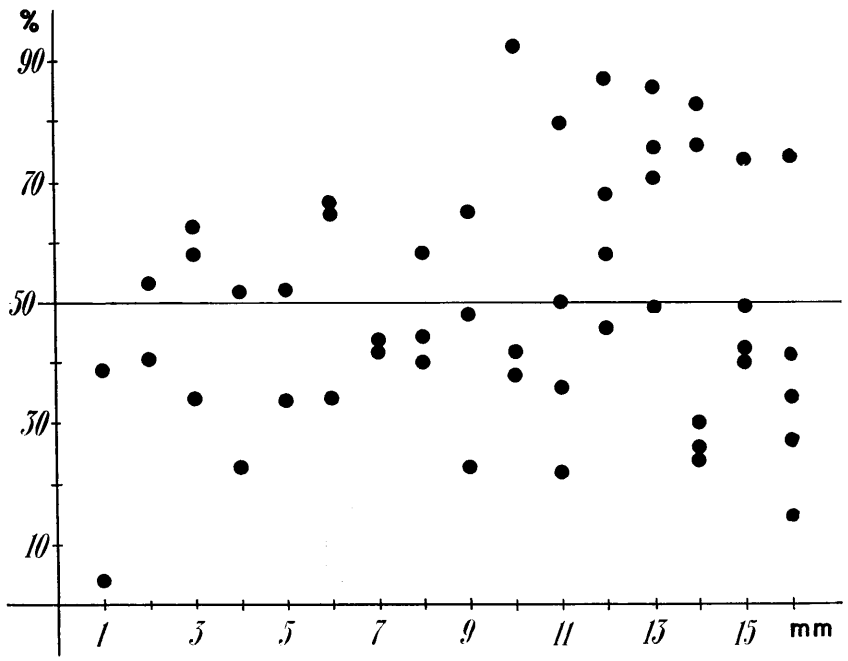

Fig. 2. Effect of excised eggplant root ends on larvae of *M. hapla*. Abscissa and Ordinate: as in Fig. 1.

As pointed out above the occurrence of the repellent agent followed a different pattern in the excised roots of each tested plant. One theory to explain these differences would be that the mode of liberation of the repellent agent actually follows a different pattern in the four plants. However, it seems more likely that the agent is produced in the same way in all the plants, but that it is superseded to a different degree and in a different manner by the attractive substance according to the distribution and the amount of the latter in the excised roots.

Thus we arrive at a picture of an interplay between attractive and repellent factors which at the end of 24 hours has reached different stages in excised roots of tomato, eggplant, soybean and bean. This situation can schematically be characterized as in Fig. 3. It must be stressed that this is a schema which shows only one aspect of the possible interplay between the two factors involved. Many modifications of the general pattern are conceivable. First of all I make the assumption that the attractive agent (a) is the same in all plants—which almost certainly is not the case. Second, I assume that the repellent agent (r) is of the same intensity throughout the experiment—which again is very unlikely. However, both modifications, the occurrence of more than one kind of attractive agent, and the change in intensity of the repellent agent, would not invalidate the main point of my argument, i.e. that the effect of the excised roots on the nematodes depends on the respective level of intensity of the attrative agent.

In tomato (T), more precisely, in the region of elongation of the tomato roots, the intensity of the attractive agent is higher than that of the repellent agent; therefore the overall effect of the roots is attractive.

In bean (B) the intensity of the attractive agent is lower than that of the repellent agent; therefore the overall effect of the roots is repellent.

The two remaining plants, eggplant (E) and soybean (S), occupy a sort of

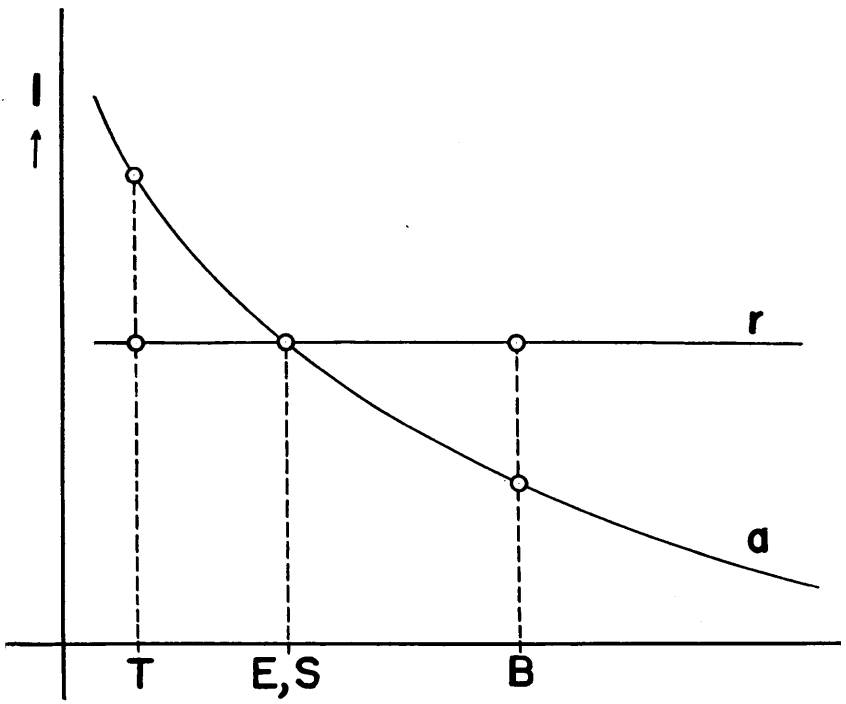

Fig. 3. Schematic representation of possible intensity levels of attractive (a) and repellent (r) agent in excised root ends of tomato (T), eggplant (E), soybean (S) and bean (B), 24 hours after excision if the presence of only one attractive agent in all four plants is assumed. Ordinate: relative intensity of agents.

intermediary position, viz. the intensities of the attractive and the repellent agent are about equal. This would result in the roots' being neutral (in which case the great variation of the dots in Fig. 2 would be entirely statistical) or they would be attractive and repellent in a random fashion according to whether the sample is—so to speak—a little to the 'left' or to the 'right' of the neutral point (in which case the variation would be caused by the actual variation of the physiological makeup of the tested roots). The distinction between these two possibilities is purely theoretical.

The variation in intensity of the attractive agent in the different roots can be expressed in terms of speed of breakdown. This would mean that, if there is only one kind of attractive agent present in the four plants, the physiological makeup of the different plants would influence the speed of breakdown of this agent; or, if there is more than one kind of attractive agent present, that each of them would break down at a different speed, that is, the one in tomato most slowly, and the one in beans most quickly. That the speed of breakdown plays a role in the effectiveness of the attractive agent is supported by the following experiment: if tomato roots are excised and left in water for 24 hours before the experiment is started, so that at the end of the experiment they have been excised for 48 hours, the roots (or rather the regions of elongation) have lost their clearcut attractive effect. Instead, they show a state of affairs which resembles that of eggplant and soybean roots 24 hours after excision, i.e. their effect varies between high attractiveness and obvious repellency. That would indicate that the tomato roots which at the end of 24 hours were at the level T of figure 3, had reached the level E, S at the end of 48 hours.

But the variation in intensity can also be expressed in terms of amount of the agent present. If there were a relatively large amount of the agent stored in the root it would—given equal speed of breakdown—disappear more slowly than if there were only little present. Applied to our case this would mean that in bean there is only a small amount of the attractive agent present, which agrees with the fact that bean is by far the fastest growing plant of the four tested and thus most likely to have only small amounts of any sort of material stored.

What the nature of the attractive and repellent agents is we do not know. I have assumed that the liberation of the repellent agent is correlated with the death of cells and the decay of root tissue or both. Whether it is due to the breakdown of chemical substances *per se,* or rather to bacterial action I do not venture to say. In a way my results are contradictory to those reached by Linford (1939), who found that excised leaf and stem tissues are attractive to root-knot nematodes. In this case one could resort to the explanation that Linford used different kinds of plant tissues (leaf and stem as against root), and probably different root-knot nematode species (in 1939 all the root-knot nematodes were still considered one species, *Heterodera marioni*). However, Gadd and Loos (1941) found that root-knot nematodes are still attracted by excised roots of *Tephrosia* and *Crotalaria*. Of the closely related *Heterodera schachtii,* Baunacke (1922) stated that the nematodes are not attracted by excised sugar beet roots, but Emanuelsson (1954) found that the latter still exert a powerful hatching stimulus on the eggs of *H. schachtii.* Thus it seems that the situation is not at all clear and the effect of excised roots and decaying tissues on the various plant-parasitic nematodes still needs closer study.

The question may be raised whether the repellent effect is not due to the

action of some 'wound substance' rather than to the breakdown of chemical compounds and the decay of plant tissues, but our knowledge as to the possible effect of this sort of substances on nematodes is so scant that the assumption should not even be raised to the status of a hypothesis.

SUMMARY

The effects of excised distal portions of the roots of bean (var. Pinto), eggplant (var. Black Beauty) and soybean (var. Bansei) on larvae of *M. hapla* were investigated. It was found that the root portions of bean had a decidedly repellent effect on the nematode. The root portions of eggplant and soybean showed a random variation of their effect, some of the roots being attractive, some repellent and some neutral.

The effect of the roots on the nematodes was interpreted as the result of an interplay between a repellent and an attractive agent, the latter being present in the living plant and the former possibly coming into play with the decay or chemical breakdown of the root. The actual effect of a given root is determined by the respective level of intensity of the repellent and the attractive agent. In tomato it is mostly the attractive, in bean the repellent, agent which is highest in intensity; therefore the overall effect is attractive in the former and repellent in the latter plant. In eggplant and soybean the levels of intensity of the attractive and the repellent agent are about equal, a condition which is responsible for the great variability of the effect of these two plants on the nematodes.

The variation in intensity of the attractive agent in the different roots can be expressed in terms of different speeds of breakdown or in terms of different amounts of the agent present before excision of the root.

LITERATURE CITED

BAUNACKE, W. 1922. Untersuchungen zur Biologie und Bekaempfung des Ruebennematoden *Heterodera schachtii* Schmidt. Arb. Biol. Reichsanst. Land-Forstwirtsch. 11, 3: 185-288.

EMANUELSSON, H. 1954. An investigation of the Sugar-Beet Eelworm, *Heterodera schachtii* (Schmidt). SOCKER, Handl. II, (Comm. Swedish Sugar Corp.) 8, 10: 101-108.

GADD, C. H. and LOOS, C. A. 1941. Host Specialization of *Anguillulina pratensis* (De Man). I. Attractiveness of Roots. Ann. Appl. Biol. 28, 4: 372-381.

LINFORD, M. B. 1939. Attractiveness of Roots and Excised Shoot Tissues to Certain Nematodes. Proc. Helm. Soc. Washington, 6: 11-18.

SASSER, J. N. 1954. Identification and Host-Parasite Relationships of Certain Root-Knot Nematodes (*Meloidogyne* spp.) Univ. Maryland, Agricult. Exp. St. Bull. A-77: 1-30.

WIESER, W. 1955. The Attractiveness of Plants to Larvae of Root-Knot Nematodes. I. The Effect of Tomato Seedlings and Excised Roots on *Meloidogyne hapla* Chitwood. Proc. Helm. Soc. Washington, 22, 2: 106-112.

Survival on Pasture of Larvae of Gastrointestinal Nematodes of Cattle

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Several studies of the survival of free-living stages of various species of nematodes parasitizing sheep have been made at the Agricultural Research Center of the U. S. Department of Agriculture, Beltsville, Maryland. That work and the results of similar studies elsewhere have been summarized by Kates (1950). Although several of the species of nematodes parasitizing sheep also occur in cattle, there appear to be strain differences within species. The species also differ in relative abundance in these two hosts, some being better adapted to one than the other. Moreover, some occurring in cattle are not harbored by sheep. The purpose of the present study was to investigate the survival on pasture, contaminated in summer, at the Agricultural Research Center, Beltsville, Maryland, of the free-living stages of the common species of gastrointestinal nematodes of cattle.

MATERIALS AND METHODS

A fenced grassy area 120 by 145 feet was used in this study. It was disked, limed, and seeded 10 months prior to being contaminated with nematode eggs. During the survival tests the ground was well covered by vegetation, which consisted of approximately 80 per cent rye-grass, 10 per cent Kentucky bluegrass, and 10 per cent clovers, grasses, and various other herbs of about 30 species. The area was shaded by 9 oak trees, each approximately 35 feet high, but not more than one-half, and usually not more than one-third, of the ground surface was in their shadow at any given time. The upper two inches of soil was a sandy loam containing a considerable amount of humus.

The grassy area was divided into three equal temporary lots. Between July 21 and August 8, 1954, each lot was contaminated successively, for 6 days, by placing on it 5 to 7 fairly heavily parasitized bovines. The reason for successive contamination of temporary lots, rather than simultaneous contamination of the entire area, was to minimize the ingestion of infective larvae by the contaminators. The feces deposited on the lots were evenly distributed by shovel. The temporary fences were then removed, and the entire area was divided into four comparable, equal plots, one-tenth of an acre each, with the fences at right angles to the former temporary ones.

The survival of nematode larvae on each of the four plots was tested by grazing on it a 3½ to 5½-month-old calf raised in a barn under conditions designed to prevent extraneous infection with helminths. Repeated fecal examinations of these calves showed them to be free of helminth parasite eggs up to the time they were placed on the test plots. A calf was placed on the first test plot 19 days after the end of the contamination period. It was allowed to graze there for two weeks and was then returned to the barn and so maintained as to prevent further infection. It remained in the barn for three weeks in order that the parasites it had acquired might have sufficient time to develop to a size which could be more readily collected, identified, and counted than larvae.

Similarly, the survival of the larvae on the second, third, and fourth plots

was tested beginning 61, 122, and 256 days, respectively, after the end of the contamination period. In all four cases the plots were closely grazed, almost all the available forage having been consumed during the test period.

At autopsy, in each case the contents of the abomasum, small intestine, and large intestine were collected separately. These organs were opened, thoroughly washed, and soaked in water several times. The small intestine was passed through the fingers several times to remove worms adhering to the mucous membrane. *Haemonchus contortus, Trichuris ovis,* and *Oesophagostomum radiatum,* because of their relatively large size and small number, were readily counted *in toto*. The numbers of other worms present were calculated from the numbers in duplicate aliquots of the sedimented contents and washings of the organ involved. After the removal of the aliquots, the remaining material was screened, using a 35 mesh screen above one with 60 meshes to the inch, to facilitate counting the larger species. These were retained by the screens, from which they were washed into clean water for counting. The few worms of the larger species recovered in the aliquots were included in the total for the respective species.

Readings of the daily air temperatures and precipitation, close to the experimental area, were taken. The maximum and minimum temperatures from the beginning of the contamination period to the first test period, and during and between test periods were noted, and the average temperature and total precipitation during each period were computed. The average temperature and total precipitation for each period were compared with the corresponding averages during an 80-year period at Washington, D. C. These weather records are presented in Table 1.

RESULTS AND DISCUSSION

Two of the contaminators died during the period of their use, and two died 11 and 13 days, respectively, after having been removed from the lots. The latter two were treated with phenothiazine after their removal from the area, and consequently the number of worms in them at autopsy was probably considerably less than it had been during the period the animals were being used as contaminators. The treatment may also account for the absence

TABLE 1.—Summary of weather data from the beginning of the contamination period to the first test period, and during and between test periods, and comparison with the normal.

Period	Dates	Temperature (Degrees F.)				Precipitation (inches)	
		During Experiment			Ave.* Wash., D.C.	During Exper.	Ave.,* Wash., D.C.
		Max.	Min.	Ave.			
Contamination to first test	July 21-Aug. 26	100	54	73.5	75.6	5.14	5.07
First Test	Aug. 27-Sept. 10	98	55	73.2	72.0	0.53	1.80
Interval between 1st and 2nd tests	Sept. 11-Oct. 7	90	35	68.0	67.7	0.92	3.02
Second Test	Oct. 8-22	89	32	58.7	57.5	1.50	1.38
Interval between 2nd and 3rd tests	Oct. 23-Dec. 7	73	16	43.0	46.5	3.67	4.08
Third Test	Dec. 8-23	53	12	31.5	36.9	1.72	1.54
Interval between 3rd and 4th tests	Dec. 24-Apr. 20	78	2	40.9	41.0	9.25	12.99
Fourth Test	Apr. 21-May 10	86	38	58.5	59.3	1.12	2.17

*80 year period.

in these animals of several species of nematodes recovered from the test calves. The following species and numbers of parasites were recovered from the four animals: *Trichostrongylus axei* 167,900; *Ostertagia ostertagi* 6,800; *Cooperia punctata* 8,640; *Trichostrongylus colubriformis* 19,630; *Cooperia oncophora* 4,300; *Cooperia* larvae 700; *Trichuris ovis* 155.

In addition to the above species, the feces of one or more of the contaminators had contained eggs of *Haemonchus contortus, Cooperia curticei, Oesophagostomum radiatum,* and *Nematodirus filicollis,* the latter two species in rather small numbers.

All of the parasites present in the contaminators, except *Nematodirus,* showed up in the first test calf. The dryness and heat of the summer may have been primarily responsible for the absence of *Nematodirus* from the first and second test calves. This genus differs from the others in that the larva develops to the infective stage within the egg and hatching occurs only under favorable conditions.

Cattle become infected with *Trichuris* by ingesting the infective, embryonated egg. In the remaining species considered here, the larva develops to the infective stage outside the egg, and it is protected by a cuticular sheath in the infective stage.

The temperature and precipitation from the beginning of the contamination period to the first test period were normal, and apparently, as evinced by the worms acquired by the first test calf, allowed a considerable number of larvae to develop to the infective stage. In spite of the precipitation being only approximately 30 per cent of average during the first test period, the test calf became heavily infected, acquiring 102,400 stomach and intestinal nematodes, so that it became sick and diarrheic. However, the above number is only approximately 0.15 per cent of the 67,500,000 eggs estimated, from weekly counts of the eggs in a composite sample of the feces of the contaminators, to have been deposited upon each of the test plots.

The number of worms acquired by the second test calf was markedly lower than that acquired by the first, the former containing only 2.2 per cent as many worms as the latter. Continued subnormal precipitation, only 55 per cent of the average, combined with the usual summer heat, from the end of the first test period to the end of the second, may have been primarily responsible for this result.

From the end of the second test period to the end of the fourth, the precipitation remained subnormal, being approximately 76 per cent of average. In contrast to the markedly fewer worms of the second test calf as compared with the first one, the second, third and fourth calves acquired similar degrees of infection, 2.2, 2.8, and 1.4 per cent, respectively, as many worms as the first calf. These results indicate that the residual contamination diminished only slightly from the beginning of the second test period to the end of the fourth. The data are recorded in Table 2.

Only *Cooperia oncophora, Ostertagia ostertagi, Nematodirus filicollis,* and *Trichuris ovis* survived the winter and were able to infect the fourth calf. The first-named species was considerably more abundant than the others.

SUMMARY AND CONCLUSIONS

The larvae of gastrointestinal nematode parasites of cattle on a Beltsville, Maryland pasture, which had been heavily contaminated in the summer, had sharply declined in number two months after the last contamination date.

TABLE 2.—Number of worms recovered from calves placed on test plots at the following periods after final date of contamination, August 8, 1954.

Parasite	19 days 8-27 to 9-10-54	61 days 10-8 to 10-22-54	122 days 12-8 to 12-23-54	256 days 4-21 to 5-10-55
Trichostrongylus axei	55,800	450	600	0
Ostertagia ostertagi	2,950	510	250	100
Haemonchus contortus	400	21	1	0
Cooperia punctata	22,450	300	100	0
Trichostrongylus colubriformis	13,500	375	0	0
Cooperia oncophora	5,060	450	1,300	1,150
Cooperia curticei	1,800	75	50	0
Cooperia larvae	200	0	100	0
Nematodirus filicollis	0	0	400	200
Trichuris ovis	246	32	40	9
Oesophagostomum radiatum	2	0	0	0

The subnormal precipitation during the test periods of this study probably accelerated the death rate of the eggs and larvae. Only four of the ten species tested survived, in rather small numbers, both the heat and dryness of the summer and the cold of the following winter.

LITERATURE CITED

KATES, K. C. 1950. Survival on pasture of free-living stages of some common gastrointestinal nematodes of sheep. Proc. Helminthol. Soc. Wash. 17(2): 39-58.

Lungworms in the Bighorn Sheep of Montana

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In response to a request by the Montana Fish and Game Commission, the lungs of 19 Bighorn Sheep were examined for lungworms by the authors during the 1954 Montana hunting season. Eighteen of these animals had been taken by hunters and were mature rams 5 to 7 years of age. Another mature ram, paralyzed in the hind quarters, was brought to the laboratory while alive. All material was obtained and brought to the laboratory by Fish and Game Commission personnel.

The sheep were taken from three geographically isolated herds. Eight lungs came from the Sun River herd in west central Montana, two from the Stillwater River herd northeast of Yellowstone National Park, and nine from the Gallatin herd northwest of the park. The only two sheep free from lungworms were from the Gallatin herd, but the samples were too small to draw any conclusions on ecological distribution of lungworms of the bighorn.

Two types of lungworm infection were encountered. In the most prevalent one, the adults were embedded in the lung tissue, and in the other the adults were found free in the bronchioles. These are termed the parenchymal

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and bronchiolar forms, respectively, and different species of *Protostrongylus* are involved.

Adults of *Protostrongylus rushi* were recovered from the bronchioles of seven specimens. The nematodes were not restricted to a specific region of the bronchioles but when large numbers, 30-50, were present, they were usually found near the tip of the diaphragmatic lobe where there was a single, large, consolidated nodule.

Lesions caused by the parenchymal lungworms were present on the surface of thirteen lungs and may have been present in the interior of others. These lesions were white and well defined usually measuring 5 to 10 millimeters in diameter, but a few as large as 22 mm. were noted. The number varied from one or two or more than thirty per pair of lungs. These lesions contained eggs, larvae, and adults. It was impossible in most cases to obtain sufficient fragments of the adults for positive identification but fragments of a male from one lesion were identified as *Protostrongylus stilesi.* At least three cases of parenchymal lungworms were accompanied by infections with *P. rushi* in the bronchioles as well. No extensive gross pathology or evidence of pneumonia was seen in any of the specimens and the animals were probably not significantly affected by the parasites.

Dikmans (1931) originally described *Protostrongylus stilesi* based on specimens taken from a bighorn sheep. Marsh (1938) examined fourteen sets of lungs over the period of 1925 to 1937 and found all lungs to be infected. The only species found was *Protostrongylus stilesi,* and there was considerable gross and microscopic pathology associated with it. Mills (1937) reported on post-mortem examination of a ewe and a ram both of which were included in Marsh's paper. Hones (1942) examined fourteen bighorn lungs, nine of which were found to be infected. Both *Protostrongylus rushi* and *P. frosti* were found, the latter being a new species closely related to *Protostrongylus stilesi.* It was his opinion that *Protostrongylus rushi* is relatively nonpathogenic. The description of *P. rushi* by Dikmans (1943) was amended.

Couey (1950) examined three hundred sixty droppings from the Sun River area over the period May, 1942 to June, 1944 and found 85% contained lungworm larvae. Adults removed from dead bighorn sheep were identified as *P. rushi* and *P. stilesi.*

The nineteen lungs examined here comprise a sample composed entirely of mature rams. Unlike other studies, no animals were taken because of obvious repiratory difficulty. In none of the lungs was there extensive pathology, but the animals from the Sun River area had a greater number of lesions as compared to those from the Gallatin herd.

LITERATURE CITED

COUEY, E. M. 1950. Rocky Mountain Bighorn Sheep of Montana. Bull. No. 2, Montana Fish and Game Commission, 90 pp.

DIKMANS, G. 1931. Two new lungworms from North American ruminants and a note on the lungworms of sheep in the United States. Proc. U. S. Nat. Mus., 79: 1-4.

DIKMANS, G. 1943. The lungworms *Protostrongylus rushi* Dikmans, 1937, of the mountain sheep, *Ovis canadensis.* Proc. Helm. Soc. Wash., 10: 8-9.

HONESS, R. F. 1942. Lungworms of domestic sheep and Bighorn Sheep in Wyoming. Univ. Wyo. Agri. Expt. Sta. Bull. No. 225, 24 p.

MARSH, H., 1938. Pneumonia in Rocky Mountain Bighorn Sheep. J. Mammal., 19: 214-219.

MILLS, H. B. 1937. A preliminary study of the Bighorn of Yellowstone National Park. J. Mammal., 18: 205-212.

Two New Species of Stomach Worms (Nematoda: Spiruroidea) from the Blue Jay, *Cyanocitta cristata L.*

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During an investigation of the parasites of the blue jay, *Cyanocitta cristata* in New England, two new species of stomach nematodes both belonging to the Spiruroidea were collected following an examination of 94 birds. The one, comprising three male and two female individuals, belongs to the genus *Microtetrameres* (Spiruridae) and was taken from the proventriculus. The other is a species of *Cheilospirura* (Acuariidae) and is represented by eight males and 10 females found between the tunics of the gizzard. The figures (1 and 2) were drawn with the aid of a camera lucida.

Through the kindness of Dr. Price of the Animal Disease and Parasitic Research Branch, U. S. Department of Agriculture, one male and one female *Microtetrameres* sp., previously collected from this same host and deposited in the Helminthological Collection (#31822), were loaned to the author to aid in the preparation of this paper. The writer wishes to express her appreciation to Mrs. M. B. Chitwood for her valuable assistance in the study of these nematodes.

This investigation was aided in part by a grant (E-725) from the National Institutes of Health, U. S. Public Health Service.

Microtetrameres spiculata n. sp.

MALE: (Fig. 1 A-C): Dark, vermiform, 2.02-2.242 mm long by 79-95 μ wide. Buccal capsule 20-24 μ deep, cylindrical somewhat broader at its distal end. Muscular esophagus (pharynx) 135-164 μ, glandular esophagus 455-540 μ in length. Nerve ring 86-100 μ and excretoryy gland 100 μ from anterior end. Cervical papillae 162-165 μ from anterior extremity. Spicules markedly unequal. Left spicule 2,312-2,576 μ long, assuming a sinuous course within the shorter body. Its proximal end 145-180 μ from anterior end, either a little anterior or posterior to the junction of the two parts of the esophagus. Distal end of left spicule bifid. Right spicule 103-147 μ in length, slightly curved; proximal and asymmetrical, distal end conical. Constriction between vas deferens and testis 600 μ distant from cloaca. Two pairs of small pre- and two pairs of post-cloacal papillae. Tail 127-147 μ long ending in a small ball-point tip.

FEMALE (Fig. 1 D-G): Red, 1.2 mm long in coiled position by 1.0 mm wide. Body coiled in one direction then in opposite direction. Buccal capsule vase-shaped, 22, 23 μ deep. Muscular esophagus (pharynx) 224-236 μ in length; glandular esophagus 1,050-1,080 μ in length. Nerve ring 109 μ from anterior end. Anus 96-103 μ from posterior end. Vulva 90-120 μ anterior to anus. Prominent cuticular fold in front of vulva. Tail ending in an inconspicuous knob. Embryonated eggs 48-50 μ long by 31 μ wide. Egg barrel-shaped bi-operculate and with sides thickened and somewhat lopsided.

HOST: *Cyanocitta cristata* L. (Blue jay)

HABITAT: Proventriculus

TYPE LOCALITY: South Hadley in Hampshire County, Massachusetts.

TYPES: U.S.N.M. Helminthological Collection No. 38015, Paratypes No. 31822.

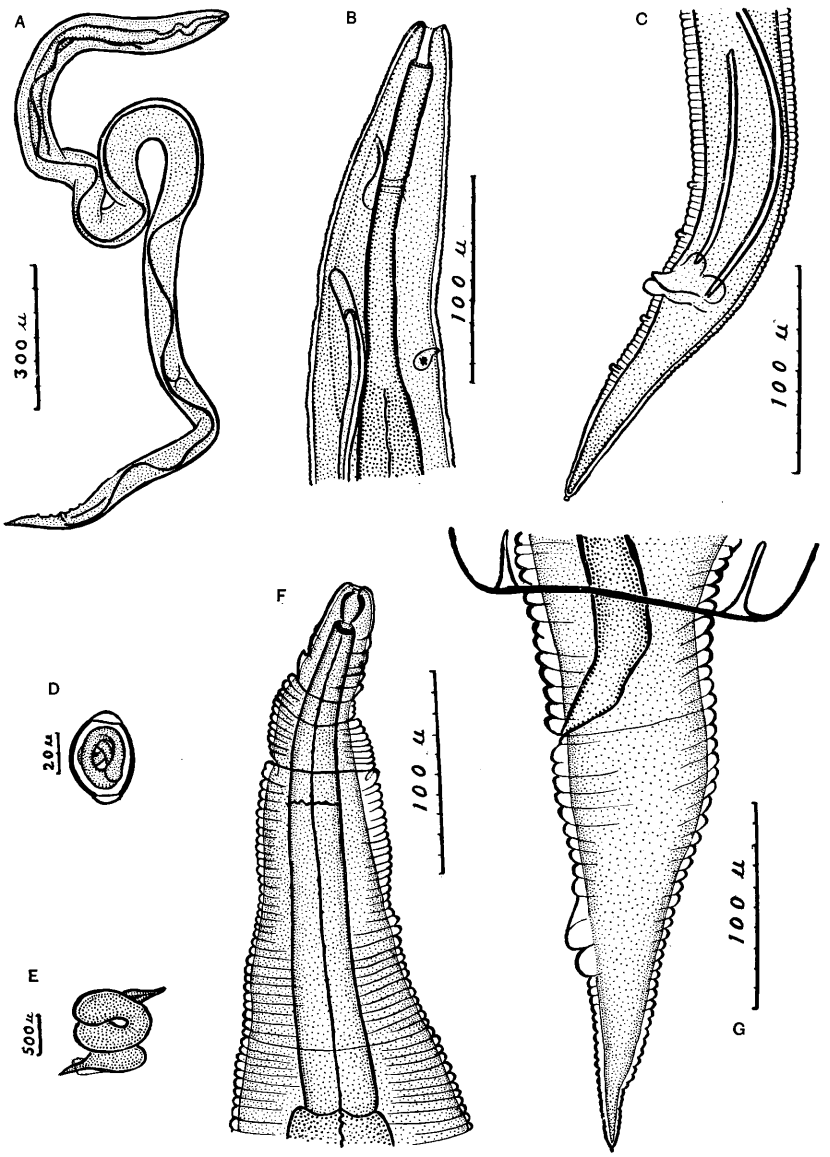

Fig. 1. *Microtetrameres spiculata* n. sp. A. Male, showing sinuous course of left spicule and constriction between testis and vas deferens. B. Male, anterior end showing buccal capsule, muscular esophagus (pharynx), excretory gland, cervical papilla and proximal end of left spicule and glandular esophagus. C. Male, posterior end, lateral view showing right spicule, distal end of left and cloacal papillae. D. Embryonated egg. E. Female, lateral view showing reversal of coiling. F. Female, anterior end showing buccal capsule, muscular esophagus (pharynx), nerve cord and proximal end of glandular esophagus. G. Female, posterior end showing cuticular fold, vulva and anus.

REMARKS: The description is based on an examination of four male and three female specimens

In her report on the nematodes of birds, Cram ('27) lists seven species of the genus *Microtetrameres* of which only one, *M. helix* (Cram) is indigenous to North America and was collected from the eastern crow. Recently four

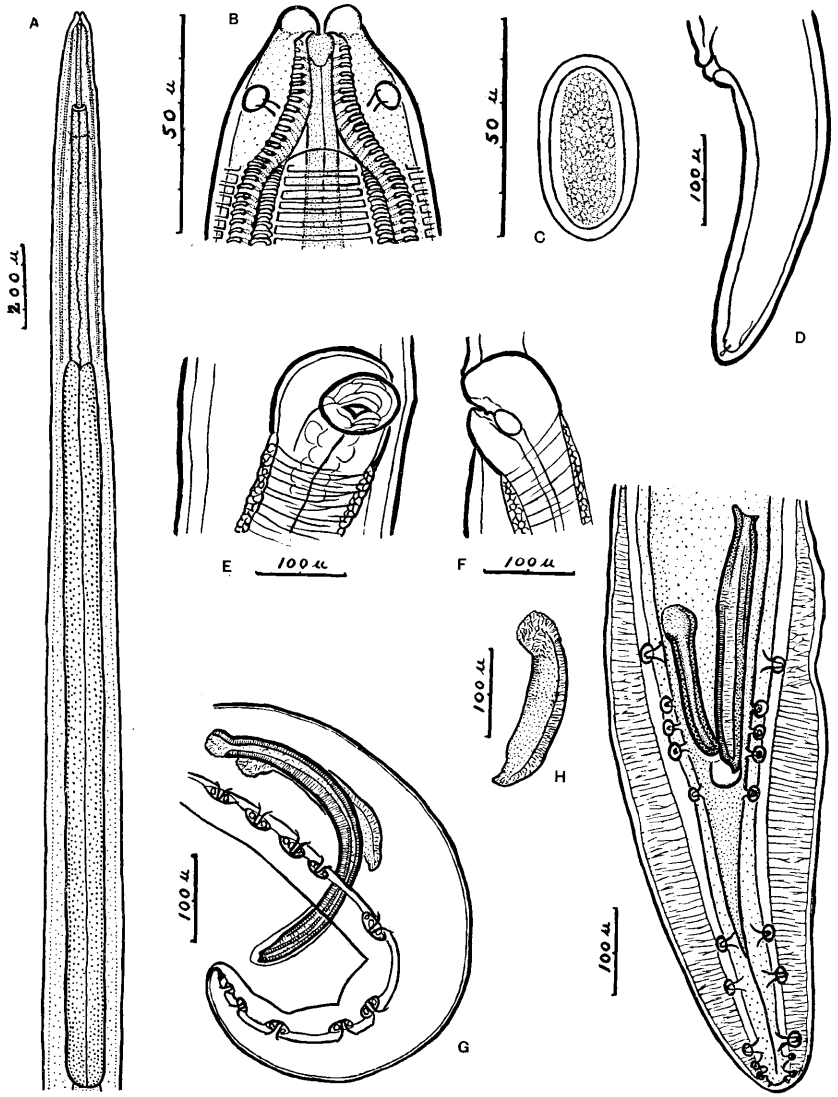

Fig. 2. *Cheilospirura cyanocitta* n. sp. A. Male, anterior end showing pharynx, two parts of esophagus, nerve ring and cordons. B. Male, head showing lips, papillae and proximal ends of cordons. C. Egg. D. Female, posterior end, lateral view showing anus and phasmid. E. Female, vulvar region, ventral view. F. Female, vulva, lateral view. G. Male, posterior end, lateral view showing both spicules and cloacal papillae. H. Male right spicule, lateral view. I. Male, posterior end, ventral view showing both spicules, alae and cloacal papillae.

additional species have been described from North American birds (Schell, '53). *M. corax* (Schell) from the raven (*Corvus corax*) shows closest resemblance to *M. helix; M. spiculata* n. sp. bears the greatest similarity to *M. corax.* The male in both species possesses four cloacal papillae and an extremely long left spicule. The females are similar to each other in body, shape and size, position of vulva and its cuticular fold, and in the bi-operculated appearance of their eggs. The most distinguishing feature between the two species is the relative length of the left spicule with regard to body length. In *M. spiculata,* the ratio of left spicule to body length is 1:0.85, whereas in *M. corax* it is 1:2.0; the proximal end of the spicule in the former arises either anterior or posterior to the junction of the two parts of the esophagus; in the latter it is consistently posterior to this junction. Other differences lie mainly in the larger size of many of the body components of *M. corax.* The male of *M. corax* measures 3.7-4.7 mm long; its muscular and glandular esophageal parts average 255 μ and 700 μ; its tail, 183 μ long. The constriction between its testis and vas deferens is 820 μ from the posterior end and the left spicule is 3.5 mm long and, unlike both *M. spiculata* and *M. helix,* its distal end is not bifid but rounded or truncated. In the female of *M. corax,* the two parts of the esophagus measure 262 μ and 1.38 mm respectively; the tail tapers to a sharp point and the egg is symmetrical in shape with a thinner shell. It is interesting to note that the above three species, each collected from a different member of the Corvidae family, bear a closer resemblance to each other than to the other three species of *Microtetrameres* so far described from North American hosts. (Golden eagle, great horned owl and goshawk) (Schell, '53).

Cheilospirura cyanocitta n. sp. (Fig. 2)

Mouth bordered by two simple lips. Near base of each lip arises a pair of mushroom-shaped papillae. The four cordons originate between the lips, separate immediately to extend posteriorly for a short distance in a straight course without anastomosing or recurring. The ratio of cordon to body length is 1:12.4 in the male and 1:13.8 in the female. Each cordon is striated transversely 13-15 μ in width with a central gutter. Cervical papillae occur a short distance (20 μ) posterior to the junction of pharynx and esophagus.

MALE: (Fig. 2 A, B, G-I): Length, 7.5-10.0 mm, average 8.4 mm. Width 160-180 μ, average 168 μ at the middle of the body. Pharynx, 185-205 μ, average 200 μ. Anterior esophagus 513-595 μ, average 563 μ. Posterior esophagus, 1,520-1,725 μ, average 1,592 μ. Nerve ring 275 μ from anterior end, 65 μ posterior to pharynx. Cordons 652-725 μ, average 687 μ terminating at junction of the two portions of esophagus or slightly anterior to this, -0-100 μ anterior. Cordons of one side may be 6-50μ from those on opposite side. Caudal extremity spirally coiled. Tail alate, 330-420 μ, average 360 μ; 1/23 of body length. Caudal alae 100 μ at widest width tapering at both ends with delicate transverse striations. Ten pairs of stalked papillae, 4 precloacal, 6 post-cloacal. The latter in three groups, one solitary pair of papillae behind the cloaca, then a set of two pairs and finally the three pairs of papillae and one pair of phasmids towards the tail tip. All the pairs of papillae prominent except the distal two pairs of small ones; right and left post-cloacal papillae may be asymmetrically arranged. The two unequal spicules curved, each with a distinct 'head' at the proximal end. The smaller stouter right spicule measuring 220-230 μ in side view with a ventral flange

giving a width of 41-48 μ. The longer more slender left spicule 315-370 μ in length, broadest 27 μ in width near its middle.

FEMALE: (Fig. 2 C-F): Length, 12.8-18.4 mm, average 17 mm. Width greatest in region of vulva, 230-297 μ, average 263 μ. Pharynx, 215-250 μ, average 236 μ. Anterior esophagus, 710-920 μ, average 787 μ. Posterior esophagus, 1,750-2,250 μ, average 1,946 μ. Nerve ring 295-363 μ, average 328 μ, from anterior extremity and 86 μ behind posterior end of pharynx. Cordons 1,095-1,390 μ, average 1,233 μ, extending to vicinty of junction of the two portions of the esophagus, ranging from 41 μ anterior to 275 μ posterior to the junction. Variation between cordon lengths of same individual, 0 to 205 μ. Tail straight or slightly curved, 275-380 μ, average 321 μ. Vulva conspicuous, dividing body into two approximately equal halves. Vulva averaging 850 μ anterior to middle of body with a range of from 190 μ posterior to 1,500 μ anterior to center of body. Egg bluntly oval, 42 μ long by 24 μ wide, shell 3 μ thick; embryonated when oviposited.

HOST: *Cyanocitta cristata* L. (Blue jay).

HABITAT: Gizzard between the tunics.

TYPE LOCALITY: South Hadley in Hampshire County, Massachusetts.

TYPES: U.S.N.M. Helminthological Collection No. 38016 and 38017.

REMARKS: Of the species of Cheilospirura listed by Cram ('27), two are parasites of North American avian hosts,—*C. hamulosa* (Diesing) in gallinaceous birds and *C. spinosa* (Cram) in the grouse. Simon ('39) has described an additional species, *C. centrocerci* (Simon) from the sage grouse. *C. cyanocitta* n. sp. differs fundamentally from other members of this genus. The most striking difference is in the short length of the left spicule (average 342.5 μ) of the male. The ratio of right to left spicule is 1:1.5. The species with the next shortest left spicule is *C. falconis* (Clapham) from a kestrel in Palestine (Clapham, '47), where the ratio is 1:2.3. The cordons of the male *C. falconis,* however, are long, 2.1-2.5 mm, unlike the short ones in the male of *C. cyanocitta* (678 μ), *C. centrocerci* (668 μ), *C. rotunda* (Stiles and Hassall) (480 μ) and *C. spinosa* (495 μ). In addition, the left spicules in each of the last three species are longer than that of *C. cyanocitta,* being 1,012, 750 and 720 μ respectively. The female of *C. cyanocitta* is 17 mm long and is characterized by an average cordon length of 1,233 μ, the central position of the vulva and an egg size of 42 μ by 24 μ. In *C. gruveli* (Cram), the vulva is also in the mid line and its cordons average 1,159 μ in length, but its egg is smaller (33 μ by 22 μ) and the worm is 34 mm long. The eggs of *C. centrocerci, C. spinosa* and *C. falconis* measure 44.5 μ by 28.4 μ, 42.0 μ by 27.0 μ and 40.0 μ by 24.5 μ, but the first two species are longer worms (47 and 36 mm) with short cordons (937 and 813 μ) and anteriorly located vulvae. *C. falconis,* on the other hand, is small, 14 mm, with longer cordons, 2.1-2.5 mm, and the vulva lies in the posterior half of the body. Thus the combination of certain morphological features distinguishes *C. cyanocitta* n. sp. from the other members of the genus *Cheilospirura.*

LITERATURE CITED

CLAPHAM, P. A. 1947. On *Viannella africana* n. sp., *Cheilospirura falconis* n. sp., and *Oesophagostomum tridentatum* Maplestone, 1932. J. Helm. 22: 29-36.

CRAM, E. B. 1927. Bird parasites of the Nematode suborders Strongylata, Ascaridata, and Spirurata. U. S. Nat. Mus. Bull. 140: 1-465.

SCHELL, S. C. 1953. Four new species of *Microtetrameres* (Nematoda: Spiruroidea) from North American birds. Trans. Amer. Micr. Soc. 72: 227-236.

SIMON, F. 1939. *Cheilospirura centrocerci,* a new nematode from the sage grouse, *Centrocercus urophasianus.* Trans. Amer. Micr. Soc. 58: 78-80.

Sphaeronema arenarium, n. sp. (Nematoda: Criconematidae), a Nematode Parasite of Salt Rush, *Juncus leseurii* Boland.

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Larvae and males of a nematode species closely related to *Sphaeronema californicum* Raski and Sher, 1952, were discovered by the author in washings obtained from soil collected about the roots of *Juncus leseurii* Boland.** in July, 1954. Additional specimens were collected May 21, 1955 from the same site on the coast of California one-half mile north of Dillon Beach, Marin County, near the edge of a shelf ending approximately thirty feet above the surf. Dillon Beach is on the mainland 10-11 miles north and across Tomales Bay from the site of the original collection of *S. californicum*, the type species, and previously the only other known member of this genus.

Specimens of adult females were not found readily at first since they normally are in a tightly coiled position and settle out with soil particles in the screening process. However, many female specimens were obtained from the roots of the plants. This was done by carefully washing the roots as clean as possible then scrubbing them vigorously in a pan of clear tap water. The roots were removed and the water cleared of silt by passing it through a 200 mesh screen. The adult females were recovered by examination of the screening residue in clear water.

It was found by close examination of the roots that these nematodes do not form colonies but most commonly occur as individual estoparasites. The females are coiled in a gelatinous matrix in which eggs and larvae were observed frequently. This is in contrast to *S. californicum* which are endoparasitic and occur more or less in colonies.

The differences between the elongate mature females of this species and the small, spherical females of *S. californicum* indicate these nematodes represent a new species for which the following description is presented.

Sphaeronema arenarium, NEW SPECIES (Pl. 1, a-g)

DIMENSIONS.— 13 ♀ ♀ L = .45-.58 mm. a = 10-16 b = 3.3-4.5 c = 12-18
47-60 1.3-2.7
V = 74-80 Stylet = 13-15 μ
8 ♂ ♂ L = .43-.57 mm. a = 30-44 b = ? c = 8-10
T = 24-39% Stylet = lacking.

59 2.1

FEMALE (HOLOTYPE).— L = .58 mm. a = 16 b = 3.8 c = 13 V = 75

Body swollen near vulva, tapering anteriorly and posteriorly. Body annules 1-1.3 μ wide. Lip region continuous with body contour, without definite annulation. Labial framework lightly sclerotized. Stylet 14 μ long, prorhabdion approximately 50% of stylet length. Stylet knobs moderate in size, rounded. Dorsal esophageal gland orifice 6 μ behind stylet base. Esophagus very strongly developed with heavily cuticularized lumen. Excretory pore at level of nerve ring, 103 μ from anterior end. Hemizonid approximately two body annules wide, immediately anterior to excretory pore. Spermatheca present. Ovary well developed, usually extending to base of median bulb, often reflexed once or twice. Posterior uterine branch very short or lacking.

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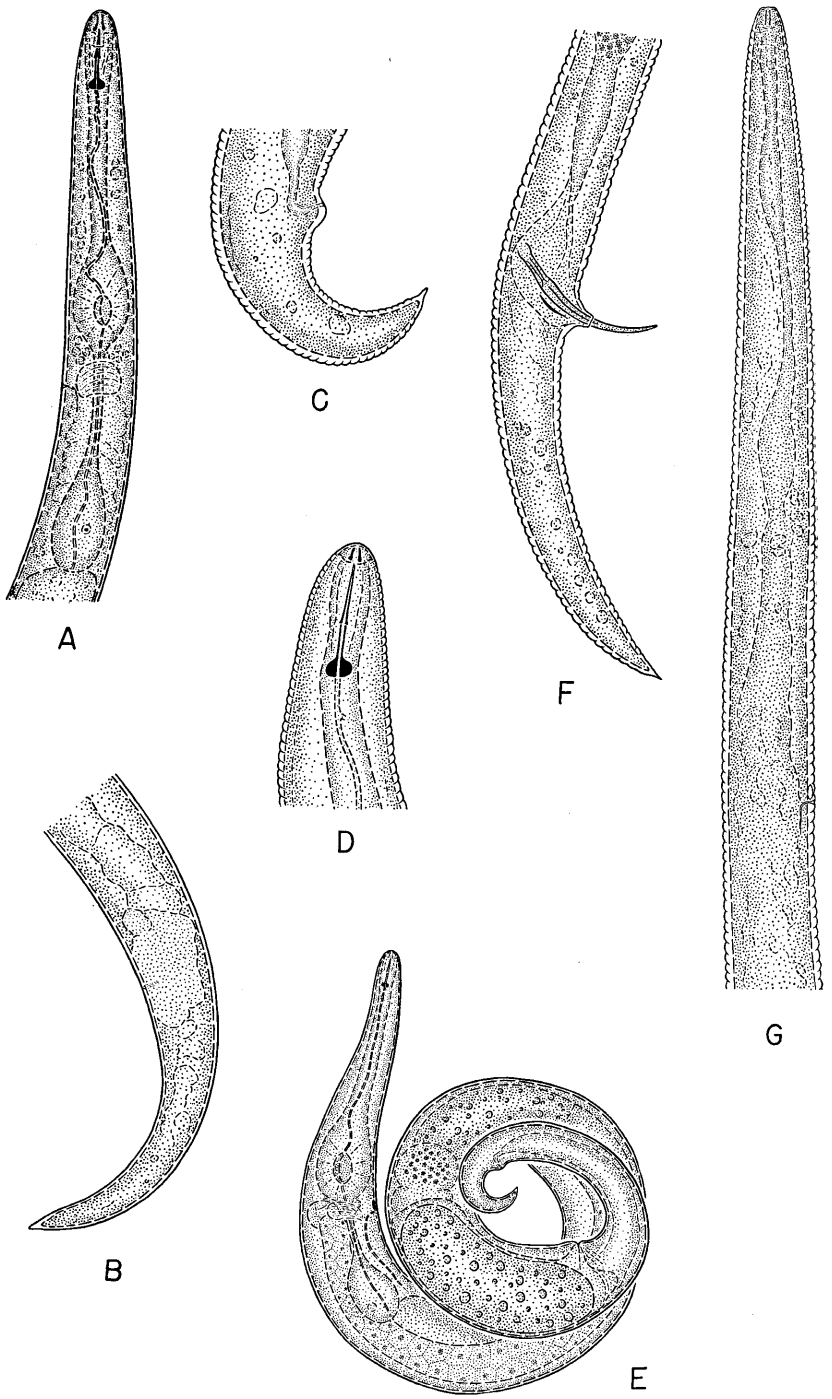
**Identification of Beecher Crampton, Agronomy Herbarium, University of California, Davis.



Anal opening obscure, located on slight elevation of cuticle. Tail conical, ventrally arcuate, tapering to a minutely rounded terminus. Lateral field not observed. Phasmids not observed.

MALE (ALLOTYPE).— L = .56 mm. a = 42.2 b = ? c = 10

Body slender, cylindrical, tapering anteriorly and posteriorly. Body annules 1-1.3 μ wide. Lip region continuous with body contour, without definite annulation. Labial framework very lightly sclerotized. Stylet lacking. Excretory pore 107 μ from anterior end. Hemizonid tow body annules wide, immediately anterior to excretory pore. Esophagus degenerate. Testis 34.4% of body length. Spicules slightly curved, 22 μ long. Gubernaculum simple, 5 μ long. Spicule sheath conspicuous. Bursa absent. Phasmids not observed. Tail conoid, slightly ventrally curved, with acute terminus.

LARVA.— L = .32-.40 mm. a = 21-29 b = 3.1-3.7 c = 10.1

Body slender, tapering anteriorly and posteriorly, generally assumes coiled position in fixative. Annulation fine and obscure. Lateral field not observed. Lip region continuous with body contour. Labial framework lightly sclerotized. Spear 12-14 μ long, prorhabdion approximately 50% of stylet length. Knobs of spear rounded swellings. Excretory pore 71-93 μ from anterior end. Anus very obscure. Tail conoid, ventrally arcuate, with rounded terminus.

HOLOTYPE.—Female collected May 21, 1955 by D. J. Raski. Catalogue number 95 University of California Nematode Survey Collection, Berkeley.

ALLOTYPE.—Male, same data as holotype. Catalogue number 96 University of California Nematode Survey Collection, Berkeley.

PARATYPES.—18 females, 26 males, same data as holotype.

TYPE HOST.—Salt Rush, *Juncus leseurii* Boland.

TYPE LOCALITY.—Dillon Beach, Marin County, Calif.

Sphaeronema arenarium can be distinguished from *S. californicum* by the elongate, tightly coiled female with conical tail and rounded terminus. In addition the male of *arenarium* is larger and has a sharply pointed tail.

Chitwood (1950) characterized the family Criconematidae Thorne, 1949, as follows: "Stylet shaft greatly elongated; cuticle coarsely striated, annulated or scaly; metacorpus greatly enlarged, elongated. (Parasites of plant roots, usually external)." The two known species of *Sphaeronema* do not conform to this concept principally on the proportions of the spear. However, the relationship of *Sphaeronema* to this family is definitely established on the female esophageal structure with enlarged metacorpus, narrow isthmus and reduced postcorpus. In addition the males of both species and the females of *arenarium* bear close resemblance to members of the Paratylenchinae. Until more material is available for comparative study it appears most logical to consider the genus *Sphaeronema* as presently assigned.

LITERATURE CITED

CHITWOOD, B. G., 1950. An introduction to nematology, Section 1, Part 1, Chapter 1:7-27. Monumental Printing Co., Baltimore, Md.

RASKI, DEWEY J. and S. A. SHER. 1952. *Sphaeronema californicum*, nov. gen. nov. spec., (Criconematidae: Sphaeronematinae, nov. subfam.) an endoparasite of the roots of certain plants. Proc. Helm. Soc. Wash. 19:77-80.

Pl. 1.—*Sphaeronema arenarium*. A—Neck region of larva, × 650; B—Tail of larva, × 1000; C—Tail of female, × 1000; D—Head of female, × 1000; E—Female, × 330; F—Male tail, × 1000; G—Neck region of male, × 1000.

A Revision of the Genus *Haliplectus* Cobb, 1913

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In 1913 Dr. N. A. Cobb described the genus *Haliplectus* with the type and only species *H. pellucidus*. In the original and only article on this genus he states that it is "found in brackish and sometimes in fresh water in the eastern states. Several species found on Atlantic and Pacific coasts of the United States." In 1955 the author asked Mr. Bickner to collect soil and root samples at Atwood Grove, Florida in the hope of finding *Atylenchus decalineatus*. A report on *Atylenchus decalineatus* will be published separately. In Mr. Bickner's first collection certain nematodes were found in soil from about the roots of *Schinus* sp. (Brazilian pepper) which we at first presumed to be a new genus, but closely related to *Haliplectus*. Since Dr. Cobb had mentioned several species but named only one we asked Dr. Steiner to loan us the original notes and sketches of *Haliplectus* from Dr. Cobb's files. These notes showed that our material is definitely congeneric with Dr. Cobb's but that his original description and illustrations were of two different species. Additional sketches are also given in Dr. Cobb's files. We have decided to publish Dr. Cobb's original notes on all species, with his sketches and attribute them to him *posthumously*, publishing our own species along with his. A revised generic diagnosis will also be given.

FAMILY—Leptolaimidae Oerley, 1880
SUBFAMILY—Haliplectinae Chitwood, 1951

Labial region without special modifications; terminal excretory duct not sclerotized. Stoma more or less tubular, esophagus terminated by a muscular bulb with valves more of a chromadoroid than plectoid type (not modified pigeon wing).

TYPE GENUS: *Haliplectus* Cobb, 1913.

OTHER GENERA: *Aplectus* Cobb, 1914; *Polylaimium* Cobb, 1920; *Linolaimus* Cobb, 1933.

The findings of a species of *Haliplectus*, together with study of the illustrations, proves that *Haliplectus* belongs in the family Leptolaimidae. Therefore the subfamily Haliplectinae Chitwood, 1951 is transferred to that family, alongside the Leptolaiminae Chitwood, 1951 and the Rhabdolaiminae Chitwood, 1951.

GENUS: *Haliplectus* Cobb, 1913

DIAGNOSIS: Subfamily Haliplectinae. Aphasmidian nematodes about 0.5 to 2.0 mm. long with cuticle striated and with scattered somatic papillae; submedian rows of hypodermal glands present; cephalic and somatic setae absent, lips inconspicuous; amphids circular to unispire, about 1 to 2 head diameters back; stoma elongate into corpus of esophagus, narrow, with or without distinct cheilorhabdions; terminated with joints in a small median esophageal bulb. Esophagus surrounding most of stoma with median bulb, isthmus and elongate rather chromadoroid posterior bulb, lining thickened, with at least two joints in posterior bulb. Esophago-intestinal valve elongate. Nerve ring at isthmus. Male with two arcuate spicules and a gubernaculum; 0 to 6 ventral preanal supplementary organs, more or less chromadoroid; with two testes. Female with two reflexed, opposed ovaries. Fresh or Brackish swamp water or soil.

TYPE SPECIES: *H. pellucidus* Cobb, 1913.

Haliplectus pellucidus Cobb, 1913

.5	7.3	11.6	'43'[29]	95.2	
1.1	2.5	2.7	3.1	2.3	1 mm.

The following description is an exact quotation from Dr. Cobb's notes:

"The thin, transparent layers of the colorless, naked cuticle are traversed by . . . transverse striae, which are not further resolvable. These striae appear to exist in the outer as well as the inner cuticle, so that the contour of the body is faintly crenate. The conoid neck becomes markedly convex-conoid near the head, so that the anterior extremity is truncate-conoid from a little behind the lateral organs. There are no cephalic setae at least in specimens killed in hot sublimate solution. The lips are thoroughly amalgamated, so that the front of the head is smooth and presents merely a narrow opening leading to the narrow tube, which passes backward through the oesophagus. It is possible to regard the beginning of this tube as a excessively minute conoid pharynx, but the worm falls naturally into the groups possessing no pharynx. The width of the mouth opening is about one-sixth as great as that of the head, and the tube leading through the anterior portion of the oesophagus is narrower still. This tube is lined with chitin, so that it is a fairly distinct feature traceable readily as far as the median bulb. Circular lateral organs are present at a point about one-third the distance from the anterior extremity to the median bulb. These organs are one-third as wide as the corresponding portion of the neck. Careful focussing in some specimens shows them to be spirals of one wind. There are no eye-spots. The oesophagus reaches nearly to the lips and begins as a tube three-fifths as wide as the head. It continues to have this diameter until it expands to form the relatively small median bulb, which is in the middle of the neck and has an ellipsoidal contour. The diameter of this median bulb is about two-fifths as great as that of the corresponding part of the neck. Behind this bulb the oesophagus again becomes tubular, but is slightly wider than before. It finally expands to form the somewhat spherical cardiac bulb, which is five-sixths as wide as the base of the neck and contains an elongated-ellipsoidal valvular apparatus of a rather simple character, having a width about one-fourth as great as that of the bulb itself. The intestine joins the middle of the posterior surface of the bulb and at that point is only about one-fourth as wide as the base of the neck. There is no distinct cardia. The intestine soon widens out so as to be two-thirds to three-fourths as wide as the body. It is moderately thick-walled and is composed of cells of such a size that probably eight to ten are required to build a circumference. These cells do not contain granules so that the worm is unusually transparent. From the slightly raised anus the rectum, which is somewhat shorter than the anal body diameter, extends inward and forward. The lateral fields are about half as wide as the body and are composed of cells of relatively large size. The nerve-ring surrounds the oesophagus somewhat obliquely. Nothing is known concerning the ventral gland or the excretory pore. The tail is arcuate-conoid to the terminus, which has a diameter about one-third as great as at the anus and bears a short somewhat apiculate, blunt, transparent, unarmed spinneret. Teh caudal glands are packed in a tandem series in the anterior half of the tail. The reflexed ovaries reach half way back to the vulva and contain six to ten ova arranged for the most part single file.

.5	6.8	11.	-M-[65]	95.7	
1.	2.4	2.6	2.9	2.6	1 mm.

The tail of the male is like that of the female in form, but is very considerably shorter and more distinctly arcuate and the anus is more prominently raised, especially the posterior lip. There are no supplementary organs in front of the anus, nor have any special papillae been seen either in front of the anus or behind it. The two equal, arcuate, uniform, yellowish spicula are about one and one-fourth times as long as the anal body diameter. Their proximal ends are not cephalated, and their distal ends taper suddenly to a point. Surrounding the distal ends of the spicula there is an accessory piece, also yellowish, from which there extends inward and forward parallel to the spicula a slender, curved, portion half as long as the spicula. Oblique copulatory muscles are present for some distance in front of the anus, a distance four to five times as great as the width of the tail. The posterior extremity of the male is evidently flattened a little on the ventral side. The ejaculatory duct is nearly half as wide as the body. Both of the outstretched testicles extend forward, one lying behind the other. The blind end of the anterior one is about twice as far behind the base of the neck as this latter is behind the anterior extremity.

HABITAT: Sand, below low tide mark, Portsmouth, N. H."

The above is reproduced from Dr. Cobb's notes. The measurements according to the demanian formula are as follows:

FEMALE: 1.0 mm.; a, 32; b, 8.6; c, 21; V, 43%; ovaries 29%; stoma (?) 73 μ.

MALE: 1.0 mm.; a, 34; b, 9.1; c, 21; testes outstretched anteriad in tandem 65%.

From the original notes it will be seen that the published illustrations do not agree. The pencil illustrations are on a sheet marked *Haliplectus floridanus* with which description they do agree.

Haliplectus floridanus Cobb, n. sp.

1.	5.3	8.7	M	95.	
9.	2.1	2.3	2.5	2.3	1.6 mm.

The following description is an exact quotation from Dr. Cobb's notes.

"The thin, transparent, colorless layers of the cuticle are traversed by plain, transverse striae, resolvable with high powers throughout the length of the body. Throughout the length of the body there occur in the cuticle longitudinal rows of circular markings or pores. These are located on opposite sides of the lateral field, and some appear also to be dorsal or dorsosubmedian. These markings have a diameter about equal to the thickness of the cuticle. Their positions as observed on the tail are shown in one of the sketches. The neck is conoid, tapering, however, much more rapidly in front of the median bulb, and ending in a rounded head apparently destitute of lips. The pharynx is tubular, and extends backward apparently to a point somewhat behind the lateral organs, but its limits are very indefinite. There are no cephalic setae. The vestibule is slightly wider than the pharynx. The chitinized walls of the pharynx end a short distance behind the lips, at a distance somewhat greater than the width of the pharyngeal tube. There are no eye-spots. The lateral organs are spirals appearing as circles about one-third as wide as the corresponding portion of the head and are situated at a distance from the anterior extremity about equal to the diameter of the head opposite the lateral organs. In front of the lateral organs there are about twelve striations on the cuticle. When these organs are seen dorso-

ventrally they break the contour of the neck being sligthly elevated. The oesophagus is at first a narrow tube about half as wide as the front of the head. This tube expands at the middle of the neck to form a somewhat fusiform but distinctly developed median bulb, whose chitinous lining is somewhat differentiated, though it is doubtful if there is a distinct valvular apparatus. Behind this median swelling the oesophagus again becomes tubular,

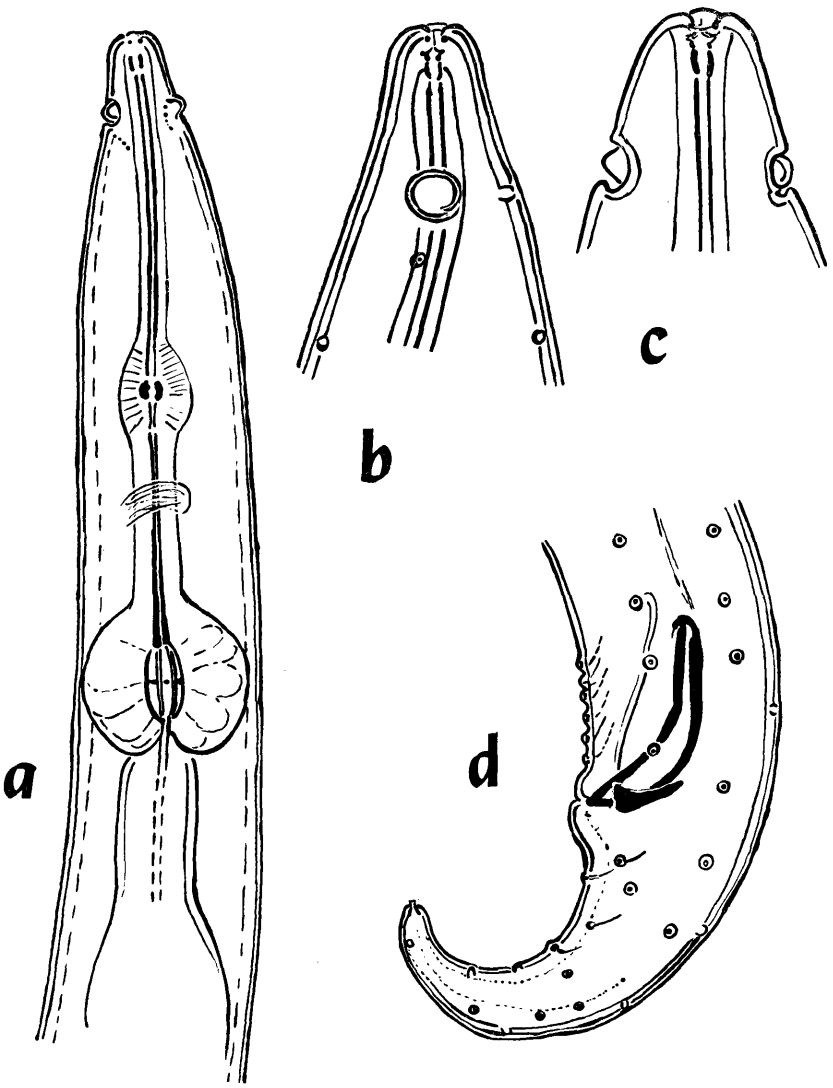

Fig. 1. *Haliplectus floridanus* n. sp., Cobb.
a, Esophageal region. (Cobb file drawing).
b, Head. (Cobb file drawing).
c, Head (Originally published as *H. pellucidus*, Cobb file drawing).
d, Male tail (Originally published as *H. pellucidus*, Cobb file drawing).

but slightly larger than in front of the median swelling. It finally expands suddenly to form an oblate cardiac bulb, which almost entirely fills the base of the neck, and which contains a very distinctly developed and strongly transversely striated chitinous threefold valvular apparatus having a somewhat fusiform contour, and about half as wide as the bulb. There is no very distinct cardia. The intestine, which is at first about two-thirds as wide as the body, is separated from the oesophagus by a distinct constriction, as well as by being smaller in diameter than the cardiac bulb. Its walls contain scattered greenish granules of variable size, the largest having a diameter about three times as great as the thickness of the cuticle, and the smallest having about one-third as great a diameter as the largest. The rectum appears to be about twice as long as the anal body-diameter. The location of the ventral gland and excretory pore remain in doubt. The nerve-ring appears to surround the oesophagus somewhat obliquely about halfway between the two oesophageal bulbs. The tail is conoid and ventrally arcuate, and ends in a distinct spinneret having the form commonly seen on *Plectus*. There is no bursa. There are papillae on the tail. In front of the anus there are six equidistant, closely approximated, bluntly conical papillae, forming a row on the ventral line, and extending forward about as far as do the spicula. The anus is distinctly raised and two-lipped. The two equal, arcuate, elongated, acute spicula are about one and one-third times as long as the anal body-diameter, and a little more than half as long as the tail. Their proximal ends are not cephalated. Their diameter is markedly less in the anterior half. They are accompanied by accessory pieces two-fifths as long, lying parallel to the distal portion. The papillae on the tail are partly ventral and partly submedian. Of the ventral papillae three may be counted near the middle of the tail, being about equidistant from each other and occupying the middle third of the ventral contour though slightly nearer to the terminus than to the anus. It is probable that a fourth papilla of this character occurs somewhat behind the anus. The lateral fields appear to be well developed, but there are no clear indications of wings to the cuticle.

HABITAT: Soil, mangrove swamp, Long Key, Florida."

The following are the measurements according to the demanian system:

MALE: 1.6 mm.; a, 40; b, 11.5; c, 20; stoma ? 85 μ.

Haliplectus conicephalum Cobb, n. sp.

The following description is an exact quotation from Dr. Cobb's notes:

0.7	4.7	6.9	12'49.'14	95.7	
0.7	1.4	1.7	2.2	1.3	1.9 mm.

"The rather thin, transparent, colorless naked cuticle is traversed by plain transverse striae rather easy of resolution, which are not altered on the lateral fields. There is a considerable amount of anastomosing of these striae. Longitudinal striae, due to the attachment of the musculature, are visible in most portions of the body, the contour of which is somewhat crenate. The body wall contains granules of the same size as those of the lateral chords and intestine. At the location of the few annules on the head end the cuticle is about twice as thick as elsewhere. The cephalic setae are reduced to almost invisible papillae, four in number, located eight annules in front of the amphids; indicated by almost the slightest possible interruption of the annule on which they occur. In front of these papillae are only about three additional annules. There are no subcephalic, cervical or somatic setae. There are two rows of exceedingly minute pores in the cuticle on each side

of the body; seen from the side, these two rows appear to be about two-thirds of a body-width. The pores themselves are about half as wide as the annules. The decidedly conoid posterior portion of the neck is succeeded by a decidedly convex-conoid, rounded, continuous head, the mouth-opening in the center of which is not depressed. There are no distinct lips; the lip region, however, is probably composed of two or three exceedingly fine, and almost invisible annules. The lips therefore have become amalgamated and the elements representing them are very minute and inconspicuous; the lip region is not set off in any way. No papillae have been distinctly seen on the six lips, but in the cuticle on the front of the head there are excessively minute, radiating cyatholaimoid elements at the mouth opening; these are believed to be vestibular, and they form a circlet one-third as wide as the front surface of the head. There is no very distinct pharnyx, but in the vestibule there are somewhat microlaimoid refractive elements of exceedingly small size, — in fact about on the limits of microscopic resolution. If the entrance to the mouth be denominated a vestibule, it must be described as excessively narrow, short and tightly closed. The distinct, strongly refractive circular amphids are without any central fleck; in reality they are spirals of about one wind, almost imperceptibly open on the dorsal side behind. Their anterior borders are removed from the anterior extremity a distance equal to a little more than the corresponding diameter of the head. They are two-fifths as wide as the corresponding portion of the head and interrupt about five annules. In other words, the amphids are about sixteen annules behind the lip region. There are no eyespots. The rhabditoid oesophagus presents a median and a cardiac swelling; the median swelling is ellipsoidal and three-fifths as wide as the corresponding portion of the neck while the oblate cardiac bulb, which is sometimes somewhat pyriform in contour, is seven-eighths as wide as the base of the neck. Behind the pharynx the oesophagus is about one-half, at the nerve-ring less than one-third, in front of the cardiac swelling one-third, and finally seven-eighths as wide as the corresponding portion of the neck. The lining of the oesophagus is a distinct feature; it seems to be in the form of a tube one-third as wide as the anterior part of the oesophagus. The wall and the lumen of the tube are distinctly visible and the diameter of the lumen is about equal to the thickness of the wall. The musculature of the oesophagus is fine and colorless. It is not known whether any oesophageal glands are present. In the anterior part of the median bulb there is a break in the lining of the oesophagus similar to that sometimes seen in the genus *Tylenchus*, and as there is granular matter in the dorsal sector of the cardiac bulb, the presence of an oesophageal gland is suggested. The median bulb contains a fusiform, simple, rather obscure valve, one-fourth as wide as the bulb itself, while the cardiac bulb contains a complicated, strongly striated valve fully one-fourth as wide as the bulb itself, marked in such a way as to remind one of the markings on diatoms belonging to the genus *Surirella*. The thick-walled intestine, separated from the oesophagus by a cardiac constriction less than one-third as wide as the base of the neck, presents a faint lumen and gradually becomes more than one-half as wide as the body; it is composed of cells of such a size that about five to six are presented in each cross section. The thick walls of the intestinal cells are rather distinctly visible. There is no prerectum. From the somewhat elevated anus the rather inconspicuous rectum leads inward and forward a distance one and one-half times as great as the anal body diameter. Scattered colorless granules of variable size, the largest of which are

one-sixteenth as wide as the body, occur in the cells of the intestine. They are not so arranged as to give rise to a tessellated effect. These granules are of about the same size as those occurring in the lateral chords. The above remarks refer, however, only to the larger refractive granules; there are numerous others packed of much smaller size among them. The chromodoroid, conoid, arcuate tail tapers from in front of the anus to the blunt, conoid, unarmed, symmetrical terminus. The simple, conoid, unarmed, symmetrical, truncate spinneret exhibits three ampullae and a rather conspicuous, more or less cylindroid, emptying tube. The three broadly saccate, or quadrate, caudal glands, extending forward to opposite the rectum and are located behind and in front of the annus in the anterior fourth of the tail. There are no caudal setae. The lateral chords are about half as wide as the body; they contain scattered spherical granules, having a diameter about equal to the width of one of the annules. The cells composing the lateral chords are of large size with prominent nuclei; along the margin of the lateral chords there are ellipsoidal nuclei, one-fifteenth as wide as the body, having diameters in the ratio of two to three. Measured transversely, these bodies are about a half of a body-width apart, and longitudinally, two-thirds of a body-width apart; they are believed to be connected with the longitudinal series of pores in the cuitcle, although an actual connection has not been seen. Observations on the pores were made on a living specimen, while the cells themselves were observed in another specimen which had been fixed in Flemming solution. The location of the excretory pore is uncertain, but it is believed to be near the nerve-ring, which is of medium size and surrounds the oesophagus obliquely. The general region of the vulva is depressed, but from the medium-sized, elevated, somewhat conspicuous vulva itself, the medium-sized vagina leads inward at right angles to the ventral surface halfway across the body. Only a single egg at a time has been seen in each uterus; these eggs are more than twice as long as the body is wide, and two and one-half times as long as wide; they are somewhat thick-shelled and smooth, and are deposited before segmentation begins. The broad, cylindroid ovaries extend nearly back to the vulva and contain numerous ova arranged irregularly.

Found about the roots of plants, salt marsh, Penzance, Woods Hole, Mass., July, 1923. Only two specimens examined. Fixed in Fleming and examined in water, unshrunken."

The following are the measurements according to the demanian system:

FEMALE: 1.9 mm.; a, 45; b, 14.5; c, 23; V, 49%; ovaries reflexed, 12 and 14% body length respectively.

Haliplectus dorsalis Cobb, n. sp.

The following description is an exact quotation from Dr. Cobb's notes:

1.5	5.9	10.1	75	M	96.6	
1.7	2.6	2.9		3.3	2.6	1 mm.

"The rather thick layers of the transparent, colorless to rather yellowish, naked cuticle are traversed by transverse striae easy of resolution, which are not altered on the lateral fields. Longitudinal striae, due to the attachment of the musculature, are visible in most portions of the body. The contour of the body is crenate. There are no cephalic, subcephalic, cervical or somatic setae. Two longitudinal series of pores exist in the cuticle, the distance between them being about equal to two-fifths of the body diameter; the longitudinal distance between successive pores is equal to two-thirds to three-fourths of the body diameter. The pores themselves have about the same

width as one of the annules of the cuticle. The neck, which is conoid posteriorly and decidely convex-conoid anteriorly, ends in a convex-conoid, continuous, rounded head, the lips of which are completely amalgamated and form a region which is not set off in any way. There are six very inconspicuous labial papillae in a single circlet a little wider than one of the amphids; these papillae have been seen in front view; it is very doubtful if they can be seen in profile. The circular fleckless amphids are in reality a spiral of one wind, the periphery of which is very nearly closed; they are about one-fourth as wide as the corresponding portion of the head. As they have been seen only in a foreshortened view, the distance back from the anterior extremity is somewhat uncertain, probably about one head diameter. There are no eyespots. The oesophagus is of typical character. The oesophagus behind the pharynx is about one-fourth, at the nerve-ring one fourth, in front of the cardiac bulb one-third, and finally more than three-fourths, as wide as the corresponding portion of the neck. The lining of the oesophagus, in fact a rather thick-walled tube, finds expression as four longitudinal refractive "lines" occupying one-fourth the width of the organ. The cardiac bulb contains a relatively large, elongated, rather simple but transversely "striated" valve, one-fourth as wide as the bulb, the markings somewhat resembling those of diatoms belonging to the genus *Surirella*. The decidedly thick-walled intestine, which becomes at once two-thirds as wide as the body, contains a rather faint somewhat refractive lumen, and is separated from the oesophagus by a cardiac collum less than one-fourth as wide as the base of the neck. Colorless granules of variable size, the largest one-twentieth as wide as the body, are scattered in the intestinal cells; they are not so arranged as to give rise to a tessellated effect. This description refers to the more refractive granules; others, much more numerous, are very faintly visible. The simple, unarmed, more or less convex-conoid, symmetrical, truncate spinneret has a distinct tube running through it as in *Plectus;* it is the truncation of this tube which gives rise to the truncate appearance of the spinneret. The granular caudal glands are packed together behind, and in front of, the anus. There are no caudal setae. The lateral chords are about one-third as wide as the body. Nothing is known concerning the renette and excretory pore. The somewhat oblique nerve-ring, which is of medium size, is accompanied by obscure nerve cells. In contour, the tail of the male is somewhat irregularly conoid to the rather blunt spinneret, the irregularly in conoid being due to the presence of papillae. The two, equal, arcuate, stoutish, rather uniform, blunt, colorless, strong, simple spicula, which at their widest part are one-fifth as wide as the corresponding portion of the body, are one and one-fourth times as long as the anal body diameter; their proximal ends, lying opposite the body axis, are somewhat cephalated by expansion. While the spicula are rather uniform in size, they taper distally at the very end only; at the distal end of the middle third, there is a slight denticle on the ventral contour. The arcuate, rather slender and rather strong gubernaculum, which lies parallel to the spicula, is one-half to two-fifths as long as these latter; it possesses no apophysis. There is a single, ventral, preanal supplementary organ opposite the proximal ends of the spicula; that is to say, opposite the proximal ends of the spicula three successive annules are slightly modified on the ventral line in the midst of a very slightly raised papillae, resulting in three contiguous, somewhat bell-shaped elements far less developed than in the typical Chromadoras. Papillae have been seen on the tail of the male as follows: Preanal: ventrally submedian—one near the anus. Postanal: lateral two—

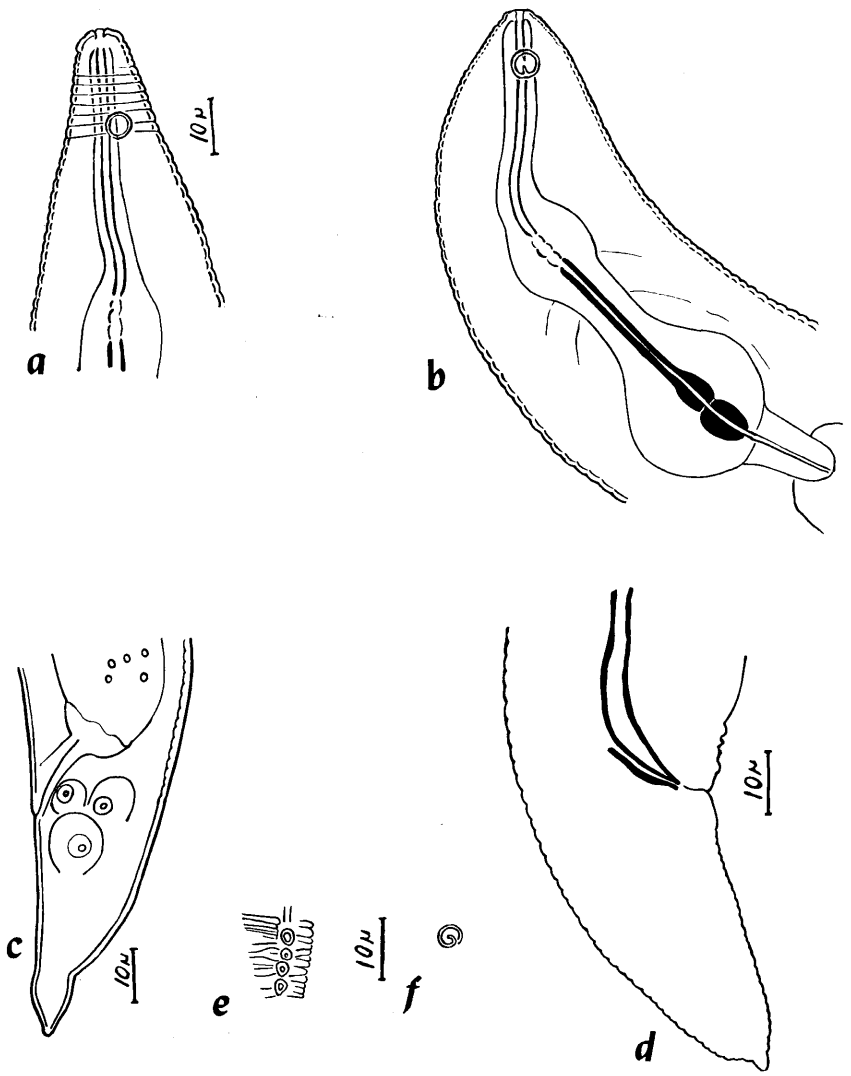

Fig. 2. *Haliplectus bickneri* n. sp.
a, Head.
b, Esophageal region.
c, Female tail.
d, Male tail.
e, Ventral view of male supplements.
f, Amphid.

one somewhat behind the middle of the tail and one on the terminus; dorsally submedian three equidistant ones—one at the terminus, one near the beginning of the second fourth and one halfway between. There is no bursa. The ejaculatory duct is two-fifths, the vas deferens one-third, the slightly cylindroid testes one-half, as wide as the corresponding portion of the body. The testes taper distally. Faint copulatory muscles exist for a distance about equal to three body-widths in front of the anus. The testes occupy six body-widths and the blind end of the anterior one is located one and one-fourth neck-lengths behind the cardia. Close to the heads of the spicula but somewhat inside the, there are two subglobular granular glands, seeming to connect backward, and between them a granular tube or duct, one-sixth as wide as the corresponding portion of the body, passing forward and probably constituting part of another gland.

HABITAT: Found at the edge of a salt marsh, east of Falmouth Heights, Mass., July, 1923. Examined in water alive. Two male specimens seen."

The following are the measurements according to the demanian system:

MALE: 1.0 mm. long; a, 30; b, 10; c, 30; testes outstretched, 15% of body length; stoma ? 59 μ.

Haliplectus bickneri: n. sp.

Amphids broken circle (unispire) nearly two head diameters from anterior extremity, about 11 to 15 μ back; about 3 to 3.5 μ across; striae 1.3-2 μ wide; stoma to base of cylindrical region 40-48 μ long, terminated in triple joint; about 1 to 1.6 μ wide. Median bulb containing 3 joints at base of stoma, elongate, not large; isthmus and elongate basal bulb, rather chromadoroid, with thickened cuticular lining consisting of 2 joints.

MALE: 760 μ to 1.05 mm. long; a, 17 to 26 (some under pressure); b, 7 to 10; c, 19 to 33; testes to within 25% of body length from head; excretory pore 14 μ from head; spicules arcuate, 35-38 μ, arcuate, not cephalated; gubernaculum 16-18 μ; 4 preanal ventral more or less chromadoroid supplements.

FEMALE: 746 μ long; a, 17; b, 7.5; c, 17; V 49%; anterior ovary 14%; posterior ovary 10% of body length.

TYPE HABITAT: Soil about roots of *Schinus* sp. (Brazilian pepper), low ground.

TYPE LOCALITY: Atwood Grove, Ellenton, Florida.

KEY TO THE SPECIES OF *Haliplectus*

1. Amphids slightly more than 1 head diameter back from anterior extremity 2
   Amphids distinctly more than 1 head diameter back from anterior extremity 3
2. Only female known, 1.9 mm. long, (amphids 2/5 as wide as head, interrupt 5 annules) ..... *H. conicephalum* Cobb, n. sp.
   Only male known, 1.0 mm. long ..... *H. dorsalis* Cobb, n. sp.
3. Male with 6 preanal supplements ..... *H. floridanus* Cobb, n. sp.
   Male with no preanal supplements ..... *H. pellucidus* Cobb, 1913.
   Male with 4 preanal supplements ..... *H. bickneri* n. sp.

LITERATURE CITED

CHITWOOD, B. G. 1951. North American marine nematodes. Texas Journ. Sc. 3: 617-672, figs. 1-17.

COBB, N. A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. J. Wash. Acad. Sc. 3 : 432-444, plate separate.

Known and Suspected Plant-Parasitic Nematodes of Rhode Island* II. *Xiphinema americanum* with notes on *Tylencholaimus brevicaudatus* n. comb.

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ATTEMPTS TO COLONIZE *Xiphinema americanum* Cobb, 1913 IN THE GREENHOUSE.

One of the most common nematode species found associated with plants throughout Rhode Island is *X. americanum,* commonly called the dagger nematode (Buhrer, 1954). In several cases of plant decline investigated, numerous specimens of this nematode have been isolated from root and soil samples taken from affected plants. Although several authors have suspected that the species is parasitic on plants (Chitwood and Oteifa 1952, Cobb 1914, Steiner 1952), there are no published accounts proving its pathogenicity (Chitwood and Oteifa 1952, Christie 1952). Chitwood and Oteifa (1952) state that this species is considered to be slow in reproducing and does not cause disease symptoms unless present over extremely long periods of time.

A series of greenhouse experiments were conducted to investigate the possible phytopathogenicity of *X. americanum.* When 300 selected females and larvae were inoculated into six-inch pots in which one-year-old elm seedlings (*Ulmus americana* L.) were growing, only an average of 137 of these nematodes were recovered eighteen months later. When 500 of these nematodes were inoculated into pots containing two-year-old boxwood plants, only 1 to 5 were recovered after 6 months.

Each of seven replicate pots in which a month-old Astoria Colonial bentgrass plant, *Agrostis tenuis* Sibth., was growing, was inoculated with either 100 or 250 selected *X. americanum* while another group of pots received nematode suspensions of several types of nematodes from bentgrass containing approximately 100 or 250 *X. americanum.* When this experiment was harvested a year later, almost all *X. americanum* populations had decreased significantly in number.

A six-week-old seedling of velvet bentgrass, *Agrostis canina* L., was placed in each of 20 3-inch pots filled with turf soil containing *X. americanum* as well as other nematodes. When the soil was screened 6 month later, *X. americanum* comprised 18 per cent of the total nematode population and was represented by numerous larvae as well as females. When picked specimens were transferred under aseptic conditions using Byar's technique (1914), to plants containing Pfeffer's agar on which red clover, *Trifolium pratense* L., and velvet bentgrass seedlings were growing, the worms perished during the 4-day observational period and were not detected feeding on the roots.

Despite the fact that no evidence of a pathogenic role was discerned for *X. americanum,* these experiments cannot be interpreted as demonstrating that the species is nonpathogenic since in no case were they really successfully colonized in these experiments. Steiner (1952) suggests that the soil offers nematodes protection against temperature extremes and sudden and rapid temperature changes. Since the greenhouse temperatures fluctuated as much

*Contribution No. 869 from the Rhode Island Agricultural Experiment Station, Kingston.

as 45 degrees F. during the time these experiments were conducted, it is possible that this, as well as the abnormal water relationships to which the greenhouse-grown plants are subjected, caused *Xiphinema* populations to decline.

DESCRIPTION OF *Xiphinema americanum* COBB, 1913.

Taxonomic studies were conducted on female specimens obtained from soil associated with roots of white pine, *Pinus strobus* L., white spruce, *Picea glauca* (Moench) Voss, dogwood (*Cornus florida* L.) American elm, bentgrass, geranium (*Pelargonium hortorum* L.) red pine (*Pinus resinosa* Ait.), boxwood, and peach (*Prunus persica* (L.) Batsch.). The male specimens were obtained from bentgrass and elm.

MEASUREMENTS: 18 ♀ : L = 1.6 mm. (1.4-1.9 mm.); a = 42.3 (33.6-46.6); b = 6.3 (4.7-7.2); c = 44.7 (36.5-52.8); V — $^{9}51^{8}$ (46-54).

3 ♂ : L = 1.6 mm. (1.5-1.7 mm.); a = 47.2 (39.7-51.6); b = 6.2 (6.1-6.3); c = 43.6 (37.8-50.1); T — 46 (40-54).

Body cylindrical, tapering gradually at each end, generally assuming a spiral position when killed by gradual heat (Fig. 1, A). Lip region set off slightly with six lips almost completely amalgamated. An inner circle of six papillae and an outer circle of 10 papillae are present in much the same manner illustrated by Chitwood and Chitwood (1950). The two lateral lips each bearing an anterior projection situated immediately adjacent to the oral opening (Fig. 1, B). Amphid apertures situated on posterior extremities of lateral lips; amphids about half as wide as lip region. Cuticle bearing fine transverse striations.

Distinctive odontostylet averaging 72 μ in length and overlapping at the point of attachment to the posterior extension averaging 47 μ in length; total average length 117 μ in females and 111 μ in males. Flanges at base of spear extension approximately 9 μ wide. Guiding ring double, almost 10μ in length, situated anterior to junction of spear and spear extension.

Intestine about six cells in circumference, filled with various sized refractive globules; intestinal lumen pronounced. Vulva a somewhat depressed transverse slit, situated near middle of body. Ovaries amphidelphic, roughly symmetrical, and reflexed (Fig. 1, D). Anterior ovary averaging 155 μ in length; posterior ovary averaging 134 μ in length. Prerectum somewhat inconspicuous; tail dorsally convex-conoid, bearing two pairs of variably situated papillae (Fig. 1, E-K).

Of the three male specimens obtained, one was found around elm roots while two were obtained from bentgrass roots. The male from elm exhibited stout, arcuate spicules of 33 μ length (Fig. 1, G) with the curvature occurring mainly near the distal end. The two pairs of caudal papillae were found to be bilaterally nonsymmetrical in position when opposing lateral views of the same specimen were inspected (Fig. 1, G, H). Besides the adanal pair of supplements, a ventro-median series of nine were observed more or less uniformly spaced (Fig. 1, I). Both of the males from bentgrass had spicules differing from those in the elm specimens in that the curvature occurred mainly near the proximal end. One male from bentgrass had spicules 30 μ long and supplements consisting of an adanal pair as well as a ventromedian series of five spaced irregularly (Fig. 1, J), while the other male had spicules

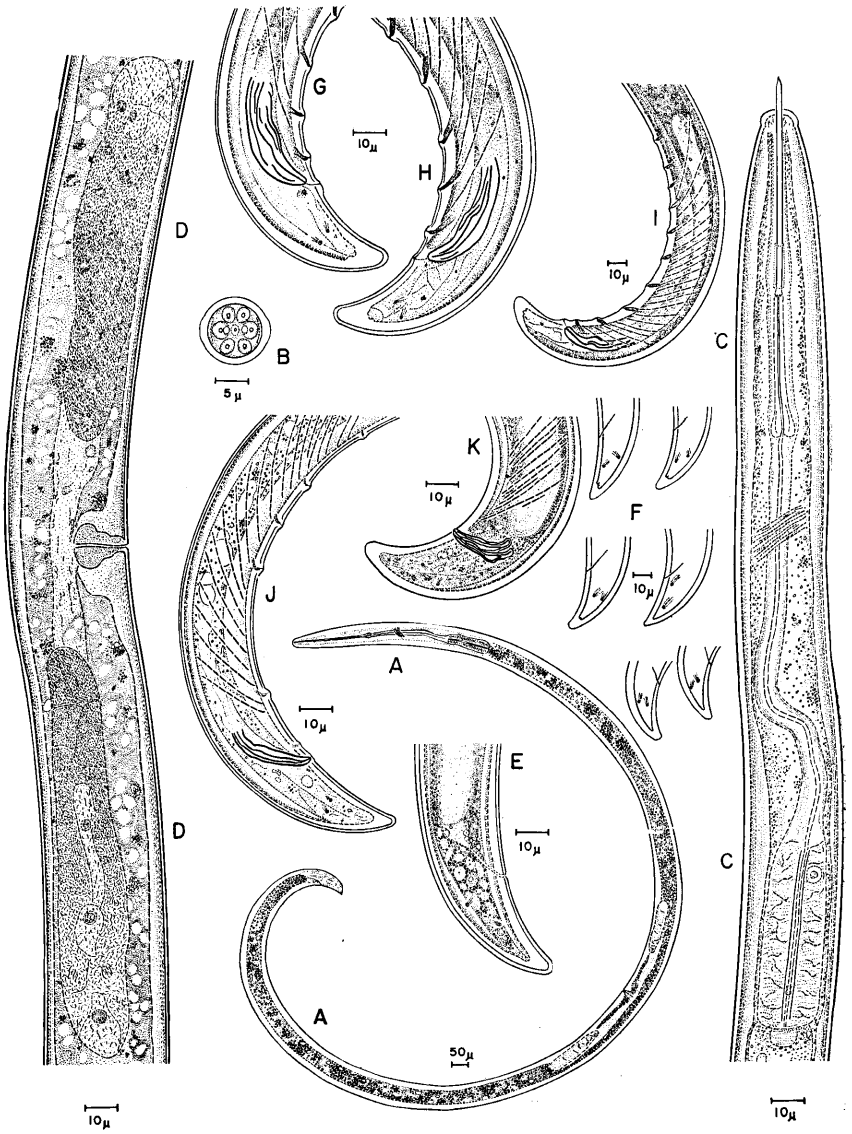

Fig. 1. *Xiphinema americanum*. A. Adult female. B. *En face* view. C. Oesophageal region of female. D. Female reproductive region. E. Posterior end of female. F. Female tails showing varying positions of caudal papillae. G. and H. Male tail showing opposing lateral views. I. and J. Male tails showing supplement arrangement. K. Male tail without supplements.

27 μ long but carried no supplements although copulatory musculature was readily apparent (Fig. 1, K).

Four female and two male specimens of *X. americanum* collected from Oregon soils and kindly loaned the author by H. J. Jensen were found to be larger than the Rhode Island specimens, the females averaging 2117 μ in length and the males 2032 μ in length. Measurements of these compared favorably to those listed by Thorne (1939), whereas measurements of the Rhode Island specimens seem to conform more closely to those presented by Cobb (1913) and by Loos (1949). The posterior end of one male from Oregon carried a total of six supplements while the other male had nine supplements, besides the adanal pair. The latter male, while exhibiting the usual two pairs of caudal papillae, also had two slightly sub-lateral papillae on one side directly above the spicules and only one papilla in somewhat the same position on the opposite side. It appears, therfore, that supplement and somatic papillae arrangements can be variable in *X. americanum*.

A NOTE ON THE IDENTITY OF SPECIMENS DESCRIBED IN A PRIOR PUBLICATION

A major portion of the first paper in this series, "Known and Suspected Plant-Parasitic Nematodes of Rhode Island, 1," was devoted to descriptions and drawings of two nematode species which were designated as *Longidorella parva* Thorne, 1939, and *Discomyctus brevicaudatus* Tarjan, 1953. In publication, the figures of *L. parva* erroneously appeared with the legend for *D. brevicaudatus* and vice versa.

Gerald Thorne has called attention to the possibility that the form identified as *Longidorella parva* might be *Dorylaimus microdorus* DeMan, 1880 or *D. penetrans* Thorne and Swanger, 1936, since Altherr (1954) had made and corrected a similar error. Thorne kindly loaned this writer specimens of the three species for comparative studies. An examination and measurement of these nematodes revealed that the Rhode Island specimens more closely resembled *L. parva* in nematode formula than they did either of the Dorylaims. The shape of the oesophagi of the Rhode Island specimens as well as the length of the stylet and overall physical appearance, however, were found to be closer to *D. microdorus;* consequently, the Rhode Island specimens originally described are redesignated as *Dorylaimus microdorus.*

It was pointed out that *Discomyctus brevicaudatus* Tarjan, 1953 had characteristics which related it to both *Discomyctus* and *Tylencholaimus,* but mainly on the basis of its non-muscular anterior oesophagus it was being placed in the former genus despite the sub-digitate tail shape which was at variance with other *Discomyctus* species. Inspection of several specimens of *Discomyctus cephalatus* Thorne, 1939 obtained from Gerald Thorne clearly revealed that the vestibular disc was unlike the type of disc observed in *D. brevicaudatus.* Another slide containing specimens of *Tylencholaimus minimus* DeMan, 1876 showed that this species had a labial region and non-muscular anterior oesphagus somewhat similar to that in the Rhode Island specimens. Accordingly, it is felt that this species is more closely related to *Tylencholaimus* than to *Discomyctus* and is redesignated *Tylencholaimus brevicaudatus* n. comb.

DIAGNOSIS (amended): Tylencholaimus with non-muscular anterior portion of oesophagus and dorsally convex, sub-digitate tail.

TYPE SPECIMENS: One paratype in University of California Collection, Berkeley; holotype and other paratypes in author's possession.

LITERATURE CITED

ALTHERR, E. 1954. Les Nematodes des Etangs de Bavois (Vaud). Bull. Soc. Vaud. Sc. Nat. 65: 33-45.

BUHRER, E .M. 1954. Common names of some important plant pathogenic nematodes. U. S. Dept. Agr., Plt. Dis. Rptr. 38: 535-541.

BYARS, L. P. 1914. Preliminary notes on the cultivation of the plant parasitic nematode, *Heterodera radicicola*. Phytopath. 4: 323-326.

CHITWOOD, B. G. and M. B. CHITWOOD. 1950. An introduction to nematology, Section I, Anatomy. Monumental Printing Company, Baltimore, Md., 213 pgs., 145 figs.

CHITWOOD, B. G. and B. A. OTEIFA. 1952. Nematodes parasitic on plants. Ann. Rev. Microb. 6: 151-184.

CHRISTIE, J. R. 1952. Some new nematode species of critical importance to Florida growers. Proc. Soil. Sci. Soc. Fla. 12: 30-39.

COBB, N. A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. Jour. Wash. Acad. Sci. 3: 432-444.

————. 1914. Nematodes and their relationships. U. S. D. A., Yearbook of Agr.: 457-490.

————. 1918. Estimating the nema population of soil. U. S. D. A. Agr. Circ. 1: 1-48.

LOOS, C. A. 1949. Notes on free-living and plant-parasitic nematodes of Ceylon—No. 5. Jour. Zool. Soc. India 1: 23-29.

STEINER, G. 1949. Nematodes that attack boxwood and their control. Proc. 25th Nat. Shade Tree Conf.: 108-118.

————. 1952. The soil in its relationship to plant nematodes. Proc. Soil Sci. Soc. Fla. 12: 24-29.

TARJAN, A. C. 1953. Known and suspected plant-parasitic nematodes of Rhode Island, 1. Proc. Helmin. Soc. Wash. 20: 49-54. (Errata. ibid: 132.)

THORNE, GERALD. 1939. A monograph of the nematodes of the superfamily *Dorylaimoidea*. Capita Zool. 8: 1-255.

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