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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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Observations on *Hymenolepis macyi* Locker and Rausch, with a Revised Diagnosis of this Cestode

BETTY S. DAVIS* AND MARIETTA VOGE**

Cestodes removed from *Sorex ornatus* caught in November, 1955, at the Hastings Reservation, Monterey County, California, were identified as *Hymenolepis macyi* Locker and Rausch 1952, a species described from *Sorex vagrans* in Oregon. Observations on this material, and a review of the type specimen and other specimens from the type locality, revealed additional structural peculiarities. A further description of this species was therefore desirable; the results of our observations are reported here.

The cestodes were fixed in Bouin's fluid. Whole specimens were stained with a dilute aqueous solution of Ehrlich's haematoxylin. Sections of gravid proglottids were stained with Gomori's trichrome. We are grateful to Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska, for the loan of specimens of *H. macyi* from Oregon.

In general appearance, the specimens from *Sorex ornatus* resemble those from the type host *Sorex vagrans*. Strobilar length is variable and depends on the number of mature and semi-gravid proglottids. When the proglottid number is relatively large, transitions between mature and semi-gravid regions do not seem as abrupt as in shorter worms. As many as 7 or 8 semi-gravid proglottids were seen in specimens from *Sorex ornatus*. Semi-gravid proglottids contain the genital ducts, which generally disappear in detached gravid proglottids. Fully gravid segments were never seen on the strobilae of preserved specimens. When removed from the intestine, the terminal segments of the worms would invariably detach. Large numbers of detached gravid proglottids occurred in the host's intestine. It is likely that after a certain stage of development has been reached, terminal semi-gravid segments break off and subsequently complete development in the intestine of the host. This may explain why fully gravid segments have not been found previously. The gravid proglottids are less elongate than semi-gravid ones and the eggs they contain measure up to 40 microns. The shells of the tightly packed eggs are very thin, so that nothing but a dense mass of onchospheres is visible under low magnifications.

The scolex bears an unarmed rostellum (Voge, 1955) measuring 24 by 38 microns. Scolex diameter ranges from 116-204 microns, sucker diameter from 57-90 by 84-150 microns. Scolex measurements of specimens obtained from *Sorex trowbridgei* and reported on previously (Voge, 1955) were as follows: scolex diameter 172-220 microns, sucker diameter 70-80 by 84-100 microns, rostellum diameter 22-32 by 40-50 microns. In five specimens, the number of semi-gravid proglottids varried from 1-5.

*Hastings Reservation, University of California, Carmel Valley.

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

Our observations on the structure of the mature proglottid (Fig. 1) differ with the original description in several ways. The cirrus armature not reported originally was found in specimens from three different host species. The spines or hairs on the cirrus are numerous, but are so slender that only very high magnification will reveal their presence. The testes were originally described as being situated in a diagonal row. In all the specimens seen by us (including the type specimen) the testes form a triangle, and the anterior testis is usually of relatively small size. The ovary is trilobed, as originally described, but we could not see the extreme posterior position of two of the lobes as illustrated in figure 2 of Locker and Rausch (1952). Rather, the ovary is in a more or less transverse position anterior to the vitelline gland.

The structure of the uterus in detached segments is unique (Fig. 2). It is an ovoid body, and in external structure reminiscent of certain plant seeds. The entire surface bears numerous fine "lines" oriented in an antero-posterior direction. From these "lines," or the spaces between them, there arise short processes that give the uterine surface a "hairy" appearance. These structures are not visible in immature gravid proglottids, and even in the detached proglottids one may see varying degrees of development of the lines and processes, which become most pronounced in segments containing the largest eggs. The regularity and consistency of these structures preclude the possibility that they are artifacts. Although the function of these peculiar structures is obscure, it is possible that the filamentous processes extending from the uterine surface serve as an anchoring device after the remainder of the proglottid has disintegrated.

Longitudinal and cross-sections of the uterus were examined to determine the nature of the "lines" and "hairs" on the uterine wall. In longitudinal section the lines on the uterine surface appear as broadly or sharply undulating tubular strands encircling the uterus antero-posteriorly. They are not uniform in width, but narrow and widen at irregular intervals (Fig. 3). Some sections of the strands contain a homogeneous material that stains a deep, clear red with Gomori's trichrome; others are colorless. In cross section the tubules are circular or oval and the wall is seen as a narrow sheath surrounding a darkly staining substance (Fig. 5). It is also apparent that the tubular strands are not a part of the uterine wall nor are they firmly fixed to it. They appear to lie on or close to the surface of the uterus in the highly fibrous and vacuolate parenchyma surrounding it and seem to bend out from and recurve narrowly toward the surface at irregular intervals (Fig. 4).

The "hairy" aspect of the uterus results from the presence of numerous short and long, delicate, spindle-shaped cells which protrude from the surface at irregular intervals (Figs. 3, 4, and 5). These cells seem to originate from the outer margin of the uterine wall and extend well out into the vacuolated parenchyma surrounding the uterus, tapering at their distal ends into narrow, twisted filaments that may reach almost to the periphery of the proglottid. The spindle-shaped cells are not evenly distributed over the surface of the uterus but are numerous in some areas and scarce in others (Fig. 2). Although they appear to arise from the "lines," as well as between them, it was found that they actually arise from the uterine surface below the "lines" rather than from the "lines" proper.

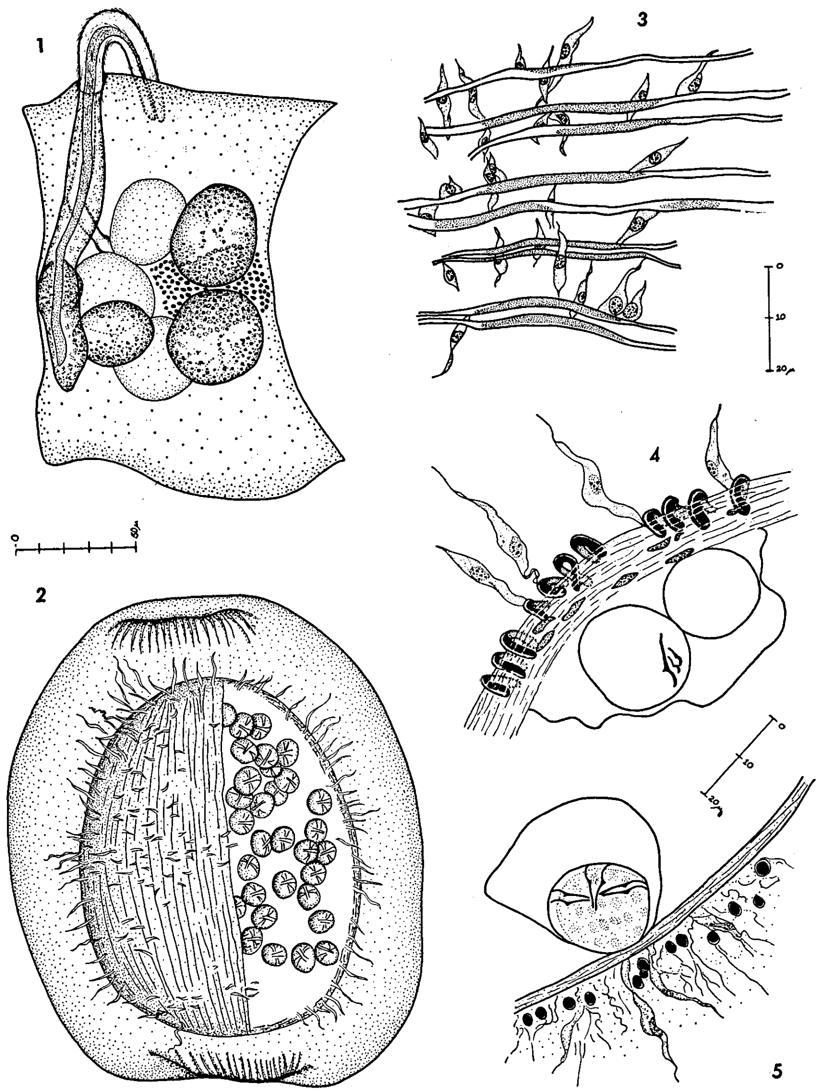

Fig. 1. Mature segment of *Hymenolepis macyi,* from *Sorex vagrans,* Oregon.
Figs. 2-5. *Hymenolepis macyi,* from *Sorex ornatus,* Hastings Reservation.
Fig. 2. Fully gravid proglottid, showing capsule-like uterus.

Fig. 3. Surface of uterus showing arrangement of tubular strands and spindle-shaped cells.

Fig. 4. Oblique section of uterine wall showing buckling strands and spindle-shaped cells.

Fig. 5. Cross-section of uterine wall, showing structure and position of strands in relation to uterus and vacuolated parenchyma.

All figures drawn with the aid of a camera lucida.

REVISED DIAGNOSIS OF *Hymenolepis macyi* Locker and Rausch, 1952.

Strobila length about 2mm; greatest width attained in semi-gravid and gravid segments, which are markedly larger than other segments. Transition from immature to mature and from mature to semi-gravid segments usually abrupt. Immature and mature segments wider than long; late mature segments nearly square; semi-gravid and gravid segments oval. Scolex with unarmed rostellum. Scolex diameter 116-230 microns; sucker diameter 57-90 by 84-150 microns; rostellum diameter 22-32 by 38-50 microns. Cirrus sac up to 140 microns long by 20 microns wide, extends nearly across mature segment. Cirrus armed with numerous, very slender spines. External seminal vesicle prominent. Testes in mature segments about 35 microns in diameter, forming a triangle. Vagina, about 8 microns in diameter in mature segments, ventral to cirrus sac. Seminal receptacle not noted. Ovary trilobed, situated near center of segment. Development of uterus abrupt; uterus in gravid segments capsule-like; surface of uterine wall with numerous encircling tubules and cellular processes which give uterine surface a hairy appearance. These structures not present in semi-gravid segments. Eggs thin-shelled, spherical, 30-40 microns in diameter.

TYPE HOST: *Sorex v. vagrans* Baird.

OTHER HOSTS: *Sorex ornatus* Merriam, and *Sorex trowbridgei* Baird.

TYPE LOCALITY: Portland, Oregon.

OTHER LOCALITIES: Hastings Reservation, Monterey County, and Humboldt County, California.

SUMMARY

Hymenolepis macyi Locker and Rausch is reported from *Sorex ornatus* in California. The mature and gravid segments of this cestode are redescribed. Observations on the unusual structure of the uterus and a revised diagnosis of *H. macyi* are included.

LITERATURE CITED

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VOGE, M. 1955. Notes on four hymenolepidid cestodes from shrews. J. Parasitol., *41*:74-76.

The Morphogenesis of the Parasitic Stages of *Trichostrongylus axei* and *Trichostrongylus colubriformis*, Nematode Parasites of Cattle*

FRANK W. DOUVRES

Trichostrongylus axei (Cobbold, 1879) Railliet and Henry, 1909, and *Trichostrongylus colubriformis* (Giles, 1892) Ransom, 1911, commonly known as the small hair worms, are trichostrongylid nematodes found in ruminants. Both species inhabit the bovine gastrointestinal tract, *T. axei* being found principally in the fourth-stomach and *T. colubriformis* in the small intestine.

Nagaty (1932) in a review of the genus *Trichostrongylus*, synonymized *T. extenuatus* with *T. axei* and *T. instabilis* with *T. colubriformis.*

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*From the Regional Animal Disease Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Auburn, Alabama.

Mönnig (1926), employing a mixed infection of *T. rugatus* and *T. colubriformis,* simultaneously studied the life histories of these two species in sheep. However, he reported no data which permitted the differentiation of the larval stages of the 2 species, prior to the attainment of the fourth lethargus. Up to the present time the parasitic development of *T. colubriformis* and *T. axei* in cattle has not been described.

Severe clinical parasitism involving *T. axei* and *T. colubriformis* together with other nematodes has been reported by Dikmans and Kates (1955) and immature specimens difficult to identify are frequently encountered in the course of post-mortem examination (Vegors, *et al.,* 1955). It is, therefore, the objective of this paper to present descriptions of the immature stages of the parasitic development of *T. axei* and *T. colubriformis* with special reference to the morphological features which could be of value in the identification and differentiation of these species.

MATERIALS AND METHODS

Eight parasite-free, grade Jersey calves obtained from local dairies within 24 hours of birth were used. They were raised in portable pens (Davis, 1949) until infected, at which time they were moved to individual stalls in a concrete-floored barn, and maintained in the manner described by Porter and Cauthen (1942).

The infective larvae administered to the calves were obtained from sphagnum moss cultures of feces (Cauthen 1940) collected from 3 culture animals; one calf having been infected with *T. axei,* the second with *T. colubriformis,* and a third calf with both species. Each of the culture animals was infected with larvae reared from eggs from identified females of each species used. The larvae were isolated with a Baermann apparatus and were administered to calves immediately thereafter or after storage in a refrigerator (4.5° C.) for one to two weeks. Infective third-stage larvae agreeing morphologically with published descriptions of *T. axei* and *T. colubriformis* (Keith, 1953 and Dikmans and Andrews, 1933) were administered by means of a blowpipe attached to a 5 cc. rubber bulb. Between 30,000 and 100,000 larvae were administered orally to 8 calves in one or two doses. Parasitic larvae were recovered at post-mortem from the 8 calves killed from about 4⅙ days to 15 days after infection, using the method described by Porter (1942).

All measurements were made from heat-killed, unstained specimens floating freely in 0.85 percent saline under the coverslip to prevent distortion. Camera lucida drawings were made from specimens treated by this method and from specimens killed with 10 percent formalin, cleared with lactophenol and mounted in glycerine.

RESULTS

Table 1 summarizes the data of the experimental infections with *T. axei* and *T. colubriformis* in the 8 calves used, and the larval stages recovered from each calf. It is noted at this time that *T. colubriformis* larvae in the third molt were not recovered from the experimental infections of the calves; therefore, the descriptions and dimensions of this stage (Table 3) are based on specimens recovered from a guinea pig that had been infected with this species 3 days prior to slaughter (Herlich, *et al.,* in press). Thus it was possible to determine the approximate time required for development for each of the stages of both species. *T. axei*: The parasitic third-stage

TABLE 1. Data on experimental infections with Trichostrongylus axei and Trichostrongylus colubriformis in 8 calves and larval stages recovered following slaughter.

Calf No.	Larvae per dose	Total larvae given	Interval, from administration of dose(s) to slaughter (days)	Major parasitic stages recovered: *T. axei*	Major parasitic stages recovered: *T. colubriformis*
1	30,000 Ta, Tc*		2	Only parasitic third	Parasitic third
	30,000 Ta, Tc	60,000	5	Only parasitic third	Fourth
2	37,500 Tc		2		Parasitic third
	37,500 Tc	75,000	5		Fourth
3	46,500 Ta	46,500	4⅙	Parasitic third Third molt	
4	30,000 Ta, Tc		6	Third molt	Fourth molt
	30,000 Ta, Tc	60,000	10	Fourth molt	Fourth molt
5	50,000 Ta		7	Fourth	
	50,000 Ta	100,000	13	Fourth molt	
6	65,000 Tc	65,000	7		Fourth molt
7	50,000 Ta	50,000	15	Fifth	
8	30,000 Tc	30,000	15		Fifth

* Ta equals *T. axei;* Tc equals *T. colubriformis*

larvae following the second ecdysis, were found as early as 2 days and as late as 5 days after infection; larvae in the third molt were recovered as early as 4⅙ days and as late as 6 days after infection; the fourth-stage larvae, following the third ecdysis were found 7 days after infection; larvae in the fourth molt were recovered 10 days after infection; and the fifth-stage worms, following the fourth or final ecdysis were recovered 15 days after infection. *T. colubriformis*: The parasitic third-stage larvae, following the second ecdysis, were found 2 days after infection; larvae in the third molt may be recovered by the third to fourth day after infection on the basis of the findings from a guinea pig (noted above) and because fourth-stage larvae, following the third ecdysis, were recovered by the fifth day after infection; larvae in the fourth molt were recovered as early as 6 days and as late as 10 days after infection; and the fifth-stage worms, following the fourth or final ecdysis, were recovered 15 days after infection.

T. axei larvae were recovered from the abomasal mucosal scrapings and washings, and only from calf 7 (Table 1) were larvae also recovered from the abomasal contents. The majority of the *T. colubriformis* larvae were recovered from the washings of the small intestines, with many specimens being collected from the contents and first rinsing of the small intestine. In addition, *T. colubriformis* larvae were recovered in small numbers from the abomasum of each calf infected with this species, whereas a few larvae of *T. axei* were recovered from the small intestine of only 2 of 5 calves.

As Tables 2 and 3 summarize the body measurements for each stage of development recovered and described for both species, it was deemed unnecessary to repeat them in the text; therefore, the descriptions of the larvae are primarily anatomical and only include measurements not given in the tables. The anatomical descriptions of the larvae are based on specimens viewed in optical sections.

PARASITIC THIRD-STAGE LARVA. Except for a difference in the structure at the tip of the tail and certain minor other differences, the larvae of the

2 species were alike. Hence the following description applies to both species, except where the differences between the two are pointed out.

This stage of development resembled the corresponding stage of related trichostrongylids; body slender and cylindrical for most of its length, tapering more posteriorly than anteriorly. The lips and papillae were barely discernible, but 2 very prominent lateral amphids (Fig. 1) were easily seen at the cephalic end of the nematode. The mouth opened into a medially located very minute buccal capsule, represented by a single thin, refractive line which terminated at an anteriorly truncated oesophagus (Figs. 1 and 2). The intestine was composed of 16 granular, uninucleated cells in *T. axei.* However, it was difficult to discern the cellular outline in most *T. colubriformis* specimens. There appeared to be 18 cells, with several cells containing more than one nucleus. Only the excretory pore, terminal canal duct, and 2 excretory gland cells of the excretory system were observed; the gland cells appeared to terminate anterior to, or at the level of the oesophageal base. In formalin fixed, lactophenol cleared and glycerine mounted specimens, a very slight depression could be seen at the location of the excretory pore; however, this depression was not evident in heat-killed specimens.

TABLE 2. Measurements[a] of parasitic larvae of Trichostrongylus axei.

Stages of Development	Parasitic third stage	Third molt	Fourth stage	Fourth molt	Fifth stage
Days after infection	2 + 5	6	7	10	15
Total length	0.83	1.09	1.46 (1.57)	2.52 (2.81)	3.70 (4.79)
Width at level of nerve ring	0.02	0.02	0.02	0.03	0.03
Width at base of oesophagus	0.02	0.03	0.03	0.04	0.04
Width at level of anus	0.02	0.03[b] 0.02[c]	0.04 (0.02)	0.07 (0.03)	------ (0.03)
Distance from excretory pore to terminal ends of excretory gland cells	------	0.29, 0.34	0.88, 0.92 (1.01, 1.05)	1.12, 1.30 (1.25, 1.44)	1.50, 1.78 (1.49, 1.79)
Oesophageal length	0.24	0.36	0.43	0.55 (0.60)	0.69 (0.72)
Distance from posterior end of genital primordium to anus	0.25	0.21[b] 0.10[c]	0.12 (0.13)	------ ------	------ ------
Distance from anus to tip of larval tail	0.08	0.07	0.06 (0.07)	0.04 (0.07)	------ (0.08)
Distance from tip of larval tail to posterior tip of sheath	------	0.01	------	0.04 (0.01)	------
Spicule length: Left	------	------	------	0.11	0.12
Right				0.08	0.09
Width at level of vulva	------	------	0.03	0.07	0.06
Combined length of vagina and muscular portions of ovijectors	------	------	------	0.15	0.27

[a]Averages in millimeters for 10 males and/or females, except where otherwise noted; where two averages are given, the one in parentheses applies to females and the one above it to males; where only one average is given it is based on 20 specimens and there were no differences between sexes, or it pertains to a sexual character.

[b]These two measurements apply to the five specimens in which sex could be determined (see Text).

[c]These two measurements apply to the 5 specimens in which sex could not be determined (see Text).

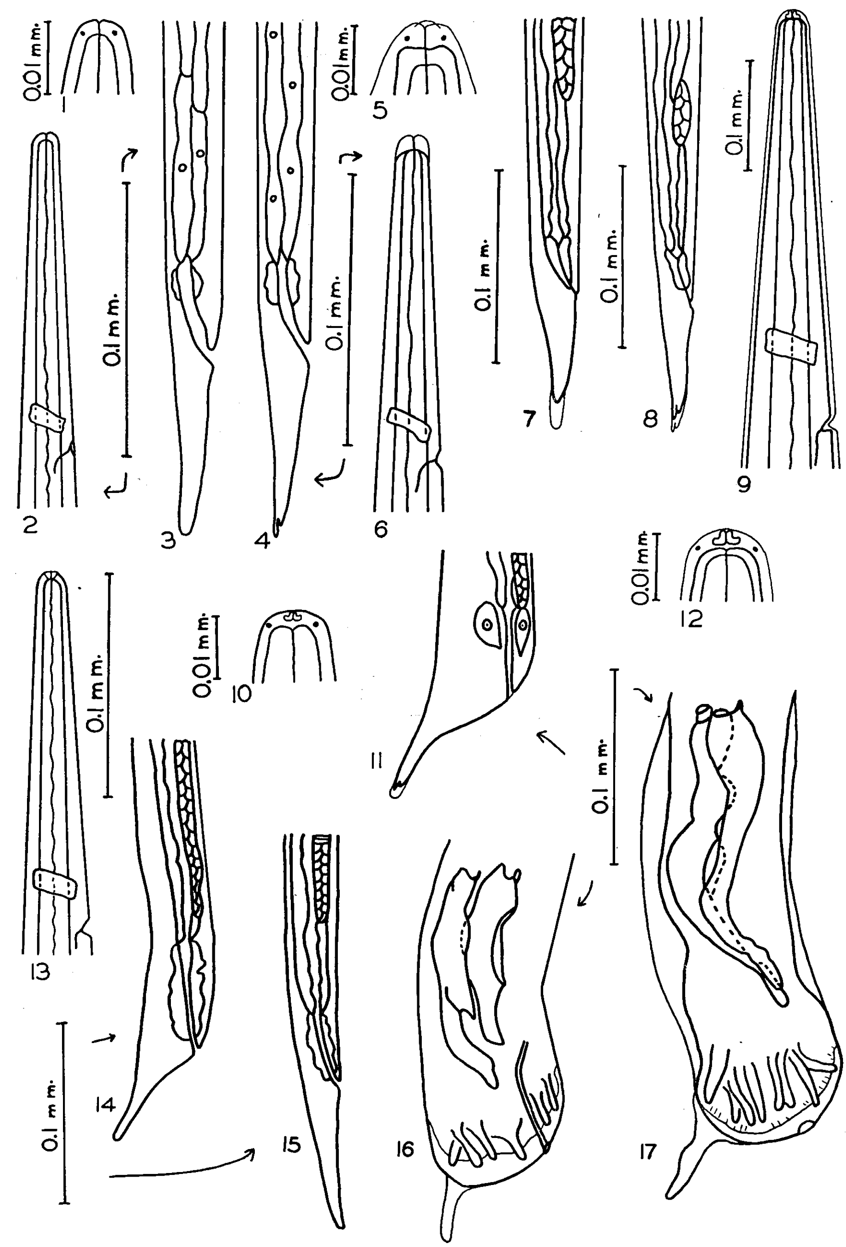

Figure 1. Cephalic end of the parasitic third-stage larva of *T. axei.* dorsal view.

Figure 2. Anterior region of the parasitic third-stage larva of *T. axei,* lateral view.*

*Drawings made from 10 percent formalin fixed, lactophenol cleared and glycerine mounted specimens.

All other drawings made from heat-killed specimens. floating freely in saline.

All figures drawn with the aid of the camera lucida.

TABLE 3. Measurements[a] of parasitic larvae of Trichostrongylus colubriformis

Stage of Development	Parasitic third stage	Third molt	Fourth stage	Fourth molt			Fifth stage
Days after infection	2	3	5	6	7	10	15
Total length	0.75	0.83 (0.90)	1.48 (1.53)	2.02[b] (1.90)	2.10 (2.33)	2.80 (3.83)	5.19 (6.56)
Width at level of nerve ring	0.02	0.02	0.03	0.03	0.03	0.03	0.03
Width at base of oesophagus	0.02	0.03	0.03	0.04	0.04	0.04	0.05 (0.04)
Width at level of anus	0.02	0.03 (0.02)	0.04 (0.02)	0.06[b] (0.02)	0.06 (0.03)	0.10 (0.03)	[c]
Distance from excretory pore to terminal ends of excretory gland cells	------	0.37 0.41 (0.36) (0.42)	0.57 0.68 (0.68) (0.82)	[c]	0.80 0.91 (0.96) (1.24)	[c]	1.36 1.44 (1.69) (1.85)
Oesophageal length	0.22	0.28 (0.29)	0.37	0.47[b] (0.45)	0.59	0.62 (0.72)	0.63 (0.67)
Distance from posterior end of genital primordium to anus	0.22	0.18 (0.10)	0.23 (0.11)	------	------	------	------
Distance from anus to tip of larval tail	0.07	0.06	0.07	0.08	0.07 (0.08)	0.06 (0.08)	------ (0.09)
Distance from tip of larval tail to posterior tip of sheath	------	0.01	------	0.01	0.01	0.04 (0.01)	------
Spicule lengths	------	------	------	------	------	0.18	0.19
Width at level of vulva	------	------	0.03	0.04	0.05	0.08	0.11
Combined lengths of vagina and muscular portions of ovijectors	------	------	------	0.09	0.14	0.17	0.39

[a] Same as (a) Table 2. [b] Based on 2 male specimens. [c] Measurements not made.

Figure 3. Posterior region of the parasitic third-stage larva of *T. axei*, lateral view.
Figure 4. Posterior region of the parasitic third-stage larva of *T. colubriformis*, lateral view.
Figure 5. Cephalic end of the larva in the third molt of *T. coulbriformis*, dorsal view.
Figure 6. Anterior region of the larva in the third molt of *T. colubriformis*, lateral view.
Figure 7. Posterior region of the larva in the third molt of *T. axei*, lateral view.
Figure 8. Posterior region of the larva in the third molt of *T. colubriformis*, lateral view.
Figure 9. Anterior region of the larva in the fourth molt of *T. colubriformis*, lateral view.*
Figure 10. Cephalic end of the fourth-stage larva of *T. axei*, dorsal view.*
Figure 11. Posterior region of a male larva in the fourth molt of *T. colubriformis*, lateral view.
Figure 12. Cephalic end of the larva in the fourth molt of *T. colubriformis*, dorsal view.*
Figure 13. Anterior region of the fourth-stage larva of *T. axei*, lateral view.
Figure 14. Posterior region of the fourth-stage male of *T. axei*, lateral view.
Figure 15. Posterior region of the fourth-stage female of *T. colubriformis*, lateral view.
Figure 16. Posterior region of a male larva in the fourth molt of *T. axei*, lateral view.
Figure 17. Posterior region of a male larva in the fourth molt of *T. colubriformis*, lateral view.

Two prominent ovoid coelomocytes, one posterior to the other, were located posterior to the oesophageal base and appeared to be indented on the ventrolateral surface of the intestine. The genital primordium consisted of 2 oval bodies, a large one adjacent and caudad to a smaller one; both were situated on the ventral surface of the intestine. The combined length of the genital primordium was 21 microns in *T. axei* and 26 microns in *T. colubriformis.* There were 2 prominent lateral caudal papillae, at equal or subequal levels, 38 to 48 microns from the tail tip in *T. axei* and 35 to 39 microns in *T. colubriformis.* In the majority of the *T. axei* specimens the tail was bent ventrad, approximately 10 microns from the tail tip which was bluntly rounded in all specimens (Fig. 3). In all the *T. colubriformis* larvae the posterior end was attenuated and terminated in 2 tubercles of unequal lengths (Fig. 4).

LARVA IN THE THIRD MOLT. In both species, third-stage larvae approaching the fourth-stage were enclosed in sheaths which were easily discerned in the tail region with an high dry (4 mm.) microscope objective and at the cephalic end only with an oil immersion (2 mm.) objective. However, in specimens viewed with medium (8 mm.) and high dry objectives, there appeared to be a hyaline-like cap present at the cephalic end, due probably to the presence of the sheath. These larvae were slightly larger in over-all size than the parasitic third-stage larvae (Tables 2 and 3).

When viewed with 8 mm. and 4 mm. objectives there were no critical differences in cephalic structure between larvae in the third molt and parasitic third-stage larvae (Fig. 6). Although differential cephalic structures likewise were not seen with the 2 mm. objective, under this objective a sheath and the body wall of the future fourth-stage larva (Fig. 5) were seen.

In both species, the oesophagus was anteriorly truncated (Figs. 5 and 6) and claviform, and the intestine was characterized by a complete lack of cellular definition, the presence of numerous nuclei and a wide lumen. All other morphologic features in this stage of development are described separately for each species.

T. axei: In approximately half the number of randomly selected 6-day-old larvae observed in lateral view, the excretory pore was located in a conspicuous depression (Fig. 6). The excretory gland cells had increased markedly in length (Table 2), and terminated in rounded ends posterior to the oesophageal base, one cell being more posteriorly extended than the other. The genital primordium was of two anatomical types: A multicellular elliptical body, which averaged 34 microns long, and a multicellular elongated or cigar-shaped body which averaged 71 microns long. Five of the 10 larvae picked at random from those recovered 6 days after infection were considered to be males because their bodies widened abruptly at the level of the anus (Table 2). It was not possible to determine the sex of the remaining 5 specimens or of any of the larvae in the third molt recovered 4⅙ days after infection. Specimens in which sex could be determined had the elongated type of genital primordium, whereas specimens of indeterminate sex invariably had the elliptical-shaped genital primordium. The tail of the larva proper and of the sheath terminated in bluntly rounded ends (Fig. 7).

T. colubriformis: When observed in lateral view, the excretory pore was located in a depression in only a very few of the 3-day-old larvae recovered from an experimentally infected guinea pig. The excretory gland cells had increased markedly in length (Table 3), and were situated as previously described for *T. axei.* Sex was readily distinguishable at this stage of development. In the males, the body was abruptly widened at the level of the anus (Table 3). The genital primordium consisted of an elongated, irregularly shaped body that appeared to have a cleft in its middle, averaged 40 microns in length and was located 182 microns anterior to the anus (Table 3). In the females there was no abrupt widening of the body at the level of the anus (Table 3), but rather a gradual tapering toward the tail end. The genital primordium consisted of an elliptical or oval body, averaged 45 microns in length and was located 96 microns anterior to the anus (Table 3). In both sexes, the tail end of the larva proper and of the sheath terminated in 2 tubercles of unequal lengths (Fig. 8).

FOURTH-STAGE LARVA. The fourth-stage larvae of the two species were found to be anatomically alike in almost all respects, except as noted below, and to vary but slightly dimensionally (Tables 2 and 3).

Except for the presence of a well-developed buccal capsule, an important feature of distinction, the structure of the cephalic region resembled that seen in third-stage larvae. No lips were evident. Cephalic papillae and amphids were present, but, while the amphids were easily seen with the 8 or 4 mm. objectives, the number of papillae could not be ascertained even with a 2 mm. objective. The mouth opened into a sclerotized buccal capsule which was medially located between an anteriorly truncated oesophagus and the cephalic tip. When specimens were viewed with 8 or 4 mm. objectives, the buccal capsule appeared as a somewhat bisected semilunar body, resting directly on the anterior end of the oesophagus (Fig. 13). However, with a 2 mm. objective it appeared as a pair of isomeric, stubby, J-shaped structures, the two being closely adjacent to one another, slightly separated from the anterior end of the oesophagus (Fig. 10).

The excretory pore was located in a conspicuous depression in both species (Fig. 13). The excretory gland cells, filled with refractive, coarse granules, had increased markedly in length (Tables 2 and 3). In both species, the longer of the 2 excretory gland cells terminated at the level of the anterior end of the genital primordium.

Sex in the fourth-stage larvae of both species was readily determined, both dimensionally (Tables 2 and 3) and anatomically. In the males, the genital primordium consisted of a multicellular elongated fusiform body, the ends of which were more bluntly rounded in *T. axei* than in *T. colubriformis*. The body was abruptly swollen at the level of the anus (Tables 2 and 3), and abruptly tapered to the caudal tip. In the females, the genital primordium extended equidistantly anteriad and posteriad from a primitive vulva, and consisted of numerous cells incipiently organized as primitive ovijectors, uteri, and ovaries. At the level of the vulva the body diameter was greater than at the level of the anus and approximately equal to that at the level of the oesophageal base (Tables 2 and 3).

Both sexes of *T. axei* had slightly tapering tails which terminated in bluntly rounded ends (Fig. 14). Lacking entirely the characteristic tubercles of the tail end described for the third-stage larvae, both sexes of *T. colubriformis* also had tails which terminated in rounded ends (Fig. 15).

LARVA IN THE FOURTH MOLT. In both species, fourth-stage larvae approaching the fifth-stage were enclosed in sheaths which were more easily discerned in the tail region than in the cephalic end.

The anatomy of the anterior ends of the larvae in the fourth molt was identical in the two species. In specimens viewed with 8 or 4 mm. objectives the anatomy of the cephalic end was identical to that seen with the

2 mm. objective in the early fourth-stage larva (Fig. 9). With the 2 mm. objective it was possible to observe in addition the following: 3 inconspicuous lips, 6 papillae, and the fact that the buccal capsule was attached to the sheath and that the cephalic structures of the future fifth-stage were not as yet differentiated (Fig. 12).

All other morphological features of the larvae of each species are described separately.

T. axei: The dimensions given in Table 2 and the following description are based on 10-day-old larvae (Table 1); those recovered from a 13-day-old infection (Table 1) were smaller and morphologically less advanced. At this stage of development the excretory pore was located in a very conspicuous notch (Fig. 9). The two excretory gland cells had increased in length (Table 2), but were still anatomically like those of the fourth-stage larvae. The reproductive systems in both sexes had developed markedly and had almost attained the structure described by other authors for the adults. In the males, the reproductive system extended 434 to 826 microns anteriad to the cloaca. Bursal rays and a pair of straw colored, unequal spicules anatomically like those of the adults were present (Fig. 16). The measurements of the spicules are given in Table 2. In the females, the vulva, vagina and muscular portions of the ovijectors were fully developed; the uteri and ovaries were recognizable by a difference in cellular organization. In both sexes, the tail end of the larva and of the sheath was bluntly rounded.

T. colubriformis: In the 6-day-old larvae (Table 1) the excretory pore was located in a conspicuous depression. Anatomically the reproductive system of the males had not altered much from that of the early fourth-stage larvae. However, in the females, the vagina and muscular portions of the ovijectors could be distinguished (Table 3). In both sexes, the tail end of the larva proper was characterized by the reappearance of 2 terminal tubercles of unequal lengths, like those observed in the third-stage, whereas the tail end of the sheath was rounded (Fig. 11).

In the 7-day-old larvae (Table 1), the spicular anlagen were present; however, their boundaries were difficult to discern and no measurement of them is given in Table 3. In the females, further differentiation of the reproductive system beyond that of the 6-day-old larvae was not possible. The tail of the larva proper and sheath of both sexes were like those of the 6-day-old specimens.

A few of the 10-day-old specimens recovered had completed the fourth or final ecdysis and were fifth-stage worms, however, the majority were still in the fourth molt. In the females, the vulva, vagina, ovijectors, uteri, and ovaries were well developed and differentiated. The bursal rays and straw colored spicules of the males were easily seen. The spicules were equal in length (Table 3) and anatomically like those of adult specimens. The tail of the larva proper and of the sheath of both sexes was like those described for the 6-day-old specimens.

FIFTH-STAGE WORMS. The fifth-stage male and female worms recovered 15 days after infection conformed with the published descriptions of *T. axei* and *T. colubriformis* adult males and females, respectively (Ransom, 1911; Nagaty, 1932).

Ninety percent of the females of *T. axei* had uteri with unfertilized eggs and 10 per cent had fertilized eggs, whereas all the females of *T. colubriformis* had uteri filled with fertilized eggs, the majority of which were in the late morula stage. This author observed two anatomical types of dorsal rays described by Nagaty (1932) for the mature males of *T. colubriformis*.

MATURE ADULTS. In other experimental studies, sexually mature *T. axei* and *T. colubriformis* were obtained from calves that had been infected with larvae of the same origin as those used in the present studies. Since the mature adults of both species have been well described by Ransom (1911) and Nagaty (1932) a recapitulation of the morphological details will not be given here.

DISCUSSION

The present study showed that larvae of *T. colubriformis* completed the third molt 2 days sooner than larvae of *T. axei.* Also, sex was determinable in larvae of *T. colubriformis* in the third molt, recovered from a guinea pig 3 days after infection, whereas the sex of *T. axei* larvae was first determinable with certainty in fourth-stage larvae recovered from a calf 7 days after infection. The general pattern of the parasitic development of *T. axei* and *T. colubriformis,* here described, appears to resemble closely the developmental pattern for *T. rugatus* and *T. colubriformis* in sheep, as reported by Mönnig (1926). Apparently, there is no appreciable difference in the time required by *T. colubriformis* for development to each successive stage in cattle, as compared to sheep (Mönnig, 1926). Although Mönnig reported no data showing that the larval stages of *T. coulbriformis* and *T. rugatus* can be differentiated prior to the fourth lethargus, the present writer has shown that *T. colubriformis* larvae can be distinguished from *T. avei* larvae in all stages of parasitic development, except the fourth-stage.

The presence of 2 tubercles at the tip of the tail of third-stage *T. colubriformis* larvae, the disappearance of these structures in the fourth-stage, their reappearance early in fourth molt, and their second disappearance in a late phase of this molt has been confirmed by examination of material obtained from a series of experimentally infected guinea pigs (Herlich, *et al.,* in press). These animals were killed at the same intervals after infection as the calves (Table 1) infected by the writer. No attempt is made to explain this phenomenon.

Hansen and Shivnani (1956) state that the tail of the third-stage infective larva of *T. axei* has two tubercle-like projections at its posterior end. In view of the observations presented here it seems possible that these investigators were dealing with *T. colubriformis* as the *Trichostrongylus* larvae examined by them were admittedly obtained from mixed cultures of fresh calf manure instead of from eggs from female worms identified as to species.

SUMMARY

1. Parasitic third-stage larvae of *T. axei* and *T. colubriformis* were recovered 2 days after the experimental infection of calves; larvae in the third molt were recovered 4⅙ to 6 days and 3 days after infection, respectively. The sexes can be differentiated in *T. colubriformis* larvae in the third molt. The following anatomical features are common to larvae of the 2 species in the third-stage and third molt: Anterior end simple; lips and cephalic papillae very difficult to discern; 2 prominent lateral amphids present; mouth opens into a medially located very minute buccal capsule, represented by a single thin refractive line; oesophagus anteriorly truncated; excretory pore may or may not be located in a conspicuous depression. The following anatomical features of the tail ends differentiate the two species: *T. axei,* tail end of larva proper and sheath bluntly rounded; *T. colubriformis* tail end of larva proper and sheath terminate in 2 tubercles of unequal lengths.

2. Fourth-stage larvae of *T. axei* and *T. colubriformis* were recovered as early as 7 and 5 days after infection, respectively; larvae in the fourth molt were recovered 10 days and 6 to 10 days after infection, respectively. Sex was readily distinguishable in the fourth-stage larvae of both species. The following anatomical features are common to larvae of the 2 species in the fourth-stage and in the fourth molt: Lips and cephalic papillae difficult to discern; 2 prominent lateral amphids present; mouth opens into a sclerotized buccal capsule that has the appearance of a bisected-semilunar body or a pair of isomeric, stubby, J-shaped structures; oesophagus anteriorly truncated; excretory pore located in a conspicuous depression or notch. Anatomical differentiation of the fourth-stage larvae of the two species was not possible; in both species the tail is rounded at the tip. However, the following anatomical features differentiate the larvae of the two species in the fourth molt: *T. axei,* tail end of larva proper and sheath bluntly rounded; *T. colubriformis,* tail of larva proper terminating in 2 tubercles of unequal lengths; tail of the sheath terminating in a rounded end.

3. Fifth-stage worms of *T. axei* and *T. colubriformis,* which had completed the fourth or final ecdysis, were recovered 15 days after infection with each species.

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Dirofilaria uniformis, n. sp., (Nematoda: Filarioidea) from *Sylvilagus floridanus mallurus* (Thomas) in Maryland

DONALD L. PRICE*
Second Army Area Medical Laboratory, Fort George G. Meade, Maryland
and
Walter Reed Army Institute of Research, Washington, D. C.

During current studies microfilariae were found in cardiac and peripheral blood of 11 of 124 rabbits, *Sylvilagus floridanus mallurus* (Thomas), collected in Maryland. The infected rabbits were collected from Andrews Air Force Base, Camp Springs, Maryland; Patuxent Research Refuge, Laurel, Maryland; and Blakistone (St. Clements) Island in the Potomac River, off Colton Point, Maryland. At necropsy, two adult female worms of *Dirofilaria scapiceps* (Leidy, 1886) and three adult male and seven adult female worms of another species of filaria were found in one of the infected rabbits examined. The unknown filarial worm was placed in the genus *Dirofilaria*, following the key proposed by Chabaud and Choquet (1953). No previous report of this parasite could be found.

DESCRIPTION

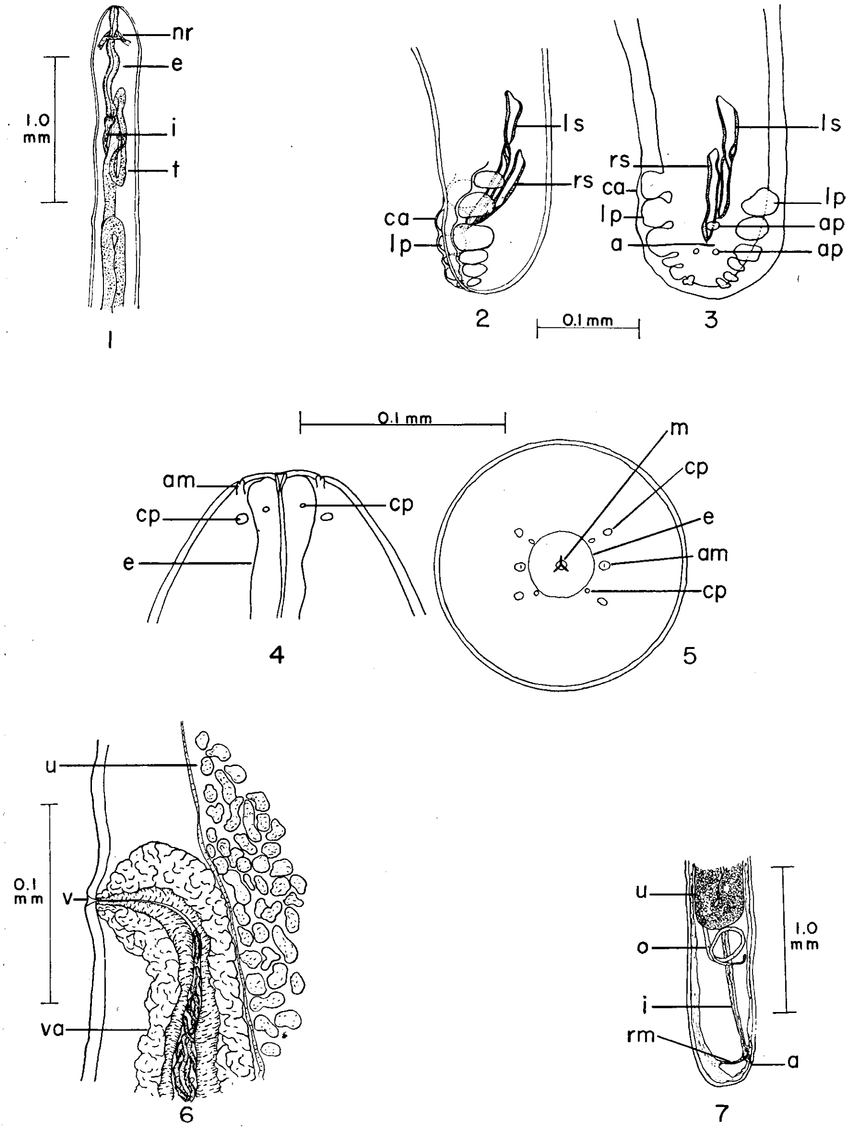
Dipetalonematidae Wehr, 1935; Dirofilarinae Wehr, 1935; genus *Dirofilaria* Railliet and Henry, 1911; subgenus *Nochtiella* Faust, 1937. Bodies of both sexes uniform in diameter, tapering only slightly at extremities. Extremities rounded, tail short, mouth without lips. Head with 8 very small cephalic papillae. Amphids lateral and clearly visible. Esophagus indistinctly divided into anterior muscular and posterior glandular portions. Microfilariae sheathed and found in blood of host.

MALE (holotype) 14.0 mm. long. Lateral width at nerve ring 0.32 mm., at excretory pore 0.29 mm., at anus inclusive of caudal alae 0.14 mm. Anus 0.06 mm. from tip of tail, nerve ring 0.22 mm. from anterior end of body. Length of esophagus 0.75 mm., left spicule 0.123 mm., right spicule 0.093 mm., caudal alae 0.36 mm. Posterior end coiled in slightly more or less than one turn. Six pairs of large, lateral pedunculate, caudal papillae, and 3 medial papillae, one anterior and 2 posterior to anus.

FEMALE (allotype) 29.0 mm. long. Lateral width at nerve ring 0.36 mm., at vulva 0.42 mm., at excretory pore 0.36 mm., at anus 0.38 mm. Dorsoventral width at anus 0.33 mm. Vulva slightly behind end of esophagus, 1.45 mm. from anterior end. Nerve ring 0.26 mm. from anterior end and anus 0.16 mm. from posterior end. Esophagus 0.96 mm. long. Opisthodelphys; viviparous.

MICROFILARIA 285 microns long, sheath 326 microns long, width 6 microns. Head rounded, cephalic space 5 microns long. Anatomical points given as average distance in microns from anterior end, followed, in parenthesis, by the average distance from anterior end expressed as percentage of entire length of microfilaria; nerve ring 65 (22.8); excretory pore 116 (40.7); excretory cell 123 (43.1); "Innen Körper," anterior limit, 145 (50.87); posterior limit, 185 (64.9); rectal cells, G_1 195 (68.4), G_2 212

*I wish to express my appreciation to the staff of Patuxent Research Refuge, Laurel, Maryland, for providing laboratory space and particularly to Dr. Carlton M. Herman and Mr. Leonard Llewellyn for their cooperation and assistance.
In addition I wish to express my appreciation to Dr. Everett E. Wehr of the Animal Disease and Parasite Research Branch, Agriculture Research Service, U.S. Department of Agriculture, Beltsville, Maryland, for his guidance and assistance throughout this project.



Dirofilaria uniformis, n. sp. Figures were drawn with the aid of a microviewscope.

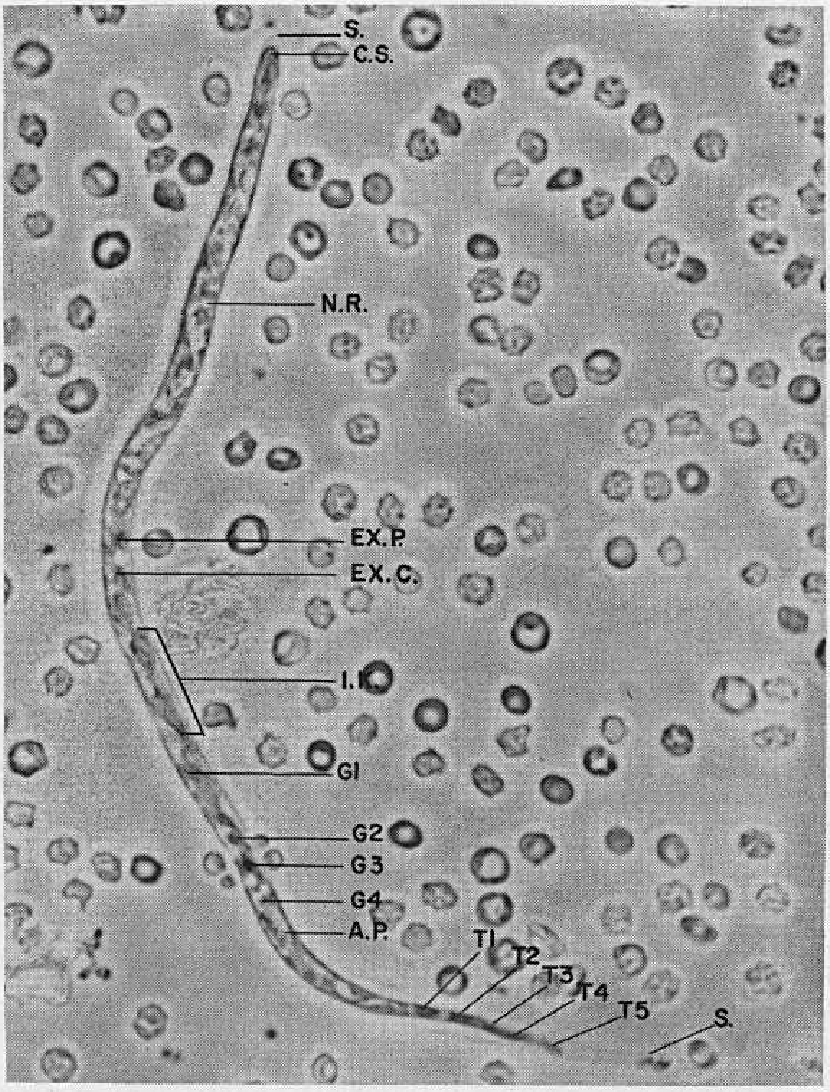
Figure 1. Ventral view of anterior end of male.
Figure 2. Off lateral view of posterior end of male.
Figure 3. Off ventral view of posterior end of male.
Figure 4. Dorsal view of anterior extremity of male.
Figure 5. En face view of male.
Figure 6. Lateral view of vulva and vagina of female.
Figure 7. Lateral view of posterior end of female.

Abbreviations used: a, anus; am, amphid; ap, anal papilla; ca, caudal alae; cp, cephalic papilla; e, esophagus; i, intestine; lp, lateral papilla; ls, left spicule; m, mouth; nr, nerve ring; o, ovary; rm, rectal muscle; rs, right spicule; t, testes; u, uterus; v, vulva; va. vagina.

(74.4), G_3 218 (76.5), G_4 224 (78.6); anal pore 232 (81.4). Tail sharply attenuated with small bulb at tip. Five tail nuclei, the first beginning at 256 from anterior end, followed by three close together, a space 10, and the final nucleus in bulb of tail. Usually a small amount of opaque material inside sheath at posterior extremity.

HOST: *Sylvilagus floridanus mallurus* (Thomas).

LOCATION: Subcutaneous connective tissue.



Microfilaria of *Dirofilaria uniformis*, n. sp. Photograph by Medical Illustration Department, Walter Reed Institute of Research.

Abbreviations used: A.P., anal pore; C.S., cephalic space; EX.C., excretory cell; EX.P., excretory pore; G, rectal cell; I.K., "Innen Körper" space; N.R., nerve ring; S, sheath; T, tail nucleus.

TYPE LOCALITY: Patuxent Research Refuge, Laurel, Maryland.

HOLOTYPE AND ALLOTYPE: U. S. National Museum Helminthological collection. No. 38159.

PARATYPES: (2 males and 6 females) U. S. National Museum Helminthological collection. No. 38160.

DISTRIBUTION: Patuxent Research Refuge, Laurel, Maryland; Andrews Air Force Base, Camp Springs, Maryland; and Blakistone (St. Clements) Island in the Potomac River, off Colton Point, Maryland.

DISCUSSION

Joseph Leidy (1886) reported and described *Filaria scapiceps* from a cottontail rabbit, *Lepus sylvaticus* [*Sylvilagus floridanus mallurus* (Thomas)]. Railliet and Henry (1911) proposed the genus *Dirofilaria* but did not include *D.* scapiceps. Later, Hall (1913) more adequately described *F. scapiceps* Leidy 1886 and Yorke and Maplestone (1926) placed it in the genus *Dirofilaria.*

Schwartz and Alicata (1931) reported microfilariae in the blood of *Lepus washingtonii* from Washington State. Highby (1943) reported *D. scapiceps* from *Lepus americanus phaeonotus* Allen, taken near Baudette, Minnesota and described the microfilaria. Penner et al. (1953) reported *D. scapiceps* in *Sylvilagus floridanus mallurus* from New England and reviewed the recorded cases of filariae from rabbits. The genus *Dirofilaria* was summarized by Anderson (1952).

One of the eleven rabbits had both *D. scapiceps* and *D. uniformis,* two had only *D. scapiceps,* and eight only *D. uniformis.* The gross morphological differences are more obvious than those observed under the microscope. *D. scapiceps* is coiled along its entire length in about three turns and tapers from the center of the body toward both ends, while the new species is straight and uniform in width. A total of 5 male and 9 female adult worms of *D. scapiceps* were recovered from the three rabbits infected with that parasite. All were recovered from the tarsal bursa, as was reported by Highby (1943), except one female worm located in the subcutaneous connective tissue of the lateral right hind leg near the knee joint. The new species were all found in the subcutaneous connective tissue mostly on the dorsal or lateral surfaces of the body.

Although the description is given for the holotype and allotype, other specimens (paratypes) compare in most respects. The range of length for six females was from 22.6 mm. to 33.7 mm. (average length 28.2 mm.) and for six males was from 11.5 mm. to 14.2 mm. (average length 12.7 mm.). The proportions within each sex were approximately the same with one exception. The spicules of four males showed some degree of variation in structure and a marked variation in length. The left spicule varied from 0.112 mm. to 0.168 mm. and the right from 0.087 mm. to 0.110 mm. More striking, the length of the right spicule expressed as percent of the left spicule varied from 61.2 to 82.1 percent. The author believes the variation in size is due primarily to the degree or rapidity of sexual development and that it will be greater in females than males.

The lateral caudal papillae of the male seem to be uniform in number in all specimens examined. In several cases one or more papillae was divided to its stem, giving the appearance of two. However, in each of these cases only 6 stems holding papillae could be found on either side. The caudal end of 4 males was examined.

Only a single uterus and ovary were visible in the intact female worms examined. These organs were teased out of one female. The uterus gradually enlarged until at about 2.33 mm. from the vulva where it divided into two branches. These branches were so tightly coiled that the division could not be seen until they were teased apart.

With the exception of length, the microfilariae of *D. uniformis* n. sp. are very similar to those of *D. scapiceps,* and might easily be mistaken for the latter without careful examination. To be certain of association of microfilariae and adult worms, some larvae were teased from the vagina of a living female worm. They were indistinguishable from those collected from peripheral and cardiac blood. The microfilariae were examined in thin and thick blood smears stained with Delafield's haemotoxylin and Giemsa' s stain, in wet mounts of whole blood, in modified Knott's technic (Herman and Price, 1955). Measurements of length made of 10 microfilariae in wet smears of whole blood compared almost exactly with 10 examined in modified Knott's solution. Final measurements were made in the latter medium for convenience and stained smears were used to locate more exactly anatomical points. The length and width are an average of the examination of 25 specimens and the anatomical points are an average of the examination of 10 specimens.

SUMMARY

A brief review of *Dirofilaria scapiceps* is given and *Dirofilaria uniformis* n. sp. is described from specimens obtained from the subcutaneous connective tissue of a cottontail rabbit. The new species is differentiated from *D. scapiceps.* The microfilariae of these two species are very similar and difficult to distinguish except under careful examination.

Some variation in total length and size of anatomical structures was observed. Also some papillae were found to be partially divided, giving the appearance of additional numbers.

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Sex Differences in the Cephalic Region of *Hoplolaimus coronatus* (Nematoda, Tylenchida)*

Harry J. Krueger** and M. B. Linford
Department of Plant Pathology, University of Illinois, Urbana

A distinctive cap-like cephalic region bearing longitudinal as well as transverse cuticular markings, and a strongly developed, yellowish cephalic framework are among the diagnostic characteristics of the genus *Hoplolaimus* von Daday, emend. Thorne, 1949. This framework seems not to have been described in detail, and published descriptions and illustrations of the cuticular pattern differ in some respects. During an investigation of an Illinois population of *Hoplolaimus coronatus* Cobb, 1923, the cephalic region was studied in some detail. This paper describes the cuticular pattern and also reports differences between sexes not only in the contour of the cephalic region, as described by Cobb (1923), but also in structure of the supporting framework.

Nematodes for this study were from 2 stock cultures that had been maintained 2 years in the greenhouse in pot cultures of red clover. These cultures were started in 1952 with 40 and 50 nematodes, respectively, from in and around roots of red clover from the Agronomy South Farm at Urbana. Morphologically, nematodes from these cultures agreed well with descriptions of *H. coronatus* except in details of variation in cuticular patterns chiefly on the tail, and in the characteristics to be described. The unusual phasmids of this species were typical in form and placement. These nematodes were larger than usually reported but they agreed fairly well in proportions as shown by the following:

Female: 1.6—2.2 mm; a = 29-33; b = 7-10; c = 50-65; v = 55%.
Male: 1.4—1.9 mm; a = 20-26; b = 5-7; c = 35-40.

Nematodes were studied in water mounts and also after fixation in Goodey's F.A. 4:10 (1951) and clearing in lactophenol or in glycerine. *En face* mounts were prepared by Buhrer's method (1949).

Form and Cuticular Pattern

The general form of the cephalic region, although subject to individual variation, tends to differ between the sexes as described by Cobb (1923) for this nematode and as illustrated by Oostenbrink (1954) for *H. uniformis* Thorne. As seen in whole mounts this region in females is a relatively low cone with nearly flat or sometimes concave sides (Fig. 1, B) while in males it is higher and hemispheroidal with convex sides (Fig. 1, A). Immature nematodes resemble the females more than males in this respect, and the cuticle cast by a male in its final molt retains the conical form in contrast with the hemispheroidal form of the male head (Fig. 1, C).

The cephalic region is 6-lobed as seen *en face*, with the lateral lobes much smaller than the two sub-ventral and 2 sub-dorsal lobes (Fig. 1, D E). These 4 are especially large in the males and much less conspicuous in females and young.

*Based chiefly upon part of a thesis submitted by the senior author in partial fulfullment of requirements for the M. S. degree in Plant Pathology from the Graduate College, University of Illinois.

**Present address: Chemical Warfare Laboratory, Directorate of Medical Research, Army Chemical Center, Maryland.

The first of the 5 to 7 annules of the cephalic region is a thin, somewhat ovoid, plate with the buccal aperture at its center (Fig. 1, D). The next few annules are interrupted by longitudinal furrows or creases between the lobes, (Fig. 1, E) that are more conspicuous in males than females. Some of them, but apparently not all, are also marked faintly by a few very unevenly spaced longitudinal striae that are difficult to observe due to the refractive properties of the underlying cephalic framework. The relatively

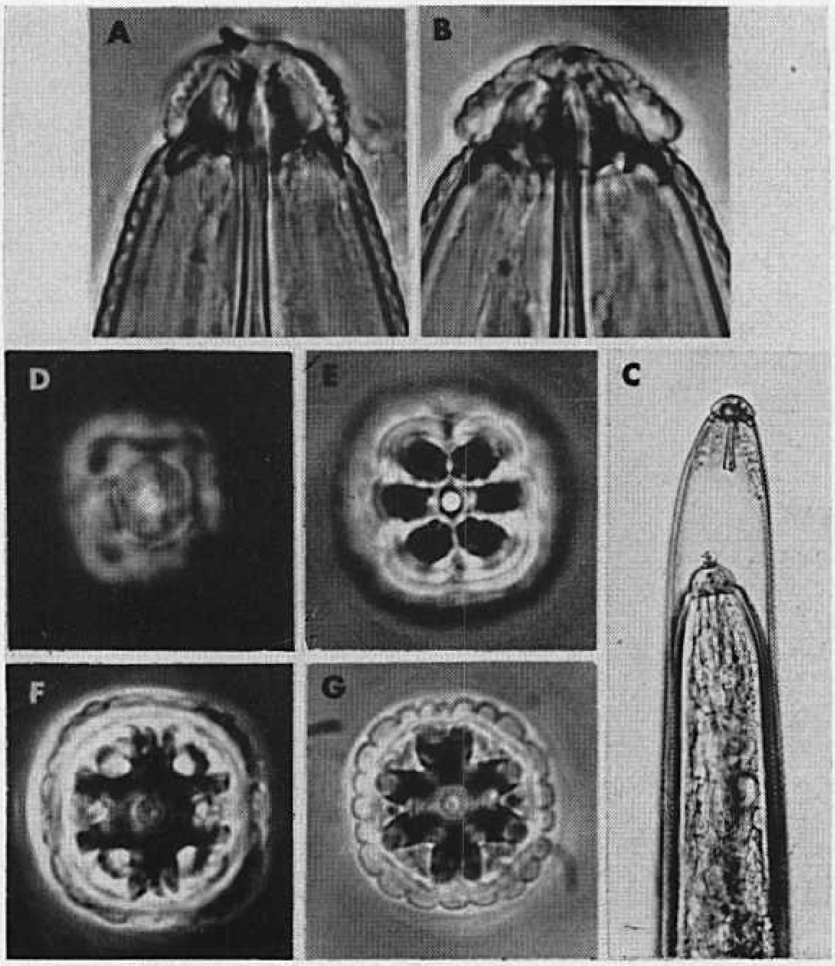

Fig. 1. Cephalic region of *Hoplolaimus coronatus* Cobb. A, male, and B, female in side view showing contour and annulation, also stylet guide, basal ring, and parts of ribs; x 1500. C, molting male, x 425. D-G, optical transections prepared from *en face* mounts, x 1800, D, male in plane of first annule with lobes out of focus; E, female at level of second annule and below, showing lobes and annulation of the cuticle, also vestibule and ribs radiating from it; F, male at level of basal plate showing 3-pronged dorsal and ventral bars, also knobs of the basal cephalic annule; G, female, at level slightly above that of F, showing 2-pronged dorsal and ventral bars.

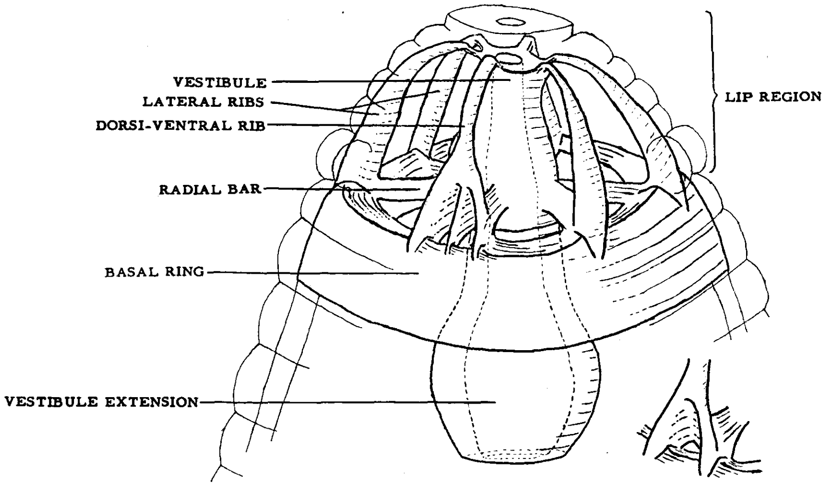

Fig. 2. Semi-diagrammatic drawing of cephalic structures of a male *Hoplolaimus coronatus,* showing the 3-pronged junction of a dorsi-ventral rib and bar. Insert, at lower right, shows the 2-pronged junction in a female.

thick basal annule, however, is distinctive (Fig. 1, F, G). It is divided into a series of rather regular knob-like protuberances similar to those described by Andrässy (1954) for *H. tylenchiformis* von Daday. The general aspect of the female head is more nearly like that shown by Cobb (1923) in the lower half of his figure 2 than in the upper half of that figure or in his figure 3.

Cephalic Framework

To aid in description of the cephalic framework, a semi-diagrammatic drawing (Fig. 2) was prepared with the help of scale drawings and photomicrographs of whole and *en face* mounts. This framework appears to be a rigid structure. It does not collapse when living nematodes are allowed to dry on a slide, and it breaks irregularly when crushed.

Axial in this framework is a heavy-walled tube, the vestibule. This together with a thin-walled lyre-shaped extension into the body cavity serves as a stylet guide. From the anterior end of the vestibule, in the latitude of the second annule, 6 ribs radiate transversely, then arch backward to fuse with the anterior edge of the basal ring and with 6 heavy bars of the basal plate that radiate transversely from the posterior end of the vestibule. These bars are in the approximate latitude of the constriction that sets off the cephalic region; the ring extends back under the first two body annules.

The arched ribs lie under the cuticular grooves that separate the cephalic lobes. Four of them are lateral, two on each side, and these are unbranched. The single dorsal and ventral ribs, however, are forked, dividing typically into 2 prongs in females and 3 in males. The bars of the basal plate with which the ribs fuse agree with the ribs in orientation and in forking (Fig. 1, F, G).

Although there are some irregularities in the forking of dorsi-ventral ribs and bars, especially in males, the observed association with sex is too consistent to be a result of chance. After recognizing this association, 10 Illinois females were examined *en face*. All had the 2-pronged condition both dorsally and ventrally. Among 17 males, 11 showed a typical 3-pronged condition dorsally and ventrally, 3 showed both 2-pronged and 3-pronged forking, and the other 3 showed still more extreme forking: one bar divided into 3 prongs while, in the other bar, the middle prong tended to divide again to make a total of 4 prongs.

Examination of 5 adult *H. coronatus* found associated with wheat roots from a field plot at Beltsville, Maryland, indicates that such forking is not peculiar to the Illinois population. Two females had the 2-pronged condition, 2 males had the 3-pronged, and the third male showed both 2 and 3 prongs.

In both sexes, a much less censpicuous forking of one or more sub-lateral ribs and bars was seen a few times. Unlike the almost equal width of prongs on the dorsal and ventral bars, those on sub-lateral bars were of very unequal size. In one Illinois male that showed this condition and that had only one of the dorsiventral bars 3-pronged, the 2 lateral cuticular lobes were much reduced while the 4 sub-lateral lobes were abnormally large.

DISCUSSION

If the sexal dimorphism of the cephalic framework described here occurs also in other species of *Hoplolaimus,* then the basal plate of *H. uniformis* shown by Thorne (1949, figure 1C) represents a female.

The bioliogical significance of these differences between sexes in details of cephalic form and structure is obscure. Males of *H. coronatus* show no degeneration of stylet or esophagus, and they have been observed to feed. Concurrent investigations show, however, that females and young are found completely enclosed within roots more frequently than are the males. Molting males have been dissected out of roots, but most of the adult males found associated with whole mounts of roots have had only the anterior end of the body within the cortex. Possibly the hemispheroidal cephalic region of the male is less suitable for deep penetration into roots than the more conical form in young and females. No such interpretation, however, seems to extend to differences in the forking of ribs and bars in the cephalic framework.

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The Recovery of Nematode Larvae by Baermann Apparatus as Affected by a Detergent

GEORGE H. ROHRBACHER, JR.
Regional Animal Disease Research Laboratory,
Agricultural Research Service, U. S. Department of Agriculture,
Auburn, Alabama

Preliminary to an ecological study of the free-living stages of cattle nematodes, the efficiency of the standard Baermann technique (Baermann, 1917) was checked with the vegetation and nematode larvae to be used. Dinaburg (1942) pointed out that the efficiency of the apparatus varied with the type of material used as substrate, and that variations in percentage recovery within his individual experiments were due mainly to the inconsistent operation inherent in the apparatus.

Lipovsky (1951) found that by adding a detergent to washwater more ectoparasites could be removed from small mammals. It was decided, therefore, to determine whether or not a nonionic detergent* could be used to increase the recovery of infective nematode larvae from forage.

Vegetation used in the experiments was cut from parasite-free experimental plots. It was weighed into liter beakers, 30 gms. each for crimson clover and dry Bermuda-grass, and 50 gms. each for orchardgrass and green Bermuda-grass. These weights gave approximately the same bulk. The forage was placed upright in the beakers. Between 50 and 100 ml. of water were added to the bottom of the beakers.

One hundred infective larvae were individually counted and placed in groups of 15 or less upon the forage by means of a pasteur-pipette. The beakers containing contaminated forage were set aside for 17 hours so that the larvae would be in a more natural state on the vegetation rather than in water droplets. When the forage was removed from the beakers, the water in the bottom was checked to determine if any larvae had fallen off the grass. Preparasitic larvae found in the water were discarded, and the number was subtracted from the total larvae used for that experiment.

The vegetation was placed on a quarter-inch mesh screen in a 10-inch diameter funnel and covered with a wet cheesecloth. Water at approximately 40° C was then poured over the cheesecloth until the vegetation was covered. In the groups where the detergent was used, 0.5 ml. detergent was added per liter of water prior to being poured over the vegetation.

Larvae were collected in 15 ml. conical centrifuge tubes attached to the funnels. The tubes were removed at the end of 24 hours. The supernatant fluid was decanted until only 10 ml. remained. One-third ml. of concentrated HCl was added to kill the free-living nematodes (Shorb, 1937). The suspension was then poured into a watch glass and the preparasitic larvae counted.

The data are summarized in Table I.

The detergent had no apparent effect upon the larvae, but the data indicate that it almost doubled the number recoverable by the Baermann technique. This was probably due to a better wetting of the vegetation by the detergent solution. While no count was made of the free-living nematodes, it appeared that many more were recovered when the detergent was used.

*Triton X-100. Rohm and Haas.

TABLE I. Recovery of infective larvae by Baermannization from different forages. Water alone versus 0.5 ml. detergent per liter of water.

Forage	Nematode	Method	Number Trials	Number Larvae Inoculated*	Total Larvae Recovered No.	%	Range in %
Crimson Clover	*T. axei*	Water alone	3	300	12	4	4
		With detergent	3	300	36	12	8-18
Orchardgrass	*O. ostertagi*	Water alone	6	562	125	22	17-30
		With detergent	6	539	208	39	27-46
Bermuda-grass Green	*O. ostertagi*	Water alone	3	298	102	34	29-42
		With detergent	3	296	134	45	32-53
Bermuda-grass Dry	*O. ostertagi*	Water alone	3	289	26	9	8-10
		With detergent	3	296	60	20	18-22

* Corrected for larvae falling off vegetation prior to Baermannization.

In two trials, one with orchardgrass and one with Bermuda-grass, maximum number of larvae recovered from water alone was more than the minimum number recovered when detergent was added. This is not considered particularly important, for in the case of all forages the total number of larvae recovered was greater when detergent was added.

The data indicate that fewer larvae were recovered from dry Bermuda-grass and crimson clover than from green Bermuda-grass or orchardgrass. Therefore, care should be taken during an ecological study in comparing the number of larvae recovered from one type of forage with the number recovered from another type.

In addition, the data reveal that under the conditions of these experiments only a small percentage of the infective larvae present are recovered from forage crops by the Baermann technique. This, too, would indicate that care should be taken in estimating the total number of larvae present on a pasture from those recovered when using this technique on field samples.

SUMMARY

A slight modification of the Baermann technique is given. A nonionic detergent added at the rate of 0.5 ml. per liter of water increased the recovery of infective larvae from cattle nematodes from Bermuda-grass, orchardgrass, and crimson clover.

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Filariasis in American Samoa
VI. Survey of Swain's Island*

LEO A. JACHOWSKI, JR.**

Brief visits were made to Swain's Island by members of the U. S. Navy's filaria research unit on 24 September 1948 and 9 August 1949. Mosquito surveys were conducted on both trips and a microfilaria survey of the total population was made on the later one.

Swain's Island is a tiny atoll located in the Pacific Ocean at 170° 55′ 15″ West longitude and 11° 05′ South latitude (fig. 1). The total land area is less than one square mile. A full description of the island is contained in a sanitary report by Stephenson (1937a), who also published a summary of its interesting history (1937b).

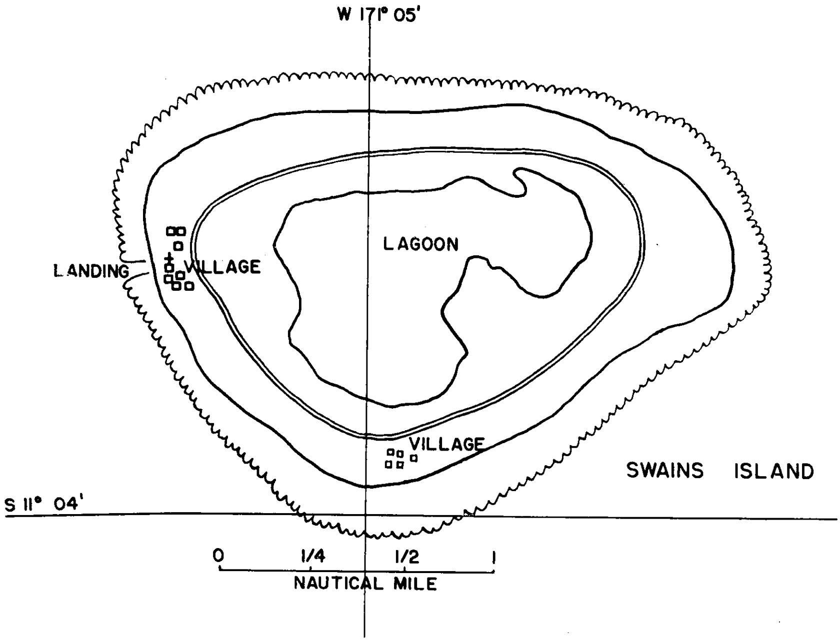

Figure 1. Outline Map of Swain's Island.

Although geographically and culturally closer to the Tokelau Islands which lie about 100 miles northward, this island (ceded to the United States in 1925 by its owner) is politically a part of American Samoa 200 miles to the south.

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*From the U. S. Naval Medical Research Institute, Bethesda, Maryland.

I wish to thank Mr. Eli Jennings, owner of Swain's Island, for his hospitality and co-operation in these studies and to acknowledge the technical assistance of Carlos Schultz, HMC, USN and Harold Marrer, HMC, USN in making these surveys.

**The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

TABLE 1.—Prevalence of Microfilaremia by Age and Sex in the Population of Swain's Island.

Age Groups (years)	Number of *Males* Exam.	Pos.	Number of *Females* Exam.	Pos.	Total Number Exam.	Pos.
< 10*	14	0	22	0	36	0
10-19	10	1	16	1	26	2
20-29	3	0	6	0	9	0
30-39	9	6	8	1	17	7
40-49	6	1	5	1	11	2
50+	6	4	5	0	11	4
Total	48	12	62	3	110	15
% Pos.	25.0		4.8		13.6	

*Infants under one year not examined.

The purpose of this report is to describe, from limited experience, the status of filariasis on Swain's Island and thereby remove another area in the South Pacific for which Iyengar (1954) listed "no available data."

OBSERVATIONS

MICROFILAREMIA. Three blood films (20 mm.^{3} each) were obtained between the hours of 1100 and 1245 on 9 August 1948 from each of the 110 residents of the island over 1 year of age. Upon examination of these films, microfilariae of non-periodic *Wuchereria bancrofti* were found in samples from 15 individuals (13.6 percent). While 12 of the 48 males examined were examined were infected, only 3 (5 percent) of the 62 females were positive for microfilariae (table 1). The highest microfilaria count was 2153 in 60 mm.^{3} of blood from a man 50 years old who had come from the Tokelau Islands 20 years previously. The youngest infected person was a girl aged 13 years. Although the numbers here are too small to be of any significance, it is perhaps of some interest to note that they suggest, just as is the case elsewhere in Samoa, that the adult males suffer a greater exposure risk than do the children and adult females.

TABLE 2.—Histories of Persons With Microfilariae

Identification Sex	Age	Birthplace	Years on Swain's	No. mf. per 60 mm.^{3} blood
F	13	Swain's	13*	40
M	14	"	14*	2
M	30	"	30*	140
M	31	"	31*	201
M	32	"	32*	2
M	36	"	**	65
M	50	"	**	68
M	52	"	**	11
F	36	Tokelau	15	22
M	37	"	15	623
M	50	"	20	2153
M	39	Samoa	17	1
F	40	"	15	198
M	41	"	14	90
M	70	"	20	250

*Thess persons never had left Swain's Island.
**These men divide their time between Swain's and Tutuila (American Samoa).

Since nearly one-third of the population was not born on Swain's Island, the possibility that the infected individuals may have acquired their infections elsewhere was considered (table 2). However, eight of the positive persons were born on Swain's and five of them had never left the island. Moreover, all of the seven with microfilaremia who were born elsewhere had resided continuously on Swain's for 14 years or more. Consequently, filariasis must be transmitted on Swain's Island.

ELEPHANTIASIS. The only case of elephantiasis observed was the bilateral enlargement of the legs in an elderly man who was microfilaria negative. Unfortunately, data were not obtained on his place of birth or on the length of residence on Swain's Island.

VECTORS. During the two mosquito surveys, larvae of one species of *Aedes* and one of *Culex* were collected. Adults reared from these larvae were identified as *Aedes polynesiensis* and *Culex* sp.*

Larvae of *A. polynesiensis* were found in broken crockery, bottles, treeholes, and coconut shells. They were found widely distributed over the island. Those of *Culex* sp. were taken from brackish pools adjacent to the central lagoon.

Adults of *A. polynesiensis* were abundant in the coconut and banana groves, but scarce in the village proper. Collectors obtained 150 females of this mosquito from human bait in 1½ hours at mid-day at the edge of the village. Dissection of the 105 mosquitoes which survived the trip back to the laboratory revealed filarial infections in three. Two contained second stage larvae in the thoracic muscles while the third had infective larvae in the mouthparts and head. No *Culex* sp. from Swain's Island were examined for filariae; however, the same species does not appear to be a vector in American Samoa.

SUGGESTIONS FOR CONTROL. Copra is the sole cash crop of Swain's Island. The coconut waste from the copra harvest is carefully collected and stacked around the roots of banana plants. This serves to support the luxuriant growth of bananas by retaining moisture and by providing humus. However, this practice also provides rat harborage and breeding places for *A. polynesiensis*. As suggested to Mr. Jennings, owner of the island, the simplest solution to the problem seems to be to cover the coconut waste with a layer of sand several inches thick. This would not reduce the value of these materials to the bananas, but would deprive *A. polynesiensis* of thousands of small water containers in which to breed.

Spraying the island with insecticides would be a needless expense of uncertain value.

Microfilaricidal drugs would be of value in reducing the human reservoir of infection. Newcomers and the men who divide their time between Swain's and Tutuila (American Samoa) would also have to be treated to prevent re-establishment of transmission.

SUMMARY AND CONCLUSIONS

Non-periodic *Wuchereria bancrofti* is endemic on Swain's Island occurring both in persons born there and those born elsewhere. Of 110 persons examined 15 (13.6 percent) had microfilaremia and one had elephantiasis. Adult males appear to suffer the greatest exposure risk.

*Adults key out to *Culex sitiens*, but the larvae more closely resemble *C. litoralis*. The same or similar species have been collected from Tahiti (Rosen, 1955) and in Samoa. Material from all three locations is currently under study.

Aedes polynesiensis is abundant and appears to be the vector. Of 105 mosquitoes dissected 3 harbored developing filarial larvae. One of these had infective larvae in its mouthparts.

Suggestions for control of filariasis on Swain's Island include the burial of coconut waste from copra operations and the periodic treatment of the entire population with suitable antifilarial drugs.

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Four New Species of Aphelenchulus (Nematoda) Parasitic in Bark Beetles in the United States*

CALVIN L. MASSEY**

The genus *Aphelenchulus* was erected by Cobb, 1920, with *A. mollis* as the type. This species is a parasite of a wood boring beetle, *Cyllene picta* Dr. belonging to the family Cerambycidae. All other known species are parasites of beetles of the family Scolytidae.

Most of the studies on nematodes of the genus have been carried on in Europe where the following species were described:

a. *Aphelenchulus diplogaster* (von Linstow, 1890) Filipjev and Shuurmans Stekhoven, Jr., 1941.

Synonyms: *Allantonema diplogaster* von Linstow 1890.
Tylenchus contortus typographi Fuchs 1914.

b. *Aphelenchulus cryphali* (Fuchs 1914) Filipjev and Shuurmans Stekhoven, Jr., 1941.

Synonym: *Tylenchus contortus cryphali* Fuchs 1914.

c. *Aphelenchulus cunicularii* (Fuchs 1929) Filipjev and Shuurmans Stekhoven, Jr., 1941.

Synonym: *Tylenchus contortus cunicularii* Fuchs 1929.

d. *Aphelenchulus laricis* (Fuchs 1929) Filipjev and Shuurmans Stekhoven, Jr., 1941.

Synonym: *Tylenchus contortus laricis* Fuchs 1929.

e. *Aphelenchulus cinerei* (Fuchs 1929) Filipjev and Shuurmans Stekhoven, Jr., 1941.

Synonym: *Tylenchus dispar cinerei* Fuchs 1929.

f. *Aphelenchulus reversus* Thorne 1935.

g. *Aphelenchulus tomici* Bovien 1937.

The male of *A. reversus* was described by Massey, 1956.

*From the Rocky Mountain Forest and Range Experiment Station, U. S. Department of Agriculture, Forest Service, with headquarters at Colorado A&M College, Fort Collins, Colorado.

**The writer wishes to thank Mr. Gerald Thorne for his review of the manuscript and the illustrations.

Descriptions of *Aphelenchulus brevicomi* n. sp., *A. spirus* n. sp., and *A. grandicolli* n. sp. are based on only parasitic females, while both males and females of *A. barberus* n. sp. are described. The presence of a spermatheca filled with spermatozoa in *A. grandicolli* n. sp. indicates that males occur in this species. All were collected during the course of a study on the parasites of the Engelmann spruce beetle and associated *Dendroctonus* and *Ips,* Massey 1956. Like other species of the genus, all were taken from the body cavities of infested insects. It is assumed that the effect on their hosts is similar to that of *A. reversus,* in that the egg laying potential of female Englemann spruce beetles is greatly reduced.

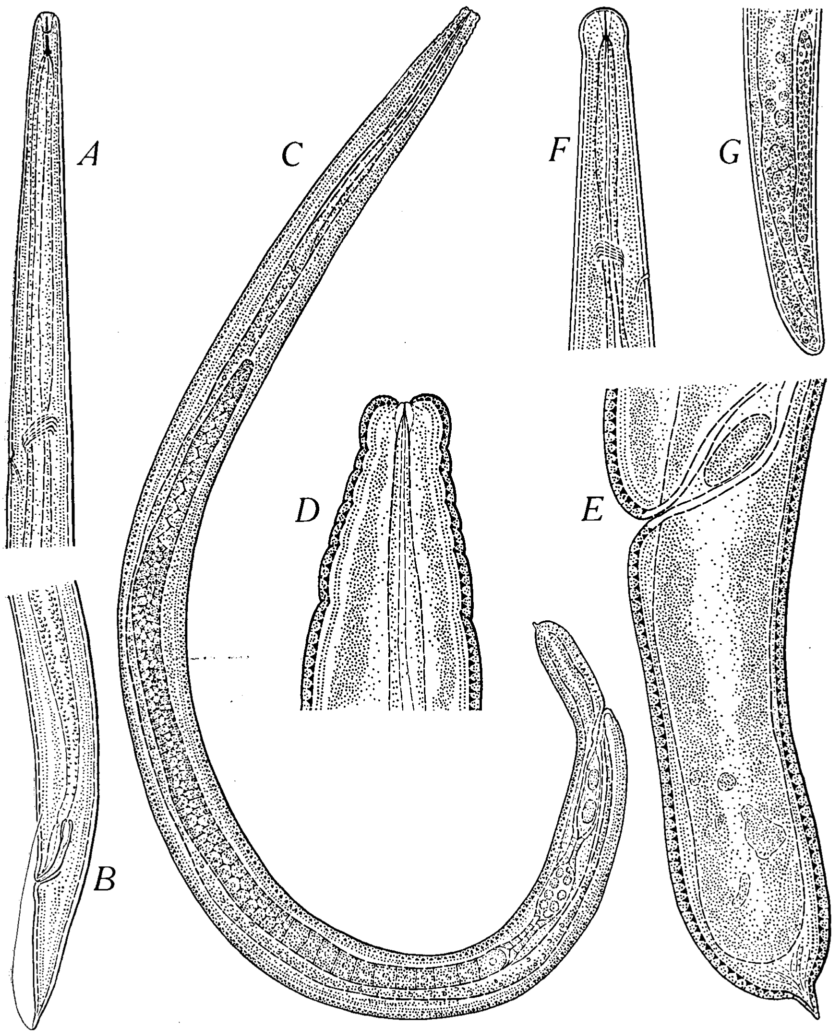

Figure 1. *Aphelenchulus barberus,* n. sp A., B.—Anterior and posterior parts of male; C.—Female; D., E.—Anterior and posterior portions of female; F., G.—Anterior and posterior ends of larva.

Aphelenchulus barberus, n. sp.

EGGS deposited before segmentation 20" x 50" laid in body cavity of infested beetles.

FIRST STAGE LARVAE: Length .4 to .5 mm. Width 20". $a = 17$, $b = 4.8$, $c = ?$. Cuticle very finely striated, lip region rounded and expanded. Spear very slender, minutely knobbed. Esophagus a narrow tube. Nerve ring prominent. Excretory pore slightly posterior to nerve ring. Genital primordia apparent. Anal opening not discernible. Fig. 1 F and G.

PARASITIC FEMALES: Length 2.1 to 3.9 mm. Width: 70" to 120". Body bent dorsally, tapering conspicuously toward head. Fig. 1 C. Cuticle thick, moderately to coarsely striated becoming annulated at the anterior end, annules more apparent in some specimens. Fig. 1 D. Body ventrally constricted at vulva. Tail broad, obtuse with a distinct mucro. Fig. 1 E. Length from posterior end to vulva 70". Spear slender, short, with or without knobs. Esophagus a straight tube. Lumen of the esophagus distinct for only a short distance from the spear. Ovary outstretched, about ¾ as long as body. Vulva a broad transverse slit. Spermatheca present filled with spermatozoa. Anal opening not discernible.

MALE: Length .65 mm. to .85 mm; width 10", $a = 56$, $b = ?$, $c = 21$, $T = 56\text{-}72$. Body much more slender than that of female tapering slightly toward the anterior end. Cuticle finely to moderately striated. Spear slender approximately as long as the body at its greatest width, knobs distinct. Esophagus a straight tube without bulb, narrowing as it passes through the prominent nerve ring. Excretory pore slightly posterior to nerve ring. Testis outstretched. Vas deferens distended with spermatozoa. Spicula curved, about ½ as long as tail. Gubarnaculum thin, trough-like, slightly curved. Bursa enveloping tail, extending forward to a point slightly anterior to spicula. Fig. 1 A and B.

The males are not parasitic and are found only in the galleries of the host.

DIAGNOSIS: *Aphelenchulus* with dorsally bent body, distinct mucro, moderately to coarsely striated cuticle, cuticle thick. Similar to *A. brevicomi* n. sp., but differs in the prominent mucro of the female tail and the shorter distance from the posterior end to the vulva. *A. barberus* average length and width less than that of *A. brevicomi.*

TYPE LOCALITY: Bandelier National Monument, New Mexico, Talladega National Forest, Alabama.

TYPE HOSTS: *Dendroctonus barberi* Hopk., *D. frontalis* Zimm. Parasitic females were recovered from both *D. barberi* and *D. frontalis.* Logs infested with the beetles were shipped to the writer from the Talladega National Forest.

Aphelenchulus brevicomi n. sp.

PARASITIC FEMALES: Length 3.2-4.2 mm. Width .130-.170 ". Body tapering slightly toward the anterior end then cylindroid to near vulva where it is ventrally constricted and bent slightly dorsally in the region of the vulva. Lip region rounded. Cuticle generally moderately striated, but annulated in many specimens in the region of the head. Fig. 2 D. Tail broad, rounded with or without mucro. When present the mucro is very small, vestigial. Fig. 2 E. Length from posterior end to vulva 90 ". Spear very short with knobs. Esophagus a straight tube, the lumen traceable for only a short distance from the spear. Gut traceable to the posterior end but anal opening not discernible. Ovary outstretched or reflexed, almost reaching the head. Spermatheca not present and spermatozoa not observed in the uterus. Vulva a broad transverse slit.

Eggs: 10 x 40 u.

Larvae and males of the species were not collected.

Diagnosis: *Aphelenchulus* with body tapering slightly in the region of the head; only slightly dorsally bent near the vulva, lip region flatly rounded. Cuticle moderately striate, differs from *A. barberus* in its greater length and in tail characteristics. Mucro usually absent, vestigial when present.

Type Locality: Salmon National Forest, Idaho.

Type Host: *Dendroctonus brevicomis* Lec.

Aphelenchulus spirus, n. sp.

Parasitic Females: Length 2.70-2.75 mm. Width 100 u. Body assumes spiral shape when killed by heat. Body more or less cylindrical throughout but narrowing conspicuously in the head region. Lip region narrowly rounded. Fig. 2 C. Tail broadly rounded without mucro. Fig. 2 B. Cuticle finely striated, annulated in some specimens at the anterior end. Four large glands occupy large portion of the head region. Outlets of glands not traceable. Spear length 10 u, slender, knobbed. Esophagus a straight tube, lumen traceable for only a short distance from the spear. Gut visible for entire length. Anal opening not discernible. Ovary occupying ¾ of body cavity reflexed one to several times. Spermatheca not present and spermatozoa not observed in uterus. Vulva a broad transverse slit. Distance from posterior end to vulva 130 u.

Eggs 16 x 35 u.

Males and Larvae: not collected.

Diagnosis: *Aphelenchulus* with body formed in a spring-like or spiral-like shape. Four large glands prominent feature of head region. Lip region narrowly rounded. Tail broadly rounded without mucro. Apparently similar to *A. diplogaster* but differs in its larger size and in the absence of a caudal mucro.

Type Locality: Uncompahgre National Forest, Norwood, Colorado.

Type Host: *Ips oregoni* (Eichh.)

Specimens of this species have also been collected from *Ips* spp. in New Mexico.

Aphelenchulus grandicolli, n. sp.

Parasitic Females: Length 1.7 to 2.3 mm; width 90 to 120 u. Body cylindroid, strongly bent dorsally until almost circular in some specimens. Fig. 3 A. Neck tapering gradually to the broadly rounded lip region. Cylindrical throughout, narrowing only slightly at the extreme anterior end. Fig. 3 B. Tail broadly rounded with a small mucro. Fig. 3 C. Cuticle thick, moderately to coarsely striated. Some specimens with annules in the region of the head. Four large glands present in head, their outlets not discernible. Spear slender, knobbed. Knobs more distinct in some specimens than in others; long, length 11 u. Esophagus a straight tube, lumen traceable for only a short distance from spear. Gut visible for entire length. Anal opening not discernible. Ovary usually outstretched but occasionally reflexed, occupying approximately ⅔ of body length. Spermatheca present, filled with spermatozoa. Vulva a broad transverse slit. Distance from posterior end to vulva 190-260 u.

EGGS: 15 x 30 ".
MALES AND LARVAE: not collected.

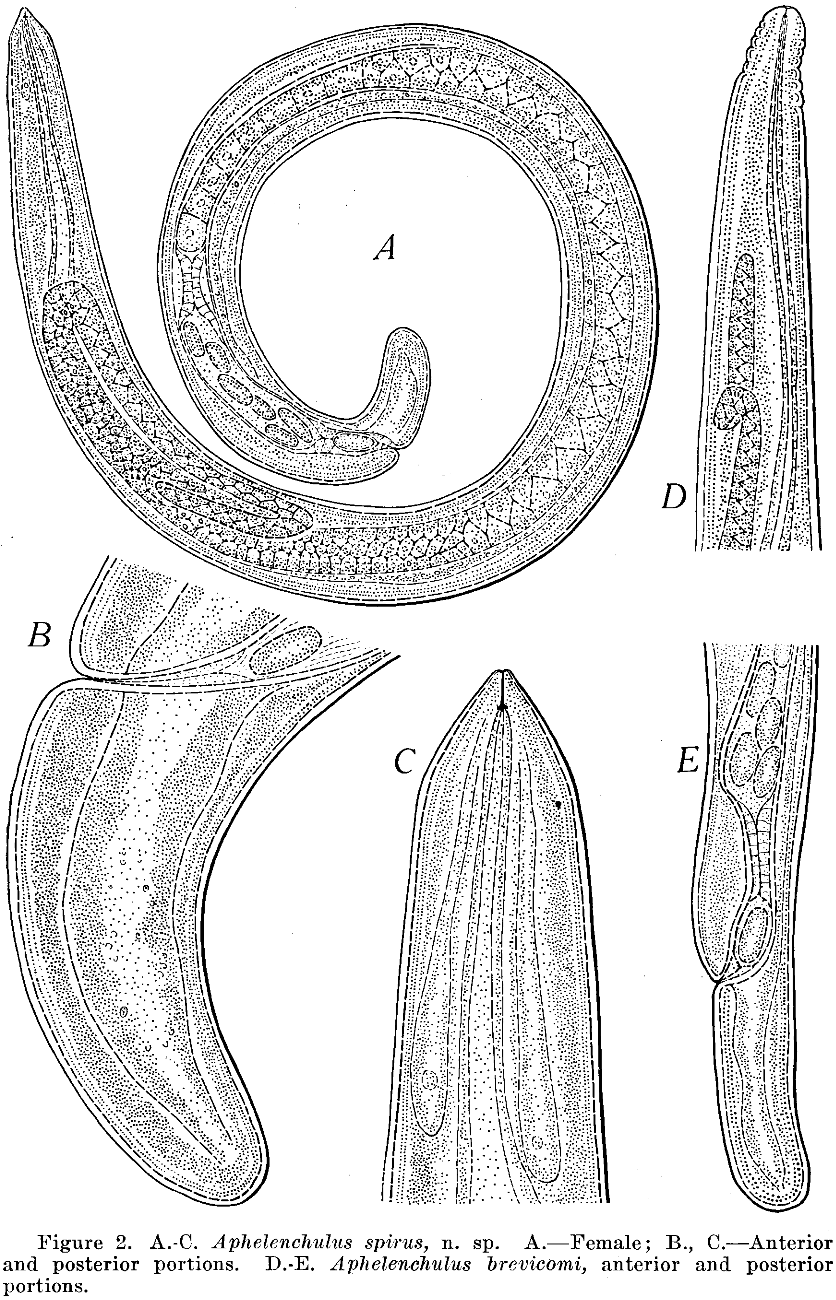

Figure 2. A.-C. *Aphelenchulus spirus,* n. sp. A.—Female; B., C.—Anterior and posterior portions. D.-E. *Aphelenchulus brevicomi,* anterior and posterior portions.

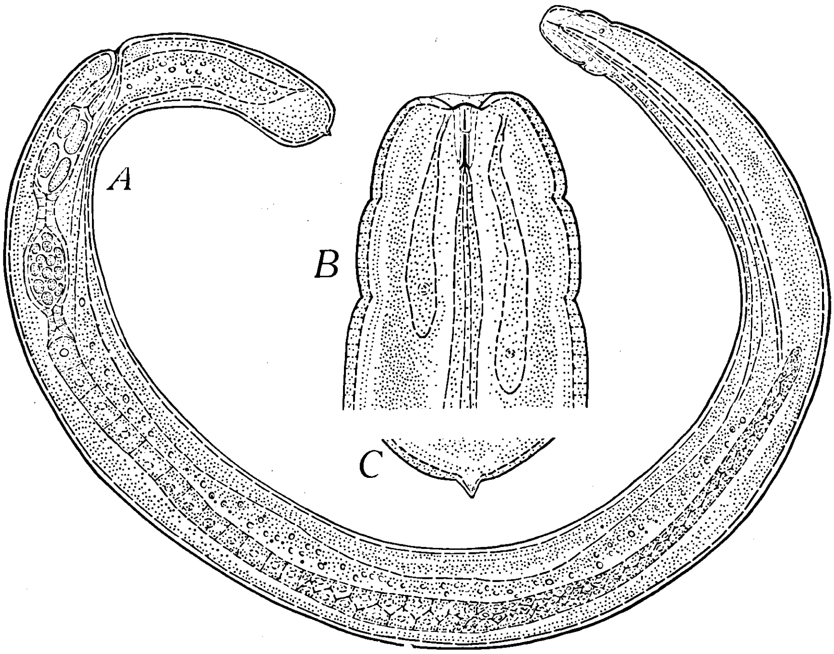

Figure 3. *Aphelenchulus grandicolli,* n. sp. A.—Female; B.—Female head; C.—Female terminus.

DIAGNOSIS: *Aphelenchulus* with body strongly bent dorsally. Lip region broadly rounded. Tail with small mucro. Cuticle thick, strongly striated. Similar to *A. tomici* Bovien, but much larger, and tail more broadly rounded with less distinct mucro. Distance from posterior end to vulva greater than in *A. tomici.*

TYPE LOCALITY: Talladega National Forest, Alabama.

TYPE HOST: *Ips grandicollis* (Eichh.)

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Trematodes of Marine Fishes of Mexican Waters. X. Thirteen Digenea, Including Nine New Species and Two New Genera, from the Pacific Coast.*

MARGARITA BRAVO-HOLLIS** AND H. W. MANTER

This paper is the tenth in a series based on studies emanating from the Institute of Biology, University of Mexico. The present collection of trematodes was made by the senior author in 1949 to 1955. She completed the study while a Guggenheim Scholar at the University of Nebraska in 1955-1956. Aid in collection was given by Eduardo Caballero y C. and Guadalupe Monsivais; hosts were identified by Rafael Martin del Campo, all of the University of Mexico.

The first six papers of this series were not numbered until listed by Bravo-Hollis (1954). The parts to date are as follows: I: Bravo-Hollis (1952); II: Caballero & Bravo-Hollis (1952); III-VII: Bravo-Hollis (1953; 1953a; 1954; 1954a; 1954b); VIII: Caballero & Bravo-Hollis (1955); IX: Bravo-Hollis & Sogandares (1956). Parts II, III, V, VII, and IX deal with Digenea.

The trematodes were killed with corrosive-acetic solution while flattened under a coverglass. Holotypes are deposited in the Helminthological Collection of the U. S. National Museum; paratypes in the Institute of Biology, University of Mexico.

All measurements are in mms. unless otherwise indicated.

FAMILY LEPOCREADIIDAE

Lepidapedon epinepheli, n. sp. (Fig. 1-2)

HOST: *Epinephelus analogus* (Gill). "grouper," "cabrilla pinta"

LOCATION: Intestine and ceca.

LOCALITY: Puerto Vallarta, Jalisco.

HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38175.

DESCRIPTION (based on 12 specimens; measurements on 4): Body very elongate; usually more bluntly rounded at posterior end; 2.52 to 7.06 long by 0.48 to 0.8 greatest width. Oral sucker 0.125 to 0.193 long by 0.193 to 0.241 wide. Acetabulum subcircular, 0.157 to 0.241 wide. Sucker ratio 1:0.8 to 1.1. Forebody 0.71 to 1.66 or 1/3.5 to 1/4.6 body length. Prepharynx 0.034 to 0.138 long; pharynx 0.135 to 0.193 long by 0.094 to 0.165 wide; esophagus 0.179 to 0.62 long, usually longer than prepharynx; intestinal bifurcation about ½ to ⅔ distance between oral sucker and acetabulum; ceca ending near posterior end of body. Genital pore sinistral, about ⅓ distance from edge of acetabulum to edge of body, usually at level between middle and anterior edge of acetabulum, in one specimen somewhat anterior to acetabulum. Gonads more or less tandem, separated. Testes smooth, subglobular, in anterior half of posterior ¼ of body; anterior testis usually slightly to left; testes separated by distance varying from ¼ to full length of testis. Post-testicular space about ⅛ body length. Cirrus sac elongate club-shaped, its posterior portion sinuous, extending posterior to acetabulum about halfway to ovary, 0.83 to 1.26 long by 0.069 to 0.19 in greatest width; anterior portion

*Studies from the Department of Zoology, University of Nebraska, No. 288.
**Current Address—Institute of Biology, University of Mexico.

⅓ to ¼ length of posterior portion, containing a small seminal vesicle, numerous prostatic cells, narrow pars prostatica and a short cirrus; posterior portion containing swollen, sinuous, tubular seminal vesicle, with very few prostatic cells, separated from anterior portion by constriction usually sharply bent. Ovary subglobular, interceeal to right side or in middle line, 0.138 to 0.207 long by 0.117 to 0.152 wide; seminal receptacle ovoid, dorsal and posterior to ovary, subglobular, interceeal to right side or in middle line, 0.138 to 0.207 long by 0.117 to 0.152 wide; seminal receptacle ovoid, dorsal and posterior to ovary, to left of median line, lying between ovary and anterior testis; 0.117 to 0.345 long by 0.097 to 0.227 wide; in contracted specimens, it overlaps partly left side of ovary. Laurer's canal present. In 9 of 10 specimens, anterior limit of vitellaria is opposite base of anterior portion of cirrus sac slightly posterior to acetabulum; in 1 specimen at midacetabular level. Vitellaria surrounding ceca, partially confluent between gonads. Uterus preovarian, with very short transverse coils extending along left side of cirrus sac; metraterm 0.276 long in 5.8 long specimen, with thick walls; eggs thin shelled, yellow; 0.066 to 0.069 long by 0.04 to 0.056 wide. Excretory vesicle extends forward to bifurcation of ceca; excretory pore with muscular walls surrounded by glands cells.

DISCUSSION: Other species of *Lepidapedon* with excretory vesicle extending to the intestinal bifurcation are *L. levenseni* (Linton, 1907) Manter, 1947; *L. nicolli* Manter, 1934. *L. hancocki* Manter, 1940; *L. trachinoti* Hanson, 1950 and *L. congeri* Manter, 1954. *L. epinepheli* is most like *L. nicolli* but differs in that the testes are separated, the body is more elongate, the oral sucker relatively larger, the prostatic cells much more scanty in the posterior portion of the seminal vesicle, the genital pore more to the left. *L. nicolli* is from a related host, *Epinephelus niveatus*, in the Gulf of Mexico and has been reported from a "grouper" from the Mexican Pacific (Manter, 1940, p. 354). *L. epinepheli* differs from *L. levenseni* in much larger size, much larger acetabulum and more anterior extent of vitellaria.

Manter (1954, p. 487) revised the description of *L. hancocki* to include a membrane around the prostatic cells of the seminal vesicle. Yamaguti's (1953) transfer of *L. hancocki* to *Lepocreadium* appears unjustified. The genus *Lepocreadium* is characterized by an external seminal vesicle lying free in the parenchyma.

GENUS *Hypocreadium* Ozaki, 1936

It seems best to recognize the genus *Hypocreadium* as distinct from *Pseudocreadium* Layman, 1930 on the basis of an intertesticular ovary and the uterus usually extending posterior to the ovary. In two species, *H. spinosum* (Manter, 1940), and *H. dampieriae* Yamaguti, 1942, the uterus does not extend posterior to the testes, and such may be the case in young specimens of any species. Trematodes of this genus are very common in *Balistes* and other plectognath fishes, a group very favorable for Lepocreadid trematodes in general. The genus *Hypocreadium* is a difficult one because of close similarity of species described and individual variations observed. Range of egg size and shape is great within a species. Other variable characters are smooth or irregular contour of gonads, and lateral extent of vitellaria. Details of the cirrus sac are probably more constant but these are somewhat affected by protrusion of the cirrus.

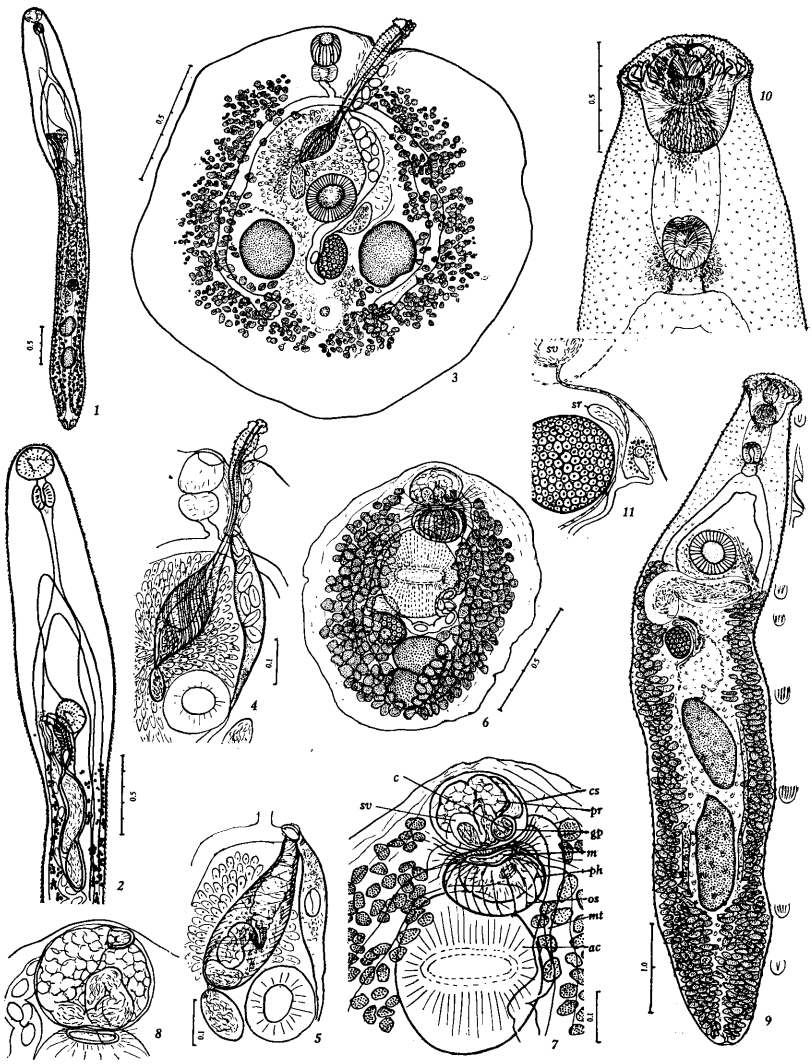

PLATE I.

Fig. 1. *Lepidapedon epinepheli.* Dorsal view.
Fig. 2. *L. epinepheli.* Enlarged view of anterior part of body. Dorsal.
Fig. 3. *Hypocreadium myohelicatum.* Ventral view.
Figs. 4-5. *H. myohelicatum.* Enlarged view of cirrus sac and metraterm. Ventral.
Fig. 6. *Guggenheimia pacifica.* Dorsal view.
Fig. 7. *G. pacifica.* Enlarged view of anterior part of body. Dorsal.
Fig. 8. *G. pacifica.* Dorsal view of anterior end of body, showing mouth and cirrus sac.
Fig. 9. *Dactylotrema squamatum.* Ventral view. Enlarged scales shown on left.
Fig. 10. *D. squamatum.* Enlarged view of anterior end. Ventral.
Fig. 11. *D. squamatum.* Diagram of ovary and adjacent organs including Laurer's canal. Dorsal.

One species was considered to be *H. scaphosomum* (Manter, 1940), although the external seminal vesicle was usually not sinuous as it was in all of the original (1940) specimens. A constant character of *H. scaphosomum* is the absence of vitellaria over the ceca, both dorsally and ventrally. In view of great variation of egg size, *H. scaphosomum* should perhaps be considered a synonym of *H. patellare* Yamaguti, 1938, which, however, appears to have a shorter prostatic vesicle.

The collection contained very numerous specimens closely resembling *H. lamelliforme* (Linton, 1907), known from *Balistes* in Bermuda and Tortugas, Florida. Some or all these specimens might well be *H. lamelliforme,* but a decision was finally made to describe them as new. These specimens agree with *H. lamelliforme* in that the vitellaria overlap the ceca ventrally, usually only in separated spots or areas, sometimes very generally along the length of the ceca. In living material these areas are sometimes seen to be elevated above the body surface, a character also occurring in *H. lamelliforme.* The new species differs from *H. lamelliforme* chiefly in possessing a long slender, muscular pars prostatica between the prostatic vesicle and the cirrus.

It is of some interest that one of the two species of *Hypocreadium* of the Mexican Pacific is extremely similar or identical to a Japanese species, the other is practically identical to a species of the tropical Atlantic.

Hypocreadium scaphosomum (Manter, 1940) Yamaguti, 1942

HOST: *Balistes verres* Gilbert and Starks. *Balistes capistratus* (Shaw).
LOCATION: Intestine.
LOCALITY: Puerto Vallarta, Jalisco and Mazatlan, Sinaloa.
NUMBER: Many in two hosts.

Hypocreadium myohelicatum, n. sp. (Figs. 3-5)

HOSTS: *Balistes capistratus* (Shaw), "botas."
LOCATION: Intestine.
LOCALITY: Puerto Vallarta, Jalisco.
NUMBER: Numerous.
HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38184.

DESCRIPTION (measurements on 4 specimens selected for range): Body broadly rounded, wider than long, length 1.3 to 1.66, greatest width 1.4 to 1.9. Oral sucker 0.116 to 0.13 long by 0.121 to 0.13 wide. Acetabulum just anterior to midbody, 0.133 to 0.22 long by 0.138 to 0.207 wide. Sucker ratio 1:1 to 1.6. Pharynx globoid, 0.066 to 0.078 long by 0.084 to 0.109 wide; esophagus 1 to 1½ times length of pharynx; intestinal bifurcation ½ distance between anterior end of body and acetabulum; ceca bowed, slightly undulate, ending at level of excretory pore. Genital pore to left near level of intestinal bifurcation, ventral to left cecum or slightly anterior or posterior to cecum. Testes more or less spherical, symmetrical, immediately postacetabular, separated by ovary and uterus. Cirrus sac elongate clavate, diagonal from genital pore to right anterior edge or acetabulum or overlapping right edge to about ¼ acetabulum length. Wall of cirrus sac with conspicuous diagonal or spirally arranged muscles. Internal seminal vesicle sac-lake; prostatic vesicle tapering anteriorly, straight, single or inconspicuously bipartite; pars prostatica a narrow tube with circular muscles, often irregularly constricted, as long as prostatic vesicle or longer; cirrus with prominent papillae, often protruded with swollen, bulb-like tip. External seminal vesicle an elongate, straight sac, lying along right side of acetabulum. Numerous gland cells around cirrus sac and external seminal vesicle. Ovary ovoid, often elongate, smooth, between testes. Seminal receptacle elongate between ovary and acetabulum, to the left. Vitellaria not reaching sides of body; some follicles, usually in scattered groups, ventral to ceca. Uterus extends posterior to ovary. Metraterm very muscular, extending to about mid-acetabular level. Eggs 0.064 to 0.077 long by 0.04 to 0.05 wide. Excretory pore dorsal, between ends of ceca, muscular, with 8 or 9 radially arranged spine-like projections.

The name *myohelicatum* is from *myo,* muscle and *helikos,* spiral. It refers to the muscles of the cirrus sac.

DISCUSSION: This species is very similar to *D. lamelliforme.* It was at first thought to be distinct because of the very conspicuous diagonal muscles of the cirrus sac. Restudy of specimens of *H. lamelliforme* from Bermuda and Florida shows such muscles occur in that species, also, although they are not conspicuous. The degree of conspicuousness probably varies. *H. myohelicatum* is distinguished by the slender, long and very muscular prostatic duct. It differs from *H. scaphosomum* in the occurrence of at least some vitellaria ventral to the ceca.

Guggenheimia pacifica, n. gen., n. sp. (Figs. 6-8)

HOSTS: *Balistes verres* (Gilbert and Starks) (type host) and *B. capistratus* (Shaw), triggerfishes.

LOCATION: Intestine.

LOCALITY: Mazatlan, Sinaloa.

NUMBER: 1; 7 others kindly loaned by Howard Winter, University of Southern California.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll. No. 38178.

DESCRIPTION (based on 8 specimens; measurements on 4): Body ovoid in outline, somewhat longer than wide, 1.073 to 1.406 long by 0.918 to 1.206 wide, broadly rounded at each end. Sides of body curved inward ventrally, with concentric cuticular rings; the entire body cup-like with ventral concavity. No spines observed. Oral sucker dorsal, embedded in body; 0.158 to 0.214 long by 0.207 to 0.289 wide. Mouth dorsal, a transverse slit, 0.168 to 0.276 from anterior end of body, posterior to genital pore which is also dorsal. Radial muscles in parenchyma between mouth and oral sucker. Acetabulum ventral, just anterior to midbody, 0.303 to 0.434 long by 0.269 to 0.365 wide, with transverse aperture, partly overlapping the dorsal oral sucker. Distance from anterior edge of acetabulum to anterior edge of body 0.296 to 0.42. Sucker ratio (widths): 1:1.26 to 1.51. Pharynx directly ventral to oral sucker, 0.113 to 0.179 long by 0.152 to 0.207 wide. Esophagus dorso-ventral, very short; bifurcation of ceca slightly posterior to mouth; ceca, bowing around acetabulum, distant from sides of body, ending at level of middle of posterior testis. Genital pore dorsal, varying from near mouth to near anterior end of body, slightly to left (as viewed from ventral surface). Testes tandem to slightly diagonal, close together, smooth, in posterior third of body. Cirrus sac large, spherical, at extreme anterior end of body, anterior to oral sucker, about same size as oral sucker, 0.165 to 0.234 long by 0.179 to 0.289 wide; containing a tubular, U-shaped seminal vesicle, mass of very large, vesicular cells, and short muscular cirrus. External seminal vesicle not seen. Ovary spheroid, to left of midline between anterior testis and acetabulum, partly overlapping left side of anterior testis; seminal receptacle preovarian, inconspicuous, overlapping left posterior edge of acetabulum. Vitelline follicles large, not reaching edges of body, from level of middle of cirrus sac to near posterior end of body, surrounding ceca; contiguous posterior to testes, slightly overlapping testes, ovary, and acetabulum. Uterus pretesticular, extending dorsally along right side of acetabulum to genital pore. No metraterm. Eggs thin-shelled, 0.066 to 0.08 long by 0.041 to 0.051 wide. Excretory system not seen.

The name *Guggenheimia* is in honor of the John Simon Guggenheim Foundation.

DISCUSSION: This trematode is related to *Pseudocreadium* and *Hypocreadium,* but is most remarkable in the dorsal position of the mouth and oral sucker. Evidently the ventral concavity of the body is used for attachment, and perhaps the oral sucker and genital pore moved forward and dorsally to be on a free surface. There is no indication of any bending or folding of the rounded body at least in the adult stage. The cercaria of this trematode should be interesting. The genus is unique in possessing the entire cirrus sac anterior to the mouth, a condition resulting from the dorsal position of the oral sucker and mouth.

DIAGNOSIS OF GUGGENHEIMIA: Lepocreadiidae. Body disc-like with ventral concavity. Oral sucker, mouth, and genital pore all dorsal in position Spherical cirrus sac at extreme anterior end of body. Pharynx and esophagus ventral to oral sucker. Ovary and uterus pretesticular. Seminal receptacle present. Metraterm absent. External seminal vesicle not observed. Otherwise similar to *Pseudocreadium.*

TYPE SPECIES: *G. pacifica.*

Dactylotrema squamatum, n.g., n. sp. (Figs. 9-11)

HOST: *Gerres* sp., "Mojarra."

LOCATION: Intestine.

LOCALITY: Zihuatanejo, Guerrero.

NUMBER: One specimen.

HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38176.

DESCRIPTION: Body elongate, 7.7 long by 1.52 wide at level of testes. Cuticula with scales, each scale with 1 to 5 spines embedded in it. Oral sucker 0.476 long by 0.407 wide, of complex structure, provided with 6 pairs of elongate, pointed processes embedded in the wall of its outer margin dorsally and laterally (Fig. 9). The pointed tips of these papillae (?) come close to the surface but none was protruded more than as a slight elevation. Central core of each papilla contains few nuclei. Mouth inconspicuous, ventral, leading to an ovoid capsule about 0.234 long and 0.195 wide, bounded posteriorly by semicircular fibers in the sucker. "Oral capsule" largely filled by broad structure with horizontal anterior edge, longitudinal bands (muscles?) in its anterior ⅔, and dorsoventral muscles in its base. Oral sucker with some radial muscles and larger dorso-ventral muscles in its posterior third. A concavity with backwardly curved anterior edge occurs on dorsal surface of posterior third of oral sucker. Acetabulum at end of first ¼ body length, 0.552 long by 0.538 wide. Forebody 1.82 long. Sucker ratio 1:1.27.

Pharynx 0.234 long by 0.22 wide, 0.269 posterior to oral sucker; prepharynx too thin-walled to be visible; longitudinal muscles connect pharynx and oral sucker; esophagus about ½ length of pharynx; intestinal bifurcation midway between suckers; ends of ceca concealed by vitellaria. Testes about 3 times longer than wide, smooth, tandem, very slightly separated, in anterior part of posterior half of body; posttesticular space 1.48. Genital pore inconspicuous, close to anterior edge of acetabulum, slightly to right. Seminal vesicle a large, mostly transverse, wide tube, just posterior to acetabulum, almost straight except that its posterior third bends backward. Pars prostatica a straight narrow tube; cirrus and cirrus sac lacking. Ovary globular, to right of midline, just anterior to middle of body, 0.468 anterior to anterior testis. Seminal receptacle narrow, elongate, curving around anterior edge of ovary. Laurer's canal opening dorsally to left of ovary at midovarian level. Uterus and eggs not developed. Vitelline follicles large, close together, from posterior edge of acetabulum to posterior end of body, surrounding ceca, confluent posterior to testes but not between gonads. Excretory pore terminal; excretory vesicle not observed.

The name *Dactylotrema* (Gr. *dactylos,* finger) refers to the finger-like papillae of the oral sucker; the name *squamatum* (L. *squama,* scale) refers to the body scales which are peculiar in being multispined.

DIAGNOSIS OF DACTYLOTREMA: Lepocreadiidae, subfamily Homalometrinae. Body elongate, with multispined scales. Oral sucker with paired marginal, elongate, pointed papillae embedded in edge of sucker laterally and dorsally. Mouth leads to small ovoid portion of oral sucker containing an elevated flattened tongue-like muscular structure. A concavity on dorsal surface of posterior half of oral sucker. Testes elongate, tandem, separated from ovary. Cirrus sac and cirrus lacking. Seminal vesicle tubular, almost straight, posterior to acetabulum. In intestine of marine fishes.

TYPE SPECIES: *Dactylotrema squamatum*

DISCUSSION: This genus is most closely related to *Homalometron* Stafford, 1904 particularly *H. elongatum Manter,* 1947 from a related host *Gerres cinereus* at Tortugas, Florida. It differs in its peculiar, multispined scales and above all in the complex oral sucker largely surrounded by pointed, finger-like papillae. These papillae suggest the tentacles of the anterior sucker of some gasterostomes but they contain a core of cells and do not appear to be protrusible. They are much less muscular than are the oral processes of *Enentereum* and related genera; nor are they homologous with oral processes occurring in the Waretrematidae. They are probably sensory. A restudy of specimens of *Homalometron elongatum* shows three pairs of papillae on the sides of the mouth (Fig. 12) which may be homologous structures.

The structure of the oral sucker, particularly the "oral capsule" suggests the oral bulb of *Sphincterostoma* Yamaguti, 1937 which is, however, composed of semicircular muscles. *Sphincterostoma* is similar to *Dactylotrema* in general structure but has a smooth cuticula. The muscular mass elevated in the oral capsule of *D. squamatum* may function as a tongue. The genus *Thysanopharynx* Manter, 1933 (Megaperidae) has a complex muscular tongue-like organ, as yet undescribed, in the oral cavity. *Dactylotrema* may eventually be found to have some affinity to the Megaperidae.

FAMILY OPECOELIDAE

Opegaster lutiani, n.sp. (Figs. 13-14)

HOST: *Lutianus aratus* (Günther); "pargo," snapper.
LOCATION: Intestine
LOCALITY: La Paz, Baja California
NUMBER: 16 specimens in one host
HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38177

DESCRIPTION (measurements on 4): Body 1.06 to 1.44 long by 0.32 to 0.4 in greatest width near midbody; almost equally wide along most of length; both ends broadly rounded. Oral sucker 0.102 to 0.117 long by 0.107 to 0.125 wide; sometimes withdrawn into body. Acetabulum 0.179 to 0.202 long by 0.179 to 0.202 wide, with 5 small papillae on each lip. Sucker ratio 1: 1:5 to 1.9. Forebody 0.22 to 0.28 long. Prepharynx very short; pharynx 0.063 to 0.083 long by 0.058 to 0.108 wide; esophagus 0.023 to 0.053 long; intestinal bifurcation near anterior edge of acetabulum; ceca uniting near posterior end of body; anus ventral a short distance anterior to excretory pore. Genital pore sinistral, at level of pharynx; halfway between midline and left edge of body. Testes tandem, close together, just posterior to middle of hind-body, wider than long. Except when pressed together, each testis is bilobed due to a median indentation at anterior and posterior end. Posterior indentation of posterior testis almost always conspicuous, but others appear to be sometimes smoothed by compression. Posttesticular distance 0.24 to 0.46. Seminal vesicle extending diagonally backward dorsal to right half of acetabulum, ending at most slightly posterior to acetabulum. Cirrus sac and prostatic vesicle lacking; male duct joins a muscular tube believed to be a tubular atrium (Fig. 14). Ovary trilobate, immediately pretesticular; Mehlis' gland immediately preovarian, sperm cells often present in early uterine coils. Vitellaria extending from level of intestinal bifurcation or base of pharynx to posterior end of body. Uterus preovarian; eggs 0.047 to 0.056 long by 0.034 to 0.039 wide. Excretory pore terminal. Extent of excretory vesicle not observed.

DISCUSSION: *O. lutiani* is similar to *Opegaster tamori* Yamaguti, 1938 in shape of testes but the latter has a tapered forebody, lacks acetabular papillae, the intestinal bifurcation is more anterior and the eggs larger. *O. acuta* Manter, 1940 has 5 pairs of papillae but the body has tapered ends, the testes are not indented and the vitellaria are entirely postacetabular. *O. pentadactyla* Manter, 1940 is more similar but its vitellaria extend only to the posterior edge of acetabulum; its testes are more rounded.

Podocotyle musculometra, n.sp. (Figs. 15-17)

HOST: *Hoplopagrus güntheri* Gill
LOCATION: Intestine and ceca
LOCALITY: La Paz, Baja California
NUMBER: 10 adult and 2 immature specimens from 2 hosts
HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38179.

DESCRIPTION: Body elongate, rather thick, 2.64 to 5. long by 0.92 to 1.34 in greatest width; almost equally wide, broadly rounded at each end. Oral sucker 0.207 to 0.31 long by 0.255 to 0.359 wide; acetabulum 0.4 to 0.621 long by 0.448 to 0.621 wide. Sucker ratio 1: 1.66 to 1.8. Radial muscles in body extending outward from edges of acetabulum. Forebody 0.78 to 1.32 long. Prepharynx 0.02 to 0.034 long; pharynx 0.138 to 0.213 long by 0.165

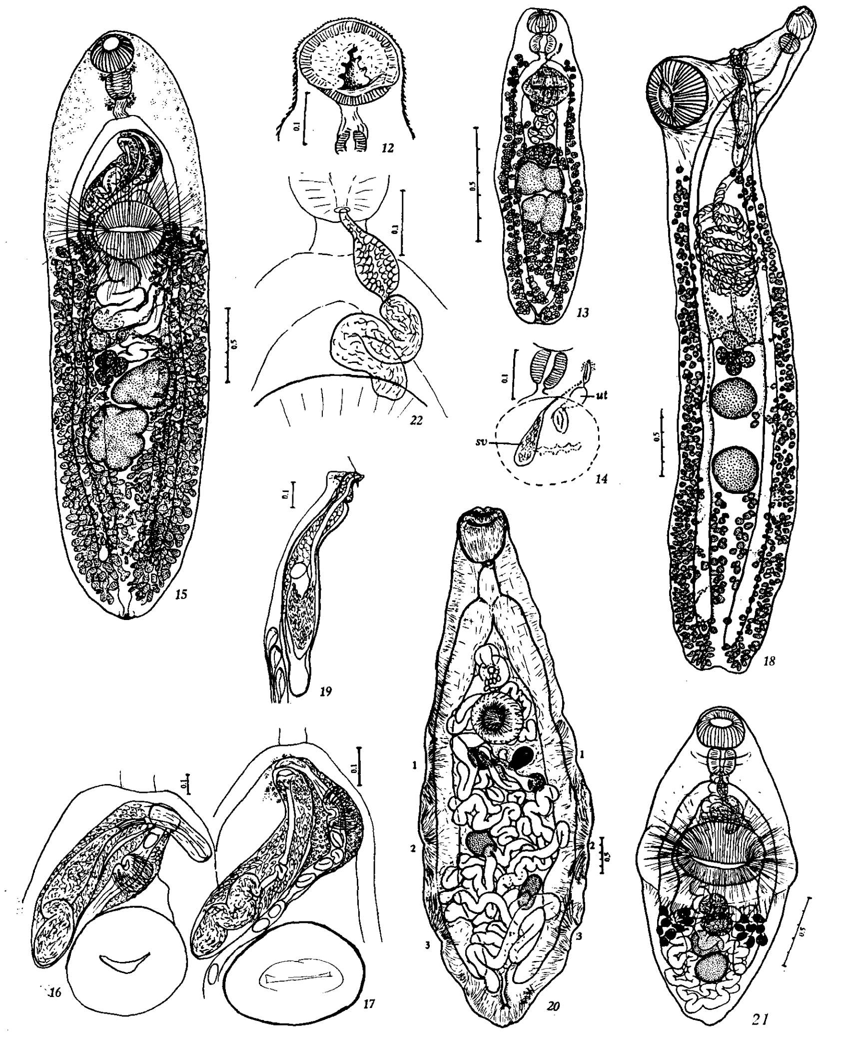

PLATE II.

Fig. 12. Anterior end of *Homalometron elongatum* Manter, 1947, showing paired papillae on each side of mouth.

Fig. 13. *Opegaster lutiani*. Ventral view.

Fig. 14. *O. lutiani*. Ventral view of terminal genital ducts. Acetabulum dotted lines.

Fig. 15. *Podocotyle musculometra*. Ventral view.

Figs. 16-17. *P. musculometra*. Enlarged view of cirrus sac and metraterm. Ventral.

Fig. 18. *Helicometra pretiosa*. Ventral view.

Fig. 19. *H. pretiosa*. Lateral view of cirrus sac.

Fig. 20. *Phyllodistomum marinae*. Ventral view. Numbers 1, 2, 3, indicate lateral indentations.

Fig. 21. *Diplangus mexicanus*. Ventral view.

Fig. 22. *D. mexicanus*. Enlarged view of seminal vesicle and prostatic vesicle.

to 0.276 wide; esophagus 0.052 to 0.138 long; bifurcation about midway between suckers; ceca extending to near posterior end of body. Genital pore median, or slightly to left, immediately posterior to intestinal bifurcation. Testes smooth or slightly lobed, diagonally placed, close together, a little posterior to middle of body; posttesticular space 0.441 to 1.6. Cirrus sac clavate, extending diagonally to right from genital pore overlapping right side of acetabulum up to midacetabular level; containing tubular, sinuous, seminal vesicle, narrow pars prostatica, and prostatic cells. Terminal portion of cirrus sac protrusible as a cirrus (Fig. 16). Ovary 4-lobed, to right of anterior testis; Laurer's canal present; seminal receptacle anterior to ovary. Vitellaria from level of anterior edge or middle of acetabulum, to near posterior end of body; dorsal, ventral and lateral to ceca; overlapping testes slightly. Uterus preovarian, usually touching anterior testis. Metraterm extending from genital pore to about middle of cirrus sac, surrounded by gland cells bounded by very thin membrane forming spindle-shaped metraterm sac. A conspicuous sphincter muscle near middle of metraterm, chiefly developed on side away from cirrus sac and producing a cup-like indentation of metraterm wall where it lies close to cirrus sac (Fig. 17). Eggs 0.054 to 0.062 long by 0.036 to 0.047 wide. Excretory pore terminal; excretory vesicle extending a little anterior to acetabulum.

DISCUSSION: This species revives the problem of separating the genera *Hamacreadium, Podocotyle,* and *Plagioporus.* Manter (1947) was inclined to emphasize the diagonal position of the testes as a chief character distinguishing *Hamacreadium* from *Podocotyle.* The long excretory vesicle of *P. musculometra* is another character in which it is more like *Hamacreadium* than *Podocotyle.* However, the anterior extent of the vitellaria, the elongate rather thick body, and the lobed ovary are more like *Podocotyle.* The glandular metraterm is unique but Yamaguti (1952) described a "muscular ring," apparently also interrupted next to the cirrus sac, in *Podocotyle gracilis* Yamaguti, 1952. *P. gracilis,* however, has tandem testes and an unlobed ovary. *Hamacreadium ositans* Linton, 1910 is similar in some respects but has a simple, non-glandular metraterm, larger acetabulum, and smaller eggs.

Hamacreadium mutabile Linton, 1910

HOST: *Mycteroperca pardalis* Gilbert, "cabrilla." (New host record)
LOCATION: Ceca
LOCALITY: La Paz, Baja California. Formerly known from Tortugas, Florida, Galapagos Islands, and the Red Sea.
NUMBER: 3 specimens in one host.

Helicometra pretiosa, n.sp. (Figs. 18-19)

HOST: *Paralabrax maculofasciatus* (Steindachner), "cabrilla pinta"
LOCATION: Intestine
LOCALITY: Puerto Vallarta, Jalisco
NUMBER: 4 specimens in one host.
HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38181.

DESCRIPTION: Body 3.64 to 4.4 long by 0.84 to 1.18 in greatest width, at level of testes; anterior end tapering to a rounded point; posterior end truncate. Oral sucker 0.172 to 0.227 long by 0.186 to 0.243 wide; acetabulum protuberant, 0.42 to 0.559 long by 0.393 to 0.517 wide, with longitudinal aperture; sucker ratio 1: 2 to 2.1. Forebody 0.54 to 0.64 or only about 1/6 body length. Prepharynx very short or lacking. Pharynx 0.117 to 0.186 long by 0.124 to 0.165 wide. Esophagus muscular, 0.186 to 0.289 long. Intestinal bifurcation at or near anterior border of acetabulum; ceca reaching to near posterior end of body. Testes usually smooth, with slight bulge in one specimen, spherical, tandem, separated by space varying from 1/4 to 5/8 diameter of testes. Posttesticular space 0.94 to 1.14. Genital pore median or submedian near mid-esophagus level, between pharynx and intestinal bifurcation. Cirrus sac usually extending little posterior to acetabulum; in one specimen only to posterior edge of acetabulum; claviform, almost straight, size 0.68 to 0.76 long by 0.117 to 0.138 in greatest width. Posterior end of seminal vesicle some distance from posterior end of cirrus sac, 0.17 to 0.195, or in one case 1/4 total length of cirrus sac. Posterior end of cirrus sac with unstained cells differing from more anterior prostatic cells. Ovary with 3 lobes and one anterior prolongation, pretesticular, slightly separated from anterior testis or overlapping it slightly. Mehlis' gland very large, preovarian; yolk reservoir and seminal receptacle immediately preovarian. Vitelline follicles almost wholly lateral to ceca with few follicles posterior to testes and median to ceca, extending from a little posterior to acetabulum to posterior end of body. Uterus spiraled between yolk reservoir and posterior end of cirrus sac, elsewhere almost straight. Eggs with long filament; eggs (less filaments) 0.065 to 0.078 long by 0.031 to 0.047 wide. Excretory pore subterminal, extent of excretory vesicle not observed.

DISCUSSION: The following four species of *Helicometra* have the vitellaria entirely posterior to the acetabulum: *H. torta* Linton, 1910; *H. bassensis* Woolcock, 1935; *H. tenuifolia* Woolcock, 1935; *H. neosebastodis* Crowcroft, 1947. In both *H. tenuifolia* and *H. neosebastodis* the vitellaria extend only slightly anterior to ovary. In *H. bassensis* the cirrus sac does not reach mid-acetabular level. *H. pretiosa* is most similar to *H. torta* but has more rounded testes, the gonads are more widely separated, the ovary very distinctly trilobed, and eggs are larger (65 to 78 by 31 to 47 microns, compared with 40 to 58 by 23 to 26 microns). The evident space within the cirrus sac between the seminal vesicle and posterior end of the sac is a unique feature of *H. pretiosa.* The name *pretiosa* is from *pretiosus,* valuable, for the pleasing appearance of this species.

GORGODERIDAE

Phyllodistomum carangis Manter, 1947

HOST: *Citula dorsalis* (Gill). "papelillo." (New host record.)

LOCATION: Posterior dorsal of cavity of body.

LOCALITY: Mazatlan, Sinaloa. Formerly known from *Caranx ruber* at Tortugas, Florida

NUMBER: 5 specimens in one host.

Phyllodistomum marinae, n. sp., (Fig. 20)

HOST: *Mycteroperca pardalis* Gilbert, "cabrilla."

LOCATION: Urinary bladder.

LOCALITY: La Paz, Baja California.

NUMBER: 2 in one host.

HOLOTYPE: U. S. Nat. Mus. Helm. Coll. No. 38182.

DESCRIPTION: Body lanceolate, 7.06 to 7.4 long by 2.38 to 2.64 in greatest width at level of anterior testis; hindbody not markedly widened. Sides of body with series of 4 lateral indentations beginning just posterior to acetabulum (Fig. 20) caused by groups of radiate muscles in body wall, resulting in shallow cup-like depressions at regular intervals; fourth depression near posterior end of body. Four slight elevations occur on each side of body between depressions. Oral sucker 0.74 to 0.78 long by 0.7 to 0.74 wide; mouth transverse. Anterior to mouth ventral surface of oral sucker bears transverse slit with diagonally transverse muscles along its posterior edge. In paratype, slit reduced to round pore near anterior edge of sucker. The name preoral pore is suggested for this structure. Acetabulum slightly sunken into ventral surface, 0.66 to 0.7 long by 0.68 to 0.7 wide. Sucker ratio 1: 0.92 to 0.97. Forebody 2.36 to 2.44. Esophagus 0.4 to 0.517 long, preceded by small globular body probably representing rudimentary pharynx; bifurcation little less than halfway between oral sucker and acetabulum; rather wide ceca ending near posterior end of body.

Genital pore median, about 0.3 anterior to acetabulum. Testes rather small, oblique, separated by uterus, in posterior half of hindbody; posttesticular space 1.58 to 1.6. Seminal vesicle sac-like, anterior to genital pore. Ovary spherical, or subspherical, smaller than testes, to right or to left, near cecum, at about midbody level. Vitelline lobes symmetrical, compact or slightly lobed, midway between ovary and acetabulum. Uterus mostly filling intercecal space, few coils overlap but do not extend lateral to ceca in hindbody, with sac-like swelling near genital pore. Eggs 0.05 to 0.061 long by 0.037 to 0.039 wide. Excretory pore subterminal and dorsal; terminal portion of excretory vesicle glandular, anterior extent not observed.

Discussion: The genus *Phyllodistomum* is a large one. *P. marinae* is unique in the paired, segmental depressions along the sides of the hindbody. The preoral pore has not been previously noted. The most closely related species is doubtlessly *P. pacificum* Yamaguti, 1951. The figure of that species shows an outline suggesting lateral indentations and there is a preesophageal bulb. *P. pacificum* has large lobed testes.

Xystretrum caballeroi Bravo, 1954

Host: *Balistes verres* Gilbert and Starks. (New host record.)
Location: Urinary bladder.
Locality: Mazatlan, Sinaloa.
Number: 2 in one host.

Discussion: These specimens agree with *X. caballeroi* except they are larger. The following measurements extend those of the original description. Body 6.2 to 8. long; 3.6 to 3.8 wide at level of the testes. Oral sucker 0.7 to 1. long by 0.74 to 0.92 wide; acetabulum 0.82 to 1.06 long by 0.86 to 1. Sucker ratio 1: 1.08 to 1.16. Eggs 0.04 to 0.05 by 0.02 to 0.025.

X. pulchrum (Travassos, 1920) differs in the contour of the acetabulum, sucker ratio (1: 1.3 to 1.5), and more anterior uterus.

Family Zoogonidae

Diplangus mexicanus, n. sp. (Fig. 21)

Host: *Balistes verres* (Gill), "pez puerco," trigger fish.
Location: Intestine.
Locality: Mazatlan, Sinaloa.
Number: 14 in one host.
Holotype: U. S. Nat. Mus. Helm. Coll. No. 38183.

DESCRIPTION: Body spindle-shaped, tapering about equally toward each end; 1.2 to 2.32 long by 0.56 to 1.06 in greatest width at acetabular level. Oral sucker 0.165 to 0.290 long by 0.207 to 0.331 wide; acetabulum near middle of body, 0.296 to 0.476 long by 0.393 to 0.6 wide, with transverse aperture. Acetabulum in middle of a large muscular expansion of body wall with radial muscles. Sucker ratio 1: 1.82 to 2. Prepharynx not visible; pharynx 0.119 to 0.193 long by 0.138 to 0.2 wide; esophagus 0.023 to 0.055 long; bifurcation about midway between sucker; ceca ending from a level opposite anterior third of posterior testis to a little past posterior testis. Genital pore median at level of pharynx. Testes globular, tandem or oblique, close together, interceeal, in posterior third of body. Seminal vesicle almost entirely pre-acetabular, more or less S-shaped; prostatic vesicle pyriform, about ⅓ length of seminal vesicle, ventral to intestinal bifurcation, tapering to a short duct opening in genital pore. Ovary smooth, immediately anterior or anterio-lateral to anterior testis, about same size as testes; seminal receptacle spherical, between ovary and acetabulum, about same size as ovary; vitelline follicles ovoid or irregular in outline, in two separated groups lateral to ovary, mostly ventral to ceca; 8 to 11 follicles on the right, 8 to 10 follicles on the left. Uterus filling most of hindbody, surrounding testes but over-lapping them very little. Metraterm lacking. Eggs 0.037 to 0.04 by 0.015 to 0.17.

DISCUSSION: *D. mexicanus* is the fifth species in the genus. All species are from American waters; three from the Gulf of Mexico, two from the Pacific. *D. paxillus* Linton, 1910 is more elongate, has a smaller sucker ratio, and more anterior vitellaria. *D. parvus* Manter, 1947 is smaller, has a more rounded posterior end and more anterior vitellaria. *D. miolecithus* Manter, 1947 has a shorter seminal vesicle, reduced vitellaria, longer ceca. The California species, *D. triradiatus* Manter and Van Cleave, 1951, is quite different.

SUMMARY

Thirteen species of digenetic trematodes are reported from marine fishes of the Mexican Coast.

The following new genera of Lepocreadiidae are named: *Guggenheimia* and *Dactylotrema.*

The following new species are named: Lepocreadiidae: *Lepidapedon epinepheli* from *Epinephelus analogus; Hypocreadium myohelicatum* from *Balistes capistratus; Guggenheimia pacifica* from *Balistes verres* and *B. capistratus; Dactylotrema squamatum* from *Gerres* sp. Opecoelidae: *Opegaster lutiani* from *Lutianus aratus; Podocotyle musculometra* from *Hoplopagrus güntheri; Helicometra pretiosa* from *Paralabrax maculofasciatus.* Gorgoderidae: *Phyllodistomum marinae* from *Mycteroperca pardalis.* Zoogonidae: *Diplangus mexicanus* from *Balistes verres.*

Other species reported are: *Hypocreadium scaphosomum* from *Balistes capistratus* (new host record) and *B. verres; Hamacreadium mutabile* from *Mycteroperca pardalis* (new host record); *Phyllodistomum carangis* from *Citula dorsalis* (new host record), *Xystretrum caballeroi* from *Balistes verres* (new host record). New locality records are: *H. mutabile,* formerly known from Tortugas, Florida, Galapagos Islands and Red Sea; *P. carangis,* formerly known from Tortugas, Florida.

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All figures were drawn with the aid of a camera lucida.

Abbreviations: *ac,* acetabulum; *cs,* cirrus sac; *gp,* genital pore; *m,* mouth; *mt,* metraterm; *os,* oral sucker; *ph,* pharynx; *pr,* prostate gland; *sr,* seminal receptacle; *sv,* seminal vesicle; *ut,* uterus.

A Redescription of *Atylenchus decalineatus* Cobb, 1913 (Nematoda: Tylenchinae) *

B. G. CHITWOOD AND A. C. TARJAN**

The genus *Atylenchus* Cobb, 1913 has been placed in the *Tylenchinae* and the *Criconematinae* by various authors since its original description. In appearance and body movement it seems to be closely related to members of the genus *Tylenchus,* and in particular to species of the subgenus *Aglenchus,* recently erected by Andrassy (1954) to include species with pronounced annulation and thin, attenuated tails. It is clearly distinguishable from all other *Tylenchina* except *Eutylenchus* Cobb, 1913 by four clearly defined setae on the head. *Atylenchus* is easily differentiated from *Eutylenchus* by 10 longitudinal ridges on the body and the absence of caudal alae on the male. The caudal alae on the male of *Eutylenchus* have been described as being

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**Florida State Plant Board, Gainesville and Citrus Experiment Station, University of Florida, Lake Alfred respectively.

shaped like a trapezium. Only one other author, Hirschmann (1954), has reported the type and only species of the genus, *A. decalineatus* Cobb, 1913, from soil around roots of *Scirpus lacuster* L. from a pond. No description was given by this author.

A study recently has been made of four females and one male of this species. Since these specimens differ somewhat in their measurements, these are presented individually, following a republication of the original description together with formula used in our present system.

ORIGINAL DESCRIPTION

"Striae about 200; interrupted by ten longitudinal grooves. Setae near the margin of the head, slender, spreading, nearly as long as the head is wide. Spear acute, shaft half as wide as the adjacent annules; its bulb twice as wide. Median bulb ellipsoidal, half as wide as the neck with small valve; posterior swelling oblong, half as wide as the neck, without valve. No supplementary organs or special male papillae or setae. Spicula arcuate, one and one-third times as long as the body diameter, tapering; constricted slightly at the proximal end. Hot sublimate to balsam.

FEMALE: 600 μ; a = 34.5; b = 5.5; c = 7.1; V = 66 per cent, ovary outstretched, 21 per cent, stylet estimated at 18.6 μ.

MALE: 600 μ; a = 35.5; b = 5.9; c = 10; testis outstretched, 45 per cent.

HABITAT AND DISTRIBUTION: On roots of cranberries (*Oxycoccus macrocarpus*), cranberry bog, New Lisbon, New Jersey, Atwood Grove, Florida."

DIMENSIONS OF OUR SPECIMENS

1. Female (neotype) from about roots of *Oxycoccus macrocarpus* Ait. L = 0.836 mm., a = 33, b = 6.5, c = 10, V = $^{26}66$, stylet = 16 μ, dorsal gland orifice = 3.2 μ behind stylet bulbs, setae = 7 μ, annules about 418 in number measuring about 2 μ wide. State Plant Board Nematology Collection F. 50. A. neotype. Gainesville, Florida.
2. Female from about roots of *Saccharum officinarum* L. L = 0.650 mm., a = 38; b = ?; c = 9.0; V = $^{28}69$; stylet = 13 μ; annules = 1.6 μ wide; egg = 40 by 10 μ. State Plant Board Nematology Collection F 282-3. Gainesville, Florida. A second female from about roots of *S. officinarum* was decapitated for head study. Slide 5, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.
3. Female from about roots of *Diospryros virginiana* L. L = 0.426 mm.; a = 26, b = 4.9, c = 6.5, V = 62, stylet = 14 μ, setae = 7 μ, dorsal gland orifice = 2 μ behind stylet bulbs, annules = 1.6 μ wide. State Plant Board Nematology Collection F 305. Gainesville, Florida.
4. Male from citrus grove soil water. L = 0.589 mm., a = 45.3, b = 6.3, c = 7.9, T = 42 per cent, stylet = 12 μ, setae = 8 μ, spicule length = 17 μ, gubernaculum length = 7 μ, annules = 1.5 μ, dorsal gland orifice = 2 μ behind stylet knobs. Slide 3, Tray 1, Cabinet C-2724, Nematode Collection, Citrus Experiment Station, Lake Alfred, Florida.

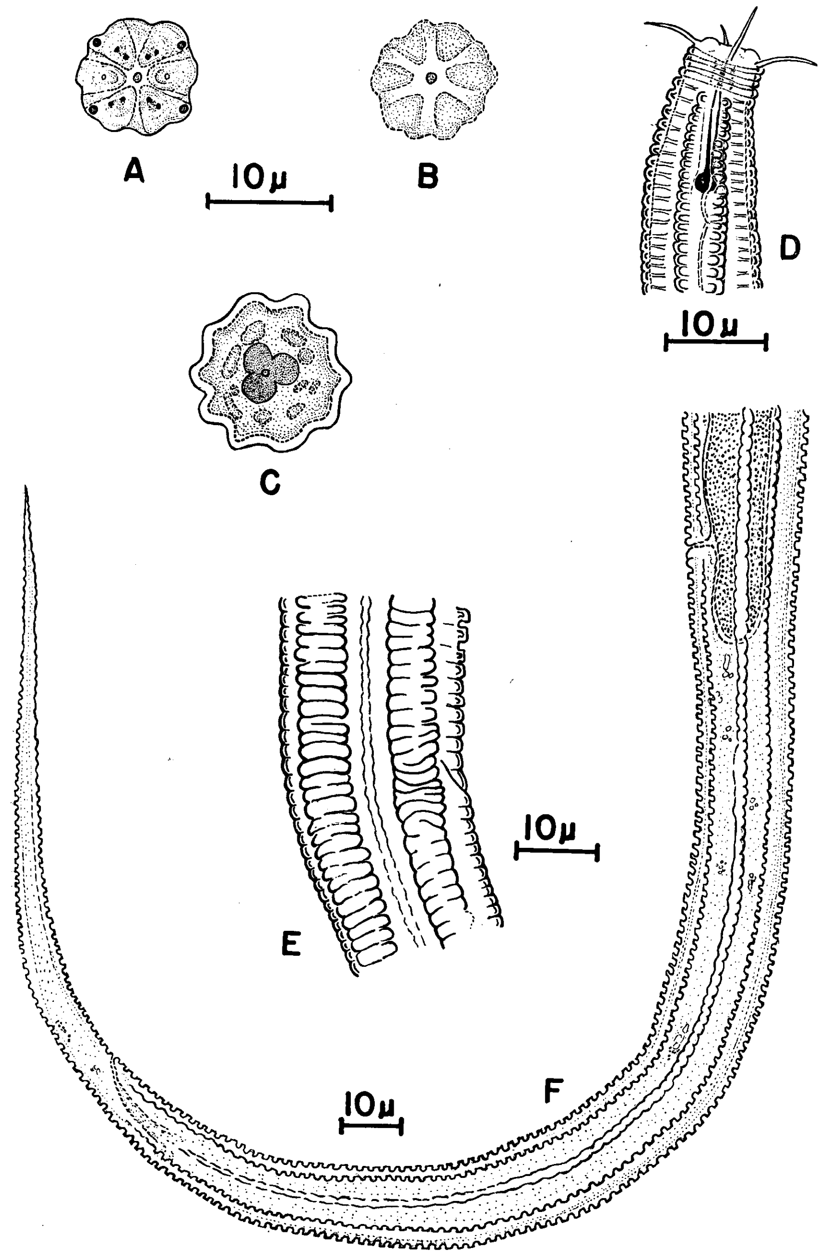

Fig. 1. *Atylenchus decalineatus.* A. Face view showing 6 lips and 4 equidistant setae. B. Area underlying face. C. Cross section of body showing stylet knobs. D. Female head. E. Annulation at vulvar region. F. Posterior portion of female body. (A, B, and C of female from *Saccharum officinarum;* D and E of female from *Oxycoccus macrocarpus;* F of female from *S. officinarum*).

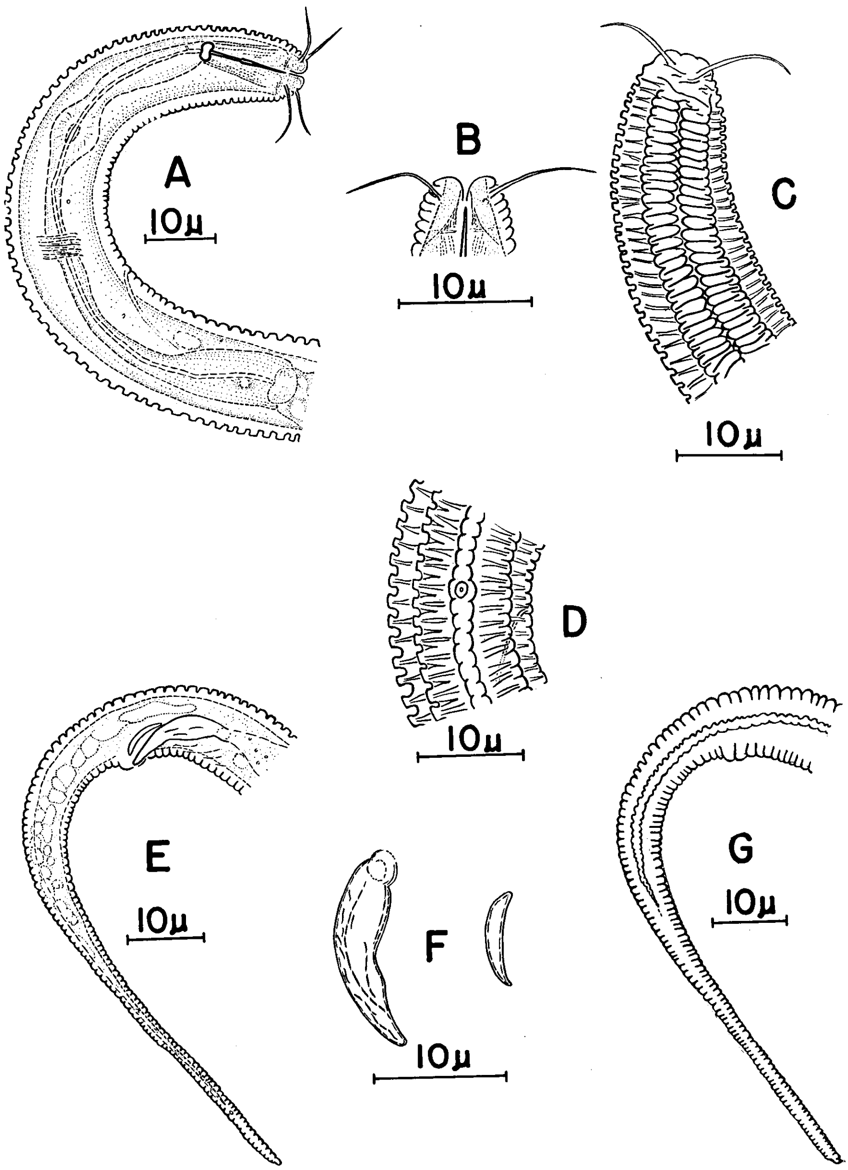

Fig. 2. *Atylenchus decalineatus.* Lateral views of single male specimen from citrus grove soil water. A. Esophageal region. B. Lip region. C. Annule arrangement, anterior part of body. D. Annule arrangement, deirid, and excretory pore, cervical region of body. E. Caudal region. F. Spicule and gubernaculum. G. Lateral field, caudal region.

DESCRIPTION EMENDED

Body cylindrical with obtuse anterior end and slender attenuated tail; terminus acute on female (Fig. 1, F), minutely rounded on male (Fig. 2, E). Cuticle coarsely annulated with distinctive, well-separated transverse annules (Fig. 1, D and E; Fig. 2, C and D) and 10 rows of longitudinal ridges (Fig. 1, C). Annules measuring about 1.5-2.0 μ at middle of body. Lateral field appearing as two irregular lines formed by union of transverse annules (Fig. 1, E and F; Fig. 2, C, D, and G).

Lips 6 in number, the two subdorsally and the two subventrally each appearing to bear 2 minute papillae and a seta presumably representing a latero-dorsal or latero-ventral papilla; papillae not observed on lateral lips but amphids appearing near oral opening (Fig. 1, A). Lip region more or less continuous with body contour, measuring about 7.5-8.0 μ wide at base, bearing 4 sublateral setae averaging 7-8 μ long (Fig. 1, A and D; Fig. 2, A); setae attached to body posterior to lips. Labial region of body (Fig. 1, B), weakly set off, bearing 4 to 6 visible annules (Fig. 1, D; Fig. 2 B and C) according to interpretation. Stylet acute, measuring about 14 μ long (12-16 μ). Stylet knobs rounded, 3 μ in diameter (Fig. 1, C). Orifice of dorsal esophageal gland about 2.4 μ (2.0-3.2 μ) behind stylet knobs (Fig. 1, D; Fig. 2, A). Metacorpus oval, 6.5 μ wide and bearing valves (Fig. 2, A). Nerve ring located about 17 μ posterior to valves of metacorpus. Esophagus expanding to form saccate posterior bulb, 7 μ wide. Esophago-intestinal valve roughly oval. Excretory pore posterior to nerve ring (Fig. 2, A). Deirid conspicuous, present in region of excretory pore (Fig. 2, D). Hemizonid not observed.

Vulva simple, transverse. Ovary outstretched. Posterior uterine sac about 13 μ long (Fig. 1, F). Testis single and outstretched. Supplementary organs, caudal papillae, phasmids, or caudal alae not observed. Spicules arcuate, true length 17 μ long and 3.6 μ at widest point. Gubernaculum simple and arcuate, 7 μ long (Fig. 2 F).

HABITATS AND LOCALITIES: One female from the roots of the type "host" *Oxycoccus macrocarpus,* from material supplied by Charles A. Doehlert, Assoc. Research Specialists, Pemberton, New Jersey designated neotype; one female from about roots of native persimmon, *Diospyros virginiana* from Monticello, Florida; 2 females from about roots of sugar cane, *Saccharum officinarum* from Lake Alfred, Florida; and 1 male collected by E. P. DuCharme from soil water (pH 4) obtained at an 8 ft. depth from a citrus grove in Lake Alfred, Florida.

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A New Species of *Xiphinemella* Loos, 1950, (Nematode) from Florida

B. G. CHITWOOD
Chief Nematologist, Florida State Plant Board, Gainesville Florida

Several times during the past year we have encountered a species of *Xiphinemella* from about roots of various plants in Florida. Usually these are larval specimens so specific identification has been impossible, but recently we have encountered adult males and females from about the roots of the Spanish oak, *Quercus falcata,* the red maple, *Acer rubrum* and the fern, *Pteris aquilina* v. *latiuscula.* These specimens appear definitely to be a new species of the genus *Xiphinemella* Loos, 1950 (Syn. *Taprobanus* Loos, 1949 not Distant, 1911) and certain points of difference indicate possible errors in the original generic diagnosis.

Xiphinemella esseri, n. sp.

Cuticle apparently smooth with internal cross-hatched fibers as in some dorylaimids and mermithids. Irregular ornamentations described as irregular longitudinal wings by Loos (1949) are apparently an internal sclerotized layer of the cuticle striated transversely and rarely if ever reaching the cuticular surface. External to this there is a possibly hollow area of cuticle, then a layer of diagonally crossing fibers and finally a layer of fine transverse fibers which sometimes appears at, sometimes under the surface of the cuticle but an optical longitudinal section gives no impression of striation. (Figs. 1 C-H). In the apparent cavity between the internal sclerotized cuticular layer and the external cuticular layers there somtimes appear to be free minute crystalloid structures. Sublateral hypodermal gland pores present, apparently beginning in the mid-esophageal region and not very regular. One of these might have been interpreted as a functional excretory pore which is also incorporated in the original generic diagnosis. Hypodermal glands or pores were not mentioned in the original description.

Labial region (Figs. 1 A-B) consisting of a circum-oral disc like structure about 3.5 by 10 microns and six fleshy well set off lips (width at this point 18 microns and height of lips about 5 microns). Apparently the disc like structure represents 6 anterior labial lobes each bearing one papilla and the posterior larger fleshy "lips" represent 6 posterior labial lobes of which the 4 submedian ones each bear 2 papillae and the 2 lateral each bear one papilla. (Fig. 1, A).

Amphids very large (10 microns wide), pocket like, immediately postlabial. Stomatal region narrow, weakly sclerotized, extending to guiding ring, without a distinct short, heavily sclerotized portion confined to labial region (such as in *X. ornatum*). Stylet normally withdrawn, guiding ring near anterior end of stylet tip, 35-38 microns from anterior extremity (8-10 microns from anterior end of stylet tip). Stylet 72-75 microns long, narrow tip portion 55-60 microns long, base with flanges. One adult male was observed with a spare stylet tip well formed in the esophageal wall. It is conceivable, therefore, that these forms molt aftr reaching adulthood. This would be supported by the cavity between cuticular layers. Anterior part of esophagus narow, not appearing very muscular, often twisted; bulb 75-85 microns long, cylindrical, containing 5 esophageal gland orifices. Protoplasm (not sclerotized) extending to cuticle as though caudal glands might exist but no spinerette valve or pore present. (Figs. 1, E & H).

MALE:—2.2-3.0 mm. long; a, 35-45; b, 7.6-11 (11.4 shortened); c, 77-120; spicules 40-45 microns long, bluntly arcuate; tail bluntly curved and rounded conoid, with two pairs of sublateral caudal genital papillae near mid-region of tail. With 1 plus 8 or 9 preanal supplements, the most caudal being immediately preanal, the remainder being in tandem over an area of about 115-125 microns beginning about 100 microns anterior to the anus. With two seminal vesicles each about 120 microns long, anterior testis extending 400 microns anterior to junction area, latter 85 microns, posterior testis extending 300 microns posteriad; anus to posterior end of posterior testis about 950 microns.

FEMALE.—2.4-3.5 mm. long; a, 40-43; b, 11.4-14 (shortened); c, 93-130; V, 42-47%. Two opposed ovaries, anterior ovary with uterus extending anterior 330 microns and posterior ovary and uterus extending 350 microns from vulva. Eggs not observed.

COTYPE SPECIMENS.—Florida State Plant Board.
Nematology Collection Slide F-589.

TYPE HOST.—*Quercus falcata* Michs.
About roots at depth of two feet.

TYPE LOCALITY.—Archer Road Village, Gainesville, Florida.

OTHER HOSTS AND LOCALITIES.—
Acer rubrum L., Flavet 3, Univ. of Florida, Gainesville, Florida.
Specimen F-407—Florida State Plant Board.
Nematology Collection.
Pteris aquilina L. v. *latiuscula* Desv., Archer Road Village, Gainesville, Florida.
Specimens F-590 Florida State Plant Board.
Nematology Collection.

DISCUSSION

Xiphinemella esseri differs from the type species *X. ornatum* (Loos, 1949) Loos, 1950 in the length of the stylet, namely 72-75 microns in our species as against 82-93 microns in Loos' species. This may be due to error on the part of the original author since the stylet is very narrow as is the stomatal lining so they could easily be confused. Loos states that there are only 3 supplementary organs in *X. ornatum* but illustrates 4 (his Fig. 1 F) the extra supplement being immediately preanal. We would prefer to characterize *X. ornatum* as having 1 plus 3 supplementary organs and our species 1 plus 8 or 9 such structures.

X. ornatum was originally described from about mango and bamboo roots, Kurunegala, Ceylon. We may well expect both species to be external, migratory plant pathogens.

The interesting finding of a spare stylet tip in an adult male of *Xiphinemella esseri* raises a number of theoretic questions*. The first and most simple explanation might be that just as human beings occasionally develop a third set of teeth after losing their adult teeth, an individual nematode might do likewise. This question can be answered by a life history study. The second possible explanation is that these are neotenic larvae, and, if so, many additional changes might be expected in the morphology in later stages. This

*A set of photographs showing this is on file at the Nematology Section, Beltsville, Md.

question can also be answered best by a life history study.

Assuming that the extra stylet in the adult is normal and signifies preparation for at least one more molt, the finding would bear on the classification of the entire Aphasmidia. Dioctophymatids, mermithids, and dorylaimids all have a multi-layered cuticle and a stylet has been described at least in larval stages in all of these groups as well as in the Trichuroidea. One author

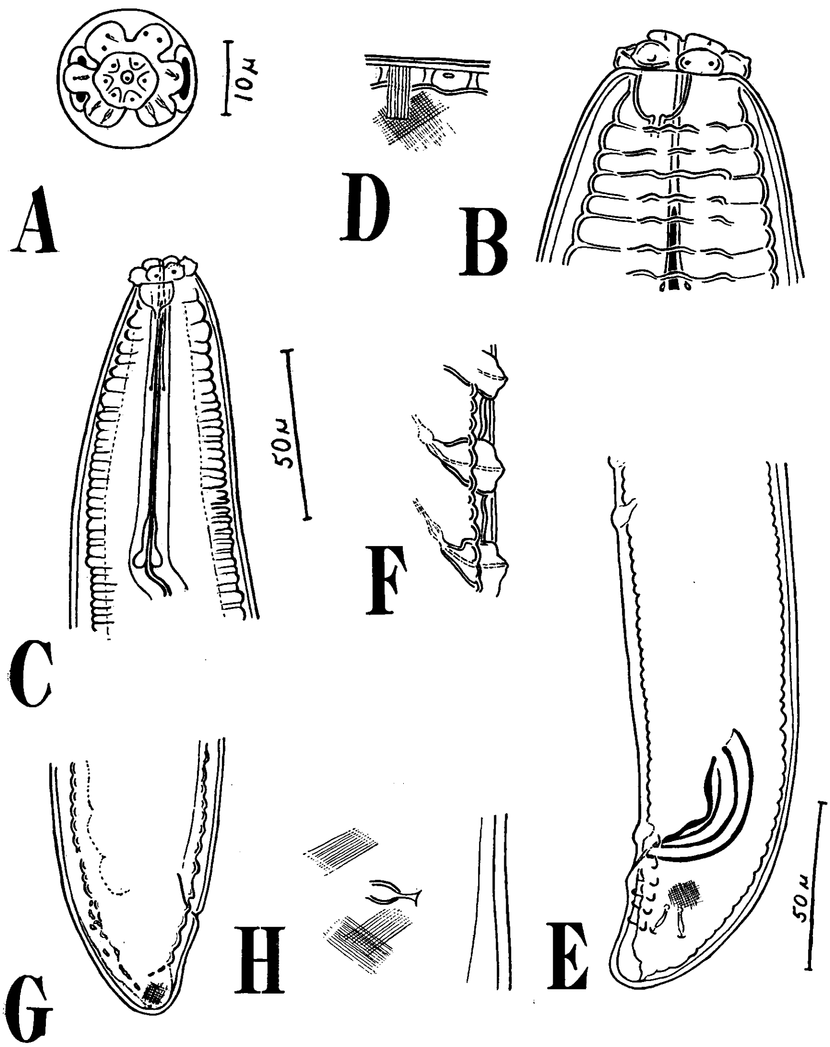

Fig. 1. *Xiphinemella esseri* n. sp. A, Head, en face; B, Head, lateral view; C, Anterior extremity, lateral view; D, Cuticle of adult, showing various superimposed layers; E, Male tail, lateral view; F, Detail of supplementary organs; G, Female tail, lateral view; H, surface view showing a hypodermal gland orifice, duct and adjoining cuticular layers (finest transverse markings not observed in this part of this specimen).

(Kreis, 1930) has described *Actinolaimus tripapillatus* as having five molts in the course of its development. It is not customary to look beyond the esophageal region when examining dorylaimoids if a spare stylet is present. Most of us have assumed such specimens are larvae, hence not identifiable to species. Observations of other workers are invited. With sufficient information we might be justified in raising the aforementioned group to ordinal rank.

In placing the genus *Xiphinemella* we note similarities in the cuticle and labial region to some genera of the Tylencholaiminae Filipjev, 1934, particularly to *Tylencholaimus* and *Enchodelus* as well as in the stylet and other characters to various representatives of the Longidorinae Thorne, 1935. Relative to a species of *Longidorella* Thorne (1939) himself states that it may belong to *Enchodelus* and in keying genera twice we used body diameter (alpha) which we would not ordinarily consider of generic rank by itself. However, since further study will probably disclose better generic characters we have not synonomized these but we have synonomized the Longidorinae with the Tylencholaiminae following year precedence as indicated by the present International Rules of Zoologic Nomenclature.

KEY TO THE GENERA OF THE TYLENCHOLAIMINAE FILIPJEV, 1934.

1. Stylet with extensions more than twice as long as labial diameter 2
   Stylet with extension lesss than twice as long as labial diameter .... *Tylencholaimus* de Man, 1876
2. Labial region, prominently set off as anterior, narrow discoid region and posterior wider posterior labial lobes 3
   Labial region not prominently set off and not clearly sub-divided into anterior and posterior labial lobes 4
3. Female tail conically attenuated to spicate; posterior enlarged glandular part of esophagus approximately one-half distance from head to base of esophagus .... *Discomyctus* Thorne, 1939
   Female tail bluntly rounded to bluntly conoid; posterior enlarged glandular part of esophagus approximately one-third distance from head to base of esophagus .... *Xiphinemella* Loos, 1950
4. Stylet extensions flanged 5
   Stylet extensions not flanged 6
5. Body short and plump (alpha 22-35) .... *Enchodelus* Thorne, 1939
   Body more elongate (alpha 25-77, usually 36 or more). .... *Xiphinema* Cobb, 1913
6. Body short and plump (alpha 19-27) .... *Longidorella* Thorne, 1939
   Body more elongate (alpha usually 50 or more) .... *Longidorus* Micoletzky, 1922

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Two New Species of the Genus *Criconema* Hofmänner and Menzel, 1914

B. G. CHITWOOD
Chief Nematologist, Florida State Plant Board, Gainesville, Florida

In the course of routine examination of plant materials in Florida we have encountered several new species of the genus *Criconema* two of which are described in the present article. The first of these, *Criconema decalineatum* was encountered in collections of fig roots from the Atwood Estate, Bradenton, Florida while seeking *Atylenchus decalineatus* Cobb, 1913. At first we thought this really was Cobb's species because of the 10 rows of spines but we have since found Cobb's species both from the type host in New Jersey (*Oxycoccus macrocarpus*) and in several collections from Florida. It really does belong to the subfamily Tylenchinae and has the setae as described by Cobb.

Two of the species recently described in the genus *Criconema* have been transferred to the genus *Criconemoides* by Goodey (1951). A key is appended to the species remaining in the genus as we conceive it, following Goodey. References to articles of taxonomic importance on the genus *Criconema* since Taylor's (1936) monograph of the group are given.

Criconema decalineatum, n. sp.

DESCRIPTION. Female 400-539 microns long; a, 9-10; b, 3.5-3.8 (contracted); c, 13-15, V, 86-88%; ovary single, anterior, reflexed or outstretched, 37-44% length of body; striae 4-5.3 microns wide, 10 longitudinal grooves or 10 longitudinal rows of blunt scales or spines (becoming more spinate posteriad); 86 counted annules, estimates of about the same number on 3 additional specimens. Stylet 66-85 microns long; knobs reflexed anteriad, 5 to 9.6 microns wide by 1.6 to 3.2 microns high. Dorsal gland orifice 5-6 microns posterior to base of stylet; median bulb isthmus and posterior glandular part of esophagus typical of genus. Excretory pore 189 microns from anterior end. Vulva on 14-15th annule from tip of tail; anterior lip of vulva with paired lateral lobes (Fig. 1C); anus at junction of 8 and 9th annules; phasmids(?) at junction of 7th and 8th annules; tail rather conoid with indications of annules nearly to tip. Head consisting of 2 or 3 non-rigid and 2 rigid annules, latter marked laterally.

TYPE HOST.—*Fiscus elasticha* Roxb. (Strangler fig or rubber tree)

TYPE LOCATION.—Roots

TYPE LOCALITY.—Atwood Estate, swampy ground, Bradenton, Florida

TYPE SPECIMEN.—Florida State Plant Board Nematology Collection F 30a; Paratypes F 30 and F 73.

Criconema spinalineatum, n. sp.

DESCRIPTION: Female 228 microns long; a, 6.2; b, 2.9, c, ? 48; V, 87%, cuticle with not over 89 annules with 8 rows of setose spines, each 2-7 microns long; stylet 40 microns long; knobs 8.3 by 4 microns; dorsal gland orifice 2 microns posterior to its base; median bulb elongate, with procorpus not distinct; corpus about as long as stylet; posterior bulbar regions short but distinct. Head consisting of two annules, first annule with several anterior rounded bumps (? lips); position of excretory pore 62 microns from anterior end vulva probably on 17-18th annule, anus on 4th annule from terminus; tip of tail rounded, with one spine.

Host.—*Zoysia matrella* Merr. (Manila grass, roots).

Locality.—Farm of Mr. Brown, Route 8, Box 330-H, Jacksonville, Florida, U. S. A.

Collector.—Ralph L. King, Jr., July 26, 1955.

Type specimen.—Florida State Plant Board Nematology Collection F-87a

This species keys out with *Criconema murrayi* (Southern, 1914) but is more slender, has more annules and the vulva appears to be located more anteriad.

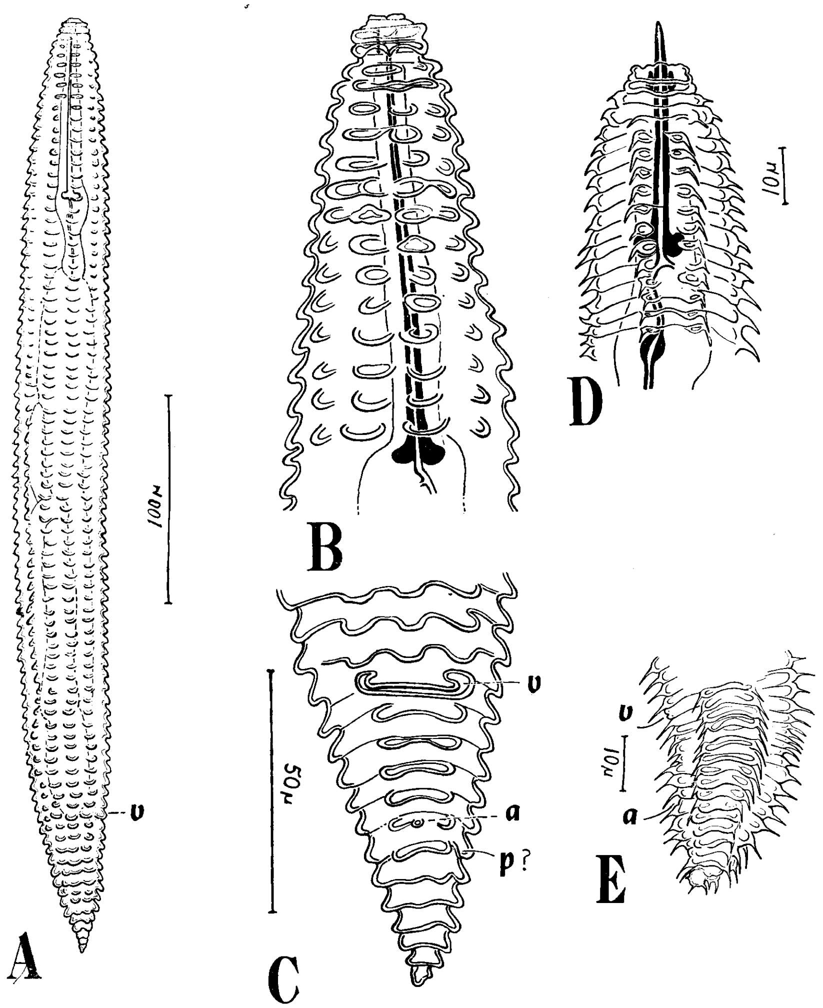

Fig. 1 A-C, *Criconema decalineatum* female; A, Full length, dorsal view; B, Anterior end; C, Posterior end, D-E, *C. spinalineatum;* D, Anterior end of female; E, Tail of female. Abbreviations.—*a,* anus; *p* ? phasmid questionable; *v,* vulva.

KEY TO GENUS *Criconema* HOFMANNER AND MENZEL 1914

1. Annules 100 or more 2
   Annules less than 100 3
2. Annules about 150; spines short and wide ....
   Criconema squamosum (Cobb, 1913) Taylor, 1936
   Syn. *Iota squamosum* Cobb, 1913
   Hopolaimus squamosus (Cobb, 1913) Menzel, 1917
   Type host.—Mango
   Type locality.—Bangalore, India.
   Annules probably 100-120; spines triangular, in 6 longitudinal rows ....
   Criconema guernei (Certes, 1889) Hofmanner & Menzel, 1914
   Syn. *Eubostrichus guernei* Certes, 1889
   Hoplolaimus guernei (Certes, 1889) Menzel,1917
   Iota guernei (Certes, 1889) Micoletzky,1925
   Type habitat.—Sediment from fresh water
   Type locality.—Tierra del Fuego.
3. Fringe continuous on each annule 4
   Fringe in strips or rows of distinct spines present (not over 16 per annule) 6
4. Annules numbering 45; 70-80 spines in continuous fringe; stylet 98 microns (vide Sveshnikova 89 microns) ....
   Criconema multisquamatum (Kirjanova, 1948), n. comb.
   Syn. *Ogma multisquamatum* Kirjanova, 1948
   Criconema fimbriatum (Cobb) Taylor of Sveshnikova, 1940.
   Annules 53 or more 5
5. Annules numbering 53; about 40 spines per annule in mid region; stylet 96 microns long ....
   Criconema fimbriatum Cobb 1936 in Taylor
   Type habitat.—Leaf mold
   Type locality.—South of Alexandria, Va., U. S. A.
   Annules numbering 60-70; about 60 spines in fringe; stylet 96-130 microns long ....
   Criconema menzeli (Stefanski, 1924) Taylor, 1936
   Syn. *Criconema guernei* (Certes, 1889) of Hofmanner and Menzel, 1914
   Hoplolaimus guernei (Certes, 1889) of Menzel, 1917
   Hoplolaimus menzeli of Stefanski, 1924
   Iota menzeli of Micoletzky, 1925
   Type habitat.—Sphagnum moss
   Type locality.—Jura Mts., near Basel Switzerland.
6. Spines in a discontinuous fringe of 32-48 per annule in 8 longitudinal bands ....
   Criconema civellae Steiner, 1949
   Type host.—*Citrus grandis*
   Type locality.—Greenhouse Beltsville, Md., U. S. A.
   Spines or scales in few (4-16) longitudinal rows 7
7. Spines in only 4 longitudinal rows: 1 dorsal, 1 ventral (2) lateral; 69-73 annules; stylet 55-60 microns long ....
   Criconema minutum (Kirjanova) 1948, n. comb.
   Syn. *Ogma minuta* Kirjanova, 1948
   Type habitat.—Soil about roots *Gossypium* sp.
   Type locality.—Kara Kul, Usbeck, SSR.

Spines or scales in 8 or more longitudinal rows 8
8. Spines or scales in 16 longitudinal rows 9
Spines in 4-12 longitudinal rows 10
9. Spines rather blunt and scale like, not very narrow even in posterior body region; annules 62-64; stylet 96 microns long ....
Criconema cobbi (Micoletzky, 1925) Taylor, 1936
Syn. *Iota cobbi* Micoletzky, 1925
Type habitat.—Sphagnum moss
Type locality.—Gribess-Moor, Denmark.

Spines bluntly scale-like anteriad, becoming narrow, spinate, even branched posteriad; annules 46-54 in number; stylet 94-100 microns long ....
Criconema coronatum (Stekhoven and Teunissen, 1938), n. comb.
Syn. *Ogma lentiforme* Stekhoven and Teunissen, 1938
Type habitat.—Forest soil
Type locality.—Albert National Park, Belgian Congo.

10. With 4-8 rounded scale rows; annules numbering 77; stylet about 52 microns long ....
Criconema lentiforme (Stekhoven & Teunissen, 1938), n. comb.
Syn. *Ogma coronatum* Stekhoven and Teunissen, 1938
Type habitat.—Volcanic soil, altitude 3770-3800 m.
Type locality.—Albert National Park, Belgian Congo.

With 8, 10, 12 or 8-12 longitudinal rows of scales or spines 11
11. Scales in 8-12 or 12 longitudinal rows 12
Scales or spines in 8-10 longitudinal rows 13
12. Scales in 8-12 rows; rounded at side dentate or wholly trifid; 67 annules; stylet 82 microns ....
Criconema tripium (Stekhoven & Teunissen, 1938), n. comb.
Syn. *Ogma tripius* Stekhoven & Teunissen, 1938
Type habitat.—Soil, Mikenov-Kerismo 3200 m. elevation
Type locality.—Albert National Park, Belgian Congo.

Scales in 12 longitudinal rows; bluntly triangular with side teeth, often somewhat tridentate; 42 annules; stylet 98.5 microns ....
Criconema tricodon (Stekhoven & Teunissen, 1938), n. comb.
Syn. *Ogma tricodon* Stekhoven & Teunissen, 1938
Type habitat.—Volcanic soil, 2,075 m. elevation
Type locality.—Albert National Park, Belgian Congo.

13. Spines in 10 longitudinal rows; annules; stylet 66-85 microns long ....
Criconema decalineatum, n. sp.
Type host.—*Ficus elastichus*
Type locality.—Atwood Estate, Bradenton, Florida.

Spines or scales in only 8 longitudinal rows 14
14. Spines mostly longer than wide 15
Spines mostly wider than long or about equal 16
15. Vulva at 15th annules from terminus; annules numbering 68-71 ....
Criconema murrayi (Southern, 1914) Taylor, 1936
Syn. *Ogma murrayi* Southern, 1914.
Hoplolaimus murrayi (Southern, 1914) Menzel, 1917
Iota murrayi (Southern, 1914) Micoltezky) 1925
Type habitat.—Moss from Beclare
Type locality.—County Mayo, Ireland.

Vulva at 17th-18th annule from terminus; annules numbering 89; stylet

40 microns long; about 89 annules ....
Criconema spinalineatum, n. sp.
Type host.—*Zoysia matrella*
Type locality.—Jacksonville, Florida, U. S. A.

16. Annules 65-75, large broad scale-like; vulva at 12th annule from terminus; stylet 63 microns long ....
Criconema octangulare (Cobb, 1914) Taylor, 1936
Syn. *Iota octangulare* Cobb, 1914
Hoplolaimus octangulare (Cobb, 1914) Menzel, 1917.

Annules 66; vulva at 10th annule from terminus; stylet 92 microns ....
Criconema zernovi (Kirjanova, 1948), n. comb.
Syn.—*Ogma zernovi* Kirjanova, 1948
Type habitat.—Soil under grassy cover
Type locality.—Summit of Mt. Ai-Petri, near Yalta.

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Species and Prevalence of Parasites in the Blood of the American Magpie (*Pica pica hudsoni* (Sabine)) in Northern Colorado

ARTHUR F. HAGEN AND O. WILFORD OLSEN
Colorado A & M College

Studies on blood parasites of magpies in this country are limited. Coatney and Roudabush (1937, Amer. Midl. Nat. 18: 1005-1030) reported on one adult bird from Nebraska, Coatney and Jellison (1940, Jour. of Parasitol. 26: 158-160) on seven adults, two immatures and six fledglings from Montana, and Wagner (1946, Bird Banding 17: 72-74) on 22 adults, five immatures and 30 fledglings from Washington.

This paper represents a study of blood smears from 200 magpies (126 adults, 56 immatures and 18 nestlings) from northern Colorado from February to September, 1955. Four genera of protozoan parasites and an unidentified microfilaria were found. The protozoa were identified tentatively as *Haemoproteus picae* Coatney and Roudabush, 1937, *Leucocytozoon berestneffi* Sambon, 1908, *Trypanosoma avium* Lavernan, 1903, and *Plasmodium* sp., which probably included *P. cathemerium* Hartman, 1927, *P. relictum* Grassi and Felette, 1891, and *P. hexamerium* Huff, 1935, (Huff,

Table 1. Prevalence of blood parasites in magpies in northern Colorado

Age	Birds: Ex-amined	In-fected	Number of birds infected with: Haemo-proteus	Leucocy-tozoon	Plas-modium	Trypa-nosoma	Micro-filaria
Adult	126	102	25	77	0	26	55
Immature	56	52	34	39	9	10	7
Nestling	18	14	13	7	0	2	0
Total	200	168	72	123	9	38	62

C. G., 1956, personal communication). Positive identification of *Plasmodium* was not attempted in the absence of gametocytes in the smears. The prevalence of infection with these parasites is given in Table I.

Leucocytozoon berestneffi, Haemoproteus picae, Plasmodium cathemerium, and microfilaria were reported from Nebraska (Coatney and Roudabush), *Leucocytozoon berestneffi, Haemoproteus picae, Plasmodium* sp. and microfilaria from Montana (Coatney and Jellison), and *Leucocytozoon berestneffi, Haemoproteus picae, Trypanosoma avium,* and microfilaria from Washington (Wagner). All of these were found in the magpies observed in this study.

Two New Freeliving Nematodes, Found in the Rain-water Reserve of *Quesnelia arvensis* (Vell.) Mez. (Bromeliaceae) from Brazil

A. H. MEYL
Zoological Institute of the Technical High School of Braunschweig, Germany

The two new nematodes described in this paper were collected by Dr. S. A. Gerlach during his one year's stay in Brazil which was made possible by an invitation of the University of São Paulo and by the financial support of the "Deutsche Forschungsgemeinschaft." My thanks are therefore due to the finder who left me three samples of nematodes isolated from the water reserve of *Quesnelia arvensis,* which collects the rain by the tight, funnel-like folding-up of the leaves. The animals were isolated by cutting off the whole plant just above the soil level and by emptying the water contents into a fine sieve (to retain the humus-particles), from which then the nematodes were rinsed off and fixed in formalin.

The nematodes found are especially remarkable since the samples have been taken from the same Bromelians which were far off from each other and because all samples contained without exception the same two characteristic nematode species.

Sample A: Plants from Cananéia, Rio Perequê, on beach sand above the tide-line, near Mangrove-vegetation.

Butlerius gerlachi: 4 ♀, 2 ♂
Dorylaimus lordelloi: 18 ♀, 3 ♂, 15 juv.

Sample B: Plants from Cananéia, Rio Nohrega, locality similar to "A"

Butlerius gerlachi: 10 ♀
Dorylaimus lordelloi: 2 ♀, 2 ♂

Sample C: *Itanhaen,* 150 km. from Cananéia, plants growing on sandy dunes, not very distant from the beach (Fig. 1).

Butlerius gerlachi: 8 ♀, 1 ♂
Dorylaimus lordelloi: 6 ♀, 1 ♂, 1 juv.

Butlerius gerlachi, n. sp. (Figs. 2A, 2B)

FEMALE: L = 1.00-1.14 mm. a = 42.0-47.7 b = 4.4-4.9 c = 5.9-7.6 V = 50.5-53.8% (n = 4)

HOLOTYPE—Female, L = 1.12 mm. a = 47.7 b = 4.9 c = 6.6 V = 53.0% G_1 = 18.0% G_2 = 14%, as No. 5601 in the nematode collection of the "Naturhistorisches Museum, Braunschweig."

TYPE LOCALITY: See sample "C."

MALE: L = 0.89-1.00 mm. a = 43.0-48.1 b = 4.1-4.4 c = 9.6-9.7 (n = 2).

HOLOTYPE: Male, L = 1.00 mm. a = 48.1 b = 4.4 c = 9.7, as No. 5602 in the nematode collection of the "Naturhistorische Museum, Braunschweig."

TYPE LOCALITY: See sample "C."

DESCRIPTION: Body tapering moderately in front, the lip width being half the diameter of the body at the end of the esophagus. Tails of both sexes filiform. Cuticle finely annulated and with ten longitudinal striae. Head not offset, flattened in front with six lips bearing six papillae with very short setae. There seem to be lateral organs (amphids) with oval openings at level of the beginning of the second mouth cavity. They could be located on only one of the females by use of the highest oil immersion objective. Mouth cavity consisting of three sections, the walls of which are well cuticularized. The foremost cavity is separated from the following one by a ringlike cuticular structure. The almost equally large second cavity is characterized by the prominent dorsal tooth and the smaller ventral one which arises a little posterior to it. The third cavity,—surrounded by the somewhat swollen esophagus-musculature,—is narrowed and possesses well cuticularized walls leading into the lumen of the esophagus. The latter is broad and almost

Fig. 1. Habitat-picture of the new nematodes found with several plants of *Quesnelia arvensis.* (Photo taken by Dr. S. A. Gerlach at location of sample "C")

uniform in diameter anterior to the distinctly developed median bulb. The nerve ring surrounds the isthmus, and only one body width behind the nerve ring the excretory pore is located ventrally. The posterior part of the esophagus is more weak in musculature than the precorpus. The small cardia differs from the esophagus in that the tissue appears to be more glandular. The female gonads are almost symmetrical, the reflexed ovaries reaching about halfway to vulva. Eggs are three times the body width long and ¾ as broad. Only one egg at a time was seen in each uterus. A distinct prerectum was not observed. The rectum is two anal body-diameters long. Female tail quickly and uniformly tapering to the threadlike and sharp end. Male testis single, reflexed anteriorly for a distance of about 1½ body width. Spicules with knobbed heads, followed by an expanded section which tapers to sharp tips. These tips glide in a cuff, formed by the anal end of the gubernaculum which is rather big and, proximally, blade-like elaborated. There are four pairs of preanal setose papillae, the first pair one and a half spicula lengths preanal, the second and third one near the anal opening, the anterior being larger, all are located subventral. The fourth lateral pair almost at level of anus. In addition there are seven pairs of postanal papillae in the following arrangement: one subventral just behind the anus; two located laterally about a spiculum-length behind anus; a group of three very small and setose papillae situated subventrally, about two spiculum-lengths behind anus; and, at the same level of the latter group, a subdorsal papilla.

Food Habits: Unknown. Probably feeds on protozoa.

Diagnosis: Species of the genus *Butlerius* Goodey 1929, differing from the next related form, *B. butleri* Goodey, 1929, in (1) being more slender (a = 42-48 against 18-23), (2) cuticle with longitudinal striations, (3) Vulva in the middle of the body (against V = 40%) (4) tails of both sexes shorter (c = 6-9.7 against 3.3-5.9), (5) Eggs longer, (6) shape of gubernaculum as illustrated. The new species differs from *B. brevispiculatus* Schuurmans Stekhoven & Teunissen, 1938, in (1) the shape of the esophagus and (2) in the form of the spicular apparatus. Differs from *B. filicaudatus* Adam, 1930: No paired cephalic setae and different shape of spicules and gubernaculum. It differs from *B. okai* Rahm, 1938, (which doubtfully belongs to this genus) among other differences in the lack of a gubernaculum in that species.

The specific name is given in honor of the finder Dr. Sebastian Adam Gerlach, Kiel, Germany.

Dorylaimus lordelloi, n. sp. (Figs. 2C, 2D, 2E)

Female: L = 2.89-3.22 mm. a = 71.0-92.5 b = 5.3-6.2 c = 26.1-31.0 V = 46.2-47.5% (n = 4)

Holotype: Female, L = 3.16 mm. a = 81.0 b = 5.7 c = 28.6 V = 46.7%, as No. 5603 in the nematode collection of the "Naturhistorisches Museum, Braunschweig," Germany.

Type Locality: See sample "C."

Male: L = 2.52-2.70 mm. a = 80.0-94.5 b = 5.0-5.4 c = 91.8-109.5 with 7-9 supplements and 5-6 subventral papillae.

Holotype: Male, L = 2.52 mm. a = 80.0 b = 5.0 c = 102, 9 supplements, 6 subventral papillae, as No. 5604 in the above mentioned collection.

Type Locality: See sample "B."

Description: Body very slender, threadlike, practically cylindrical. Lip width about ⅓ of the body diameter at level of cardia. Cuticle smooth, without longitudinal striations or annules. Lateral fields one fourth of the

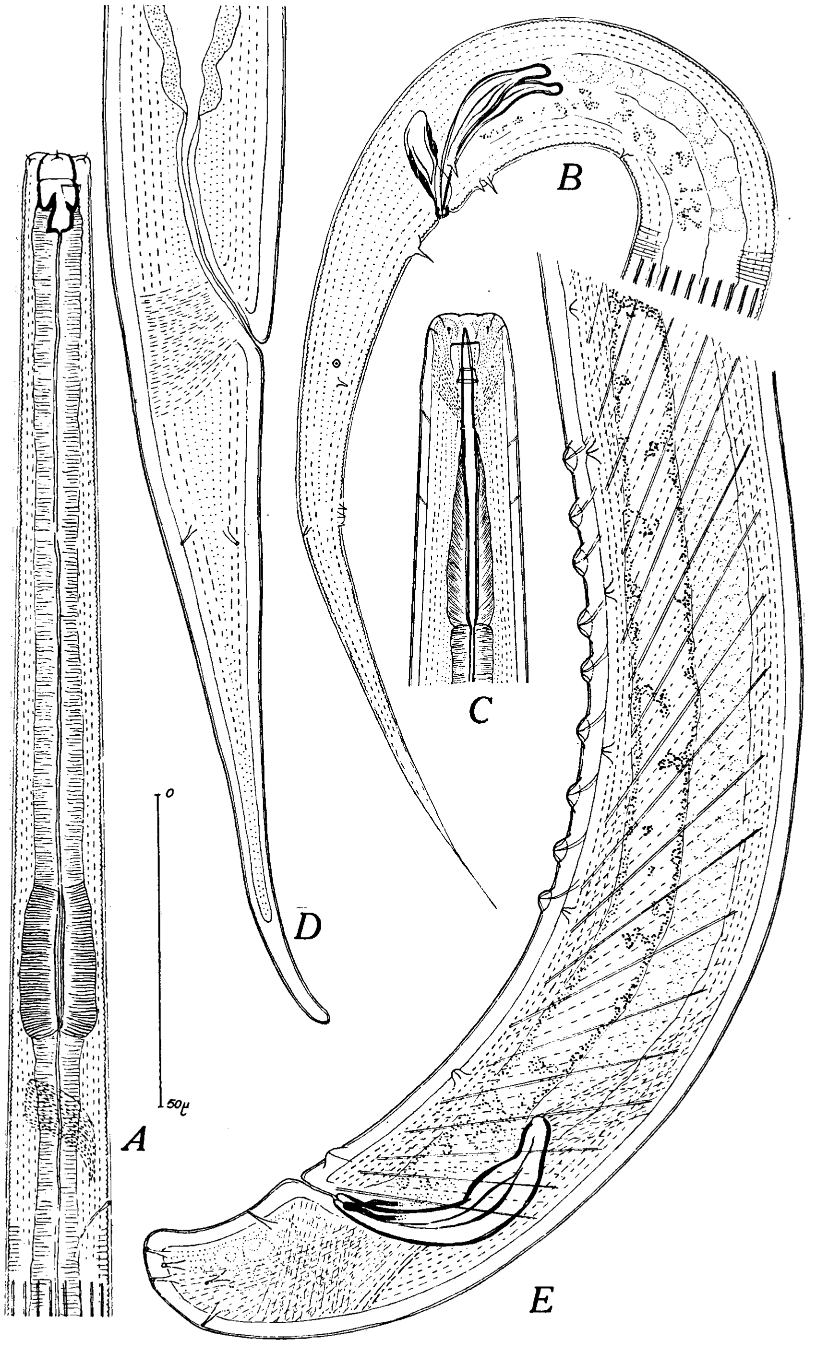

Fig. 2. *Butlerius gerlachi*, n. sp. A—Anterior portion and excretory pore. B—Tail and spicular apparatus of the male. *C-Dorylaimus lordelloi*, n. sp. Head end. D—Female tail. E—Copulatory apparatus and tail of the male. All figures drawn with the aid of the camera lucida.

body width. Lips continuous with neck contour with the usual two circlets of rather obscure papillae. Spear length 1¼, spear aperture 2/5 and spear width 1/6 of the width of lip region. Guiding ring double, weakly cuticularized. Amphids pouchlike, their slits one-third as wide as the corresponding head-width. Spear extensions moderately cuticularized, surrounded by the esophagus, the beginning of which has at level of extensions an obliquely striated musculture. Esophagus to 56-57% of its total length (from head-end) a moderately slender tube,—at 28-32% crossed by the nerve ring,—then gradually widening until twice its original width. Cardia conical, 1½ times as long as broad. The female with two almost symmetrical ovaries of 13-17% of total body length, which may be reflexed to as much as 9/10 of their length. Eggs (with females of more than 3 mm. body length): 124-150 by 34-38 μ, cylindrical in shape and bluntly rounded at the ends. Vulva transverse, well cuticularized, vagina reaching ⅔ of body width. All females contained sperms. Rectum 1¾ to 2 anal body diameters, prerectum 9 to 10 anal widths long. Anal musculature distinct. Female tail uniformly attenuated, the last 1/5 almost cylindrical with rounded end. Two papillae located at the end of the first third of the tail. Head and neck of the male are of the same structure as those of the female. Testes typical, beginning at 34% of body length from the head end. With 7 to 9 supplements (of 6 males 3 had nine, two seven and one 8 preanal supplements), which are not quite regularly spaced; mostly, however, spaced 1½ to 2 organ-widths from each other. The series begins two spicule lengths preanally. The usual adanal pair of papillae is present. The normal number of subventral papillae seems to be 6; two males showed only 5; the postanal papillae are obvious and typically located (see Fig. E). Spicula moderately bent and cuticularized, without a gubernaculum but with lateral guiding pieces, the anterior ends of which are narrowed. Tail of the male convex-conoid with somewhat subtruncated end.

FOOD HABITS: Probably feeds on cell contents of host plant.

DIAGNOSIS: Extremely slender Dorylaimus-species with the above mentioned characteristics. Most closely related to *D. intervallis* Thorne & Swanger, 1936, but differing in (1) being much more slender, and longer ($L = 2.5$-3 mm. against 1.3-1.6 mm. and $a = 71$-94 against $a = 40$) (2) lips continuous with neck contour (the species compared has lips set off by a slight depression), (3) spear 1¼ lip widths (against one lip width) long and 1/6 as broad (against about 1/5), (4) female prerectum 9-10 anal body diameters (against 4), (5) male with 7-9 supplements (against 11) and 5-6 subventral papillae (against 10).

The specific name is given in honor of Dr. Luiz Gonzaga E. Lordello, Piracicaba, State of São Paulo, Brazil.

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Studies on the Anthelmintic Value of 3,5-Dimethyl-4-Chlorophenol in Dogs

H. M. MARTIN

In screen-tests with a variety of compounds, several chemicals seemed promising as anthelmintics for removal of certain intestinal parasites of dogs. One of these, 3,5-dimethyl-4-chlorophenol, because of activity reported by the writer (Am. J. Vet. Res., 11:58-69, 1950), has been further studied as a vermifuge for dogs.

MATERIAL AND METHODS

The compound is a white, granular solid with a mild phenolic odor. It is non-irritating but has a slight anesthetic action when placed on the oral mucosa. Its melting point is 114-117° C, and it is insoluble in water.

The drug was administered in an unaltered state in hard gelatin capsules at various dose levels. The dogs used in the critical tests (Series 1) were fasted 18 to 24 hours before treatment and 2 to 6 hours after treatment. The dogs in Series 2 were fed as usual in the late afternoon preceding the morning when the compound was administered. In Series 3 the dogs were given milk before each treatment.

The 95 dogs used in these studies were of mixed breeds and varied in age from two months to slightly over a year. Animals used in critical tests were kept in individual cages. Feces were collected for two or more days preceding administration of the medicament to determine the extent of spontaneous loss of helminths and the kinds of helminths present in each animal. Feces were collected for at least three days following administration of the drug to recover all worms evacuated. The animals were necropsied and examined for parasites from three to six days after treatment.

The dogs employed in Series 2 and 3 were caged in groups. All feces were not examined for parasites following treatment. Subsequent to medication, feces from each group were examined for helminth eggs by flotation. Thirty of the dogs in Series 2 were necropsied within six months following treatment. Those in Series 3 were observed for nine to sixteen months, but they were then necropsied and examined for parasites.

All organs were examined for gross lesions and sections were prepared from the stomach, intestine, liver and kidneys of some of the dogs of Series 1 for histopathologic study. In Series 2 and 3, the above tissues, together with others were examined histopathologically.

RESULTS

SERIES 1. The compound was administered to 19 dogs in doses from 0.05 Gm. to 1.0 Gm. per pound of body weight. Each dog harbored one or more species of helminths at the time of treatment. Sixteen of the 19 dogs in this group harbored a total of 153 ascarids. One of 2 dogs that received 0.05 Gm. per pound of body weight of the compound passed all of its 4

From the School of Veterina·y Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
The investigations were supported by a grant from the Koppers Company, Inc., Pittsburgh, Pa. The Research Department of Koppers Company furnished the chemical compound used in the tests here repo ted. The author is indebted to Mr. H. G. Guy of Koppers Company for his cooperation and helpfulness in this work.

ascarids while the other evacuated only 6 (40%) of 15. The 14 dogs that received the medicament in larger doses (5 dogs, 0.1 Gm.; 3 dogs, 0.2-0.3 Gm.; 6 dogs, 0.5-1.0 Gm. per pound of body weight) expelled 134 (99%) of 135 ascarids. The animal retaining the single roundworm received a dose of 0.1 Gm. per pound.

The value of the compound for removal of hook worms (*A. caninum*) is limited since only 2 of 7 infected dogs passed hookworms following treatment. One dog harboring 2 hookworms passed both, the other expelled 53% of 390. Each of these was given 0.1 Gm per pound of body weight. The 5 remaining dogs, harboring only a few (1 or 2) *A. caninum,* failed to expel any hookworms after receiving doses up to 0.8 Gm. per pound.

Four dogs in this Series harbored several hundred whipworms. Two dogs with 54 and 115, respectively, failed to pass any of these worms following the administration of 0.05 Gm per pound of body weight of the compound. A third dog, infected with several hundred, passed only 16 after receiving 0.1 Gm. per pound of body weight. It appears that the compound is of little value against *Trichuris.*

The action of the chemical against cestodes was not determined since only one dog passed a few *Taenia* segments following treatment and no tapeworms were found at necropsy in any of the test animals.

SERIES 2. This group comprised 50 young, healthy dogs treated in groups of 3 to 5 animals each. Composite fecal samples were examined for parasitism and all specimens contained ascarid eggs. Each dog, remaining on normal diet and not fasted, was given 0.2 Gm. of the chemical per pound of body weight. There was no ill effect following the administration of the compound. Post-treatment examination of composite fecal specimens revealed ascarids of various sizes in all cages. The stools were later examined and found to be free of ascarid eggs.

The 30 dogs necropsied within a period of 6 months following treatment were found to be free of ascarids. The 20 remaining animals were not examined for parasites.

Since no controls were used and only a portion of the feces was collected, absence of ascarids at autopsy cannot be said to have been entirely due to the administration of the drug. However, the expulsion of ascarids immediately after treatment was presumably due to the action of the drug.

SERIES 3. Twenty-nine dogs were divided into groups of 3 to 5 per cage. Composite fecal samples were collected from all cages and in each instance the examinations revealed ascarid eggs. Each dog was given 0.1 Gm. of the chemical per pound of body weight. The feces from all cages contained ascarids following treatment. A month after treatment, feces showed ascarid eggs but in reduced numbers. The dogs were again treated, following a feeding of milk, but this time were given 0.5 Gm. per pound of body weight. Following this treatment, the feces contained adult ascarids. The feces collected several weeks later were found to be free of ascarid eggs. None of the dogs showed ill effects after the administration of the compound. All 29 dogs were necropsied within a period of nine to sixteen months following the last treatment and found to be free of ascarids.

Since controls were also omitted from this series, definite conclusions concerning the value of the compound cannot be drawn. However, the findings indicate that 0.1 Gm. per pound of body weight removed some ascarids and that 0.5 Gm., which was probably in excess of the dosage required, removed all ascarids without producing ill effects.

TOXICITY. The compound, 3,5-dimethyl-4-chlorophenol, when given to dogs *per os* in doses up to 1.0 Gm. per pound of body weight, seemed to have no toxic effect upon the animals. The dog given 1.0 Gm. per pound weighted 9.5 pounds and received a total of 9.5 Gms. of the compound within a four-hour period. Other dogs that received single doses up to 0.8 Gm. per pound of body weight showed no toxic reaction.

Necropsy examinations of 75 dogs sacrificed 2 days to 16 months after receiving the drug, were negative, i.e.; there was an absence of gross tissue changes in all instances.

Histopathologic studies were made on the liver and kidneys of 6 of the dogs used in the critical test series. The small intestine and pancreas were also studied in 4 of the animals. In Series 2, the liver, kidneys, bladder, stomach, small and large intestines, spleen, adrenals and thyroids of 14 of the dogs were examined histopathologically. The ovaries and uteri of 10 females and the testes and prostates of four males were likewise examined. In Series 3, the testes and prostates of eight males and the ovaries and uteri of nine females, and the brain, lungs, heart, liver, stomach, small and large intestines, adrenals and kidneys of all 17 were also studied. The results of these studies were all negative except for a few non-specific changes which were unrelated to the administration of the compound.

CONCLUSIONS

In anthelmintic tests with 3,5-dimethyl-4-chlorophenol, 143 (93%) of 153 ascarids were expelled from 16 dogs which were fasted 18 to 24 hours before treatment. The 10 ascarids remaining at autopsy were found in two dogs. The dogs receiving 0.1 Gm. or more per pound of body weight passed all but one of the 134 ascarids present at the time of treatment.

Apparently all of 50 dogs eliminated their ascarids after receiving a single dose of 0.2 Gm. per pound of body weight after "over-night" fasting; worms were found in the feces and later examination of the stools were negative. Thirty of these dogs were necropsied and found free of helminths.

The dogs of another trial failed to pass all their ascarids when given 0.1 Gm. per pound of body weight immediately after a meal of milk but eliminated the remaining ones after receiving a dose of 0.5 Gm. after a meal of milk.

The data suggest that the compound is effective for ascarids when given either to fasted or unfasted dogs.

From one dog infected with 390 hookworms, 53% were expelled following administration of 0.1 Gm. per pound of body weight.

The medicament seemed to have no action on *Trichuris*.

There was no evidence that the compound is toxic when given *per os* in the dosages employed.

Effect of Mycothricin Complex on the Nematode, *Rhabditis briggsae**

JACK D. TINER AND G. RANGASWAMI

Few antibiotics are known to possess selective toxicity for nematodes. Briggs (1950) tested penicillin, streptomycin, and streptolin against eggs, larvae, and gravid females of *Rhabditis briggsae (Caenorhabditis briggsae)* Dougherty, a saprophytic nematode. Of the three antibiotics tested, streptolin was comparatively more effective, the threshold concentration for inhibition of motility of larvae and acute toxic level respectively being 180 and 40,000 units/ml. Levine (1953) found that among the several antibiotics tested only chlortetracycline at a concentration of 0.1 per cent prevented the development of horse strongyle larvae. Levine *et al.* (1956) found that filipin had no effect on strongyle larvae, even at a concentration of 0.4 per cent.

Two mycothricin complexes, related to the streptothricin group of antibiotics, were recently isolated in this laboratory (Rangaswami *et al.* 1956). They have wide antimicrobial spectra, and it was thought desirable to evaluate their action on nematodes.

MATERIALS AND METHODS

Two preparations of mycothricin, complex A and complex B, each of 400 units/mg, obtained from strains of the *Streptomyces lavendulae* group, were used in this study. The nematodes were derived from cultures of *Rhabditis briggsae,* kindly provided by Dr. E. C. Dougherty, Department of Physiology, School of Medicine, University of California. The organism was maintained on nutrient agar medium together with cells of *Escherichia coli.* After five days of growth the culture was suspended in sterile phosphate buffer at pH 6.0 and diluted to contain a total of 30 eggs, larvae, and adults per 1/10 ml. The antibiotic was included in the buffer solution and additional *E. coli* cells were added to nourish the nematodes.

RESULTS

Data obtained from the tests are summarized in Table I. Both the mycothricin complexes are inhibitory to the growth of larvae at 100 and 10 mcg/ml.** There was inhibition of growth and motility in the early stages resulting in the death of the nematodes within five days. Two-fold or better population increases occurred in each of the dishes treated with 1 mcg/ml mycothricin or with streptomycin, and in the untreated controls. Two related antibiotics, streptothricin and pleocidin, were obtained through the courtesy of Dr. Carl P. Schaffner and tested for activity against *R. briggsae.* Both the substances were found to inhibit the nematode at 100 and 10 mcg/ml. Lower concentrations of these last mentioned antibiotics have not yet been tested.

*From the Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, N. J., U.S.A.
Work supported in part by a research grant from Merck Co. Inc.
**Micrograms per milliliter.

TABLE 1.—Action of mycothricin complex on *Rhabditis briggsae*. Number of larvae and adults developing from an inoculum of about thirty eggs, larvae, and adults per dish.

Antibiotic concentration	Average No. of worms per dish 2 days	5 days
Mycothricin complex A:		
100 mcg/ml	7	0
10 mcg/ml	31	1
1 mcg/ml	38	90
Mycothricin complex B:		
100 mcg/ml	1	0
10 mcg/ml	21	1
1 mcg/ml	24	55
Streptomycin sulfate:		
100 mcg/ml	22	92
10 mcg/ml	38	81
1 mcg/ml	28	100
Untreated controls	23 - 46	100

DISCUSSION

The inhibitory concentration of the two mycothricin complexes is comparatively much lower than that of streptolin and chlortetracycline, which were previously reported to possess some effect on nematodes. This may be due to the techniques used. Brigg's study was concerned with the threshold and acute toxic levels of the antibiotics on free-living nematodes in the absence of food material. Levine used growing larvae of parasitic nematodes, but his system was relatively complex. It consisted of a number of species of strongyles growing in horse manure. The present study was designed to measure the inhibitory effect of chemicals on growth and reproduction of a single nematode species feeding on *E. coli* cells. The antibiotic preparations used in these studies were only partially purified. It is hoped that the anti-nematode potency of the substances can be increased by further purification.

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