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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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The Axenic Cultivation of *Rhabditis briggsae* Dougherty and Nigon, 1949 (Nematoda: Rhabditidae). I. Experiments with Chick Embryo Juice and Chemically Defined Media

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INTRODUCTION

In a recent paper (Dougherty and Calhoun, 1948) studies on the cultivation of the nematode species *Rhabditis pellio* and related soil-dwelling rhabditoids have been discussed in some detail, and a general historical survey of nematode culturing techniques has been presented. Subsequently to the writing of this paper the senior author spent a period of collaborative research with Dr. Victor Nigon at the laboratory of the Travaux Pratiques de Biologie Animale, Faculté des Sciences P.C.B., Paris (Ve); and, largely as a result of experiments conducted there, it became evident that certain hermaphroditic species of the family Rhabditidae and genus *Rhabditis* (*sensu lato*) offer practical as well as theoretical advantages over a dioecious species such as *R. pellio* in general nutritional studies and in any contemplated program of investigations on the genetics of nematodes. One important practical reason is that inbreeding in dioecious nematodes results in a marked decrease in fertility within as few as three generations (Nigon, 1949b; Dougherty, unpublished observations on *R. pellio*). On the other hand, in the case of self-fertilizing hermaphrodites, genes leading to infertility and possibly present in the dioecious ancestry of such forms must, of necessity, have been lost in order for this type of monoeciousness to be successfully established. Therefore, it is possible to deal with consistently small populations of such species as *Rhabditis elegans* Maupas, 1900, *R. briggsae* Dougherty and Nigon, 1949, etc., without having to concern oneself with problems of infertility. Moreover, it is a considerable convenience to be able to use only one larva in initiating new cultures.

In the course of their work Nigon and Dougherty (in press) were able to isolate the first morphological mutant in the Nematoda and to study the inheritance of the mutation. It was discovered that the species in which this mutant appeared and which was identified as *R. elegans* in papers by Dougherty and Calhoun (1948)

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and Nigon (1949a, b) actually represented a closely related, new species, which Dougherty and Nigon (1949) have named *Rhabditis briggsae* (see also Nigon and Dougherty, 1949). This species is easily cultivable and of rather good fertility. It consists largely of self-fertilizing hermaphrodites and thus has promising genetic features (see Dougherty, 1949; Nigon and Dougherty, in press). Accordingly it has been used in the investigations reported here. A brief note (Dougherty, 1950) has already reported that it is indefinitely cultivable under *axenic* conditions (*i.e.*, in the absence of other living organisms) on sterile pieces of chick embryo.

The ultimate aim of these studies is to devise a medium for the cultivation of *R. briggsae* and other rhabditoid nematodes that will consist only of known, relatively simple chemical substances, *i.e.*, amino acids, vitamins and growth factors, purine and pyrimidine bases (or derivatives thereof), sugars, etc. In the entire animal kingdom (if one ignores the plant-like flagellates) species of only one genus have been cultivated indefinitely on a medium in which all but one factor is chemically known—ciliates belonging to *Tetrahymena* (see Kidder and Dewey, 1949a-c; Kidder *et al.*, 1949, for the most recent of their many papers on the requirements of these forms); in this case the chemical structure of one growth factor, protogen, has not yet been described, although the substance has been considerably purified (see Stokstad *et al.*, 1949). Similar work on the fruitfly *Drosophila melanogaster* (see Wagner and Mitchell, 1948; Begg, 1949) and on larvae of the mosquito, *Aedes aegypti* (see Trager, 1948; Golberg and DeMeillon, 1948a, b), has progressed somewhat less far. A recent, preliminary note by Stoll (1948) suggests that much of the nutritional picture of the rhabditoid nematode, *Neoaplectana glaseri* (family Steinernematidae), may soon be revealed; he reports a measure of success in growing this species on defined media, but has not yet described their exact constitution.

The rhabditid nematodes appear to present certain difficulties not encountered with the species mentioned in the foregoing paragraph, all of which can be grown on autoclaved media. Dougherty and Calhoun (1948) were able to show that *R. pellio* requires a heat-labile factor (or factors) that can be provided in unheated, Seitz-filtered, aqueous liver extract. Our experience with *R. briggsae* has been consistent with the picture of one or more heat-labile requirements by the rhabditids; whatever these factors may be, they are admirably provided for by chick embryo tissue (Dougherty, 1950) or by juice prepared by the sterile pressing of chick embryos and supplemented with known biochemicals (the present studies).

With continued success in the axenic cultivation of *R. briggsae* on pieces of chick embryo it seemed reasonable to replace a part of this highly complex food with known substances—in other words, to reduce, if possible, the proportion of chick embryo substance to trace amounts. Moreover, it seems worthwhile to make an attempt to substitute an entirely synthetic medium; the possibility existed, we felt—although it seemed unlikely—that the heat-labile needs of the rhabditids, when grown on complex organic media, were due to the presence of toxic or inhibitory substances, which could be detoxified, or of which the action could be reversed, by the presence of the heat-labile factor or factors. The experiments described herein were designed with these ends in mind. A brief note on some of the results reported here has already appeared (Dougherty, Raphael, and Alton, 1949).

MATERIALS AND METHODS

A. Use of Cultures

Most of our experiments have been carried out with larvae from two-membered cultures of *R. briggsae* and the bacterium, *Escherichia coli;* in a single experiment larvae from an axenic culture of *R. briggsae* on chick embryo was used. In general it appears preferable to use larvae from the former source because of

the fact that the latter cultures are more difficult and, in particular, more expensive to maintain in such a way that tiny larvae will be available on demand; moreover, in the one case in which larvae from an axenically reared culture were used, certain anomalous results were obtained, which are discussed under "Miscellaneous Experiments."

The production of sterile larvae from two-membered (N) cultures has been described by Dougherty and Calhoun (1948). The rearing of axenic larvae on pieces of chick embryo (A-cultures) has been discussed by Dougherty (1950).

B. Preparation of Media

Experiments with two chemically defined basal media, which may be termed Media I and II, have been carried out (and in addition a few preliminary experiments with media similar to I); the growth of *R. briggsae* larvae on these alone and with the addition of chick embryo juice (CEJ) in various proportions has been studied. The media have been based, more or less arbitrarily, largely on those used by Kidder and associates (Kidder and Dewey, 1949a–c; Kidder *et al.*, 1949) and by Trager (1948) for *Tetrahymena* and *Aedes* respectively. Medium I, that of Kidder and Dewey (1949a), Medium II, and that of Trager are presented in Table I. For further remarks on the relationship of these media, see "Discussion."

Media I and II have been prepared as indicated in this and the following paragraphs. In both cases sufficient quantities of the amino acids were weighed out to make up one liter of final medium. To the pooled dried powders in a 250 ml. volumetric flask were added 4 ml. of 0.1 N HCl and 246 ml. of distilled water. In the case of Medium II neutrality was readjusted, after all amino acids had dissolved, merely by adding 0.1 N KOH. This resulted in deviations from the salt values used by Kidder and Dewey, as indicated in Table I. In the preparation of Medium I, however, this discrepancy was avoided by neutralizing the amino acid solution with 11 mg. $Ca(OH)_2 \cdot 2H_2O$, a Ca^{++} and Cl^- equivalence of 26 mg. of $CaCl_2 \cdot 2H_2O$ in solution thus resulting; and the later addition of 24 mg. of this salt to the mixture produced the total amount thereof required by the Kidder-Dewey formula. To the amino acid solutions in the case both of Medium I and Medium II was then added 0.1 ml. of protogen concentrate[4] (= 0.5 mg. of the substance, or 1000 units) and 2 ml. of a 50% ethanol solution of the methyl ester of biotin (= 25γ/ml.).

The preparation of growth factors (other than protogen and biotin) in the two media differed somewhat. In the case of Medium I all were weighed out in amounts for 10 liters of final medium and dissolved in 100 ml. of distilled water. With Medium II, however, enough growth factors (except cholesterol) for one liter of final medium were weighed out and dissolved in 100 ml. of water. This mixture was sterilized by Seitz filtration to avoid destruction of the heat-labile ascorbic acid and glutathione. The cholesterol was dissolved in a few milliliters of ethanol and put into the protogen-biotin-amino acid mixture along with the other ingredients (except the growth factors) as will be described shortly.

The nucleotides and related compounds were weighed out in amounts sufficient for 1 liter of medium in the case of both media and dissolved in 100 ml. of water.

The salts were weighed out similarly (except for the $CaCl_2 \cdot 2H_2O$) and also dissolved in 100 ml. of water. The $CaCl_2 \cdot 2H_2O$ was weighed separately and added to the final medium as indicated in the recipes for final compounding in the next two paragraphs. The other ingredients—dextrose, acetate, and tween—were similarly weighed out in appropriate amounts.

[4] See Table 1, footnote f.

TABLE 1.—*Basal media as used in the present investigations for* R. BRIGGSAE *and by Kidder and Dewey (1949a) for* TETRAHYMENA *and Trager (1948) for* AEDES. *(All values are in γ/ml. of final medium.)*

	Medium I	Kidder & Dewey Medium[a]	Medium II	Trager Medium
Amino acids[b, c]				
L-Arginine HCl	163	163[a]	163	.....
L-Histidine HCl	66	66[a]	66	.....
DL-Isoleucine	213	213[a]	213	.....
L-Leucine	247	247[a]	247	.....
L-Lysine HCl	196	196[a]	196	.....
DL-Methionine	214	214[a]	214	.....
DL-Phenylalanine			300	
L-Phenylalanine	110	110[a]		.....
DL-Threonine	238	238[a]	238	.....
L-Tryptophane	60	60[a]	60	.....
DL-Valine	96	96[a]	96	.....
DL-Serine	317	317[a]	317	.....
L-Glutamic acid	233	233	233	.....
L-Aspartic acid	61	61	61	.....
Glycine	5	5	5	.....
DL-Alanine	55	55	55	.....
L-Proline	175	175	175	.....
L-Hydroxyproline	75	75	100[d]	.....
L-Tyrosine	67	67	67	.....
L-Cysteine	3.5	3.5	3.5	.....
L-Cystine				200
Growth factors				
Ca pantothenate	1.0	0.1[a]	6.0	6.0
Nicotinamide	1.0	0.1[a]	10.0	10.0
Nicotinic acid			2.0	2.0
Pyridoxine HCl	1.0	2.0[a] (1.0[e])	4.0	4.0
Pyridoxal HCl	0.1	(0.1[e])	0.02	
Pyridoxamine 2HCl	0.1	(0.1[e])	0.02	0.02
Riboflavin	1.0	0.1[a]	2.0	2.0
Pteroylglutamic acid	0.1	0.01[a]	0.6	0.6
Biotin (methyl ester)	0.05	0.0005	0.05	0.05
Thiamine HCl	1.0	1.0[a](?)	3.5	2.0
i-Inositol			40.0	40.0
p-Aminobenzoic acid			2.0	2.0
Ascorbic acid			3.0	
Glutathione			10.0	10.0
Choline chloride	1.0	1.0	22.0	20.0
Cholesterol			31.0	30.0
Protogen[f]	0.5	0.375[a]	0.5	
Nucleotides or their bases[g, h]				
Adenylic acid		(20)	50	.....
Guanylic acid		(30)	50	.....
Cytidylic acid	25	(25)	50	.....
Uracil	10	(10)	50	.....
Thymine	50		50	
Yeast nucleic acid (hydrolyzed)		100[a, g]		
Carbon sources				
Dextrose	1000	1000	1000	
Sodium acetate	1000	1000	1000	
Sucrose				2000

Salts				
$MgSO_4 \cdot 7H_2O$	100	100[a]	100	200
K_2HPO_4	100	100[a]	100	600
KH_2PO_4				600
$CaCl_2 \cdot 2H_2O$	50	50[a]	50	
$CaCl_2$				12
$Fe(NH_4)_2$ $(SO_4)_2 \cdot 6H_2O$	25	25[a]	25	
$FeSO_4 \cdot 7H_2O$				12
$CuCl_2 \cdot 2H_2O$	5	5[a]	5	
$FeCl_3 \cdot 6H_2O$	1.25	1.25[a]	1.25	
$MnCl_2 \cdot 4H_2O$	0.05	0.05	0.05	
$MnSO_4 \cdot 4H_2O$				12
$ZnCl_2$	0.05	0.05	0.05	
NaCl				12
KCl			15[i]	
Detergent				
Tween 85	500	500	500	

[a] Essential nutrients or nutrilites for *Tetrahymena;* those not marked in this column by a, e, or g are generally stimulatory, but never essential.

[b] Kidder and Dewey in later papers (1949b, c) have used lower levels of amino acids than those in column two.

[c] Trager (1948) used purified "vitamin-free" casein (30–40 mg. per tube) in his medium to supply amino acid requirements (except for addition of cystine.).

[d] Used inadvertently for 75.

[e] Values as used by Kidder *et al.* (1949) in their most recent paper.

[f] Protogen concentrate very kindly supplied by Dr. E. L. R. Stokstad, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

[g] In more recent papers Kidder and Dewey have replaced hydrolyzed yeast nucleic acid with purine and pyrimidine bases or derivatives thereof. Of these, guanine (or its nucleoside or nucleotide) is essential; and either uracil or cytidylic acid (but not cytosine) can supply the essential pyrimidine requirement. Values in parentheses are those used in Kidder *et al.*'s most recent paper (1949).

[h] Trager used yeast nucleic acid (unhydrolyzed).

[i] Present because of excess HCl and KOH; see text.

A final compounding of Medium I was in the following proportion:

Protogen-biotin-amino acid stock	250 ml. (full amount)
Growth factor stock	10 ml. (out of a total of 100)
Nucleotide (etc.) stock	100 ml. (full amount)
Salt stock	100 ml. (full amount)
$CaCl_2 \cdot 2H_2O$	24 mg.
Dextrose	1.0 gm.
Acetate	1.0 gm.
Tween	0.5 gm.
Water	40 ml.
Total volume	500 ml.

The foregoing mixture was autoclaved and stored in a frozen condition, constituting a stock 2X the concentration of the final basal medium.

The final compounding of Medium II was in the following proportions:

Protogen-biotin-cholesterol-amino acid stock	250 ml.
Nucleotide (etc.) stock	100 ml.
Salt stock	100 ml.
$CaCl_2 \cdot 2H_2O$	50 mg.

Dextrose	1.0 gm.
Acetate	1.0 gm.
Tween	0.5 gm.
Water	50 ml.

The foregoing mixture was autoclaved and to it was added, with precautions to ensure sterility:

Growth factor stock	100 ml.
Total volume	600 ml.

The total mixture was stored in a frozen state, constituting a stock 5/3 × the concentration of the final basal medium.

The chick embryo juice[5] (CEJ) has been routinely prepared in the following manner: 20–30 embryos are removed from their shells sterilely and put in a sterile press; the pulp is collected in 40 ml. centrifuge tubes, with 10 cc. of pulp allowed to each tube, 10 ml. of Tyrode's solution are added to each, and the mixture stirred, allowed to extract for at least 30 minutes in the refrigerator, and then centrifuged for 20 minutes at 2500–3000 r.p.m.; the supernatant is poured into sterile flasks and stored in the frozen state.

C. Experimental Equipment and Procedure

All experiments reported here were carried out in *Neurospora* "growth tubes," 15 cm. long at the base and 1.5 cm. in diameter. These tubes were plugged at both ends with cotton, placed on a specially constructed rack accommodating 12 tubes, and autoclaved. Basal media and CEJ, water, or other ingredients were added in proportions to make, as a rule, 2 ml. of final medium per tube. Larvae were sterilized from two-membered cultures with streptomycin, penicillin also being included to safeguard against chance contamination, and transferred with capillary micropipettes to the experimental tubes; small larvae (of the 1st and 2nd instar) were used, approximately 50 worms per tube.

The cultures were maintained at 18° C and observed each day. Visible evidence of maturation and reproduction was the only criterion of growth used. In these survey experiments no effort has been made to use more precise methods, such as the adenosine deaminase technique for measuring growth suggested by Dougherty and Mitchell (1948).

EXPERIMENTAL RESULTS

A. With Medium I

Three series of tubes were run using Medium I brought to final dilution with water and with different strengths of CEJ. In all cases streptomycin-sterilized larvae were used.

In the first series three tubes were set up with the stock basal medium diluted to final concentration (1: 1) with CEJ, and three with water. Within four days the worms in the former tubes had matured and laid eggs, and by eight days there was a massive culture in each, with many growing larvae of the II and III generations. In the control tubes without CEJ, however, there was little if any growth. The tubes were discarded after two weeks.

In a second series 22 tubes were set up, each containing 1.0 ml. of the stock basal medium, 0.8 ml. of CEJ, and 0.2 ml. of water (containing, for the most part, varying concentrations of "guanazolo"). In all these tubes the larvae were again mature in four days, and by eight days there were massive cultures in all 22.

[5] Very kindly made by Mrs. Elsa Zitcer, Department of Biochemistry, University of California, Berkeley.

In two control tubes containing only undiluted CEJ, growth of the larvae introduced occurred as rapidly as in the other tubes, but very few II generation larvae were produced. At eight days, when the experimental tubes had numerous larvae, the original worms in the two CEJ tubes were very large, but almost no increase in number had taken place; these tubes were thus in striking contrast to the other 22.

In a third series with Medium I (also involving an experiment with "guanazolo"—see "Discussion"), six tubes were set up with 1.0 ml. of stock basal medium, 0.1 ml. of CEJ, and 0.9 ml. of water (with "guanazolo" at two levels—4 tubes, and without "guanazolo"—2 tubes). In all six tubes there was some growth, but even after two weeks the larvae were little more than half-grown, and, when all tubes were discarded after one month, none had matured.

B. With Medium II

Two experiments were run with Medium II. In both cases streptomycin-sterilized larvae were used.

In the first series six tubes were set up with basal medium; in three the final dilution was achieved with CEJ (3: 2), and in three with water. In the first group maturation of larvae was rapid—taking 4 days, but no reproduction occurred, even after a week. In the second group little or no growth took place.

A second series with Medium II (actually done, in point of time, before the experiment already described) was designed to test the growth-promoting properties of Medium II alone and with various supplements. In each case 1.2 ml. of stock basal medium per tube were diluted with 0.8 ml. of supplement. The latter included: 1) Sørenson buffer (pH 7.2); 2) liver extract prepared as described by Dougherty and Calhoun (1948) except that Sørenson buffer was used in place of distilled water; and 3) filtered or centrifuged medium from a broth culture of an unidentified *Streptomyces* sp.[6] (see subsection C). The tubes were set up in triplicate, but all failed to show maturation of the worms after two weeks; in fact, little, if any, growth took place. A control tube, with larvae inoculated onto a sterile piece of chick embryo, showed a massive culture at this time. When, however, 1 ml. of CEJ was added to each of three tubes supplemented with *Streptomyces* medium, the larvae rapidly matured—in four days; but no reproduction took place. By seven days, when the tubes were discarded, the worms were very large.

C. Miscellaneous Experiments

Preliminary experiments to those already described deserve recording. These were carried out with a medium similar to I, but differing by the inadvertent use of certain of the growth factors at levels one-tenth as concentrated as in the Kidder-Dewey medium. No supplementation with CEJ was made, and with one exception negative results were obtained.

In the case of this exception, larvae from A-cultures were used and at a somewhat more advanced age (probably in the 3rd instar) than has generally been true with sterilized larvae from N-tubes. They were transferred directly from the chick embryo medium to sterile water in a Petri dish. In all cases, even with Sørenson buffer controls, maturation occurred in 7 days, and some II-generation larvae were produced. Moreover, in one tube (basal medium brought to final concentration with buffer) these II-generation larvae were numerous and proceeded to mature and produce young, a vigorous culture being obtained by the tenth day. At that time the spherical colony of a single contaminant was noted; upon subculture it proved to be an actinomycete—the species of *Streptomyces* already men-

[6] Generic determination kindly supplied by Professor Selman A. Waksman, Department of Microbiology, Rutgers University, New Brunswick, N. J.

tioned in subsection B. Exhaustive tests for other contaminants were negative. When an attempt to repeat the foregoing experiment was made with younger larvae sterilized from N-culture and introduced into the same medium with actinomycete colonies, maturation did not occur. Furthermore, as already described, experiments with Medium II were negative. This phenomenon has not yet been studied further.

DISCUSSION

Media I and II were devised for the work reported here for the reason that similar media had permitted the axenic cultivation of *Tetrahymena* and *Aedes* respectively. That for *Tetrahymena* has, in fact, permitted indefinite cultivations, although that for *Aedes* has only been used for rear single generations.

Medium I is similar to that of Kidder and Dewey (1949a), the most important exceptions being the higher levels (by tenfold) of calcium pantothenate, nicotinamide, riboflavin, and pteroylglutamic (= folic) acid and substitution of cytidylic acid, uracil, and thymine for ribosenucleic acid hydrolysate. The omission of purine bases or their derivatives from Medium I was due to the fact that it was originally designed to test the effect of "guanazolo" (= 5-amino-7-hydroxy-1-H-*v*-triazolo[d]pyrimidine) on the growth of *R. briggsae;* this compound has been found by Kidder and Dewey (1949d) to possess markedly inhibitory action of the growth of *Tetrahymena.* A full account of the experiments with "guanazolo" will be the subject of a separate communication.

Medium II was made up with the Trager medium (1948) for *Aedes* as a basis for the levels of vitamins and growth factors used and with the Kidder-Dewey medium for the other factors. For minor deviations see Table 1. In addition, however, two growth factors not present in the Trager medium—one present in, and one absent from, the Kidder-Dewey medium, were included, *i.e.,* protogen and ascorbic acid respectively. The general object was to include as many likely requirements in this medium as possible.

It seems evident from experiments with Media I and II that *R. briggsae* requires a factor (or factors) provided in CEJ and that on the basis of the present and past work (Briggs, MS; Dougherty and Calhoun, 1948) at least one such factor is heat-labile. It is therefore not identical with any of those present in the Kidder-Dewey medium for *Tetrahymena* or the Trager medium for *Aedes,* both of which are heat-sterilized and neither of which ordinarily support reproduction of the nematode. In the single experiment in which growth and reproduction occurred in an unsupplemented medium similar to I, the positive results followed the use of somewhat older larvae than usual, transferred from an axenic medium on chick embryo rather than sterilized from a two-membered culture with *E. coli.* The most likely explanation would seem to be that the older larvae used had stored up sufficient nutrients to permit maturation and reproduction even in buffer (!); quite possibly the small amounts of the chick embryo medium carried over as microcontaminants aided this phenomenon.

The most significant aspect of the results recorded here is that, for the first time, a rhabditid nematode has been grown as well axenically as would ordinarily be the case in the presence of other living organisms suitable as food; Medium I diluted from double to normal strength with equal volumes of CEJ has been the substrate permitting this to take place. It would therefore appear that in Medium I, which is very similar to that used for species of *Tetrahymena,* no strongly inhibitory nutrients occur and that it is suitable for maximal or near-maximal axenic growth of *R. briggsae* when properly supplemented.

On the other hand, it is strikingly evident that Medium II, which contains all the ingredients of I (although in most cases in higher concentrations as regards the growth factors) and, in addition, a number of other substances, has an inhibitory effect on some phase of reproduction. Thus, when supplemented with CEJ,

it permits rapid maturation of *R. briggsae,* but the adults so produced, which become very large, nevertheless do not produce viable II-generation larvae. Whether this is due to the toxicity of some substance or substances not present in Medium I, *i.e.,* of nicotinic acid, *i*-inositol, *p*-aminobenzoic acid, ascorbic acid, glutathione, or cholesterol, or to the toxic effect of the higher concentration of some ingredient or ingredients also present in Medium I, cannot at this time be said.

The fact that equal parts of CEJ and double strength Medium I permit the establishment of excellent cultures of *R. briggsae,* whereas, if the CEJ is reduced in amount to one part in 10 of the final medium, but little growth is supported, shows that the essential ingredient or ingredients in CEJ are either required in high concentrations, or, if potent growth-promoters as trace substances, are already very dilute therein.

It is curious, in view of the fact that Dougherty and Calhoun (1948) were able to get maturation and reproduction of *R. briggsae* (called by them "*R. elegans*") in a complex medium supplemented with Seitz-sterilized aqueous liver extract, that the same substance did not support appreciable growth when used to supplement Medium II. This matter bears further investigation.

The results reported herein obviously represent only a beginning in the determination of the basic nutritional requirements of *R. briggsae.* Medium I supplemented with CEJ does, however, present an excellent basis for further work. Initially the primary problem is to attempt to fractionate the CEJ and determine the general properties of the essential factor(s) contained therein. It would not seem unlikely, if one analogizes this problem with that already studied to some extent in the ciliate genera, *Paramecium* and *Colpoda,* in which certain species, at least, require one or more heat-labile factors—shown by Johnson and Tatum (1945) in the case of *P. multimicronucleatum* to be intimately associated with a protein or proteins and thus possibly to be the protein(s) themselves—that similar requirements may exist for rhabditid nematodes. This problem is now under investigation.

When the CEJ factor or factors are prepared in somewhat purified form, it should be possible to determine whether certain of the constituents of Medium I are essential to the growth of *R. briggsae.* It may also become practical to investigate further the *Streptomyces* factor that appeared highly stimulatory in the one experiment described.

SUMMARY

1. Experiments on the successful axenic cultivation of *Rhabditis briggsae* Dougherty and Nigon, 1949, are described.

2. When supplemented with chick embryo juice (CEJ), a medium (I) based on the Kidder-Dewey formula for ciliates of the genus *Tetrahymena* has been shown to permit axenic growth of *R. briggsae* comparing favorably with growth in the presence of living bacteria. This is the first such axenic medium for a rhabditid nematode.

3. A medium (II) based (for growth factors) on the Trager formula for mosquito larvae of the genus *Aedes* and (for other ingredients) on the Kidder-Dewey medium and similarly supplemented with CEJ permits rapid growth, but II-generation larvae are not produced. Some mechanism of inhibition of reproduction is obviously involved.

4. It is suggested that the essential factor or factors provided by CEJ may be protein in nature. This problem is now under investigation.

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Cacopaurus epacris, new species (Nematoda: Criconematidae), a Nematode Parasite of California Black Walnut Roots

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In April, 1949, the Tulare County Agricultural Extension Service and the Visalia Growers Association conducted a disease survey of 25 walnut orchards in

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Tulare County, California. One of the main objectives of the survey was to determine the distribution of the root-lesion nematode, *Pratylenchus* sp., in the walnut orchards of the county. Samples of roots and soil from trees in each orchard were submitted to Berkeley for nematode examination. A new species of nematode belonging to the genus *Cacopaurus* Thorne 1943 was found attacking the walnut roots submitted in one of the samples. The trees in this orchard were also infested with the root-lesion nematode. The presence of the two root parasites makes it impossible to estimate the damage that might be attributable to the new species. However, judging from the damage done to walnuts on English roots by *Cacopaurus pestis* Thorne 1943 (Fig. 1) it seems likely that this new species might con-

FIG. 1. Right, walnut tree, variety Mayette on English roots, showing injury associated with attack by *Cacopaurus pestis* Thorne 1943. Left tree shows little or no injury from attack. (Photograph 1949.)

tribute to the disease symptoms observed in the Tulare County orchard.

Numerous specimens of *C. epacris* were observed feeding on the roots of the infested walnut trees. Adult females and larvae were observed with their stylets imbedded in the root tissues. There appears to be a definite tendency, as has been observed with *C. pestis*, for the new species to occur in colonies on the roots. Eggs were observed associated with the debris usually present around the colonies. Males of the new species were obtained by washing infested soil through a series of graded screens and from scrapings made from the bark of infested roots. It is possible to observe this nematode feeding on the roots but diagnosis of the infestation is made more readily by examining root scrapings in water under a dissecting microscope. The females of this species have a tendency toward formation of the thickened yellowish cuticle that is so characteristic of the senile females of *C. pestis*. However, the cuticle must be less resistant to disintegration as we did not find the empty yellowish cuticles of *C. epacris* in either root scrapings or soil.

The discovery of a new species of *Cacopaurus* on the roots of California Black Walnut is especially interesting in view of the fact that Black Walnut roots appear to be immune to *C. pestis*. Thorne (1943) reported examining samples of *Juglans hindsii* Jepson seedling plants in areas where English walnuts had failed because

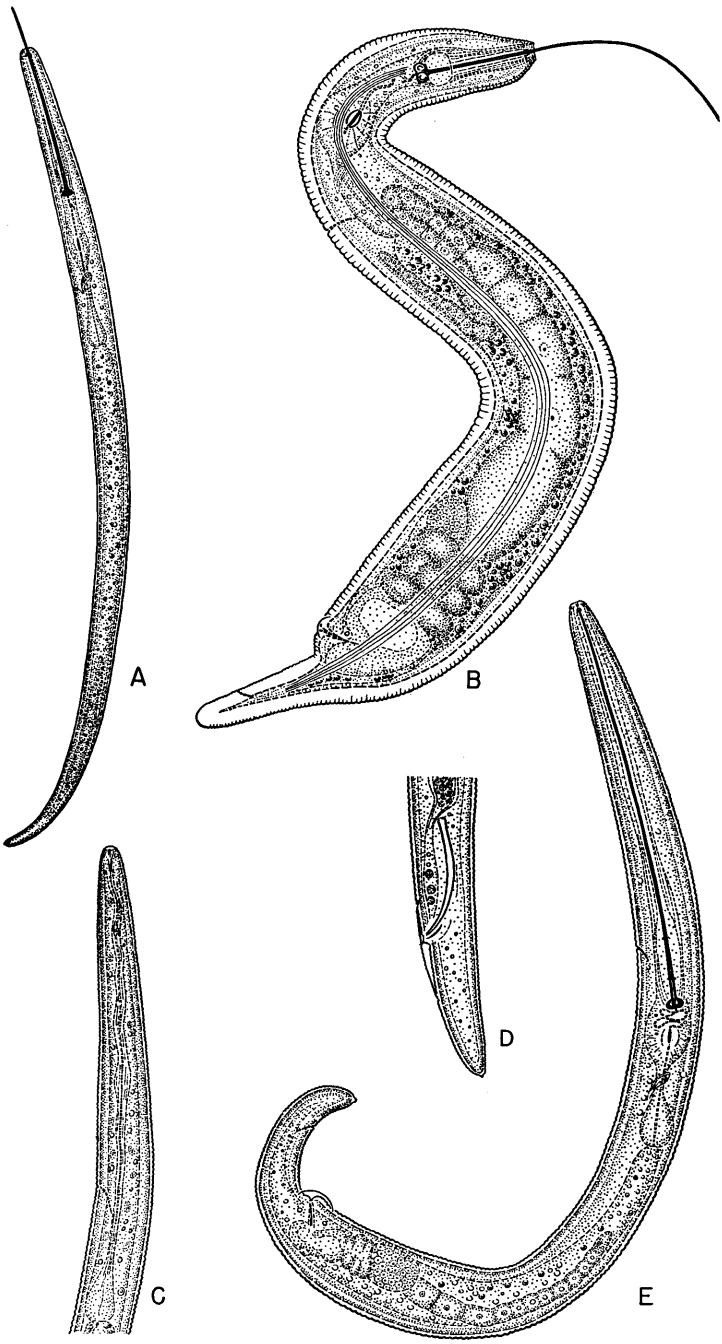

FIG. 2. *Cacopaurus epacris,* n. sp. A—Young larva; ×500. B—Mature female; ×500. C—anterior end of male; ×660. D—Male tail; ×1000. E.—Young female; ×500.

of *C. pestis* infection. He was unable to find any evidence that *C. pestis* could attack the roots of Black Walnut. It remains to be determined if *C. epacris* can attack English roots.

Cacopaurus epacris, new species

Description.—Larvae (Fig. 2, A). Length 0.20–0.26 mm.; width 22 μ. Body cylindrical. Lip region continuous with neck. Esophagus occupying one-third body length. Excretory pore slightly posterior to nerve ring. Tail conoid to a blunt terminus, sometimes with an obscure mucro. Cuticle marked by obscure lateral striations. Lines of wing area not observed. Spear 42 μ long, basal knobs well developed, conspicuous. Anal opening obscure.

Young female (Fig. 2, E). Length 0.28–0.29 mm.; a = 13–14; b = 2.8–3.0; c = 18–20; Vulva = 83–84%. Lip region continuous with neck. Transverse annulation of cuticle conspicuous. Spear long, well developed sometimes about 100 μ in length. A sclerotized spear-guiding apparatus visible in the lip region. Position of excretory pore variable in relation to esophagus depending upon extent spear is extruded. Valvulated postcorpus not set off from corpus of esophagus. Terminal esophageal bulb glandular, distinctly set off from intestine. Intestine granular. Rectum and anal opening inconspicuous. Vulva large with slightly protruding lips, guarded laterally by small membraneous flaps. Ovary single. Uterus with heavy walls. Anterior portion of egg duct a modified spermatheca. Tail conoid cylindrical, terminus rounded, frequently slightly digitate.

Adult female (Fig. 2, B). Length 0.24–0.32 mm.; a = 7.5–8.0; b = 2.6–3.6; c = 16–20. Vulva = 85–87%. Spear length 82–98 μ. Body obese, straight, curved or variously bent. Lip region continuous with neck contour. Cuticle marked by well defined transverse annules which average about 1.1 μ in width near the middle of the body. Cuticle plain, not ornamented with refractive dots. Wing area marked by four lines extending from the neck region to the vicinity of the anal opening. Body diameter reduced posterior to vulva. Body posterior to the vulva cylindrical conoid. Tail bluntly rounded. Vulva conspicuous, guarded laterally by small membraneous flaps. Basal knobs of spear well developed. Corpus of esophagus thick, gradually broadening into the valvulated postcorpus. Isthmus of esophagus distinct, surrounded by the nerve ring. Posterior bulb of esophagus well defined but usually obscured by the ovary and intestine. Excretory duct opening in vicinity of posterior esophageal bulb. Intestine granular. Ovary single, frequently doubly reflexed in older females and extending anteriorly to the region of the postcorpus. Uterus heavily walled. Modified spermatheca present at anterior end of egg duct.

Male (Fig. 2, C, D). Length 0.24–0.30 mm.; a = 17–22; b = 4.0–4.1; c = 12. Lip region continuous with neck contour. Cuticle marked by transverse annules. Body rather uniformly cylindrical. Tail bluntly rounded, sometimes faintly digitate. Bursa or bursa-like structure extremely obscure. Spicules ventrally curved, cephalated, slender, about 16 μ in length. Gubernaculum slightly curved. Testis single. Esophagus degenerate, details obscure. Phasmids not seen. Spear absent in adult males.

Type host.—Roots of California Black Walnut, *Juglans hindsii* Jepson.

Type locality.—Visalia, Tulare County, California.

Diagnosis.—*Cacopaurus epacris*, n. sp., differs from the type species *C. pestis* Thorne 1943 in the shape of the body posterior to the vulva. Longer tail. Absence of punctate ornamentation on the cuticle. Presence of lateral vulvular flaps. Four lines in the wing area as compared to three lines in the type species.

Cacopaurus epacris, n. sp., probably represents less specialized development than is exhibited by *C. pestis*. Certain morphological characters indicate its relationship to the genus *Paratylenchus* Micoletzky 1922. The flaps at the lateral margins of the vulva are typical of most females in the genus *Paratylenchus*. *C.*

epacris, n. sp., does not have the punctate ornamentation present on the cuticle of *C. pestis*. However, the youngest larvae observed had a well developed stylet which is characteristic of the larvae of *Cacopaurus*. Young larvae of *Paratylenchus* have no stylet. The general body shape of young females is similar to females of *Paratylenchus* but mature females show a close relationship in body shape to the type species *C. pestis*.

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The Male of *Dracunculus insignis* (Leidy, 1858) Chandler, 1942

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In 1932 Benbrook reported the occurrence of *Dracunculus medinensis* females from *Vulpes fulva* taken in Iowa. In 1933 the writer reported similar specimens from *Procyon lotor* from Ontario and New York and from *Putorius vison* from Nebraska. At that time he identified the material with that described by Leidy (1858), i.e. *Filaria insignis*, type host *Procyon lotor;* but, since he was unable to distinguish between specimens of female guinea worm from the Old and New Worlds, the question was left unsettled. Travassos (1934) reported *Dracunculus fuelleborni* as a new species from *Didelphis aurita* in Brasil. Chandler (1942) found the guinea worm of raccoons rather common in Texas and elevated it to specific rank on the basis of cephalic differences and size differences in the female. Having had the privilege of studying Moorthy's (1937) specimens of *D. medinensis* we are dubious of the differentiation of the species on these bases. Moorthy's material was obtained by the artificial infection of dogs with guinea worm of human origin. The size of the females was up to 53 cm., while our materials from North American mammals were from 16 to 36 cm. and Chandler's from 7.5 to 28 cm. Records on the size of females from the Old World are usually between 45 and 70 cm. long but Cinotti (1906) reported a female from an Egyptian dog as 36 cm. long. The females in Travassos' material were 46 to 49 cm. long. Recent reports of *Dracunculus* females from North American mammals are summarized by Dikmans (1948).

The present material comprises two male specimens collected by Ernest Ediger from subcutaneous fascia of inner thigh of *Procyon lotor* in Dorchester County Maryland marshlands, Sept. 23, 1949.

The males measure 17 to 22 mm. in length with a maximum diameter of 240 μ. The esophagus is 13 to 14 mm. long, the anterior muscular part 300 μ long; latero-ventral commissure 620 μ from anterior end; cervical papillae (deirids) 700 μ and excretory pore 800 μ from anterior end of body; tail 350 μ long; spicules equal, setaceous, 460 to 495 μ long; gubernaculum 119 μ long. Preanal genital papillae 5 pairs, oblique; postanals in transverse row of 2 pairs subventral and 1 pair ventrolateral, followed by 2 pairs of subventral papillae, then the paired lateral phasmids.

This description differs but little from that given by Moorthy (1937). The phasmids were incorrectly identified in the former description. Actually they are in the same position as in the present material. However, there were clearly 6 pairs of preanal genital papillae in Moorthy's specimens as against only 5 pairs in the present. Also Moorthy describes the gubernaculum as 200 μ long while it is only 119 μ long in the present material. Travassos describes the males of *D. fuelleborni* as having spicules 380 to 420 μ long and a gubernaculum 88 to 100 μ long. Unfortunately his illustration of the genital papillae does not appear plausible. Hence the identity of *D. fuelleborni* must remain in doubt.

On the basis of the above comparisons, it would appear that *D. insignis* may be separated from *D. medinensis* on the basis of length of gubernaculum and number of preanal genital papillae. However, it is quite possible that a more extensive series of specimens would disclose an overlap in these characters.

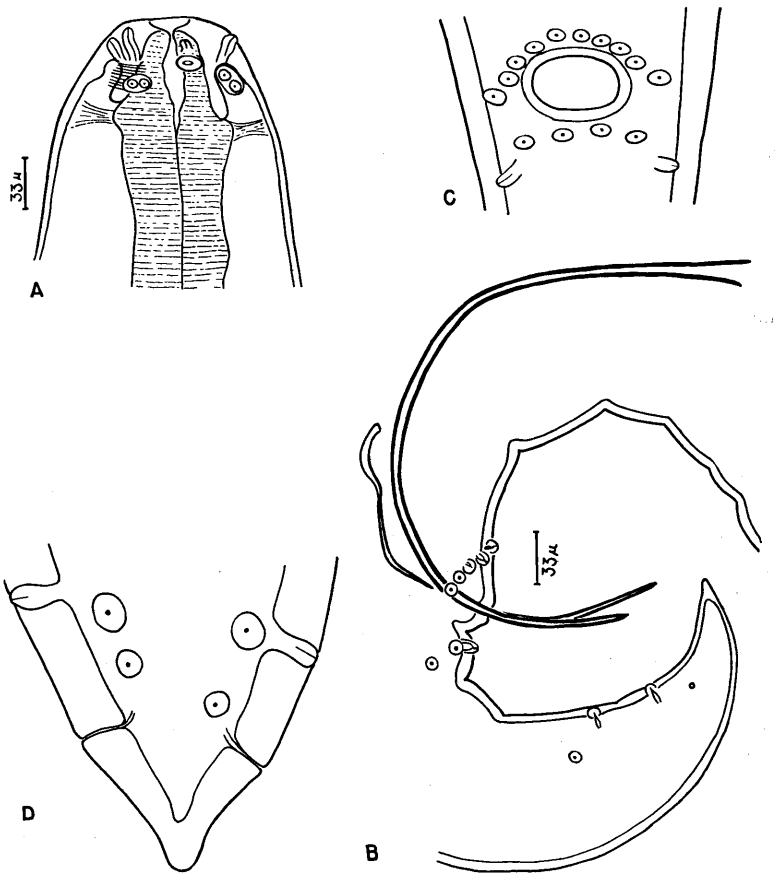

FIG. 1. *Dracunculus insignis* male. A—Head, lateral view. B—Male tail, lateral view. C—Cloacal region, ventral view. D—Tip of tail, ventral view.

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The Lungworm, *Protostrongylus rufescens*, Found in Domestic Sheep, *Ovis aries*, in the United States

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Lungworms of the genus *Protostrongylus* have previously been reported from various wild ruminants—mountain sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), deer (*Odocoileus virginianus*), varying hares (*Lepus* spp.) and the Black Hills cottontail (*Sylvilagus nuttali grangeri*) in different parts of North America, but, thus far, lungworms of this genus have not been reported from domestic sheep in this country or Canada.

The nematodes discussed in the present paper were collected from the lungs of domestic sheep at Ithaca, New York, by C. R. Mapes and referred to the Zoological Division of the U. S. Department of Agriculture for identification. They were identified as *Protostrongylus rufescens* (Leuckart, 1865) Kamenskii, 1905. This lungworm has been reported as a parasite of domestic sheep in Europe, North Africa and Asia, but as it has not previously been found in North America, no adequate description or figures of this parasite are available in American parasitological literature. It is the purpose of the present paper to supply that description and figures.[1]

Examination of lungworms collected from sheep, deer and rabbits in the United States and deposited in the U. S. National Museum Helminthological Collection has failed to reveal any specimens of *P. rufescens*.

Protostrongylus rufescens

The dimensions of the specimens of *Protostrongylus rufescens* recovered from domestic sheep at Ithaca, New York, are as follows:

Males		Females	
Length	25 to 35 mm.	Length	30 to 45 mm.
Width		Width	
At head end	0.035 to 0.040 mm.	At head end	0.045 to 0.050 mm.
Middle of body	0.130 to 0.150 mm.	Middle of body	0.140 to 0.160 mm.
Immediately anterior to bursa	0.070 to 0.075 mm.	In region of vulva	0.080 to 0.085 mm.
Esophagus	0.300 to 0.350 mm.	Vulva to anus	0.150 to 0.165 mm.
Nerve ring	approx. 0.160 mm. from head end	Anus to tip of tail	0.085 to 0.090 mm.
		Vagina	0.900 to 1.5 mm.
Excretory pore	approx. 0.280 mm. from head end	Provagina	Absent
		Eggs	0.070 to 0.075 × 0.035 to 0.375 mm.
Spicules	0.225 to 0.290 mm.		
Gubernaculum	0.125 to 0.150 mm.		
Capitulum			
Corpus	0.075 to 0.090 mm.		
Crura	0.045 to 0.050 mm.		
Number of papillae on dorsal ray	6		
Arcus	Well developed		

[1] There are, in American parasitological literature, references to *Protostrongylus rufescens* as a lungworm parasite of sheep, deer and rabbits, presumably in North America but the reports of its occurrence in sheep and rabbits lack substantiating evidence, and the report of its presence in deer was apparently due to a misdetermination.

The dimensions of parts of the body vary somewhat with the size of the complete worms.

The rays of the bursa are similar to those of other species of this genus, namely, ventral rays united except at the tips, anterio-lateral ray separated from the medio- and postero-lateral rays, medio- and postero-lateral rays arising from a common stem and separated for about half of their length, postero-lateral shorter than the medio-lateral. Externo-dorsal rays arising independently from the dorsal ray and rather widely separated from the postero-lateral rays. Dorsal ray rounded, mound-like, with 6 papillae on the ventral surface.

There is no provagina in the female but the cuticle in the region of the vulva is slightly inflated. The posterior lip of the vulva is rounded and quite prominent.

Dougherty and Goble (1946), recognize and list 9 species of *Protostrongylus* from ruminants and 3 from rabbits and hares. Those listed from ruminants are: *Protostronglylus rufescens, P. unciphorus, P. cameroni, P. skrjabini, P. brevispiculum, P. stilesi, P. rushi, P. coburni* and *P. rupicaprae. P. rufescens* differs from *P. stilesi, P. rupicaprae* and *P. coburni* in the absence of a provagina; from *P. skrjabini* in size and conformation of the crura of the gubernaculum, [*P. skrjabini* was described by Dikmans (1945) under the name of *P. gracilis,* the paper by Shult's and Boev (1940) in which the nematode had been named *P. skrjabini* not being available at that time]. *P. rufescens* differs from *P. brevispiculum* in the length of the spicules and from *P. unciphorus* and *P. rushi* in the conformation of both the corpus and crura of the gubernaculum. The available description and figures of *P. cameroni* are, unfortunately, inadequate to make a reasonably accurate comparison. *P. rufescens* appears to differ from all the above mentioned species in the number of papillae on the ventral surface of the dorsal ray.

The 3 species of Protostrongylus from rabbits and hares are *Protostrongylus pulmonalis, P. kamenskyi* and *P. boughtoni.* The females of *P. kamenskyi* and *P. boughtoni* are provided with provaginae and *P. rufescens* can therefore be differentiated from these species by the absence of this structure.

Some European investigators have reported that *P. rufescens* occurs in both small ruminants, that is, sheep and goats, and rabbits and hares, but they have also described another species, *P. pulmonalis* (v. Frölich, 1802) Goble and Dougherty, 1943.

The Russian authors, Shult's, Orlov and Kutas (1933), disagree with other European authors and state specifically that *P. rufescens* does not occur in hares.

In the absence of comparative material definitely known to have been collected from sheep and hares in Europe, it is not possible for us to settle the question. However, it may be pointed out that the worms called *Strongylus rufescens ovis* and *S. rufescens leporis* by Lutz (1926), can be surely differentiated on the basis of the figures produced by this author.

Scott (1943), described as a new species of *Protostrongylus* a lungworm found in the Black Hills cottontail, *Sylvilagus nuttali grangeri,* in Wyoming. He named it *P. sylvilagii.* Dougherty and Goble (1946), made *P. sylvilagii* a synonym of *P. pulmonalis.* An examination of specimens of *P. sylvilagii* available in the U. S. Nat. Museum Helm. Coll. shows that these worms can be readily differentiated from *P. rufescens* collected from sheep at Ithaca, New York. Whether or not *P. sylvilagii* is identical with *P. pulmonalis* is a question that should receive some further consideration.

There are 4 rather striking morphological features which have led the present writers to call the worms collected from the lungs of sheep at Ithaca, N. Y., *Protostrongylus rufescens,* namely, (1) the morphology of the ventral rays, (2) the beaded appearance of the corpus of the gubernaculum, (3) the presence of 6 papillae on the dorsal ray, and (4) the shape of the terminal portions of the crura of the gubernaculum. In all these features the worms collected at Ithaca resemble

the worms described and figured as *Strongylus rufescens ovis* (= *P. rufescens*) by Lutz (1926); *Synthetocaulus kochi*[2] (= *P. rufescens*) by Shult's, Orlov and Kutas (1933), and by Shult's and Boev (1940); and *P. rufescens* by Joyeux and Gaud (1946). The disposition of the ventral rays is particularly interesting. At first glance, these rays appear to be completely fused but careful examination shows that they are bent over ventralwards at the tips and that the tips are separated. These features are well illustrated in the figures of *Strongylus rufescens ovis* (= *P. rufescens*) presented by Lutz (1926), and those of *Synthetocaulus kochi* (= *P. rufescens*) presented by Shult's, Orlov and Kutas (1933), and Shult's and Boev (1940).

The specific identity of *Protostrongylus rufescens* (= *Strongylus rufescens* Leuckart, 1865) has been a matter of controversy for many years, due largely to (1) inadequate original description, (2) lack of adequate figures, and (3) confusion with other lungworms from sheep, deer and rabbits. According to Schmid (1939), *P. rufescens* was considered to be identical with (1) *P. pulmonalis* (v. Froelich, 1802) Goble and Dougherty, 1943, a lungworm of rabbits and hares, by Mueller, Goldbeck, Jerke, Schlegel, Joest-Nieberle, Hueber and Fiebiger, (2) with *Muellerius minutissimus* (Cameron, 1927) Dougherty and Goble, 1946, the hair lungworm of sheep, by von Richters. This same author also reports that Cameron (1927) expressed the opinion that *P. rufescens* was perhaps identical with *P. pulmonalis*, and that Schlegel (1899) considered *Cystocaulus ocreatus* (Railliet and Henry, 1907) Mikačić, 1939 [= *Protostrongylus nigrescens* (Jerke, 1911) Gebauer, 1932] as identical with *P. pulmonalis* vel *P. rufescens*, and Fiebiger (1936) considered *Varestrongylus sagittatus* (Mueller, 1890) Dougherty, 1945 [= *Protostrongylus sagittatus* (Mueller, 1890) Cameron, 1927] as identical with *P. rufescens*. This indicates that there was considerable confusion not only concerning the identity of the lungworm called *Strongylus rufescens* by Leuckart but also concerning the specific identities of other protostrongyline lungworms of sheep, deer, rabbits and hares.

It was undoubtedly the inadequate description by Leuckart of the worm he called *Strongylus rufescens* and the subsequent confusion concerning its specific identity, referred to by Schmid (1939), that caused Shult's, Orlov and Kutas (1933), to propose a new name, *Synthetocaulus kochi*, for the lungworm of sheep, called *Protostrongylus rufescens* in the present paper, and to make of *Strongylus rufescens* Leuckart, 1865, a "species inquirenda." They contended that the original description of *Strongylus rufescens* by Leuckart does not contain any specific details by which it can be differentiated from other species of *Protostrongylus* known at the present time, and that there is no evidence that other authors who subsequently dealt with what they called *Strongylus rufescens*, were dealing with the worm seen and described by Leuckart. The action of these Russian authors is contrary to procedure generally followed in dealing with similar problems of zoological nomenclature, and, if permitted to stand, would have the unfortunate result of replacing a specific name that has been universally accepted, as shown by pertinent literature, with a new and comparatively unknown name.

[2] In the papers cited, Shult's and coworkers use the name *Synthetocaulus kochi* for the nematode which they had previously called *Protostrongylus rufescens*. Dougherty and Goble (1946), point out that since 1933 Soviet helminthologists, almost alone, persist in the use of the generic name *Synthetocaulus* Railliet and Henry, 1907, in place of the earlier name *Protostrongylus* Kamenskii, 1905. They note that since the genotype of *Synthetocaulus*, namely, *Strongylus commutatus* Diesing, 1851 (= *Filaria pulmonalis* v. Froelich, 1802) as selected by Railliet and Henry (1907), and the genotype of *Protostrongylus*, named *Strongylus rufescens* Leuckart, 1865, as selected by Cameron (1927) are clearly congeneric, the former generic name must by the Rules of Zoological Nomenclature be considered as a synonym of the latter. Leiper (1926) also pointed out that the generic name *Protostrongylus* has priority over the generic name *Synthetocaulus*.

Dougherty and Goble (1946), however, considered *Synthetocaulus kochi* Shult's, Orlov and Kutas, 1933, as a synonym of *Protostrongylus rufescens* (Leuckart, 1865) Kamenskii, 1905. In the paper cited the authors stated that the historical data and evidence upon which they based their action were to be given in a later paper. The present writers are inclined to agree with Dougherty and Goble in considering

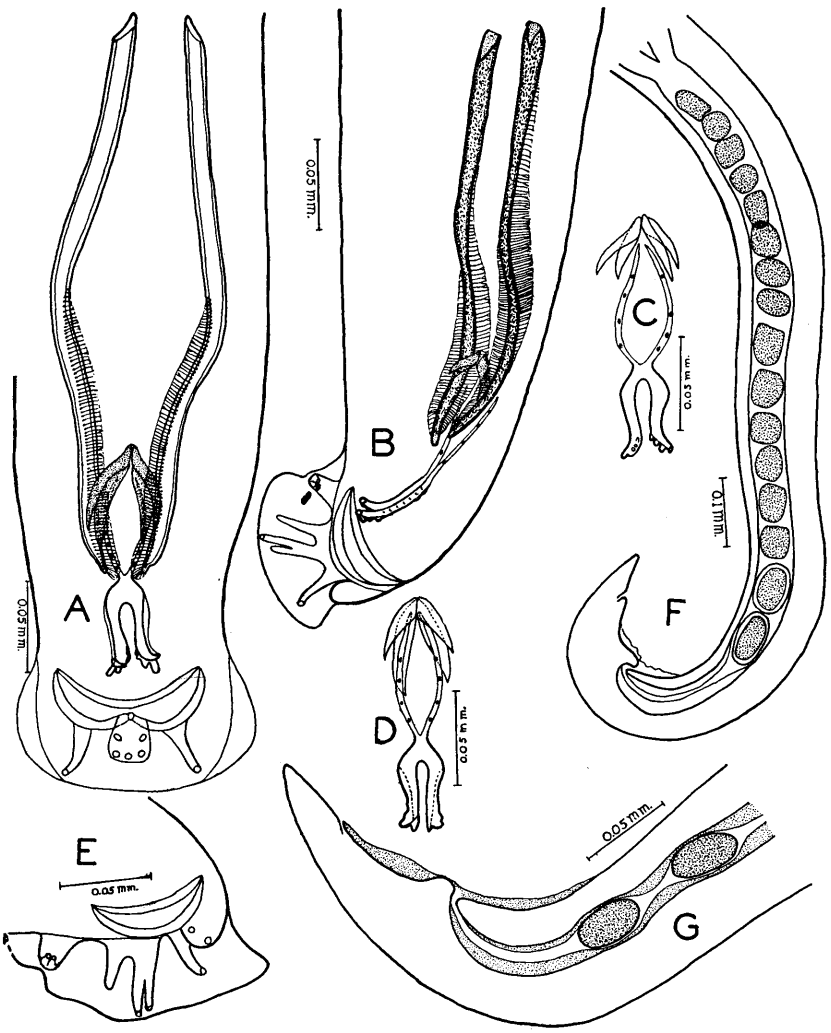

FIG. 1. *Protostrongylus rufescens.* A—Caudal region of male, ventral view, showing papillae on dorsal ray. B—Caudal region of male, lateral view, showing spicules and gubernaculum. C and D—Gubernaculum. E—Bursa. F—Posterior portion of female, showing length of vagina. G—Posterior portion of female, showing relative positions of vulva and anus.

Synthetocaulus kochi Shult's, Orlov and Kutas, 1933, a synonym of *Protostrongylus rufescens* (Leuckart, 1865) Kamenskii, 1905, and since Dougherty advises us (in litteris) that he will not in the foreseeable future have the opportunity to prepare the above-mentioned paper, we propose to review briefly the evidence upon which our opinion is based.

As previously noted the Russian authors advance two reasons for making *Strongylus rufescens* Leuckart, 1865 a "species inquirenda," namely, (1) that the original description of *Strongylus rufescens* lists no specific characters by which it can be differentiated from other species of *Synthetocaulus* (= *Protostrongylus*) known at the present time, and (2) that there is no evidence that other authors who dealt with *Strongylus* or *Synthetocaulus* "*rufescens*" were dealing with the same parasite that Leuckart had before him.[3]

It should be noted that while these authors expressed the opinion that the specific identity of *S. rufescens* cannot be established from Leuckart's original description, they conceded, as may be inferred from their statements quoted above, that Leuckart's parasite was a species of *Protostrongylus* as now known. Further evidence to that effect is that they listed Leuckart's species as a probable synonym of their *S. kochi.*

Leuckart (1865) used the name, *Strongylus rufescens,* for a nematode that he had collected from the lungs of sheep. He called it a new species but he neither described nor figured it at that time. In 1866, he described it briefly as follows: Head unarmed, with three small lips, esophagus short. Thin, female about "Spannenlänge," male shorter. Vulva immediately anterior to anus. Only a few eggs in first stage of development in uterus. Bursa of male short with stout rays. Two long, rather strongly curved spicules.[4]

A reading of this description shows that Shult's, Orlov and Kutas were correct in stating that Leuckart's original description of *S. rufescens* contained no specific characters by which it can be differentiated from other species of *Synthetocaulus* (= *Protostrongylus*) known at the present time. It should be noted, however, that Leuckart's description is sufficient to eliminate from consideration as candidates for the name of *S. rufescens* other lungworms of sheep known to occur in that area. Three other genera and species of lungworms are definitely known to occur in sheep in the area in which Leuckart was located, namely *Dictyocaulus filaria, Muellerius minutissimus* and *Cystocaulus ocreatus.* Leuckart's mention of the presence of a bursa with stout rays in the male of *S. rufescens* eliminates *M. minutissimus* because such a structure is lacking in the male of the latter nematode. The description of the location of the vulva in the female of *S. rufescens* shows that he was not confusing *S. rufescens* with *D. filaria,* and the fact that he does not mention the presence of a provagina in the female of *S. rufescens* indicates that he was not dealing with *Cystocaulus ocreatus.* By a similar process of elimination it can also be shown that, in all probability, he was not dealing with any genus of the subfamily Protostrongylinae other than the genus *Protostrongylus.*

Therefore, one of the two following propositions must have been true: (1) Leuckart had before him and, for the first time, recognized and named a particular species of *Protostrongylus* from sheep; or (2) he had before him and, for the first time, recognized and named a complex of species of *Protostrongylus* from sheep. In either event the International Rules of Zoological Nomenclature, or well-estab-

[3] The exact words of the Russian authors are as follows: "Die Originalbeschreibung des *Strongylus rufescens* Leuck. 1865 enthält keine spezifischen Merkmale, durch die dieser von den zur Zeit bekannten Synthetocaulus-Arten unterschieden werden konnte. Es liegen auch keine Beweise vor, dass es die anderen Autoren, die den *Strongylus* oder *Synthetocaulus* "*rufescens*" behandelten, mit demselben Parasiten zu tun hatten, den namentlich Leuckart im Auge hatte."

[4] After he mentioned *Strongylus rufescens* as a new species found in the lungs of sheep together with *Strongylus filaria* (= *Dictyocaulus filaria*), Leuckart's actual description is as follows: "Die Charactere dieser Art, deren namen ich von dem braun durchschimmerden Darm entlehne, sind folgende: Kopf unbewaffnet, mit drei kleinen Lippen und kurzem Oesophagus. Dünn, das Weibchen fast von Spannenlänge, Männchen kürzer. Die Vulva dicht vor dem After; im Uterus nur Wenige Eier in Furchung. Die Schwanzkappe des Männchens kurz mit plumpen Rippen und zwei langen, ziemlich stark gekrümmten Spiculae."

lished precedent, indicate that the trivial name *rufescens* must be used for some one of the species of *Protostrongylus* from sheep. If he had a single species before him and did not describe it adequately, the first recognizable redescription stated by the author of that redescription to apply to *Strongylus rufescens* Leuckart fixes the zoological entity to which that name must henceforth be attached, provided the redescription (1) does not do violence to the original description, (2) is based on specimens from the type host and locality, and (3) cannot be demonstrated to rest upon a misidentification. Koch (1883), provided such a redescription. Obviously, since Leuckart's description lacks sufficient detail to show definitely which species he had before him, misidentification on Koch's part can only be proven by recourse to Leuckart's type specimens, if such are available. Misidentification on Koch's part has, thus far, not been proven.

There is rather strong indirect evidence that Leuckart dealt with a single species instead of with a complex of species, and the matter of the correct procedure to be followed when a complex of species is to be divided into one or more restricted species need not to be considered here.

In his paper Koch described and figured what he called "Die rötlichbraune Palissadenwurm, *Strongylus rufescens* (Leuckart)." He stated that this worm was first described by Leuckart and that it was later (1876–1877) collected from sheep lungs at Bern, Switzerland, by Bugnion who sent him (Koch) a detailed description of the worm. Koch sent the posterior portion of a male specimen of the worm which he called *Strongylus rufescens* (Leuckart) to Leuckart and the latter informed him that it was possibly (möglicherweise) identical with the worm described by him (Leuckart) in 1866 as *S. rufescens*. The only thing that seems to have made Leuckart somewhat hesitant to state positively that the worm sent to him by Koch was definitely identical with the worm which he (Leuckart) had called *Strongylus rufescens* in 1865, was the comb-like structure of the spicules.[5]

In spite of Leuckart's somewhat guarded opinion that Koch's *S. rufescens* was identical with the worm called *S. rufescens* by him in 1865, Koch stated that he considered his *S. rufescens* to be the same as Leuckart's *S. rufescens*.

He published a detailed description of this worm he called *Strongylus rufescens* (Leuckart) and that description was accompanied by figures that are clearly recognizable as representing a species of the genus now known as *Protostrongylus*. The publication of this description and of these figures had the effect of establishing the trivial name "*rufescens*" even if Leuckart's original *Strongylus rufescens* should be considered a *nomen nudum*. Kamenskii (1905), created the genus *Protostrongylus* for the group of lungworms with morphological characters similar to those of *Strongylus rufescens* Leuckart, 1865. Therefore, the correct and, at present, generally accepted name of the lungworm of sheep described by Leuckart (1866) and Koch (1883) as *Strongylus rufescens* is *Protostrongylus rufescens* (Leuckart, 1865) Kamenskii, 1905.

Whether subsequent authors who discussed or dealt with *Strongylus rufescens* Leuckart, 1865, actually were or were not dealing with the parasite which "Leuckart im Auge hatte" has no bearing on the question of the validity of the trivial name "*rufescens.*"

Koch (1883), reported that Bugnion (1875), mentioned *Strongylus rufescens* and that Bugnion had sent him an illustrated description of this worm, which had often been collected from the lungs of sheep at Bern, Switzerland, in 1876–1877. Koch in turn sent to Bugnion a drawing of the posterior portion of a male specimen of the worm he called *Strongylus rufescens* and Bugnion confirmed Koch's

[5] "Prof. Leuckart hatte die Güte, ein männliches allerdings nicht völlstandiges Exemplar, welches ich ihm zur Ansicht zusendete, als für ein möglicherweise mit *St. rufescens* identisch zu bestimmen, nur fiel demselben die kammartige Bildung der Spicula dieses Praparates auf."

identification. Railliet (1884), reported finding *S. rufescens* in sheep in France. Noting that he was dealing with a worm which differed from *D. filaria* and also from the worm which Megnin, 1878, had called *Strongylus minutissimus*, he was inclined to give his worm a new name until Neumann called his attention to Koch's paper. After seeing that paper he recognized the similarity between the worm that he (Railliet) had collected from the lungs of sheep and the worm described by Koch as *Strongylus rufescens* (Leuckart) and he concludes his paper with the following words, "la pneumonie vermineuse (est causée) par le *Strongylus rufescens* Leuck."

While this does not prove that Bugnion and Railliet were dealing with the parasite which "Leuckart im Auge hatte" it furnishes adequate evidence that Koch, Bugnion and Railliet were dealing with the same parasite, and since Koch

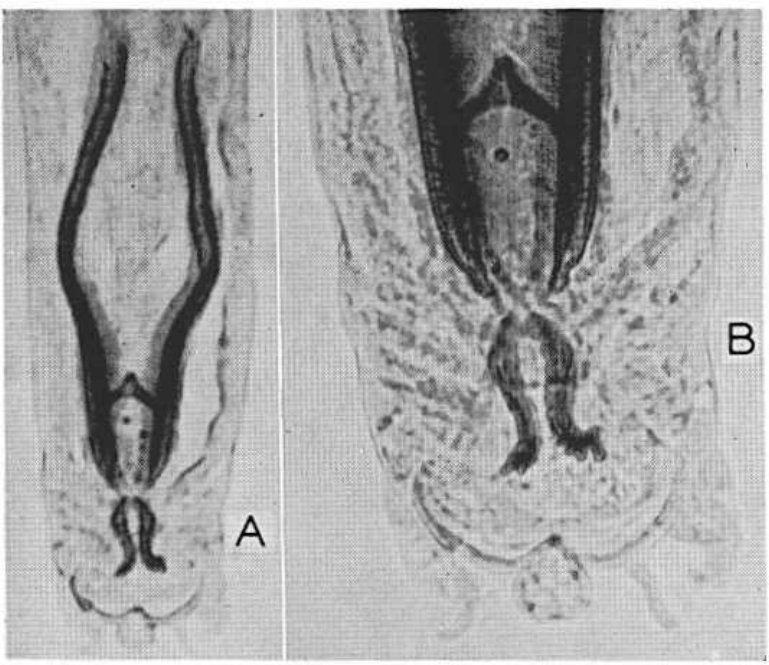

FIG. 2. *Protostrongylus rufescens.* A—Posterior portion of male, showing "beading" of corpus of gubernaculum. B—Posterior portion of male, showing conformation of terminal portions of crura of gubernuculum.

was apparently convinced that he was dealing with the parasite described by Leuckart, as shown by the retention of the name, *Strongylus rufescens*, given to the parasite by Leuckart, the assumption that Leuckart, Koch, Bugnion and Railliet were all dealing with the same parasite is not unwarranted.

Additional circumstantial evidence to the effect that Leuckart, Koch and later Lutz (1926) and Hueber (1928) were dealing with the same worm, consists in the fact that what is now known as *Protostrongylus rufescens* appears to be the most prevalent, if not the only, species of *Protostrongylus* occurring in the lungs of sheep in the area in which these authors were located. As previously noted, Dougherty and Goble (1946), include 9 species in the genus *Protostrongylus* namely, *P. rufescens, P. unciphorus, P. cameroni, P. skrajabini, P. brevispiculum, P. stilesi, P. rushi, P. coburni* and *P. rupicaprae.* Four of these, namely, *P. rufescens, P. unciphorus, P. brevispiculum* and *P. rupicaprae* are known to occur in western and central

Europe. The exact geographic distribution of *P. unciphorus* is not known, but it has been reported from Western Europe and from Russian Turkestan (Central Asia). Schult's, Orlov and Kutas (1933), described this nematode under the name of *Synthetocaulus hobmaieri* n. sp.; Dougherty and Goble (1946), however, considered *S. hobmaieri* to be the same as *P. unciphorus*. *P. rupicaprae* was described by Gebauer (1932), as a parasite of the chamois, *Rupicapra rupicapra*, and has not been reported from sheep. *P. brevispiculum* Mikačić, 1939, is a recent addition to the subfamily Protostrongylinae, and its occurrence outside of Yugoslavia is unknown. Furthermore, a comparison of the figures of *P. rufescens* published by Koch (1883), Lutz (1926), Hueber (1928), Joyeux and Gaud (1946), with the figures representing *P. unciphorus*, *P. rupicaprae* and *P. brevispiculum*, as published by Shult's and coworkers, Gebauer and Mikačić, shows very clearly that the first named authors were not dealing with any of these 3 species.

The fact that the places at which the above mentioned authors presumably collected their material were located within relatively short distances of each other also lends support to the idea that they were dealing with the same lungworms of sheep. While Leuckart does not indicate specifically the place of origin of the sheep from which he collected his *S. rufescens*, it is not unreasonable to assume, especially when travel facilities available in Leuckart's time are considered, that those sheep originated close to Leipzig. The sheep examined by Koch were located at Pressburg, near the Czechoslovakian border, a short distance east of Vienna. Lutz collected his specimens from the lungs of sheep slaughtered at Augsburg, Bavaria, and Hueber wrote his dissertation at Munich. A glance at the map of Central Europe shows that those places are fairly close together.

Therefore, while the specific identity of the lungworm of sheep called *Strongylus rufescens* by Leuckart in 1865 cannot be determined with absolute certainty from Leuckart's original description, it can be determined with relative certainty from Koch's (1883) figures and redescription. There is also adequate evidence that at least some of the authors who subsequently dealt with *Strongylus rufescens* dealt with the same parasite that "Leukart in Auge hatte."

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A Note on an Early Record and a Synonym of the Tropical Rat Mite, *Liponyssus (Bdellonyssus) bacoti* (Hirst)

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Entomologists and others who are interested in quarantines find it of particular importance to know when and where a pest of foreign origin was first recorded in their country. Such knowledge is indispensable in the study of the dispersal of a pest, and is of value in other ways. In the years following World War I the tropical rat mite, *Liponyssus* (*Bdellonyssus*) *bacoti* (Hirst), began to appear in great numbers in the United States. The first reports of its attacks came largely from the Atlantic seaboard and the southeastern states. Now, the pest is known to occur in all sections of the United States.

In a paper published in 1932[1] the present writer established the fact that this mite was present in this country as early as 1916, for in that year, or before that year, specimens of the tropical rat mite had been acquired by the United States National Museum which bore the following data, "on mouse, St. Louis, Missouri, from Dr. Loeb." This record has been considered the oldest for the United States. Now, however, a definite record, taking us back several years, is reported.

When the writer was working as a graduate student in the Iowa Agricultural Experiment Station in 1910 he collected a mite from *Mus rattus*, that was described by Ewing and Stover in 1915 as new under the name of *Haemogamasus sanguineus* in a contribution from the Department of Zoology and Physiology of the Oregon Agricultural College.[2] In those days it was not the practice to clear and stain mite specimens. Since coming to Washington the type of *Haemogamasus sanguineus* has been cleared, stained, remounted, and again studied. It proves to be

[1] Proc. Ent. Soc. Wash., Vol. XXXIV, p. 14.

[2] Ent. News, Vol. XXVI, p. 109.

not a *Haemogamasus* but no other than *Liponyssus bacoti* (Hirst). Hirst's description had not yet appeared when *sanguineus* was identified as new, but his name has priority over *sanguineus*.

Liponyssus bacoti (Hirst) was described in 1913 from *Mus norvegicus* taken at Assiût, Egypt, there being no collecting date given. The specimens collected by the writer, which consisted of an adult female and an immature individual, were taken at "Ames, Ia., June 25, '10, by myself. From *Mus rattus* 13." Thus this record from one of the Central States appears to be the earliest known for our country. Undoubtedly it also must be one of the earliest, if not the earliest, for the world.

In view of the fact that the second oldest record for our country also comes from the Central States, one can see little justification for assuming that the tropical rat mite was first established in our country on the Atlantic seaboard, the region from which a majority of the early records came. Also since this mite, as well as many of its near relatives, may live for many days off its host it is not necessary to assume that it was introduced on rats.

CHIEF POINTS OF PAPER

1. Apparently the earliest record for the United States of the tropical rat mite, *Liponyssus* (*Bdellonyssus*) *bacoti* (Hirst), is for Ames, Iowa, where specimens were taken from *Mus rattus* (now *Rattus rattus*) by the writer on June 25, 1910. The specimens were reported as belonging to a new species which was given the name of *Haemogamasus sanguineus* Ewing and Stover.

2. *Haemogamasus sanguineus* Ewing and Stover (1915) is a synonym of *Leiognathus bacoti* Hirst (1913), now known as *Liponyssus* (*Bdellonyssus*) *bacoti* (Hirst).

Selecting Experimental Groups of Chicks by Weight

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In setting up an experiment involving several groups of chicks it is desirable, for comparison of the different groups when assaying the results of the experiment, that the weights of the individual birds in each group and the average of each group be comparable at the beginning of the experiment. Since the range in weight between the lightest and the heaviest chicks in a hatch is considerable, even at an early age, the following method of distribution is suggested for saving time and still achieving the closest possible uniformity.

After banding the chicks, weigh them individually and record the weights in columns, each column having a range of 10 grams. Write the weight first, and the number of the bird after it, either in parentheses or separated by a dash, as follows:

114 (330)	122 (398)	136 (329)	147 (322)	153 (373)	167 (321)	177 (355)
118 (347)	130 (374)	131 (365)	141 (368)	152 (342)	170 (302)	177 (328)
112 (325)	126 (390)	135 (364)	147 (334)	153 (400)	166 (386)	180 (336)
114 (319)	125 (312)	136 (381)	150 (391)	157 (317)	163 (392)	
111 (308)	130 (385)	131 (351)	148 (396)	152 (370)	162 (305)	

Some columns will be longer than others, of course, since it is very unlikely that there will be the same number of chicks in each 10-gram category. For purposes of illustration, however, only a few chicks in each category are listed.

After weighing all the chicks from which selection is to be made, count the number in each column. Assuming that the experiment requires 6 groups of 10

chicks each, a total of 60, the next step is to determine the maximum and minimum limits of weight necessary to fill this quota from the available material. The larger the number of chicks from which to choose, the better, as greater uniformity can be attained from a larger group.

In the particular case used for illustration here, there were 12 chicks in the 121–130 gram group, 7 in the 131–140 group, 22 in the 141–150 group, 11 in the 151–160 group, and 6 in the 161–170 group, a total of 58, which was 2 less than the necessary 60. In addition, there were available some unusually large chicks at the one extreme and some small chicks in the 111–120 gram category at the other extreme. The 2 chicks needed for the necessary 60 had to be taken from one or both of these groups. In this case it was considered more desirable to use two chicks weighing 118 grams each because the weight-gap to be bridged was less with these birds than with heavier ones. This decision is a matter of judgment, however, for various factors may be involved.

The 60 chicks to be used having now been selected, the next step is to assign each chick to its group in such a way as to attain the most uniform distribution possible. To do this, list the chicks in a continuous column by weight, starting with the lightest and continuing to the heaviest. Then assign the first 6 chicks to the first 6 groups in regular order. Assign the seventh chick to group 2, the eighth to group 3, etc., until the twelfth, which is assigned to group 1. Chick no. 13 should then be assigned to group 3, no. 14 to group 4, etc., no. 17 being placed in group 1 and no. 18 in group 2. Continuing through the list in this manner, the result will be as follows:

118 (347)–1	130 (385)–3	141 (383)–5	147 (339)–1	153 (343)–3
118 (367)–2	130 (369)–4	143 (348)–6	148 (376)–2	154 (315)–4
122 (398)–3	131 (365)–5	144 (309)–1	149 (307)–3	156 (377)–5
122 (349)–4	131 (351)–6	145 (354)–2	149 (338)–4	157 (317)–6
123 (301)–5	132 (357)–1	146 (394)–3	150 (391)–5	157 (399)–1
125 (312)–6	135 (364)–2	146 (378)–4	150 (395)–6	158 (326)–2
126 (320)–2	136 (329)–4	146 (323)–6	150 (337)–2	162 (305)–4
127 (350)–3	136 (381)–5	146 (333)–1	151 (371)–3	163 (392)–5
127 (352)–4	137 (340)–6	147 (322)–2	152 (342)–4	165 (397)–6
128 (331)–5	141 (368)–1	147 (334)–3	152 (370)–5	166 (386)–1
130 (335)–6	141 (316)–2	147 (310)–4	153 (373)–6	167 (321)–2
130 (374)–1	141 (382)–3	147 (314)–5	153 (400)–1	170 (302)–3

There remains only to place each chick in its proper cage, which is easily done by reference to the final list.

In the example used for illustration, the average weight per bird for each group was as follows: group 1, 143.4 grams; group 2, 143.5 grams; group 3, 143.6 grams; group 4, 142.5 grams; group 5, 142.7 grams; and group 6, 143.7 grams. The range between the lightest average weight per bird, 142.5 and the heaviest, 143.7, proved to be only 1.2 grams, and the agreement among the individual chicks was as close as could be obtained by any arbitrary method of distribution.

An Improved Method of Mixing Fecal Suspensions for Nematode Egg Counts

LEE SEGHETTI

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When sheep are suspected of clinical parasitism, fecal egg counts are a definite aid in diagnosis. In the past few years this laboratory has examined large numbers of fecal specimens and the necessity arose for increasing the ease and speed of preparing feces for counts.

The egg-counting technic used at this laboratory is a modification of the Lane direct centrifugal flotation method. Ten grams of sheep feces are triturated with a small quantity of water in a mortar and pestle. The resulting suspension is poured into a 500 cc. wide mouth Erlenmeyer flask. Then the mortar and pestle are washed with several changes of water, pouring each of the washings into the flask. Sufficient water is added to bring the total volume to 400 cc. and mixed thoroughly. A 10 cc. sample is quickly withdrawn with a pipette having a large terminal opening and placed in a plain 20 cc. centrifuge tube with a ground lip. The suspension is centrifuged for 2 minutes at 1,500 r.p.m. after which the supernatant fluid is poured off. The tube is nearly filled with sugar solution (100 grams cane sugar in 100 cc. water) and the fecal material is re-suspended. Then the tube is completely filled by adding sugar solution with a bulb pipette. An 18 mm. square cover slip is placed over the tube and the sample is again centrifuged at 1,500 r.p.m. for 2 minutes. The cover slip is then carefully lifted and transferred to a slide for counting.

In this method, trituration and mixing of fecal specimens are time consuming. It was found that by placing sheep feces in the container of an electric blendor and adding sufficient water to make 400 cc., a homogeneous suspension could be obtained in a minute or less. Considerable foaming results, but when fecal egg counts were compared with the method generally used, no significant discrepancy was noted, as shown below.

Method	Sheep Number									
	1	2	3	4	5	6	7	8	9	10
Trituration by Hand	120	3290	890	1280	1250	2420	1440	1680	1030	1330
Electric Blendor	120	4230	1050	1180	1310	2650	1130	1280	1190	1610

In using this method no significant distortion or crushing of eggs has been observed as the result of high speed mixing. The use of the electric blendor has greatly reduced the time required in preparing fecal suspensions and we are of the opinion that it does a more efficient job of producing a homogeneous suspension of nematode eggs. The blendor has also been found useful in making suspensions of large quantities of feces or in obtaining large numbers of nematode eggs for culture work.

Paratylenchus hamatus n. sp. and *Xiphinema index* n. sp., Two Nematodes Associated with Fig Roots, with a Note on *Paratylenchus anceps* Cobb

GERALD THORNE[1] and MERLIN W. ALLEN[2]

Specimens of fig roots, *Ficus carica* L., were sent to the Salt Lake City, Utah, station of the Division of Nematology, on August 17, 1943, by Mr. E. F. Serr, Extension Specialist in Deciduous Fruits, University of California. These roots had been collected from five orchards near Planada, California, three of them affected by a condition locally known as "leaf drop" (Fig. 1). Examination of these roots, and the soil accompanying them, revealed that a nematode, *Paratylenchus hamatus* n. sp. was present in all three of the lots which were from trees

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affected by "leaf drop." Two of these were from the variety White Adriatic and one from Calimyrna.[3] One lot from roots of the Kadota variety contained *P. hamatus* but the tree did not exhibit the characteristic "leaf drop," while the fifth lot, from an unaffected Black Mission tree, did not contain the nemas. Dis-

FIG. 1. Calimyrna fig tree exhibiting typical "leaf drop." Twig dieback and general decline are not yet evident.

[3] Standardized Plant Names states "This is not a variety name, though frequently so used. It is a trademarked term for Smyrna-type Figs grown in California. Since Lob Ingir is the only one of this type grown there, the name Calimyrna is commonly, though incorrectly, applied to that variety."

tribution of affected trees in the orchards is not uniform and the scattered pattern indicates that the nemas were introduced with the trees from the nurseries in which they were grown.

Conversations with men familiar with conditions in the affected orchards, and observations made by the junior author, indicate that the first symptom observed is a lighter color of the leaves, which gradually becomes more pronounced until the leaves die and fall. Fruit on these trees is undersized and generally falls along with the leaves. A slow decline of the entire tree takes place, culminating in die-back of twigs and small limbs. Conditions in the orchards observed may have been aggravated by inadequate irrigation water during the hot summer months. However, high nematode populations almost invariably accompanied the leaf drop and decline in the area observed.

Almost all of the *Paratylenchus hamatus* in the samples were immature forms lying dormant in the soil. A request was made that additional lots be collected after the winter rains and on March 17, 1944, Mr. E. A. Davey, Assistant Pomologist, University of California, made 11 collections from the two orchards previously found to be most severely infested, and populations of *P. hamatus* in these ranged from 6 to 3,000 per pound of soil. *Xiphinema index* n. sp. appeared in 5 lots from the Calimyrna orchard with populations of approximately 20, 60, 150, 250 and 700 per pound. Many specimens of *P. hamatus* were observed attached by their spears to the rootlets in these 11 lots, but no *X. index* was observed feeding. However, the absence of other plant roots in the samples indicates that the xiphinemas probably were dependent on the fig roots for their sustenance. The combined circumstantial evidence suggests that *P. hamatus* is a contributing, if not the primary, factor in this fig tree decline, and it is possible that *X. index* also is involved. Following are technical descriptions of the two species.

Paratylenchus hamatus n. sp. (Tylenchida, Criconematidae)

Figure 2

♀ : 0.35–0.40 mm.; a = 17; b = 4.3; c = 15; V = 50 84

♂ : 0.35–0.40 mm.; a = 24; b = 5.1; c = 13; T = 34.

Cuticle marked by rather coarse striae which are interrupted by the lateral fields which are about ¼ as wide as the body and marked by four incisures which appear as bright lines. The rounded lip region is continuous with the body contour and bears an inner circlet of four papillae grouped closely about the oral opening and an outer circlet of six which are located on the outer margin of the lips. Amphid apertures appear as minute refractive dots at the apices of the lateral lips when observed from a face view. Excretory pore and deirids located near the base of the neck. Phasmids not seen.

The strong, heavily knobbed spear of the female averages about 28 μ long. Dorsal esophageal gland opening into the esophageal lumen about 8 μ behind the spear. The strongly developed median esophageal bulb contains the usual refractive valvular apparatus. Basal esophageal bulb elongate-pyriform, joined to the intestine by a small, conoid cardia. Intestinal cells well filled with small, uniform, refractive granules. The intestine ends in a short rectum which opens through the very obscure anus; in fact it is frequently so difficult to see the rectum and anus that it appears possible that they actually are not functional.

The ovary begins with a cap cell followed by four or five oögonia arranged in single file, after which there is a short region of multiplication from which the developing oöcytes proceed in tandem until they become eggs. The eggs range in width from 12–16 μ and are 40–53 μ long. Vulva a broad, transverse slit with lateral membranes which from a ventral view are observed to extend inward and over the vulvar opening. These heretofore undescribed membranous structures have

been observed on all *Paratylenchus* females in the Salt Lake City, Utah, collection and probably constitute a generic character (Fig. 2, F).

The body of the male is more slender than that of the female and the spear is

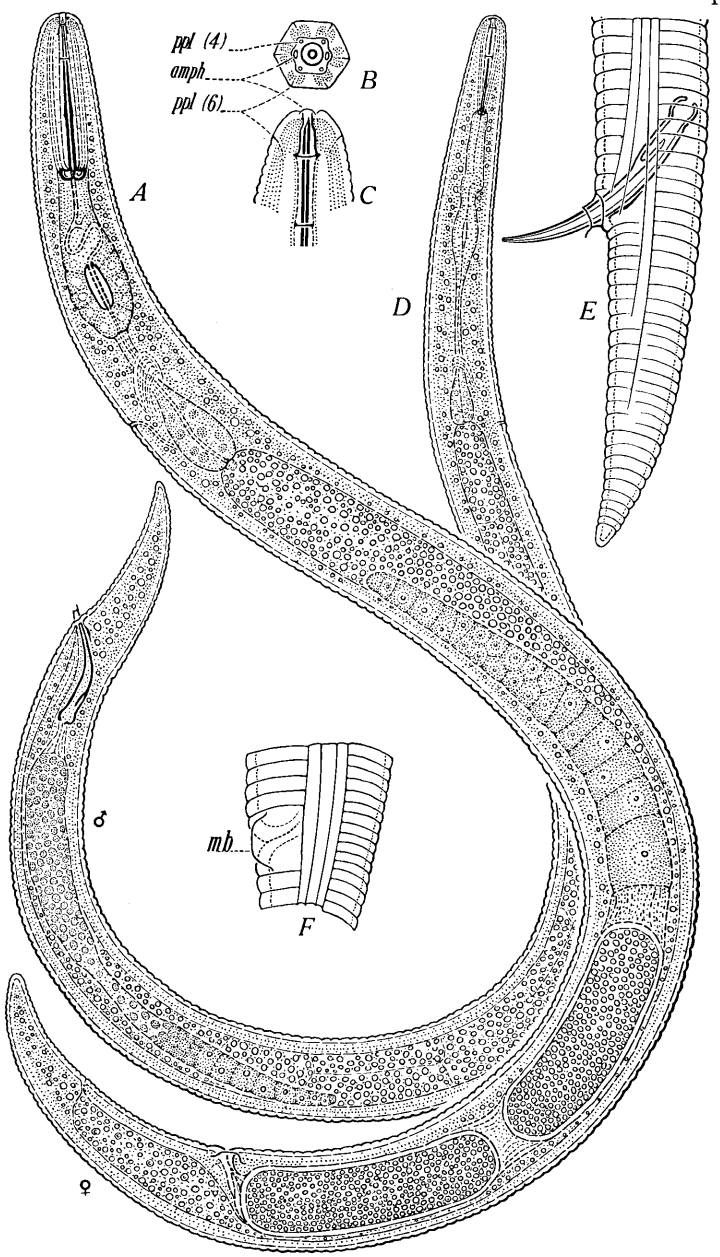

FIG. 2. *Paratylenchus hamatus.* A—Female; × 800. B—Face view showing the two circlets of cephalic papillae, *ppl* 4 and *ppl* 6, and amphid apertures, *amph;* × 1,600. C—Head; × 1,600. D—Male; × 800. E—Male tail; × 1,600. F—Vulvar region showing membrane, *mb;* × 1,600.

only moderately developed. The median bulb is weak and contains an obscure ovate valvular apparatus. Details of the testis are as illustrated (Fig. 2, D). The simple arcuate spicula rest on a thin trough-like gubernaculum. However, the most striking characteristic of the species is a sheath surrounding the spicula and protruding from the anal opening when the spicula are extruded. From the posterior margin of this sheath projects a hook-like process; hence the specific name, *hamatus*. In the region of the anal opening, the male tail is ventrally flattened or somewhat concave.

Diagnosis.—*Paratylenchus* with the above measurements and general description. Distinctive because the male retains its spear and possesses a protrusile anal sheath which surrounds the spicula and bears a posterior hook-like process.

Habitat.—Ectoparasitic on the roots of figs, *Ficus carica*, and inhabiting the soil surrounding them in orchards near Planada, California.

Relationships.—The Genus *Paratylenchus* includes species which may be placed in three groups:

1. Species in which the male possesses a normal spear somewhat smaller than that of the female: *P. macrophallus* (deMan, 1880) Goodey, 1934, and *P. hamatus*. The latter is distinguished by the presence of an anal sheath bearing a posterior hook-like process and by the absence of a narrow bursa which deMan figured for *P. macrophallus*. However this bursa illustrated by deMan may have been the margin of the ventrally concave tail seen from a slightly subdorsal angle.

2. Species in which the male spear is degenerate or missing: *P. besoekianus* Bally and Reydon, 1931, *P. minutus* Linford, 1949, and *P. elachistus* Steiner, 1949.

3. Species in which the male either does not occur or has not been collected: *P. bukowinensis* Micoletzky, 1922, *P. anceps* Cobb, 1923, and *P. nanus* Cobb, 1923. Of these species, *P. anceps* is distinctive because in the immature stage it has a well developed spear, as well as a most unusually long neck (Fig. 4).

Identification of *Paratylenchus* females unassociated with males is difficult because of their general similarities, except for *P. bukowinensis* which possesses a short posterior uterine branch.

Xiphinema index n. sp. (Dorylaimina, Dorylaimidae)
Figure 3

♀ : 3.4 mm.; a = 58; b = 7.6; c = 76; V = 15 38 16

♂ : 3.6 mm.; a = 63; b = 7.3; c = 88; T = 49

Lip region continuous with the neck contour, bearing the usual two circlets of 6 and 10 papillae. Amphids almost as broad as head with slender tubes leading back to the sensillae pouches. Minute fibrils are sometimes visible in the amphidial pouch. Lateral fields very narrow on the neck but gradually becoming broader until they are about one-fourth as wide as the neck. A series of lateral pores is present, appearing in a single line on the neck, then branching into two lines. Dorsal and ventral pores present near the head. Four pairs of caudal pores present on both males and females. Caudal cuticle radially striated.

Spear typical of the genus with the usual extensions and flanges, its total length averaging about 90 μ. Esophagus beginning as a slender tube which is reflexed when the spear is retracted. Just anterior to the nerve ring, a tiny spear can usually be seen in the esophageal tissues which marks the location of the cell from which the spear originated. Just before a moult, the fully developed spear occupies almost the entire length of the slender anterior portion of the esophagus (Fig. 3, E). Frequently the basal portion of the esophagus is shifted in position until the conspicuous dorsal gland may appear in a lateral or ventral position. The anterior pair of submedian gland nuclei generally is easily visible but the posterior pair frequently is very obscure.

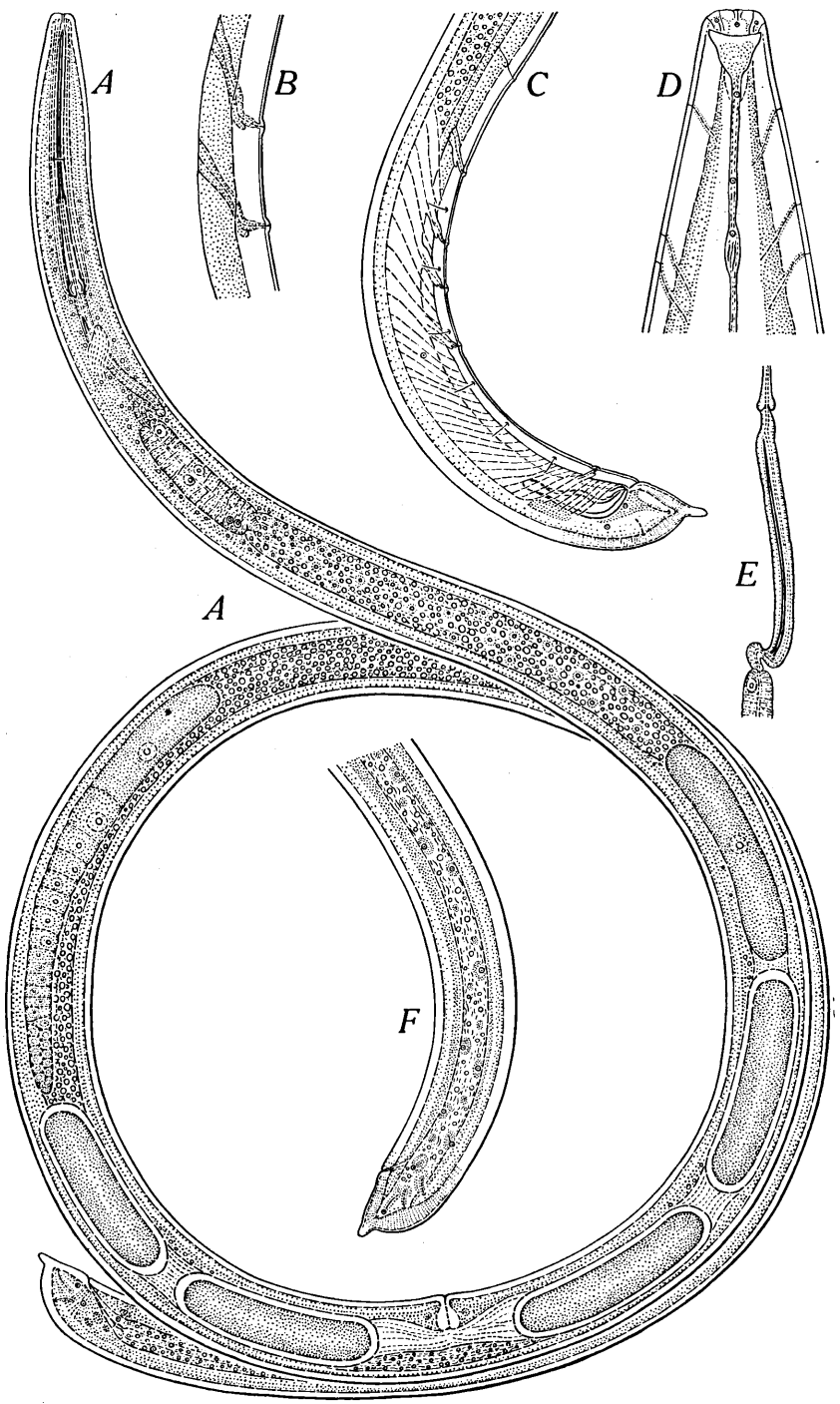

FIG. 3. *Xiphinema index*. A—Female; × 170. B—Detail of supplements; × 500. C—Posterior portion of male; × 250. D—Head showing form of amphid; × 665. E—Spear developing in anterior portion of esophagus of young specimen; × 250. F—Posterior portion of female; × 250.

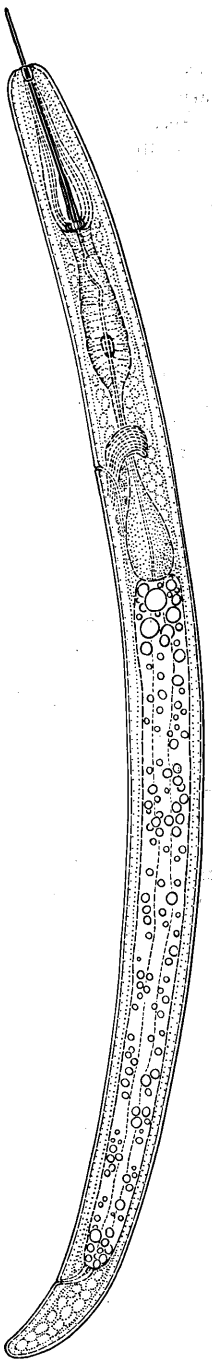

FIG. 4. *Paratylenchus anceps.* Immature specimen; ×800. (Drawn from pencil sketch made by W. E. Chambers in 1912.)

Cardia conoid. Intestine about 8 cells in circumference, generally filled with dark, uniform sized granules; sometimes the cell nuclei are prominent. Female prerectum 8 to 10 times as long as body width, its cells filled with granules similar to those of the intestine. Frequently the junction of the intestine and prerectum is difficult to observe because the tissues of the two organs are so similar.

Vulva a depressed, transverse slit. Ovaries approximately symmetrical, reflexed, but varying greatly in location because of the large eggs which crowd them out of position. Usually the anterior ovary lies on the right, the posterior on the left side of the body. Details of the development of the ova are as illustrated (Fig. 3, A).

Male tail bearing the usual adanal pair of supplements, a ventromedian series of four, and an innervated organ anterior to the supplement series. There also is a ventro-submedian series of seven pairs of innervated papillae. Musculature of the posterior portion of the male is a prominent feature. Spicula strong, arcuate, with small lateral guiding pieces.

Diagnosis.—Xiphinema with above general description and measurements. Most closely related to *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, from which it is distinguished by the more anterior position of the vulva, 38%: 47.7%; four pairs of male caudal pores compared with six for *diversicaudatum;* greater width of female, a = 58: 72; and greater length of neck, b = 7.6: 10.1. *X. index* is slightly smaller, 3.4: 4.0 mm., but this can scarcely be considered as a character of specific importance.

Habitat.—Hundreds of females and two males from soil about the roots of fig, *Ficus carica,* collected near Planada, California.

Paratylenchus anceps Cobb

The identity of *Paratylenchus anceps* Cobb, 1923, has been somewhat in question because illustrations were not published with the description and the measurements and characters given were rather indefinite from a diagnostic standpoint. Because of this Goodey, 1934, made *P. anceps* a synonym of *P. macrophallus* (deMan, 1880) Goodey, 1934.

Original notes by Cobb on the species from the files of the Division of Nematology include certain measurements and a pencil sketch by W. E. Chambers which throw new light on its identity, and these are here presented in an attempt to give the species more definite standing. Cobb's original description is as follows:

"*Paratylenchus anceps n. sp.*

$$\frac{24.\quad 28.\quad 34.\quad Y\quad 92.6\ (?)}{5.3\quad 5.3\quad 5.3\quad 4.6\quad 3.6}\ 0.28\ \text{mm.}$$

P. anceps so closely resembles *P. nanus* that only the differences need be here noted. The striae are one micron apart. The optical expression of the wings is a pair of refractive parallel lines whose distance apart is about equal to the width of two annules of the cuticle. The conoid neck becomes convex-conoid at the head, at the front of which the lip region is about four microns wide. The spear guide is six microns long, and the spear about half as long as the neck, the long slender anterior part comprising three-fourths or four-fifths of the whole. The three-lobed, flattish basal bulb of the spear is about one-fourth as wide as the corresponding portion of the neck, that is about four microns wide. The somewhat elongated-pyriform or pineapple-shaped posterior bulb is three-fifths as wide as the base of the neck. The deirids are near the base of the neck. The tail is slightly conoid to the broad, rounded terminus, which is half as wide as the base of the tail. The vulva was about to appear at the same relative position as in *P. nanus.* In all other respects almost precisely as in *P. nanus.*

Habitat: Roots of *Umbellularia californica,* Riverside, California, 1912."

The formula given above is on one work sheet in the file but the sketch from which it was prepared is not there. Three other formulae made from immature

specimens are present and these had been averaged to give the following measurements:

18.2	29.5	39.5	Y	95.0	0.2 mm.
4.8	5.2	5.5	4.9	3.2	

Transposed to the deMan system of proportional measurements these are:

Y: 0.28 mm.; a = 19; b = 2.9; c = 13.5 (?) Cobb, 1923

Y: 0.20 mm.; a = 18; b = 2.5; c = 20.0 Cobb notes, 1912

Y: 0.22 mm.; a = 17; b = 2.5; c = 15.0 Figure 4

The most striking characteristics of this species are the very long neck and the well developed spear which appear to be sufficiently important to separate it from immature specimens of other known species. Immature specimens of the genus rarely possess such well developed spears. Possibly the species is a *Cacopaurus*, not a *Paratylenchus*.

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A New Species of *Ascoschöngastia* from North Burma (Acarina: Trombiculidae)[1]

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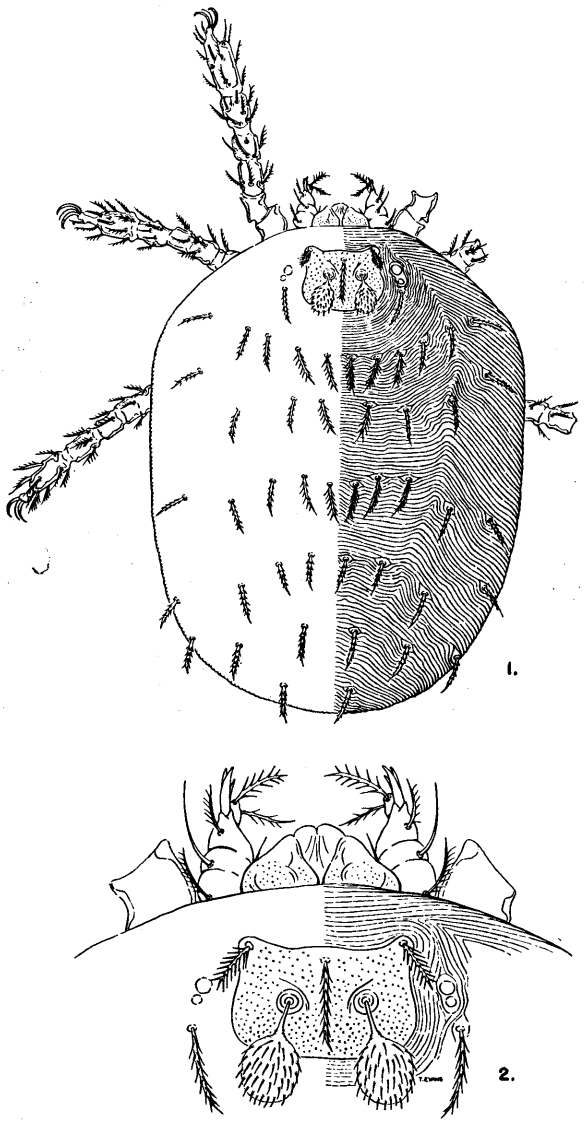
Among the larval trombiculid mites or chiggers collected by the United States of America Typhus Commission in Burma in 1944–1945 is a new species of *Ascoschöngastia* collected from *Rattus rattus* and from shrews.

Ascoschöngastia masta sp. nov.

Diagnosis.—Although the generic status is readily determined by the fact that the posterolateral setae are off the scutum or dorsal plate, the species is readily

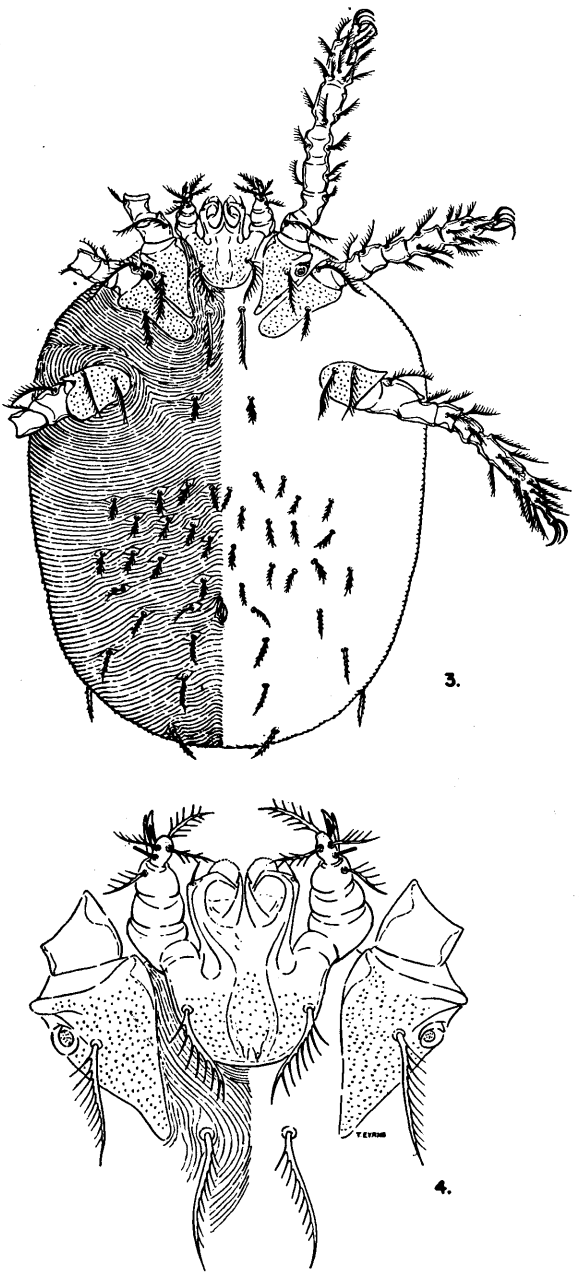
[1] Published under the auspices of The Surgeon General, U. S. Army, who does not necessarily assume responsibility for the professional opinions expressed by the authors.

separable from *A. malayensis* Gater, the genotype. In the new species there are about 45 dorsal setae, not 25; the third coxae are bisetose, not unisetose; the palpal claw is trifurcate, not bifurcate; the anterior margin of the scutum in the new species is slightly concave, not convex.



FIGS. 1–2. *Ascoschöngastia masta* sp. nov. 1—Dorsal aspect of larva. 2—Dorsal aspect of gnathosoma and scutum.

Larval description (Figs. 1–4).—*Body:* outline oval, often with two faint constrictions on the abdomen; length 287 μ; width 220 μ. *Dorsal plate:* small; broader than long; anterior margin somewhat concave, but middle portion slightly sinuate, although not extending as far anterior as shoulders. Lateral margins almost straight except where curving to straight posterior margin. Sensillae (pseudo-



FIGS. 3–4. *Ascoschöngastia masta* sp. nov. 3—Ventral aspect of larva. 4—Ventral aspect of gnathosoma and scutum.

stigmatic organs) clavate or subglobose with petiole almost as long as head. Bases of sensillae inserted slightly anterior to midline of scutum. With a faint crista above sensillae. Anterolateral bristles much shorter than anteromedian and posterolateral bristles. Anteromedian bristle inserted just too caudad to be in line

with anterolaterals. Posterolateral bristles in line with the eyes and at a level of the posterior eighth of scutum. *Eyes:* two pair; fairly well developed; inserted on a level slightly anterior to that of sensillae; diameter of anterior eye greater than that of posterior eye. *Chelicerae:* acuminate, with a single vestigial dorsal subapical tooth. *Palpal claw:* trifurcate; lateral prongs much shorter than middle prong. *Palpal tarsus:* about twice as long as broad at base; with an apical plumose bristle and two similar ventral ones and, in addition, a proximal ventral spur-like process. *Palpus:* with a proximal dorsal plumed bristle on palpal trochanter and femur; with a dorsal nude seta on genu and a dorsal and ventral frayed bristle on tibia. *Galea:* with a pair of plumed proximal ventral setae. *Dorsal setae:* essentially similar to posterolaterals in size and shape. Humerals not distinguishable from bristles of first row; about 46 in number, arranged 12(11)–8–10–8–6–2–. *Coxae:* I and II unisetose, III bisetose. *Sternal setae:* a pair of plumose setae between bases of coxae I and II and between coxae III. *Ventral setae:* about 36 in number; somewhat shorter and thinner than dorsal setae. *Legs:* I—0.22 mm.; II—0.17 mm.; III—0.19 mm. Sensory setae as follows: I—two genualae, one microgenuala, two tibialae, one microtibiala, one spur, one microspur, one pretarsala; II—two tibialae, one spur, one microspur, one pretarsala; III—apparently lacking specialized bristles. *Tarsal claws:* subequal, elongate, acuminate, curved, resembling the blade of a scythe.

Slide Number (Cotypes)	*Standard measurements (in microns)*										
	AW	PW	SB	ASB	PSB	A–P	AM	AL	PL	Sens.	DS
401–1	.....	70	21	14	13	.....	.....	.....	24	32	27
401–4	.....	66	19	.....	.....	.....	.....	.....	24	30	24
401–3	38	70	24	18	14	32	28	.....	24	.....	24
401–2	42	74	21	19	16	32	28	14	24	.....	24
Mean	40	70	21	17	14	32	28	14	24	31	25

Type material.—Cotypes (slide numbers 401–2 to 401–4 and 401–7 to 401–18 incl.), ex *Rattus rattus* collected at Myitkyina, North Burma, by members of the United States of America Typhus Commission, 23 November 1944. Deposited in the U. S. National Museum, Washington, the Rocky Mountain Laboratory of the U. S. Public Health Service, the British Museum and the South Australian Museum. Additional specimens from a tree-shrew, *Tupaia belangeri,* 24 December 1944 and a shrew, *Crocidura* sp., 2 February 1945, *ibid.*

Remarks.—We are indebted to Mr. Thomas Evans of the Department of Parasitology, Army Medical Department Research and Graduate School, for preparing the illustrations and to Lt. Vernon Tipton of the same institution for reviewing the descriptions.

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CORRECTION

The sixth entry in the Table of Contents on the back cover of Vol. 16, No. 2 was listed incorrectly. The paper beginning on page 104 is by Christie and not by Basir. Change the first line of this entry to read: "Christie, Jesse R. Host-parasite Relationships of the Root-Knot Nematodes, ".