VOLUME 7 JANUARY, 1940 NUMBER 1

PROCEEDINGS

of

The Helminthological Society of Washington

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

Subscription $1.00 a Volume; Foreign, $1.25

Published by
THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 7 JANUARY, 1940 NUMBER 1

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This number issued March 28, 1940.

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A review of the trematode superfamily Opisthorchioidea. EMMETT W. PRICE, U. S. Bureau of Animal Industry.

In the past trematode classifications have been based almost exclusively on adult characters. These classifications, while serving a practical purpose, often fail to show natural relationships; this is due to the difficulty experienced in distinguishing between characters of genetic significance and those resulting from convergent evolution.

During the past two decades increasing attention has been directed toward solutions of life histories and to studies of larval stages of trematodes. Accordingly, considerable data of value in indicating natural relationships have been accumulated. Unfortunately, however, too few life histories are known in many groups to permit of an entire revision of the existing classifications.

Owing to the importance, both in human and veterinary medicine, of the flukes comprising the Opisthorchiidae and Heterophyidae, more complete life histories are known in these families than in most others, and it is now possible to determine the components of the superfamily group to which these flukes belong.

HISTORICAL RÉSUMÉ

Faust (1929) proposed the superfamily "Opisthorchoidea" for the family "Opisthorchidae" and the superfamily Heterophyoidea for the family Heterophyidae. With regard to the Heterophyoidea, Faust stated that "further information may possibly justify the inclusion of the family Lecithodendriidae Odhner, 1910, and the subfamilies Microphallinae Ward, 1907, and Gymnophallinae Odhner, 1905." The principal character used for distinguishing these superfamilies was the miracidium, which was stated to be bilaterally symmetrical in the Heterophyoidea and asymmetrical in the "Opisthorchoidea." The cercariae were noted to be similar in both groups, those of the "Opisthorchoidea" lacking the "spinose armature" present in those of the Heterophyoidea.

In a paper appearing shortly after Faust's proposed classification, Witenberg (1929) erected the superfamily "Opisthorchoidea" for the families "Opisthorchidae" and Heterophyidae. The inclusion of both of these families in a single superfamily was based on "a common scheme of anatomical structure, both in adults and cercariae, and similar life histories." This proposal was concurred in by Vaz (1932).

In 1932 Faust definitely included in his superfamily Heterophyoidea the families Heterophyidae Odhner, Microphallidae Viana, and Lecithodendriidae Odhner, leaving the superfamily "Opisthorchoidea" to contain only the family Opisthorchidae Lühe. In his diagnosis of these superfamilies Faust (1932) again emphasized the importance of the symmetry and asymmetry of the miracidia as superfamily characters. He also considered the flame cell pattern as a character of superfamily value, stating that the fundamental pattern for the Heterophyoidea was $2((1+1)+(1+1))$ and that for the "Opisthorchoidea" was $2(2+2+2+2+2+2)$.

Ciurea (1933) accepted Faust's superfamilies and proposed a revision of the Heterophyoidea. In this classification Ciurea included in the Heterophyoidea the

families Heterophyidae Odhner, Cryptogonimidae Ciurea and Microphallidae Viana; the Lecithodendriidae Odhner was rejected because of the position of the vitelline glands. Ciurea's classification was based for the most part on the position of the reproductive organs. In the Heterophyidae were included the subfamilies Heterophyinae Ciurea, Metagoniminae Ciurea, Apophallinae Ciurea, Centrocestinae Looss, Cryptocotylinae Lühe, and Sigmaperinae Pöche. The latter subfamily, based on the single genus *Sigmapera* Nicoll, was already rejected, and rightly so, from the Heterophyidae by Witenberg (1929) because of the presence of a well developed cirrus pouch. Ciurea distinguished his new family Cryptogonimidae from the Heterophyidae because the uterus was largely posttesticular. To the Cryptogonimidae were assigned the subfamilies Cryptogoniminae Osborn, Neochasminae Mueller and Van Cleave, Galactosominae Ciurea, Haplorchiinae Looss, and Adleriellinae Witenberg. The family Microphallidae Viana included the subfamilies Microphallinae Ward, Maritreminae Nicoll, and Gymnophallinae Odhner. The inclusion of the Microphallidae in the same superfamily as the Heterophyidae by various authors, including Faust (1932) and Ciurea (1933), is not surprising since the adult forms closely resemble the heterophyids; the flame cell pattern is also identical with that of some heterophyids. This resemblance, however, is apparently a result of convergence since there are distinct differences both in morphology of the adults and in the life histories. Rothschild (1937), in a comprehensive review of the life histories and larval stages of the Microphallidae, has pointed out the resemblance of the cercariae of members of this family to those of the Plagiorchiidae, both families having larvae of the same type, that is, Xiphidiocercariae. It now seems certain that the Microphallidae, Lecithodendriidae, and Dicrocoeliidae should be included in the superfamily Plagiorchioidea Dollfus. That the cercariae of members of the Microphallidae were of the xiphidiocercarial type was recognized by Faust as early as 1924, but this fact was apparently lost sight of by him in later publications (Faust, 1929; 1932).

Vogel (1934) is in agreement with Witenberg (1929) in including in the same superfamily the families Heterophyidae and Opisthorchiidae, proposing for them the new superfamily name Opisthorchioidea. This abolition of the superfamily Heterophyoidea Faust was based on the fact that the cercariae in both families were of the same type. Vogel also points out that the asymmetry of the miracidia of the Opisthorchiidae, one of the characters which Faust considered as important in separating the opisthorchiids and heterophyids into separate superfamilies, is in fact only an asymmetry of the secretory gland cells; such asymmetry was noted by him in the miracidia of *O. tenuicollis* and also on one occasion in the miracidium of a heterophyid, *Apophallus mühlingi*. A comparison of the illustration of the miracidium of *Clonorchis sinensis* as given by Faust and Khaw (1927) with that of *Opisthorchis tenuicollis* given by Vogel (1934) shows great similarity, there being a single sausage-shaped cell on one side only. This unusual position of the cells suggests that the miracidia of these species had been drawn in lateral view, and that the asymmetry is apparent rather than real. This suggestion is supported by the fact that in the illustration of the egg of a heterophyid, *Cryptocotyle lingua*, given by Stunkard (1930) the miracidium is drawn in lateral view showing the two cells in a lateral position, one partly obscuring the other, a condition approaching that of miracidia of *C. sinensis* and *O. tenuicollis*.

CERCARIAL TYPE AS EVIDENCE OF SUPERFAMILY RELATIONSHIP

A review of the life histories of the heterophyid and opisthorchiid trematodes shows that all have cercariae sufficiently similar in type to indicate close relationship. These cercariae belong to the Pleurolophocerca and Parapleurolophocerca groups as established by Sewell (1922); they develop in rediae which are provided

with short intestinal ceca but without collars and locomotor appendages. Up to the present more or less complete life histories have been described for at least 20 flukes having cercariae of these types, as follows: *Stamnosoma formosanum* Nishigori (= *Centrocestus formosanus* (Nishigori)), by Nishigori (1924a); *Monorchotrema taihokui* Nishigori (= *Haplorchis pumilio* (Looss)), by Nishigori (1924b) and Faust and Nishigori (1926); *M. taichui* Nishigori (= *H. taichui* (Nishigori)), by Nishigori (1924a) and Faust and Nishigori (1926); *Clonorchis sinensis* (Cobbold) by Faust and Khaw (1927) and Yamaguti (1935); *Cercaria floridensis* McCoy (= *Acanthostomum floridensis* (McCoy)), by McCoy (1929); *Stamnosoma armatum* (Tanabe) (= *Centrocestus armatus* (Tanabe)), by Takahashi (1929a) and Yamaguti (1938a); *Metagonimus yokogawai* (Katsurada), by Takahashi (1929b) and Yamaguti (1933); *M. takahashii* (Suzuki), by Takahashi (1929b); *Exorchis major* Hasegawa (= *Pseudexorchis major* (Hasegawa)), by Takahashi (1929b); *Cryptocotyle lingua* (Creplin), by Stunkard (1930); *Kasr aini* Khalil (= *Haplorchis pleurolophocerca* (Sonsino)), by Khalil (1932); *Opisthorchis felineus* (Rivolta) (= *O. tenuicollis* (Rudolphi)), by Vogel (1934); *Metagonimoides* (?) *oregonensis* Price, by Ingles (1935); *Apophallus venustus* (Ransom), by Cameron (1937); *Heterophyes heterophyes* (Siebold), by Khalil (1937); *Metorchis intermedius* Heinemann, by Heinemann (1937); *Cryptocotyle jejuna* (Nicoll), by Rothschild (1938a); *Cercaria coronanda* Rothschild (= *Acanthostomum coronandum* (Rothschild)),[1] by Rothschild (1938b); *Euryhelmis monorchis* Ameel, by Ameel (1938); and *Caecincola parvulus* Marshall and Gilbert, by Lundahl (1939).

An analysis of the characters exhibited by the cercariae of these species (Table 1) shows sufficient similarity as to leave little doubt that all belong to a single superfamily group. All except the cercaria of *Euryhelmis monorchis* possess eyespots; all have rudimentary acetabula; all are apparently provided with oral spines;[2] and all except species of *Centrocestus* are provided with tail fin-folds.

In those species possessing tail fin-folds, these folds are dorsoventral (ventral only in *Metagonimoides* sp. (Ingles, 1935)) except in the species of *Haplorchis*. In species of *Haplorchis* the fin-folds are lateral and this position has been regarded by Rothschild (1938b) as of possible family significance. Whether all Parapleurolophocerca cercariae belong to the genus *Haplorchis*, as Rothschild suggests, or whether more than one subfamily or possibly family is represented remains to be determined. In the cercariae of the three species of *Haplorchis* for which the adults are known, one character (not given in table 1) seems to be correlated with the lateral type of fin-fold; this character is the extent of the penetration glands posteriorly. In the cercariae of *Haplorchis pumilio*, *H. taichui*, and *H. pleurolophocerca* the penetration glands are linear in arrangement and extend to the posterior part of the cercarial body, and the gland ducts are not grouped in bundles as in the other species listed. In none of the other parapleurolophocercous cercariae does this gland arrangement occur, and it may be that when the cercariae of the other species of the genus *Haplorchis* are known this combination of characters may be found to have definite taxonomic significance.

The other cercarial characters show such wide variation that little more than specific value can at present be ascribed to them. Even the excretory system, which has been regarded by several investigators as having great taxonomic value,

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[1] *Cercaria coronanda* was regarded by Rothschild (1938b) as belonging to the subfamily Neochasminae, an error which has been subsequently corrected (Rothschild, 1940).

[2] Faust and Khaw (1927) state that oral spines are absent in the cercaria of *Clonorchis sinensis;* this is an error since Yamaguti (1935) has shown them to be present and similar to those of *Opisthorchis tenuicollis.*

TABLE 1.—*Characters of cercariae of known species of Opisthorchioidea*

Species	Eyes	Acetabulum	Oral spines	Penetration glands	Penetration gland ducts	Excretory system			Tail fin-folds
						Bladder	Duct pattern	Flame cell pattern	
Opisthorchis tenuicollis	present	rudimentary	present	20	4, 6, 6, 4	reniform	stenostoma	2((5) + (5 + 5 + 5 + 5))	dorso-ventral
Clonorchis sinensis	do	do	do	14	3, 4, 4, 3	triangular	do	[a]	do
Metorchis intermedius	do	do	do	14	3, 4, 4, 3	round	[a]	[a]	do
Acanthostomum coronandum	do	do	do	20	4, 6, 6, 4	butterfly-shaped	mesostoma	2((2 + 2) + (2 + 2))	do
A. floridensis	do	do	do	14	3, 4, 4, 3	Y-shaped	[a]	[a]	do
Pseudexorchis major	do	do	do	14	3, 4, 4, 3	V-shaped	stenostoma	[a]	do
Caecincola parvulus	do	do	do	14	(?) 3, 4, 4, 3	round	[a]	2((2 + 2) + (2 + 2))	do
Heterophyes heterophyes	do	do	[a]	14	(?) 3, 4, 4, 3	[a]	[a]	[a, b]	do
Metagonimus yokogawai	do	do	present	14	3, 4, 4, 3	round	[a]	[a]	do
M. takahashii	do	do	do	14	3, 4, 4, 3	do	[a]	[a]	do
Metagonimoides sp.	do	do	[a]	[a]	[a]	Y-shaped	[a]	[a]	ventral
Cryptocotyle lingua	do	do	present	18	4, 5, 5, 4	reniform	stenostoma	[a, c]	dorso-ventral
C. jejuna	do	do[d]	do[d]	14	3, 4, 4, 3	do[d]	do[d]	[a]	do
Apophallus venustus	do	do	[a]	16	4, 4, 4, 4	V- or Y shaped	[a]	[a]	do
Euryhelmis monorchis	absent	do	[a]	12	(?) 3, 3, 3, 3	V-shaped	[a]	2(2 + 2 + 3 + 2)	do
Centrocestus armatus	present	do	present	8	2, 2, 2, 2	reniform	mesostoma	2((2 + 2) + (2 + 2))	absent
C. formosanus	do	do	do	[a]	[a]	butterfly-shaped	[a]	[a]	do
Haplorchis pumilio	do	do	do	14	linear	triangular	stenostoma	[a]	lateral
H. taichui	do	do	do	14	do	do	do	[a]	do
H. pleurolophocerca	do	do	(?) do	14	(?) do	do	[a]	[a]	do

[a] Not given.
[b] 2((3 + 3) + (3 + 3)), according to Looss (1894).
[c] 2((3 + 7 + 7) + (7 + 7 + 7)) in metacercaria, according to Stunkard (1929).
[d] According to Rothschild (personal communication).

shows greater variation than one might reasonably expect. The excretory bladder varies from round to Y-shaped, with all intermediate shapes. The collecting duct pattern is in general of the "stenostoma" type but in *Cercaria coronanda* (Acanthostomidae) and in the cercaria of *Centrocestus armatus* (Heterophyidae) it is of the "mesostoma" type. The flame cell pattern varies from 2((5) + (5+5+5+5)) in the cercaria of *Opisthorchis tenuicollis* (Vogel, 1934) and 2((3)+(3+3+3+3+3)) in the adult of *Opisthorchis pedicellata* (Verma, 1927) in the Opisthorchiinae (Opisthorchiidae); to 2((2+2)+(2+2)) in *Cercaria coronanda* (Acanthostomidae) (Rothschild, 1938b), 2((2+2)+(2+2)) in *Caecincola parvulus* (Cryptogonimidae) (Lundahl, 1939), 2((3+3)+(3+3)) in *Heterophyes heterophyes* (Heterophyinae; Heterophyidae) (Looss, 1894), 2(2+2+3+2) in *Euryhelmis monorchis* (Apophallinae; Heterophyidae) (Ameel, 1938), 2((2+3)+(3+2+3)) in the metacercaria of *Apophallus donicus* (Apophallinae) (Hsü, 1935), and 2((3+7+7)+(7+7+7)) in the metacercaria of *Cryptocotyle lingua* (Cryptocotylinae; Heterophyidae) (Stunkard, 1929). The flame cell patterns of the other species of Opisthorchioidea are not known, and in view of the above it appears unwise to attempt to base major groups on this character. This is especially true, since in the case of *Pseudamphistomum truncatum* (Metorchiinae; Opisthorchiidae) the collecting duct pattern, as figured by Dollfus (1936), suggests that the anterior and posterior groups of flame cells are equal in number instead of unequal as in *Opisthorchis*.

From the above discussion it appears that it is the *type of cercaria*, and not the characters exhibited by any of the larval organ systems, which is of importance in uniting the Opisthorchiidae, Heterophyidae, Acanthostomidae, and Cryptogonimidae into a single major group or superfamily.

ADULT CHARACTERS AS EVIDENCE OF SUPERFAMILY RELATIONSHIP

The adult flukes comprising the Opisthorchioidea as constituted in this paper are fundamentally very similar in organization, although superficially some of them appear quite different. The cuticula is usually provided with scale-like spines and the musculature is in most instances weakly developed, giving the worms a translucent appearance. The excretory system consists of a Y-shaped bladder and the usual duct system. The shape of the bladder is of two general types; one type is characteristic of the Opisthorchiidae and Heterophyidae, and consists of a short stem and short branches; the other type is characteristic of the Acanthostomidae and Cryptogonimidae, and consists of a relatively long stem with spacious branches extending into the anterior part of the body. In the Opisthorchiidae the stem is usually more or less sigmoid, branching at or near the level of the posterior margin of the ovary, forming short limbs extending anteriorly no farther than the level of the anterior margin of the ovary. In the Heterophyidae the stem of the bladder is short and frequently quite wide, with short limbs, the entire bladder often approaching a triangular shape. In the Acanthostomidae the stem of the bladder is relatively long and straight, branching in the vicinity of the middle of the body; the limbs are long and spacious and extend to or near the level of the pharynx. In the Cryptogonimidae the stem of the bladder is relatively short and the limbs long and spacious as in the Acanthostomidae.

The reproductive system is fundamentally similar in all four families and is characterized particularly by the absence of a cirrus pouch, the presence of a seminal receptacle, and the union of the terminal portions of the male and female ducts to form a hermaphrodytic duct. The ovary is in general pretesticular, although in some genera, notably *Pachytrema* and *Microtrema* (Opisthorchiidae), the ovarian and testicular zones partly or completely overlap. The position of

the testes, however, is so variable that this character is frequently of little more than specific value.

In the Heterophyidae and Cryptogonimidae there is, in practically all cases where a careful study has been made, a small structure known as the gonotyl or genital sucker situated in the region of the genital pore. This structure assumes a variety of shapes, but is usually papilla-like. The presence of this structure has been taken by some investigators, including Van Cleave and Mueller (1932, 1934) Mueller and Van Cleave (1932), Manter (1934), and Srivastava (1939), as evidence of family relationship and as a result many of the genera comprising such subfamilies as Neochasminae, Cryptogoniminae, and Siphoderinae have been included in the Heterophyidae. While gonotyls appear to be more or less characteristic of the Heterophyidae and Cryptogonimidae, it is possible that they may also occur in modified form in members of the Opisthorchiidae and Acanthostomidae, especially in the immature stages. This possibility is suggested by the fact that Rothschild (1938b) has reported a gonotyl-like structure in the metacercaria of *Cercaria coronanda* (Acanthostomidae).

In considering the relationships of the genera comprising the superfamily Opisthorchioidea the one character in the adult flukes that seems to be of particular value in determining affinities is the shape of the excretory vesicle. Taking this character as a basis, the genera *Opisthorchis, Cyclorchis, Pachytrema, Metorchis, Heterophyes, Metagonimus, Apophallus,* and *Centrocestus,* in which the excretory vesicle does not extend anteriorly beyond the level of the ovary, must be regarded as more closely related than such genera as *Acanthostomum, Oesophagicola, Cryptogonimus,* and *Metadena,* in which the limbs of the vesicle extend to the region of the pharynx. As further indication of this relationship, it may be noted that the opisthorchiids and heterophyids, with few exceptions, are parasites of warm blooded vertebrates, while acanthostomids and cryptogonimids are usually parasites of cold blooded vertebrates. Moreover, there is a persistence of eyespots in so many of the acanthostomids and cryptogonimids that it appears likely that if a careful examination were made it would be found that all members of these two groups would show at least remnants of these structures. So far as the writer is aware eyespots have not been reported as present in adult opisthorchiids and heterophyids.

The separation of the Opisthorchioidea into families on the basis of the excretory vesicle seems to be reasonably satisfactory. Difficulties are experienced, however, when an attempt is made to find an entirly satisfactory character or combination of characters for subfamily groups, since several possible groupings may be effected depending upon the characters selected. Thus, for example, in the Opisthorchiidae the genera *Opisthorchis, Clonorchis, Amphimerus, Cyclorchis, Cladocystis, Pachtrema* and *Diasia* may be regarded as belonging to one subfamily, since the uteri and vitellaria are confined to the postacetabular region, and *Metorchis, Parametorchis, Pseudamphistomum, Holometra* and *Microtrema* to another subfamily because the uteri and vitellaria do extend to some extent beyond the acetabulum. On the other hand, *Pachytrema* and *Microtrema* might be regarded as forming the nucleus of a subfamily, since the primary excretory ducts lie entirely in the intercecal field and because the ovarian and testicular zones partially or completely overlap. Furthermore, *Cladocystis, Pseudamphistomum,* and *Holometra* could be regarded as belonging to one subfamily because the ovary is relatively far removed from the testes and separated from them by uterine loops. Similar situations occur in the other families and because of this it appears that subfamily groupings must be based for the most part on characters that are largely subjective and arbitrary.

SUGGESTED REVISION OF THE OPISTHORCHIOIDEA

OPISTHORCHIOIDEA (Faust, 1929) Vogel, 1934

Synonyms.—Opisthorchoidea Faust, 1929; Heterophyoidea Faust, 1929.

Diagnosis.—Body usually spiny, with poorly developed musculature; eyes present or absent; excretory vesicle Y-shaped; cirrus pouch absent; ovary pretesticular; seminal receptacle present; metraterm and ejaculatory duct united to form hermaphroditic duct; eggs small and containing miracidia. Cercariae pleurolophocercous or parapleurolophocercous larvae provided with eyespots, rudimentary acetabula, without stylets but with 2 to 3 short transverse rows of oral spines, developing in simple rediae provided with short intestine and without ambulatory appendages.

I. OPISTHORCHIIDAE Braun, 1901

Synonym.—Opisthorchidae Lühe, 1901.

Diagnosis.—Medium sized to small flukes, usually elongated, with suckers more or less weakly developed; eyes absent; excretory vesicle Y-shaped, with short branches not extending anteriorly beyond level of ovary; genital aperture immediately preacetabular; without distinct genital sinus and without gonotyls; vitellaria usually extracecal and pregonadal.

Type genus.—*Opisthorchis* R. Blanchard, 1895.

1. OPISTHORCHIINAE Looss, 1899.

Diagnosis.—Uterus and vitellaria not extending beyond level of acetabulum.

Genera.—*Opisthorchis* R. Blanchard, 1895 (syns. *Notaulus* Skrjabin, 1913; *Gomtia* Thapar, 1930); *Clonorchis* Looss, 1907; *Amphimerus* Barker, 1911; *Cyclorchis* Lühe, 1908; *Cladocystis* Poche, 1926; *Pachytrema* Looss, 1907; *Diasia* Travassos, 1922.

The genus *Clonorchis*, as Morgan (1927) has shown, does not differ sufficiently from *Opisthorchis* to be regarded as a distinct genus; it has been retained only because it has become so firmly established in medical literature.

2. METORCHIINAE Lühe, 1909

Diagnosis.—Uterus and vitellaria extending anteriorly beyond level of acetabulum.

Genera.—*Metorchis* Looss, 1899; *Parametorchis* Skrjabin, 1913 (restricted to contain only *P. complexus* Stiles and Hassall, 1894); *Pseudamphistomum* Lühe, 1908; *Holometra* Looss, 1899; *Microtrema* Kobayashi, 1915.

3. RATZIINAE (Dollfus, 1929)

Synonym.—Ratzinae Dollfus, 1929.

Diagonsis.—Rudimentary cirrus pouch present; vitellaria extending anterior to acetabulum and into testicular zone.

Genus.—*Ratzia* Poche, 1926.

4. PHOCITREMATINAE Yamaguti, 1933

Diagnosis.—Vitellaria extending from level of base of seminal vesicle to posterior end of body; seminal receptacle preovarial.

Genera.—*Phocitrema* Goto and Ozaki, 1930; *Witenbergia* Vaz, 1932.

The genus *Phocitrema* bears great resemblance to the Heterophyidae and was included in that family by Price (1932); it apparently differs from the heterophyids only in the absence of a gonotyl. The genus *Witenbergia* is included provisionally in this subfamily; its resemblance to the Heterophyidae is as great as

to the Opisthorchiidae, and except for the apparent absence of a gonotyl it might well be included in the former family. In the shape of the oral sucker and in its host relationship (parasite of fish), *Witenbergia* also resembles some members of the Acanthostomidae; however, it may be retained in the Opisthorchiidae until a more complete description of the type species, especially of its excretory vesicle, is available.

The subfamily Delphinicolinae, which was proposed by Yamaguti (1933) for *Delphinicola tenuis*, from the bile ducts of a cetacean, is excluded from the Opisthorchiidae since it undoubtedly belongs to the Campulinae (Fasciolidae). If *D. tenuis* were restudied, especially sectioned material, the anteriorly directed ceca and a cirrus pouch would probably be found present.

II. HETEROPHYIDAE Odhner, 1914

Synonyms.—Coenogonimidae Nicoll, 1907; Cotylogonimidae Nicoll, 1907; Haplorchidae Travassos, in Viana, 1924; Stictodoridae Poche, 1926.

Diagnosis.—Small to very small flukes, usually oval to piriform in outline; acetabulum usually inclosed in genital sinus; eyes absent; excretory vesicle Y-shaped, sometimes almost triangular, limbs not extending anterior to ovarian level; genital sinus variously modified and containing a cirrus-like body or gonotyl (genital sucker of authors); 1 to 2 testes, in posterior end of body; vitellaria usually inter- and extracecal.

Type genus.—*Heterophyes* Cobbold, 1866.

1. HETEROPHYINAE Ciurea, 1924

Diagnosis.—Acetabulum not enclosed in genital sinus; gonotyl postero-lateral to acetabulum, bearing a row of chininoid rodlets.

Genera.—*Heterophyes* Cobbold, 1866; *Heterophyopsis* Tubangui and Africa, 1938 (syn. *Pseudoheterophyes* Yamaguti, 1939); and *Knipowitschiatrema* Isaichikov, 1927, according to Yamaguti (1939).

2. METAGONIMINAE Ciurea, 1924

Diagnosis.—Acetabulum lateral, enclosed in genital sinus; gonotyl inconspicuous, in form of 1 or 2 papilla-like bodies.

Genera.—*Metagonimus* Katsurada, 1913 (syns. *Loossia* Ciurea, 1915; *Yokogawa* Leiper, 1913; *Dexiogonimus* Witenberg, 1929); *Metagonimoides* Price, 1931; *Acetodextra* Pearse, 1924.

3. CRYPTOCOTYLINAE Lühe, 1909

Diagnosis.—Acetabulum median, rudimentary, in anterior wall of the spacious, more or less muscular genital sinus; genital aperture postacetabular; gonotyl single, papilla-like.

Genera.—*Cryptocotyle* Lühe, 1899 (syns. *Tocotrema* Looss, 1899; *Hallum* Wigdor, 1918; *Ciureana* Skjabin, 1923); *Scaphanocephalus* Jägerskiöld, 1903; *?Taphrogonimus* Cohn, 1904.

4. APOPHALLINAE Ciurea, 1924

Diagnosis.—Acetabulum relatively well developed, inclosed in small, non-muscular genital sinus; genital aperture preacetabular; gonotyl single or double, papilla-like.

Genera.—*Apophallus* Lühe, 1909 (syns. *Rossicotrema* Skrjabin and Lindtrop, 1919; *Cotylophallus* Ransom, 1920); *Euryhelmis* Poche, 1926 (syn. *Eurysoma* Dujardin, 1845, nec. Gistl. 1829); *Tauridiana* Isaichikov, 1925; *Ponticotrema* Isaichikov, 1927; *Pricetrema* Ciurea, 1933.

Ponticotrema Isaichikov (1927) is probably identical with *Tauridiana* Isaichikov (1925) which was based on an immature specimen; the internal organization of the two forms, so far as can be determined, is essentially similar.

5. Galactosominae Ciurea, 1933

Synonym.—Cercarioidinae Witenberg, 1929.

Diagnosis.—Acetabulum greatly reduced or absent; gonotyl globular, usually with spines; uterus largely postovarial.

Genera.—*Galactosomum* Looss, 1899 (syns. *Microlistrum* Braun, 1901; *Cercarioides* Witenberg, 1929; *Tubanguia* Srivastava, 1935); *Stictodora* Looss, 1899 (syn. *Cornatrium* Onji and Nishio, 1916); *Acanthotrema* Travassos, 1929.

6. Centrocestinae Looss, 1899

Diagnosis.—Anterior end with 1 or 2 rows of circumoral spines; acetabulum relatively well developed, included in genital sinus; gonotyl, when present, consisting of 1 or 2 papilla-like bodies.

Genera.—*Centrocestus* Looss, 1899 (syn. *Stamnosoma,* Tanabe, 1922; *Stephanopirumus* Onji and Nishio, ?1924); *Ascocotyle* Looss, 1899; *Pygidiopsis* Looss, 1907; *Phagicola* Faust, 1920 (syns. *Parascocotyle* Stunkard and Haviland, 1924; *Metascocotyle* Ciurea, 1933).

7. Haplorchiinae Looss, 1899

Synonyms.—*Haplorchidinae* Pratt, 1902; *Haplorchinae* Poche, 1926.

Diagnosis.—Circumoral spines absent; acetabulum rudimentary; gonotyl relatively large, armed with chitinous rodlets or other armature; expulsor absent; one testis.

Genera.—*Haplorchis* Looss, 1899 (syn. *Monorchotrema* Nishigori, 1924; *Kasr* Khalil, 1832).

8. Stellantchasminae Price, 1939

Diagnosis.—Acetabulum, when present, small, lateral, inclosed in genital sinus; gonotyl, when present, relatively large and spiny; distal portion of seminal vesicle in form of prominent muscular "expulsor"; 1 or 2 testes.

Genera.—*Stellantchasmus* Onji and Nishio, 1916 (syn. *Diorchitrema* Witenberg, 1929); *Procerovum* Onji and Nishio, ?1924.

9. Adleriellinae Witenberg, 1930

Synonym.—Adleriinae Witenberg, 1929.

Diagnosis.—Acetabulum absent; gonotyl prominent, bearing large spines; testis anterior to ovary and seminal vesicle.

Genus.—*Adleriella* Witenberg, 1930 (syn. *Adleria* Witenberg, 1929, nec Rohwer and Fagan, 1917).

III. ACANTHOSTOMIDAE Poche, 1926

Synonym.—Acanthochasmidae Nicoll, 1914.

Diagnosis.—Body elongate, usually slender; suckers relatively well developed; circumoral coronet of spines present or absent; eyes usually present; excretory vesicle Y-shaped, with long stem and spacious branches extending anteriorly to about level of pharynx; genital aperture preacetabular; gonotyls absent in adults.

Type genus.—*Acanthostomum* Looss, 1899.

1. Acanthostominae Nicoll, 1914

Synonyms.—Acanthochasminae Nicoll, 1915; Anoiktostominae Nicoll, 1915.

Diagnosis.—Circumoral spines present; ovary without lobes; vitellaria and uterus pretesticular.

Genera.—*Acanthostomum* Looss, 1899 (syn. *Acanthochasmus* Looss, 1900; *Caimanicola* Freitas and Lent, 1938); *Anoiktostoma* Stossich, 1899.

2. OESOPHAGICOLINAE Yamaguti, 1933

Diagnosis.—Circumoral spines absent; ovary without lobes; vitellaria extending to posterior end of body.

Genus.—*Oesophagicola* Yamaguti, 1933.

Yamaguti (1933) places this subfamily in the Opisthorchiidae, but owing to the fact that the branches of the excretory vesicle extend into the anterior part of the body it cannot be retained in that family.

3. ANISOCOELIINAE Looss, 1901

Diagnosis.—Circumoral coronet of spines present or (?) absent; ovary lobed or entire; uterus extending into posttesticular region.

Genera.—*Anisocoelium* Lühe, 1900; *Anisocladium* Looss, 1902 (syn. *Anisogaster* Looss, 1901, nec Deyr, 1863).

4. ISOCOELIINAE Price, 1939

Diagnosis.—Circumoral coronet of spines absent; eyespots present; ovary deeply lobed; uterus entirely pretesticular.

Genera.—*Isocoelium* Ozaki, 1927; *Paraisocoelium* Ozaki, 1932.

IV. CRYPTOGONIMIDAE Ciurea, 1933

Diagnosis.—Small flukes, oval to piriform, rarely linguiform, in outline; anterior end with or without circumoral coronet of spines; eyespots frequently, if not always, present; excretory vesicle short Y- or V-shaped, limbs spacious and extending into vicinity of pharynx; goontyls present in many, if not all, species; uterus extending into posttesticular part of body.

Type genus.—*Cryptogonimus* Osborn, 1903.

1. CRYPTOGONIMINAE Ward, 1917

Synonym.—*Exorchiinae* Yamaguti, 1938.

Diagnosis.—Circumoral coronet of spines usually absent; eyespots present; gonotyls present in some, and probably all species; ovary rosette-like; vitellaria largely pretesticular.

Genera.—*Cryptogonimus* Osborn, 1903; *Caecincola* Marshall and Gilbert, 1905; *Centrovarium* Stafford, 1904; *Aphallus* Poche, 1926; *Paracryptogonimus* Yamaguti, 1934; *Mehrailla* Srivastava, 1939; *Exorchis* Kobayashi, 1921; *Pseudexorchis* Yamaguti, 1938; *Biovarium* Yamaguti, 1934; *Metadena* Linton, 1910 (syn. *Stegopa* Linton, 1910).

2. NEOCHASMINAE Van Cleave and Mueller, 1932

Diagnosis.—Circumoral coronet of spines present; gonotyls present; ovary usually follicular; vitellaria preovarial, in equatorial or preequatorial zones.

Genera.—*Neochasmus* Van Cleave and Mueller, 1932; *Allacanthochasmus* Van Cleave, 1922; *Opisthometra* Poche, 1926 (syn. *Lacerdaia* Travassos, 1931).

Opisthometra Poche is provisionally included in this subfamily although it bears considerable resemblance to members of the Centrocestinae (Heterophyidae). In general the topography of the organ systems suggests affinities with the Neochasminae; however, the two species belonging to this genus are from bird hosts, which suggests heterophyid affinities, but it is retained here pending a description of the excretory vesicle.

3. SIPHODERINAE Manter, 1934

Diagnosis.—Circumoral coronet of spines absent; remnants of eyespots present; gonotyl in form of pseudosucker surrounding acetabulum; testes 2 or more, in equatorial zone; ovary rosette-like; vitellaria pretesticular.

Genera.—*Siphodera* Linton, 1910; *Siphoderina* Manter, 1934.

4. POLYORCHITREMATINAE (Srivastava, 1939)

Synonym.—*Polyorchitreminae* Srivastava, 1939.

Diagnosis.—Gonotyl anterior to acetabulum; testes numerous, in posterior third of body; ovary compact or slightly lobed; vitellaria pre- and postovarial; uterus pretesticular.

Genus.—*Polyorchitrema* Srivastava, 1937.

Srivastava (1939) places this subfamily in the Heterophyidae, noting that it stands nearest to *Siphodera* Linton (1910). Neither Polyorchitremainae nor Siphoderinae (Manter, 1934) can be included in the Heterophyidae since the excretory vesicles of representatives of both subfamilies are of the type characteristic of the Crypotogonimidae.

APPENDIX

The subfamily Maseniinae, which was proposed by Chatterji (1933) for *Masenia collata* and placed in the Acanthostomidae, cannot be included at present in either the Acanthostomidae or the Cryptogonimidae because of the presence of a cirrus pouch. While in general this fluke has characters relating it to the Acanthostomidae, the nature of the cirrus pouch suggests affinities with the Acanthocolpidae.

The family Monodhelminthidae Dollfus (1937b) which is based on the single genus *Monodhelmis* Dollfus (1937a), may possibly belong to the Opisthorchioidea. The absence of a cirrus pouch, presence of a complicated genital sinus, and a V-shaped excretory vesicle with spacious limbs extending forward to the region of the esophagus are suggestive of such relationship.

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A note on the systematic position of *Cercaria coronanda* Rothschild, 1938.

MIRIAM ROTHSCHILD, London, England.

Dr. H. A. Baylis and Dr. E. W. Price have kindly drawn my attention to certain morphological similarities between the metacercaria of *Cercaria coronanda* and species of the family Acanthostomidae. McCoy (1929, Jour. Parasitol. 16(1): 29–34) had tentatively assigned *Cercaria floridensis* McCoy, 1928, a pleurolophocercous larva, to this group, basing his belief on characters of the metacercaria which are now known to be common to both this family and more recently discovered heterophyoid trematodes (*Neochasmus*, Van Cleave & Muller, 1932, Roosevelt Wild Life Ann. 3(1): 5–51), *Allacanthochasmus* (Van Cleave, 1922, Proc. U. S. Natl. Mus. 61 (Art. 9): 1–8), etc.

The type of circumoral spine, and more especially the position of the testes and the course of the uterus point to *Cercaria coronanda* pertaining to the Acanthostomidae rather than the Heterophyidae. It will also be recalled that the main excretory ducts showed very unusual features which were discussed in some detail in the original description. In view of Yamaguti's (1938, Ztschr. Parasitenk. 10(2): 293–296) recent description of the cercaria of *Centrocestus armatus*, in which he shows an identical excretory system for this larva, this apparently aberrent arrangement cannot be used as evidence one way or another.

The main character upon which *Cercaria coronanda* and its metacercaria were assigned to the Neochasminae was the presence of a genital sucker or gonotyl. In the mounted type specimen this structure does not appear very noticeable but in the living animal it is exceedingly conspicuous. It should also be stated that at this stage the vas deferens and hermaphroditic duct are poorly developed and represented merely by a chain of cells, and that the only structure of the reproductive organs which can be clearly seen in the living metacercaria is the genital sucker.

It is of course possible that in the fully matured trematode this organ is not very noticeable and that further examination of the Acanthostomidae may yet reveal the presence of an inconspicuous gonotyl. It is a curious fact that several organs in the pleurolophocercous cercariae show a fluctuating degree of development. Thus, for example, the ventral sucker is visibly more developed in very immature cercariae when a lip is discernible, but gradually disappears after the larvae leave the radiae to appear once again in the metacercarial stage.

At present I am inclined to consider my original confidence regarding the systematic position of this species as misplaced. It appears more likely that *Cercaria coronanda* represents a form midway between the Acanthostomidae and the Heterophydiae (Neochasminae) but inclining toward the former. The type and detailed morphology of the cercariae can leave no doubt as to its inclusion in the Opisthorchioidea. Should the adult form eventually prove to pertain to the Acanthostomidae it is difficult to see how this family could then be excluded from Vogel's superfamily Opisthorchioidea.

***Pseudapatemon aldousi*, new species (Trematoda; Strigeidae) from the American Woodcock, *Philohela minor*.** ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

In 1938 (Tran. 3 North Amer. Wildlife Conserv. p. 844) C. M. Aldous, in connection with woodcock-management studies in Maine, reported "flukes of the family Strigeidae" from woodcock killed in the fall of 1936 and 1937. The birds, from which the intestines of over 200 specimens were examined for parasites, were collected near St. Stephens, New Brunswick, and across the river in Washington County, Maine. The parasites from these birds (77 vials) were forwarded, through Dr. J. E. Shillinger, U. S. Bureau of Biological Survey, to the Bureau of Animal Industry, for identification. From the records, it appeared that about 5 per cent of the birds harbored from 1 to 20 flukes. This interesting trematode was found to be an undescribed species belonging to the genus *Pseudapatemon* Dubois, 1936, and is named for Mr. C. M. Aldous of Orono, Maine.

Pseudapatemon aldousi, n. sp.

Description.—Body slender, 1.35 to 1.72 mm long, increasing in width towards extremities, bisegmented, with anterior segment often reflexed on posterior segment; anterior segment comprising from $\frac{1}{4}$ to $\frac{1}{5}$ of body length; posterior end of body usually set off from remainder of posterior segment by a definite constriction at level of caudal margin of posterior testis. Oral sucker subterminal, 70 μ by 80 μ; acetabulum large, 120 μ in diameter. Tribocytic organ large, cup-shaped, posterior and ventral to acetabulum. Pharynx from 30 to 40 μ in diameter; esophagus short; intestinal ceca simple, terminating at level of genital atrium. Testes in distal half of posterior segment, somewhat variable in shape and size; margins entire; anterior testis asymmetrical, about 200 μ in greatest diameter; posterior testis from 200 to 280 μ in diameter. Ejaculatory pouch 60 μ by 120 μ, situated caudal to posterior testis and antero-ventral to copulatory bursa; the posterior end of the pouch continues as the ejaculatory duct extending along the dorsal side of the uterus, uniting with the latter and forming a single duct which soon penetrates the genital protuberance on the postero-ventral wall of the genital sinus. The copulatory bursa opens subterminally on dorsal surface of body. Ovary subspherical, 90 μ to 110 μ in diameter, pretesticular, near equatorial level of posterior body segment. Oviduct arising from dorsal side of ovary and after extending a short distance posteriorly a short Laurer's canal is given off which opens on dorsal surface of body; the oviduct then continues posterior to the oötype and Mehlis' gland which lie between the testes. The uterus extends in a cephalic direction to a point

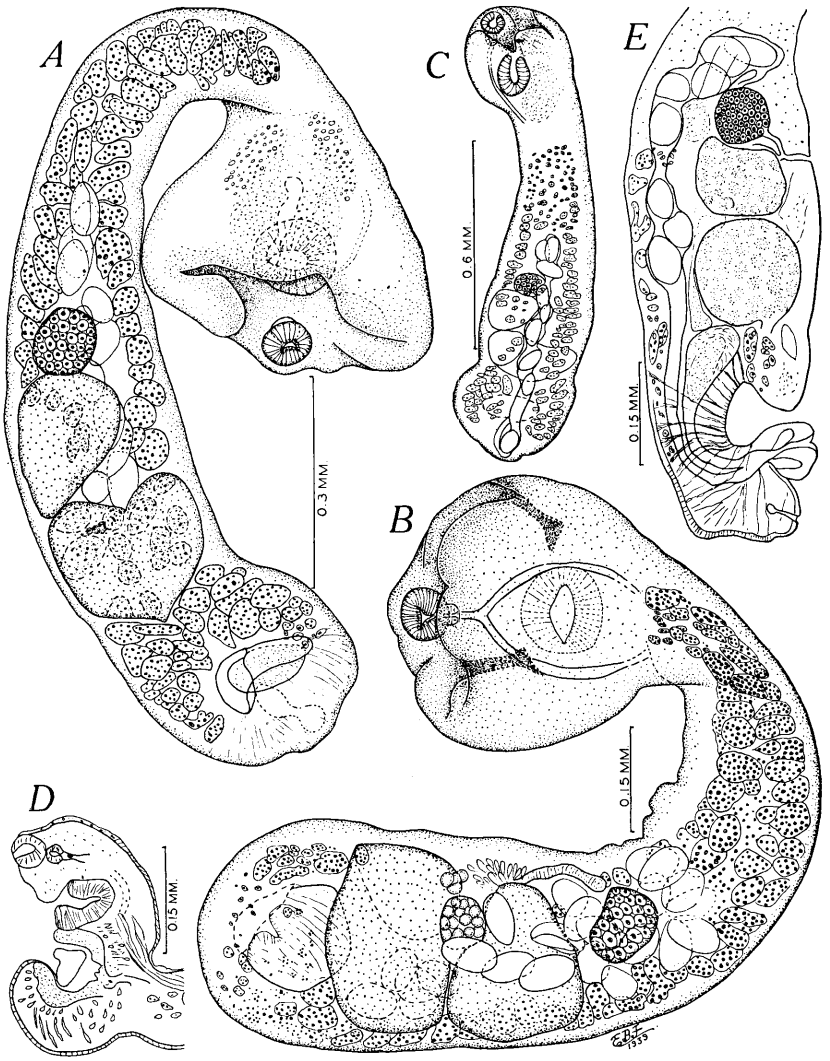

FIG. 1. *Pseudapatemon aldousi*, n. sp. A—Entire specimen, showing anterior segment in ventral aspect and posterior segment in dorso-lateral aspect. B—Similar to A, with posterior segment in ventro-lateral aspect. C—Entire specimen, showing prebursal constriction. D—Section through anterior segment showing cup-shaped tribocytic organ. E—Section through posterior half of body showing arrangement of reproductive organs.

cephalad of the ovary, then turns toward ventral surface of body and proceeds posteriorly to near end of body, curving toward dorsal surface and penetrates the posterior wall of the genital atrium. Vitellaria consisting of large follicles, extending almost entire length of posterior segment. Eggs 80 μ by 50 μ, usually from 10 to 20 present.

Habitat.—Intestine of American woodcock, *Philohela minor* (Gmelin).

Distribution.—North America (Maine and New Brunswick).

Specimens.—U. S. N. M. Helm. Coll. Nos. 44153 (type) and 44154 (paratypes).

Remarks.—Pseudapatemon aldousi is the third species to be assigned to the genus and the first to be reported from the American continent; all three species are from related birds belonging to the suborder Charadrii. *P. aldousi* differs from the genotype, *P. elassocotylus* (Dubois, 1934) Dubois, 1936, in general body shape and especially in the position of the ovary. In the genotype the ovary is in the anterior portion of the posterior segment, while in *P. aldousi* it is near the equatorial level of the posterior segment. In the other member of the genus, *P. mamilliformis* (Tubangui, 1932) Dubois, 1936, the ovary is in a position similar to that found in *P. aldousi*; these two species appear to differ in the anterior extension of the uterus, the turning point being at the ovarian level in the former and at some distance cephalad of the ovary in the latter. A very pronounced character of the new species, and one that should serve to differentiate it from the other members of the genus, is the constriction near the caudal margin of the posterior testis, which delimits the large, muscular, copulatory bursa.

The efficacy of crude unconditioned phenothiazine for the removal of gastro-intestinal parasites from sheep. ROBERT T. HABERMANN, PAUL D. HARWOOD, and W. HAYWARD HUNT, U. S. Bureau of Animal Industry, Washington, D. C.

In an earlier paper (Habermann and Harwood, Vet. Med. in press) it was pointed out that recrystallized phenothiazine is much more effective than crude phenothiazine, which had been conditioned for use as an insecticide, for the removal of nematodes from the gastro-intestinal tract of sheep. Since recrystallization adds greatly to the expense of producing phenothiazine for use as an anthelmintic, it seemed advisable to determine if the lowered efficacy noted in crude, conditioned phenothiazine was due to the conditioning agent, or to impurities produced in the process of manufacturing the chemical in question.

The crude, unconditioned phenothiazine was obtained from commercial sources. The manufacturer stated that this crude drug contained at least 98 per cent phenothiazine. Aside from the chemical, the materials and methods employed in this investigation were identical with those described in another paper (Habermann and Harwood, Vet. Med. in press). Details of the experiments conducted and the results obtained are set forth in table 1.

Crude phenothiazine was tested in 6 experimental animals in doses of 25 grams per animal. The drug removed at least 90.2 per cent of *Bunostomum,* 84.3 per cent of *Oesophagostomum,* 95.9 per cent of *Haemonchus,* 48.1 per cent of *Ostertagia,*[1] 76.8 per cent of *Trichostrongylus,* 14.9 per cent of *Cooperia* and 0 per cent of *Nematodirus.* In addition, a total of 15 large nematodes (either *Bunostomum* or *Oesophagostomum*) and 5,581 small nematodes (either *Trichostrongylus* or *Cooperia*) were eliminated following treatment. Phenothiazine failed to remove any of 183 *Strongyloides,* 90 *Capillaria,* 1 *Trichuris,* and 8 *Moniezia* present in these animals. A comparison of these results with those obtained earlier with recrystallized phenothiazine indicates that there is no significant difference between the effectiveness of recrystallized phenothiazine and of a good grade of unconditioned crude phenothiazine.

No pathology that could be associated with the drug was observed in any of the 6 host animals at necropsy. One hundred grams of crude, unconditioned phenothiazine was given to one ewe and temperatures were taken daily thereafter;

[1] Probably the drug removed a much greater percentage of *Ostertagia,* but it is impossible to estimate accurately the actual percentage of worms removed because many of these worms are digested after being killed by the drug. For a complete discussion of this point see Habermann and Harwood (*loc. cit.*)

TABLE 1. *The efficacy of crude unconditioned phenothiazine for the removal of gastrointestinal strongyles from sheep*[a]

Designation of animal	Weight	Date of treatment	*Worms eliminated following treatment*								
			Bunostomum	*Oesophagostomum*	Large, unidentifiable	*Haemonchus*	*Ostertagia*	*Nematodirus*	*Trichostrongylus*	*Cooperia*	Small, unidentifiable
	Pounds	*1939*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	87	Aug. 31	15	11	8	14	0	0	0	1,062	1,787
2	72	Sept. 12	14	1	1	0	84	0	219	56	253
3	75	Sept. 22	12	3	3	702	295	0	0	1,352	1,581
4	84	Sept. 27	7	10	0	0	1	0	3,129	76	225
5	79	Oct. 3	4	2	1	1	0	0	229	0	0
6	87	Oct. 10	3	16	2	33	170	0	902	381	1,735
Totals			55	43	15	750	550	0	4,470	2,927	5,581

Designation of animal	Weight	Date of necropsy	*Worms removed at necropsy*						
			Bunostomum	*Oesophagostomum*	*Haemonchus*	*Ostertagia*	*Nematodirus*	*Trichostrongylus*	*Cooperia*
	Pounds	*1939*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	87	Sept. 7	0	2	0	193	10	40	3,960
2	72	Sept. 22	0	0	0	40	0	60	10
3	75	Sept. 29	0	0	0	10	340	10	260
4	84	Oct. 4	0	4	12[b]	180	0	20	0
5	79	Oct. 18	1	0	0	0	0	0	20
6	87	Oct. 19	5	2	20	170	476	1,220	15,460
Totals			6	8	32	593	826	1,350	19,710

[a] The dose was 25 grams of the drug per sheep, administered in a hard gelatin capsule without a preliminary fast.
[b] Immature specimens.

no clinical signs of intoxication were observed. Three days after administration of the drug, this animal was necropsied but no significant gross pathology was observed.

As all the sheep used in these experiments were cull animals, conspicuous lesions were present in some of them. Sheep 2, 5, and 6 exhibited at necropsy extensive adhesions among the abdominal organs. Apparently these lesions were the result of an extension of inflammation from the numerous *Oesophagostomum* nodules in the intestines of these animals. Since this pathology was more extensive in sheep 6 than in any other animal, it is possible that the condition of this animal may explain in part the relatively poor results obtained following treatment.

An experiment with crude, unconditioned phenothiazine not reported in table 1 is of interest. An old ewe, weighing 55 pounds, was given 25 grams of phenothiazine on September 9, 1939. At the time of treatment, this ewe was in such poor condition that she had to be supported while the treatment was being administered; however, she was treated in the hope that information of some value might be obtained, provided she survived for a sufficiently long time. Apparently because of ill health this ewe ate very sparingly for the first 5 days after treatment. However, 7 *Bunostomum*, 68 *Oesophagostomum*, 3 *Chabertia*, 13 large unidentifiable worms, 40 *Ostertagia*, 236 *Trichostrongylus*, 563 *Cooperia* and 816 small unidentifiable worms were eliminated in the feces. On the 6th and 7th days after treatment the sheep ate more freely and seemed much improved. Twenty-seven *Bunostomum*, 90 *Ostertagia*, 2,870 *Trichostrongylus*, 14,670 *Cooperia*, 10 *Capillaria*, and 9 *Moniezia* were recovered when this animal was necropsied on September 17, 1939. In this case, as in other experiments with sheep which were obviously ill at the time of treatment, phenothiazine was relatively ineffective. However, a number of nematodes were removed, and the animal seemed somewhat improved following treatment. Therefore, it is possible that an additional treatment would have resulted in complete recovery.

SUMMARY

Six cull sheep treated with 25 grams of crude, unconditioned phenothiazine gave results which indicated that this type of the chemical was as effective for the removal of nematodes from the gastro-intestinal tract of sheep as recrystallized phenothiazine. Apparently phenothiazine is less effective in sheep which are obviously ill than in healthy ones.

Preliminary observations on the effectiveness of crude, unconditioned phenothiazine for the removal of worms from horses. PAUL D. HARWOOD, ROBERT T. HABERMANN, ERWIN H. ROBERTS, and W. HAYWARD HUNT, U. S. Bureau of Animal Industry.

Previous experience with phenothiazine as an anthelmintic (Habermann and Harwood, 1939, Vet. Med. 35: 24–30; Swanson and Harwood, 1940, Jour. Amer. Vet. Med. Assoc., in press) suggested this drug is very effective for the removal of Strongylata from the gastrointestinal tracts of herbivorous animals, especially when these worms inhabit that portion of the gut where the movement of the food materials is relatively slow. These conditions are fulfilled in the horse, since all of the numerous species of bursate nematodes which occur in equines, with the exception of *Trichostrongylus axei*, are located in the colon and cecum. Theoretically, phenothiazine should be a particularly valuable anthelmintic for the removal of these nematodes from horses, and in order to determine this point tests were carried out involving 3 horses and a mule. The results of these tests are reported at this time in order that the information so far obtained may be available to other investigators who may have an opportunity to carry out more extensive experiments on the administration of phenothiazine to equines.

The drug, commercial phenothiazine not conditioned as an insecticide, was administered usually in hard gelatin capsules in doses varying from 80 to 90 grams, but the mule received ½ its dose in a mixture of ground feed. At the time of treatment the animals were confined in a stall having a smooth concrete floor from which all fecal material could be collected daily. The animals were not fasted at any time, but during the course of the tests their diet was limited to a mixture of oats, bran, and a very small quantity of alfalfa hay, as the task of separating the worms from the feces is somewhat easier if the animals are kept on a restricted diet. Approximately one week after treatment each horse was killed and the entire gastrointestinal tract examined for any parasites that might be present. For the detection of *Strongylus* spp., all the fecal material passed daily, as well as the entire contents of the gastrointestinal tract, were examined, but in order to estimate the numbers of cylicostomes present in the experimental animals, it was necessary to employ the sampling technique described by Harwood, Underwood, and Schaffer (1938, North Amer. Vet. **19**(7) : 44–46). The results obtained in these experiments are as follows:

Horse 1.—Male; weight, 1,200 pounds; given 90 grams of phenothiazine on November 8, 1939. Thirty-seven *Strongylus* spp. and 37,579 cylicostomes (estimated number) were recovered from the feces following treatment. At necropsy, November 14, 1939, only 3 unattached specimens of *Strongylus* spp. were found. The appearance of these worms suggested that they had been killed by the drug.

Horse 2.—Female; weight 1,115 pounds; was given 80 grams of phenothiazine on November 20, 1939. One strobila of *Anoplocephala perfoliata* and 14,211 cylicostomes (estimated number) were recovered from the feces. At necropsy on November 27, 1939, only one female ascarid was found.

Horse 3.—Male; weight 1,150 pounds; was given 80 grams of phenothiazine on November 29, 1939. Nine *Strongylus* spp. and 51,531 cylicostomes (estimated number) were recovered from the feces passed subsequent to treatment. At necropsy on December 6, 1939, 65 bots were the only gastrointestinal parasites found.

Mule 1.—Female; weight, 1,125 pounds; given 80 grams of phenothiazine, part mixed with the feed on December 12, 1939, and part in gelatin capsules, December 13, 1939. Four *Strongylus* spp. and 8,930 cylicostomes (estimated number) were recovered from the feces following treatment. At necropsy, on December 19, 1939, 8 bots were found.

According to these records phenothiazine in the doses employed removed 94.5 per cent of 53 *Strongylus* spp., and 100 per cent of 112,241 cylicostomes. It is noted, however, that the 3 *Strongylus* spp. not removed by the treatment from horse 1 were not attached to the mucosa and appeared to be dead. It is thought possible that had the animal been kept a day longer these 3 worms might have been eliminated in the feces. Accordingly, horses 2 and 3 were kept one more day after treatment before they were killed. Horse 3 eliminated one *Strongylus* sp. on the last day before it was killed, indicating that *Strongylus* spp. may be removed by phenothiazine as late as 7 days after treatment. The drug was ineffective for the removal of ascarids or bots. No other helminths were present in the gastrointestinal tracts of the treated animals.

Additional investigations may prove that much smaller doses of phenothiazine are effective for the removal of nematodes that parasitize the colon and the cecum of equines.

No symptoms of intoxication were observed in any of these animals following treatment.

SUMMARY

In doses of 80 to 90 grams per adult equine phenothiazine removed 94.5 per cent of 53 *Strongylus* spp., and 100 per cent of more than 100,000 cylicostomes

from 3 horses and a mule. Reasons are given for accepting the view that the efficacy of the drug in removing *Strongylus* spp. was actually 100 per cent. Possibly the doses employed were much larger than necessary.

Experimental infections of swine with the red stomach worm, *Hyostrongylus rubidus*.[1] DALE A. PORTER, U. S. Bureau of Animal Industry.

INTRODUCTION

The red stomach worm, *Hyostrongylus rubidus*, a small trichostrongylid parasitic in the stomach of swine, has a rather wide distribution, having been reported from Asia, Australia, Europe, Central America and North America. The presence of this parasite in swine has been frequently associated with a capricious appetite, diarrhea and loss of weight, accompanied by gastritis and ulceration of the gastric mucosa. The symptoms have been observed in the case of swine naturally infected and where the infection may have been complicated by the presence of other parasites and pathogenic bacteria. There is very little published information relative either to the course of the infection with or to the effect of the parasite on the host under rigidly controlled experimental conditions. In view of this, a series of experimental infections in pigs was studied to ascertain the effect of this parasite on the host under conditions where extraneous infections with other helminths were, for the most part, excluded, and where complicating bacterial infections were absent, so far as this could be determined without recourse to culture methods. The information obtained in this study, including observations on the prepatent period of the worms, course of egg production, length of life of the parasites and the lesions associated with the infections are discussed in this paper.

MATERIALS AND METHODS

Twelve pigs of mixed breeding were used as host animals. The pigs were farrowed and raised in well-drained cement-floored pens under conditions of strict sanitation that precluded extraneous infections with all helminths except *Strongyloides*. The procedure followed in keeping the animals free from extraneous parasites was a modification of that used by Spindler (1933) in raising pigs free from nodular worms. This procedure, as used in the present experiments, was as follows:

Each morning the manure adhering to the feet and bodies of the animals was carefully removed by washing with warm water, and the pens were scrubbed with soap and boiling water. In addition, accumulated manure was removed from the pens several times each day. By using this method, it was possible to maintain test and control pigs in the same pen.

The pigs were weaned 6 to 8 weeks after farrowing and these hosts were maintained subsequently on a diet composed of the following ingredients by weight: Cracked yellow corn, 54 parts; wheat middlings, 20 parts; meat meal (tankage), 20 parts; powdered yeast, 1 part; powdered limestone, 3 parts; steamed bone meal, 1 part; common salt, 1 part. The food was kept in troughs and a plentiful supply of clean fresh water was kept before the animals at all times.

Infective larvae of *Hyostrongylus rubidus* were obtained by culturing on moist bone charcoal for 7 to 10 days eggs obtained by cutting up female worms with scissors or by allowing the gravid females to oviposit in physiologic saline. When the larvae reached the infective stage they were recovered from the charcoal culture by means of the Baermann apparatus.

[1] These investigations were carried out in Moultrie, Georgia, during the period from November 1936 to February 1938.

Since Goodey (1924) and Alicata (1935) failed to demonstrate penetration of baby rat skin by infective larvae of *H. rubidus*, the pigs in the experiments discussed in this paper were infected by mouth. The larvae to be fed to each animal were first counted by the dilution method; the larvae were then placed far back in the mouth of the animal by means of a metal pipette attached to a 5 cc rubber bulb. Immediately after administration of the larvae the animal was fed to insure that the larvae were swallowed.

Fecal examinations, involving the salt flotation technique were made on both test and control animals at frequent intervals. In the case of the test animals, beginning about 15 days after experimental infection, examinations were made daily until eggs appeared in the feces. In most cases the salt-flotation examinations were then made at intervals of 2 to 4 days for a period of about 2 weeks and then at intervals of 10 to 15 days until necropsy. In the case of 2 host animals the course of egg production of the parasites was followed by means of the Stoll dilution egg-count technique, counts being made of the numbers of eggs in representative samples of 24-hour accumulations of feces. When the number of eggs passed in the feces of an animal became too small to be detected by the dilution method, the salt flotation technique was employed.

Test and control animals were weighed at weekly intervals and their general condition and activity noted. Post-mortem examination of test and control animals concluded each test. The stomach, as well as other viscera, were examined for lesions associated with the presence of *H. rubidus*, and any worms present were counted. Portions of affected tissues were preserved in formalin; sections of these tissues were later stained with hematoxylin and eosin for histological examination.

DATA OBTAINED

The results of the experiments involving 9 test and 3 control pigs are summarized in table 1. As shown by the data, eggs of *H. rubidus* appeared in feces of the pigs from 20 to 25 days following experimental infection; in all cases these eggs were demonstrable in the feces of the infected animals until necropsy. The maximum periods over which *Hyostrongylus* eggs were demonstrable in the absence of reinfection were 6 months in the case of pig 1A and at least 8 months in the case of pig 5A. The number of days necessary for females to reach a level of egg production high enough for eggs to be detected in feces by salt flotation is in agreement with what is known of the development of the parasite, in other than the normal host. In this connection, Alicata (*loc. cit.*) noted fourth-stage larvae of both sexes undergoing the fourth or final molt 13 days after experimental infection and he found the adult males and females 17 and 19 days, respectively, after experimental infection of guinea pigs.

In the case of each of 2 pigs (14C and 19D), the course of egg production of the parasites was followed by means of the Stoll dilution technique; the data from pig 14C are regarded as typical and are shown graphically in figure 1. As can be seen from the figure, eggs were first detected in the feces of this animal by salt flotation 21 days after an initial infection of 4,600 larvae. Two days later the number of eggs in the feces was estimated to be 1,400 per gram, but 6 days later the number had decreased to 200 per gram (Fig. 1). After varying between 100 and 200 eggs per gram of feces over a period of about 2 months, the numbers of eggs became so small that their presence could be detected only occasionally even by the salt flotation technique. Consequently, on September 16, 115 days after the initial infection, 2,000 additional larvae of *H. rubidus* were administered. Salt flotation examinations made on feces collected 16, 22 and 24 days later revealed the presence of eggs (Fig. 1); however, the eggs were too few in number to be detected by the dilution technique and no counts were made. On October

13 and 14, 27 and 28 days, respectively, after the second administration of larvae the numbers of eggs had increased to 200 per gram. However, from this time until necropsy (November 9) the numbers of eggs were so small that their presence could be detected only by the salt flotation technique. The increased numbers of eggs noted October 13 and 14 may have been coincident with the maturing of female worms from the second infection. Considering the low egg production that followed, and the small number of worms recovered at necropsy in relation to the number of larvae administered (Table 1), it is possible that the host had developed a resistance to reinfection.

In the case of pig 19D that had been given a single infection of 4,800 larvae (see Table 1) the course of egg production by the parasites was similar to that in the first infection of pig 14C, already described. Eggs first appeared in the feces of this animal 21 days after infection and 6 days later (27 days after infection) reached a peak of 1,500 per gram. Following this, the number decreased rapidly to a low level but small numbers of eggs were observed in salt flotations made at intervals of approximately 10 days until necropsy 85 days after infection.

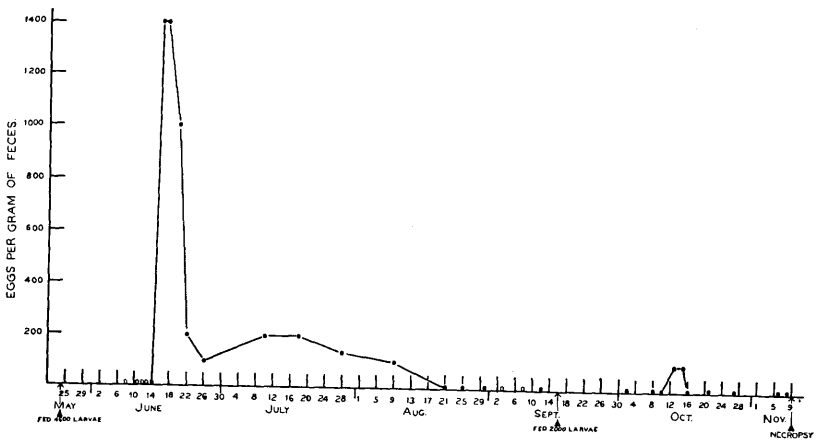

FIG. 1. Egg counts obtained on feces from pig 14C exposed to infection as indicated. Points on base line indicate samples positive to salt flotation, open circles indicate negative findings.

In addition to the case of pig 14C in which eggs were found in the feces 115 days after the first infection, 2 other cases illustrating the longevity of this parasite are of interest. Following their first appearance in the feces as determined by salt flotation, 21 days after infection, eggs were found in the feces of pig 1A until necropsy 171 days after infection when 291 or 12.6 per cent of the number of larvae fed originally were recovered as adult worms. In the case of pig 5A fed 9 doses of larvae (Table 1), eggs appeared 21 days after the first dose of larvae was fed and eggs from females established by feeding these doses of larvae were found continually from then on over a period of 243 days after the last dose of larvae was given. At that time the pig was turned out on pasture and thus exposed to possible natural reinfection. These data would indicate that an infection with *H. rubidus* may persist 6 to 8 months or longer after larvae are ingested. Since neither control pig 17C kept in the same pen with pig 14C, nor 18D, another control which was kept with pigs 19D and 25D, became infected during the tests, it appears that self reinfections in the concrete-floored pens did not take place under the conditions of these experiments.

As can be seen from the table the number of worms recovered at necropsy decreased as the duration of this parasitism increased. For example, in the case

TABLE 1.—*Data on experimental infections of pigs (from 4 litters) with Hyostrongylus rubidus*

Pig No.	Age when infected	Estimated number larvae fed		Interval between first dose of larvae and appearance of eggs in feces	Days from administration of larvae to necropsy		Worms recovered	Percentage of worms recovered in terms of larvae given	Percentage of female worms recovered
					First dose	Last dose			
	Days	*Date*	*Number*	*Days*	*Days*	*Days*	*Number*		
2A	37	11–19–36	1300	21	49		416	32.0	57.6
5A	37	11–19–36	300	21	414	302	112	1.7	not determined
		12–16–36	600				[a]		
		12–30–36	600						
		2– 2–37	850						
		2–10–37	1050						
		2–15–37	450						
		2–26–37	800						
		3– 4–37	750						
		3–11–37	1050						
1A	104	1–25–37	2300	21	171		291	12.6	58.4
3A	167	3–29–37	3800	24	24		1836	48.3	60.0
11B	28	[b]	0		[c]		0	0	0
12B	28	3–29–37	3800	20	25		1671	43.9	not determined
17C	47	[b]	0		[c]		0	0	0
14C	47	5–24–37	4600	21	169	54	232	3.5	not determined
		9–16–37	2000						
18D	35	[b]	0		[c]		0	0	0
25D	35	9–23–37	1000	22	123	76	765	15.9	not determined
		10– 1–37	400						
		10– 8–37	1000						
		10–14–37	400						
		11– 9–37	2000						
19D	35	9–23–37	4800	21	85		298	6.2	64.0
24D	118	12–15–37	6350	25	34		2444	38.4	not determined

[a] On pasture after 243 days, hence, exposed to possible reinfection.
[b] This animal was a control, consequently, no larvae were fed.
[c] Necropsy performed on same day as animal following.

of pigs 3A and 12B, the numbers of worms recovered at necropsy 24 and 25 days, respectively, after infection, were estimated to be 48.3 and 43.9 per cent, respectively, of the numbers of larvae fed. Since the peak of egg production found to occur 23 to 27 days after infection in pigs 14C and 19D, was followed by a sudden drop in the number of eggs passed, it is possible that a loss of worms began at about this time or shortly thereafter. The fact that approximately 60 per cent of the worms recovered at necropsy 24, 49, 85, and 171 days after the infection of pigs 3A, 2A, 19D, and 1A, respectively, were females, indicates that the males and females of this parasite may be about equally long lived. In this connection, Alicata (*loc. cit.*) found the percentage of females to be slightly less (53.4 per cent) in a count of 150 fourth-stage larvae recovered from a guinea pig 10 days after experimental infection.

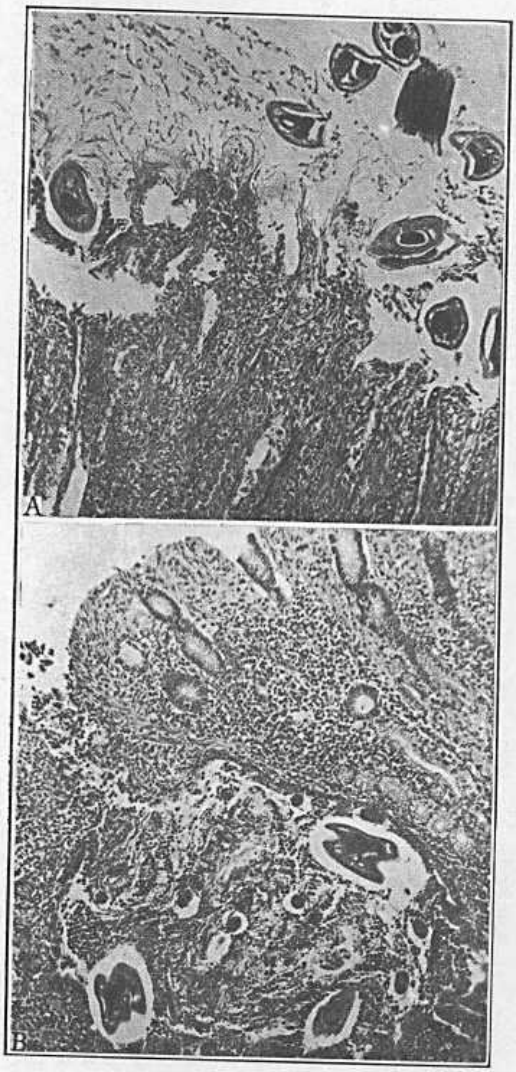

FIG. 2. A—Erosion of the gastric mucosa associated with *H. rubidus*. B—Section of stomach showing worms and eggs in an exudate deep within a gastric gland.

At necropsy of the experimental host animals, the majority of the worms were localized on the mucosa of the fundus and along the lesser curvature of the stomach; a small number of worms was generally scattered over the remainder of the stomach mucosa. Gross lesions consisting of alterations in the gastric mucosa varied from slight erosion and hyperemia to definitely eroded areas or ulcers. These lesions were observed in locations where worms were abundant in the case of pigs 2A, 3A, 12B and 24D, examined from 24 to 49 days after infection. Ulceration was also observed in pig 25D that had been fed a total of 4,800 larvae in 5 doses between 76 and 123 days before necropsy. Some ulcers measured from 6 to 15 mm in diameter; their craters were filled with an accumulation of flocculent mucus in and under which worms were found to have nested in large numbers. Erosion of gastric mucosa and ulceration with mucus formation has been previously observed in the stomachs of naturally-infected swine (Hassall and Stiles, 1892; Opperman, 1905; Crocker and

Biester, 1920; Skrjabin and Bekenskii, 1925; Clay, 1938). In the investigation herein reported histological examination of tissue from the experimentally-infected animal revealed the worms on the surface of the mucosa or penetrating into the gastric pits. Considerable destruction of the mucosa was evident, the worms being enmeshed in mucus composed mostly of disintegrating cells (Fig. 2A). Worms were also found deeper in the gastric glands (Fig. 2B) and in one instance not illustrated in this paper they were observed to have penetrated as far as the muscularis mucosa. A moderate to heavy leucocytic infiltration, particularly of eosinophiles in the mucosa and submucosa, also was observed. Thickening of the mucosa was quite evident. These observations are similar to those reported by Crocker and Biester (*loc cit.*) in the case of swine naturally infected with these worms. The investigators named found parasites embedded in the tissue, with the microscopic folds of the mucous membrane in the vicinity of the parasites degenerated and necrosed; in the more severe cases, necroses of larger areas of the mucosa, with worms in masses of fibrin and cellular detritus covering such areas, were observed. Clay (*loc. cit*) also reported a chronic inflammation with thickening of the mucosa and leucocytic infiltration in natural infections.

Small circumscribed depressions resembling the actively eroded areas in shape, size and location, in what appeared otherwise to be normal gastric mucosa, were also found in the stomachs of pigs 1A, 5A, 14C and 19D, examined 171, 302 to 414, 54 to 169 and 85 days, respectively, after experimental infection. From their gross as well as microscopic appearance, these areas were considered to be sites of former ulcers in which the mucosa had regenerated in the process of healing. The stomachs of the uninfected controls (Pigs 11B, 17C and 18D, Table 1) raised free of parasites in the same pens with infected litter mates were entirely normal at necropsy. In view of this finding, the association of the parasite with the lesions observed is particularly significant.

Observations on the physical condition and activity of the hosts during the course of infection failed to disclose any symptoms of injury, as indicated by the growth rate of the test animals as compared to the controls. For example, pigs 12B, 14C, and 25D gained an average of 3.5, 5.7 and 2.2 pounds per week, respectively, during infection periods of 25, 169 and 123 days, respectively. During the same periods the average gains of their controls (11B, 17C and 18D) were 2.7, 6.7 and 2.2 pounds, respectively. Pig 19D gained on an average of 4.4 pounds weekly during the 85 days of its infection in comparison with the 2.4 pounds gain per week in the case of the litter mate control (18D) during the same period. In view of these findings it does not appear that the parasites exerted any evidently marked deleterious effect on the host animals.

DISCUSSION

From the cases described in this paper it is evident that *H. rubidus* was associated with the gastric lesions of the type described. Correlating the type of lesion with the course of infection, the data indicate that ulcers may be produced as early as a month after experimental infection, when the worms are most numerous, but may heal as the infection grows older and the number of parasites decreases. This is borne out by the presence of what appeared to be healed ulcers in the older infections (Pigs 1A, 5A, 14C and 19D). The only exception to this was the finding of ulcers in the case of pig 25D in which reinfection was continued between 123 and 76 days before necropsy. Although infections may persist from 6 to 8 months, healing of ulcers was observed as early as 85 days after single exposure to larvae and as early as 54 days after reexposure. However, in the last instance the small numbers of eggs observed in the feces indicate that the host (Pig 14C) had developed considerable resistance to reinfection and the healed

lesion, therefore, possibly dated from the primary infection given 169 days before necropsy.

Diarrhea, variable appetite, emaciation and occasional deaths have been reported as a result of severe natural infections in sows (Opperman, *loc. cit.;* Castle, 1932). Emaciation (Skrjabin and Bekenskii, *loc. cit.*) and loss of weight and death (Clay, *loc. cit.*) have also been observed in pigs showing extensive erosion of the gastric mucosa in association with *H. rubidus*. It has been suggested, however, that this parasite is not particularly pathogenic unless it is present in association with other disease processes (Crocker and Biester, *loc. cit.;* Hoogland and Seijffers, 1928) or invades animals showing depleted vitality such as young nursing sows (Castle, *loc. cit.*). None of these symptoms were observed in the animals used in these tests. Since young pigs, free of disease, were used as host animals, the lack of any noticeable effects may have been due either to the generally light infections or possibly to the absence of other complicating disease factors.

SUMMARY

Experimental infections with the red stomach worm, *Hyostrongylus rubidus*, were studied in swine raised free from parasites, with the exception of *Strongyloides*. The work was carried on at Moultrie, Georgia, from November, 1936, to February, 1938.

The eggs of the nematode appeared in the feces in from 20 to 25 days after experimental infection. In the case of these animals the maximum number of eggs per gram of feces occurred 23 to 27 days after the administration of larvae, after which the number of eggs decreased until they could be found only by salt flotation during the remainder of the course of infection.

The maximum number of worms in relation to the number of larvae fed were recovered from animals examined 24 to 25 days after infection, whereas the number of worms recovered after longer periods of infection were correspondingly smaller. The post-mortem data obtained indicated that both sexes may be about equally long lived. Observations on the course of infection, as adjudged by egg production of this parasite, indicate that infections may persist at least 6 to 8 months in the absence of reinfection.

At necropsy of the test animals the only lesions noted were in the stomach. The parasites were localized for the most part on the mucosa of the fundus and along the lesser curvature. In this location small ulcers were found. Healed ulcers, appearing as circumscribed craters in the mucosa, were found in cases of pigs necropsied later than 85 days following experimental infection. This indicates that repair of lesions may follow the loss of some of the parasites.

Histological examination of stomach tissue showed that the worms may penetrate the mucosa, this penetration producing erosion of the mucosa with attendant inflammatory changes. Clinical evidence of injury to the host by the worms was not observed.

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A simple medium for the cultural diagnosis of *Trichomonas foetus* infection in cattle. JOHN L. AVERY and GEORGE G. GARLICK, U. S. Bureau of Animal Industry.

The medium used in the Zoological Division to maintain cultures of *Trichomonas foetus* has also been used for the cultural diagnosis of the infection in cattle. This medium is the Ringer's-egg-blood medium used by Rees (1938, Vet. Med. **33** (7): 321–334) except for the substitution of blood serum for whole defibrinated blood. In order to determine the comparative efficiency for diagnostic purposes of this culture medium with a more easily prepared medium, a number of comparative tests were conducted. The results of these tests are given in this paper.

The modified medium consisted of egg-slants made by thoroughly beating a mixture of 3 eggs and 15 cc of 0.75 per cent sodium chloride solution. This mixture, in 2 cc quantities, was placed in standard bacteriological culture tubes, and inspissated in an ordinary steam pressure cooker, the tubes being inclined so as to form slants about 1½ inches long. To obtain smooth slants, it was necessary to close the air vent of the cooker before the heat was applied. Immediately upon reaching a pressure of 15 pounds per square inch the heat was turned off and, without opening the vent, the pressure was allowed to diminish gradually by cooling. After inspissation, the tubes were cooled and the water of condensation drained off. About 10 cc of a 0.75 per cent solution of sodium chloride was then added to each tube, after which the latter were plugged with cotton and autoclaved at 15 pounds pressure for 20 minutes.

The experimental tests to determine the comparative diagnostic efficiency of this modified medium were made on animals known to be infected with *T. foetus*. The vaginal pipette described by Andrews and Miller (1938, Amer. Jour. Hyg. **27** (2): 235–249) was used for flushing the vagina or prepuce of the test animal with physiological saline solution. A drop of the recovered washings was immediately examined microscopically for the presence of trichomonads. The remainder of the washings was divided equally among 3 tubes of media; 2 of Ringer's egg-blood serum medium and 1 tube of the saline-egg medium. One tube of the Ringer's egg-blood serum medium was incubated at 37° C. and the other kept at room temperature (20–25° C.). The saline-egg diagnostic medium was kept at room temperature. After 24 hours a microscopical examination was made of the sediment in each tube. The results of these examinations are given in table 1.

These data show that of 35 examinations of known positive animals, all except 5 (14 per cent) were detected by the new diagnostic medium. The Ringer's-egg-blood serum medium gave an error of 40 per cent when incubated at 37° C. and an error of 21 per cent when kept at room temperature. Direct microscopical examination of the genital washings detected all but 14 per cent of the positives. A combination of the results of direct examination and of the saline-egg medium gave the most accurate results. Of the 5 positives not detected by the saline-egg medium, 4 were not detected by the other culture medium. Three of these samples were from animal No. 150, a bull having a low-grade infection. A comparison of the

TABLE 1.—*Comparison of Ringer's-egg-blood serum and of saline-egg as a diagnostic medium for the detection of trichomonad infection in cattle*

Animal designation	Date of examination (1939)	Results of examinations			
		Direct smear	Ringer's-egg-blood serum medium		Saline-egg medium
			Incubated at 37° C.	Room temperature	Room temperature
Heifer 190	May 24	+	[a]	+	+
Heifer 190	May 26	+	+	+	+
Bull 150	May 26	+	+	−	−
Bull 150	May 27	+	−	−	−
Bull 150	June 1	−	+	+	+
Bull 150	June 3	+	−	+	+
Heifer 161	June 6	+	[a]	[a]	+
Heifer 185	June 13	+	−	−	−
Bull 150	June 13	+	[a]	−	−
Heifer 185	June 16	+	+	+	+
Heifer 185	June 19	+	+	+	+
Heifer 161	June 19	+	+	+	+
Bull 150	June 20	+	−	+	+
Heifer 161	June 20	+	+	+	+
Heifer 185	June 20	+	+	+	+
Heifer 185	June 26	+	[a]	+	+
Bull 150	June 26	+	[a]	−	−
Heifer 187	June 26	+	[a]	+	+
Heifer 189	June 26	+	[a]	+	+
Heifer 187	June 29	+	−	−	+
Heifer 189	June 29	+	+	+	+
Heifer 189	July 5	+	−	+	+
Heifer 189	July 6	+	−	+	+
Heifer 189	July 7	+	[a]	+	+
Heifer 189	July 10	+	+	+	+
Bull 150	July 10	−	−	+	+
Heifer 189	July 12	−	−	+	+
Heifer 185	July 17	+	+	+	+
Cow 174	July 17	+	+	+	+
Heifer 185	July 21	+	+	+	+
Heifer 185	July 25	−	[a]	+	+
Heifer 187	July 25	+	[a]	+	+
Cow 174	July 26	+	+	+	+
Heifer 185	July 26	−	−	−	+
Heifer 187	July 28	+	+	+	+

[a] No tests made on this date.

results obtained after incubation at 2 different temperatures, using Ringer's-egg-blood serum medium, shows that 6 positives not detected by this medium incubated at 37° C. were detected when the medium was kept at room temperature, while of only one positive was the reverse true. The saline-egg medium was more efficient for detecting infections when kept at room temperature than the Ringer's-egg-blood serum medium kept either at room temperature or at 37° C.

The saline-egg diagnostic medium was found to be unsuitable for the continued cultivation of the trichomonads, as the organisms failed to survive the second or third transplant.

SUMMARY

1. The preparation of a simple medium for the cultural diagnosis of *Trichomonas foetus* is described. The medium consists of egg-slants covered with a 0.75 per cent solution of sodium chloride.

2. A comparison of the efficiency of this medium with the Ringer's-egg-blood

serum medium used for the continuous cultivation of *T. foetus* showed the saline-egg medium to be more accurate for diagnostic purposes only.

3. The Ringer's-egg-blood serum medium incubated at room temperature gave more accurate results than when incubated at 37° C.

4. Although satisfactory for use in cultural diagnosis, the saline-egg medium is not suitable for the continuous cultivation of *T. foetus*.

Coccidiosis in a litter of pigs. LEONARD E. SWANSON and KENNETH C. KATES, U. S. Bureau of Animal Industry.

In September, 1939, one of us (L.E.S.) was consulted concerning a litter of 9 Poland China pigs, about 4½ months of age, which were being raised under special sanitary conditions by some employees of the stockyards at Moultrie, Ga. The pigs had failed to gain weight as rapidly as expected, considering the amount of feed available and excellent care that had been given them.

The total weight of the 9 pigs was only 540 pounds, the individual animals showing a wide variation in size. The pigs had ravenous appetites but they were definitely undernourished; the hair coat was rough; and for some time past a profuse diarrhea had been present. No other external symptoms were noticeable.

Since parasitism was suspected as a possible cause of the observed malnutrition, salt flotation examinations of the feces were made for eggs of internal parasites. Small numbers of ascarid and nodular worm eggs and large numbers of coccidia oöcysts were found.

It was decided to treat the pigs with phenothiazine, which has been found by Harwood, Jerstad and Swanson (1938, Jour. Parasitol. 24(6, suppl.): 16–17) to be an effective roundworm anthelmintic. After the pigs had been fasted for 36 hours, they were given 270 grams (9.6 oz.) of the drug with 1,080 grams (38 oz.) of mixed grain feed. All the feces from the 9 pigs were examined for worms for a period of 48 hours, allowing 2 days after for the drug to act. Only 6 ascarids and 29 nodular worms were recovered.

After this treatment the pigs seemed to be doing well, but were still scouring. The feces of all pigs still contained many oöcysts. Although the peak of oöcyst production was probably on the decline, oöcyst counts were made to obtain data on the degree of infection.

METHOD AND RESULTS OF OÖCYST COUNTS

Twenty-four hour samples of fecal material were collected from each of the 9 pigs. Each fecal sample was separately and thoroughly mixed with a spatula, and from each gross sample a representative 5-gram sample was taken. The 5-gram samples were placed in 300 cc flasks with 200 cc of N/10 sodium hydroxide and glass beads. The samples were allowed to stand over night, and the next day each preparation was thoroughly shaken and a dilution count of each was made. The oöcysts in each of two 1/10-cc samples were counted from each flask, and the number of oöcysts per gram of moist feces was calculated. The results of the counts are summarized in table 1.

DISCUSSION

It may be noted that the largest pig (number 3) had a comparatively small number of oöcysts and the 2 smallest pigs (numbers 1 and 6) had the largest number of oöcysts. Considering the possibility that the oöcyst counts were made some time after the peak of oöcyst production (this is probably true, as these pigs had made poor gains for some time before they were brought to the authors' attention) these counts are rather high.

TABLE 1.—*Results of oöcyst counts on nine samples of feces from as many pigs*

Pig number	Oöcysts per 1/10 cc sample		Oöcysts per gram
	Number	*Average number*	*Number*
1	(1) 251 (2) 218	234.5	93,800
1	(1) 251 (2) 164	234.5	65,800
3	(1) 108 (2) 97	102.5	41,000
4	(1) 240 (2) 227	233.5	93,400
5	(1) 142 (2) 163	152.5	61,000
6	(1) 384 (2) 341	362.5	145,000
7	(1) 84 (2) 90	87	34,800
8	(1) 36 (2) 42	39	15,600
9	(1) 222 (2) 197	209.5	83,800

The evidence indicated definitely that the worm infestation was light, and that the poor condition of the pigs was due mainly to coccidiosis. In the light of these findings the history of these pigs is interesting.

On May 14, 1939, a Poland China sow was sold to a local packing plant for slaughter and the following day she farrowed the 9 pigs in question in a stockyard pen; all pigs were in excellent condition. Within 2 days after farrowing the sow and pigs were moved to a clean pen having a concrete floor. The pen floor was washed thoroughly either daily or every other day during the 4½ months that the pigs were kept in the pen. The concrete floor had one break about 3 feet square where the pigs could root and sleep.

The pigs were weaned 7 weeks after farrowing. At the end of the first month the pigs were given the following feed and this ration was continued until September 21, 1939: (1) Corn soaked in copper-iron solution, supplied in self-feeders; ½ to 1 pound of dry buttermilk made into a slop with 2 ounces of cod-liver oil added for the morning feed, while buttermilk slop alone was fed at night; (3) protein supplement supplied in self-feeders; (4) tankage and minerals in self-feeders.

Buttermilk has often been recommended as a treatment for coccidiosis in pigs, but in this case it apparently had no beneficial effects. The pigs were not given iodine and potassium iodide, as recommended in connection with the buttermilk treatment.

SUMMARY

1. A litter of 9 pigs was found to be suffering from coccidiosis which was causing a profuse diarrhea. The pigs were making poor gains, although they had ravenous appetites and were supplied with excellent rations, and had good care.

2. The largest pig of the litter had a comparatively small oöcyst count, while the 2 smallest pigs had the largest öocyst counts.

3. The feeding of buttermilk, as recommended for coccidiosis (minus iodine and potassium iodide) apparently had no beneficial effects on the 9 pigs considered in this paper.

Studies on oxyuriasis. XXIV. Comparative findings in the white and Negro races. ELOISE B. CRAM, National Institute of Health, U. S. Public Health Service.

For the past two and a half years, in the Division of Zoology of the National Institute of Health, the incidence of pinworms, *Enterobius vermicularis,* in man has been investigated; an improved technique has been used, with collection of pinworm eggs from the perianal region by the NIH swab first described by Hall (1937). The findings to date as regards the general population of Washington, D. C., were summarized briefly in the summer of 1939 (International Congress for Microbiology; Proceedings in press), as follows: Of about 2800 persons, 39 per cent were positive for pinworms. Information relative to race was not available for about 100 of those persons; in the remainder there were 2150 white persons with an incidence of 43 per cent and 570 Negroes with an incidence of 16 per cent. In the ensuing months, to December 1, 1939, additional examinations have brought the total of persons, whose race was known, to 3371, of whom 1203 persons were positive, an incidence of 36.7 per cent; in this total there were 2582 white persons, of whom 1081, or 41.9 per cent, were positive, and 789 Negroes, of whom 122, or 15.5 per cent, were positive for pinworms. It is desired here to report in detail the results of examination of certain selected samples included in that total.

It has been pointed out in earlier papers that most of the anal swabs were made in the homes, the children being swabbed by the parents and the latter, as well as other adults, swabbing themselves, immediately upon arising in the morning. This procedure introduces the personal equation to an extent not true in the examination for most other parasites, different persons having made the swabs probably with a more or less varying technique in spite of specific directions. This factor is one of several variables which render negative results of examinations less conclusive evidence of the *absence* of pinworms than are positive results of their presence. In addition, it has been more difficult on the whole to persuade Negroes, than it has been to persuade white persons, to swab members of their families concerning whom there were no suspicions of pinworm infection. This fact in itself would tend to load the Negro sample with suspected positive cases and would increase the incidence figure proportionately over that of the whites. However, on the other hand it has also been more difficult to persuade Negroes to repeat the swabbing, so that whereas we have attempted to obtain at least 4 swabs made on different days, in the case of Negroes the number of examinations has fallen short of 4 to a greater extent than in the case of white persons; this fact would lead to inclusion among the Negro "negatives" of persons who might have proven positive had additional examinations been made, and consequently the incidence reported in Negroes would be lower than actually exists.

Efforts have therefore been made to obtain a sample of the two races with elimination of these two factors, that is, on the one hand the tendency to include a larger number of suspidious positive cases and on the other hand the more frequent failure to reexamine negative cases in one group than in the other.

For this purpose two groups of children, one of preschool age, 2 to 5 years, and one of school age, 6 to 12 years, were examined in nursery schools and summer camps, respectively. There are included for consideration here only children on whom 4 swabbings were made, in case the results of examination were negative.

The nursery schools were among those conducted by the WPA (Works Progress Administration) for the children of persons on relief. Examinations were made in 2 nurseries for whites and in 2 nurseries for Negroes; although in separate schools, members of the two races were of the same general economic level and resided in the same parts of the city, congested residential areas not far from The Mall in the southwest and northwest sections of the city. Of the summer camps, the one for

white children was conducted by the Jewish Community Center for Jewish children, and that for Negroes by the Family Service Association; both were for children of families who could afford to pay only a small fee. The residences of the camp children were much more widely scattered throughout Washington than were those of the nursery school children. Both boys and girls were examined in the white camp; only boys were examined in the Negro camp.

The swabbing was standardized as much as possible. In the nursery schools a trained nurse made all swabs soon after the arrival of the children at 9 o'clock in the morning; in the camps a physician and a nurse made the swabs on the 3 successive days after arrival of the children at camp, and the 4th swab 2 days later, as was done by Bozicevich and Brady (1938).

RESULTS OF EXAMINATIONS

In table 1 are shown the results of the examinations and the distribution by

TABLE 1.—*Incidence of Enterobius vermicularis in white and Negro children as result of 4 NIH swab examinations*

Race	Age	Number examined	Number positive	Per cent positive
White	2 to 5	34	19	55.9
White	2 to 5	28	13	46.4
		62	32	51.6
Negro	2 to 5	36	9	25.0
Negro	2 to 5	32	4	12.5
		68	13	19.1
White	6 to 12	147	37	25.2
Negro	6 to 12	63	13	20.6
Total:				
White	2 to 12	209	69	33.0
Negro	2 to 12	131	26	19.8

race. Pinworm infections were found in 69 of 209 white children, or 33.0 per cent, and in 26 of 131 Negro children, or 19.8 per cent. The difference in incidence in the two races was more pronounced in the nursery school children than in the camp children; in the former it was 2.7 times as great in the whites as in the Negroes. The individual nurseries showed some variation; in the two nurseries for white children, respectively, slightly more than $\frac{1}{2}$ and slightly less than $\frac{1}{2}$ the children were infected; in the two Negro nurseries, respectively, $\frac{1}{4}$ and $\frac{1}{8}$ of the children were infected.

The findings in the two sexes were almost identical, or actually identical, in both races (Table 2). As regards the time of appearance of the positive cases on consecutive swabs, in the white children of both age groups $\frac{1}{2}$ or more than $\frac{1}{2}$ of the positive cases came to light on the first swab, as shown in table 2; among the Negroes this is true of the older age group but in the nursery children the first swab revealed only 2 of 5 positive males and 2 of 8 positive females. As regards the numbers of positive swabs, in a few cases swabbing was discontinued when a positive result was obtained, so that there were not 4 swabs from all the positive children. In the nursery group, on the 32 white children proving to be infected with pinworms, 116 swabs were made, of which 57 were positive; on the 13 Negro children who proved to be infected, 44 swabs were made, of which 16 were positive. There were therefore relatively more positive swabs in white than in Negro children;

TABLE 2.—*Distribution of E. vermicularis cases found in white and Negro children*

Race	Age	Distribution by sex				Initial finding of pinworm eggs								Number of swabs on positive cases	
		Male		Female		1st swab		2nd swab		3rd swab		4th swab		Positive swabs	Negative swabs
		Number examined	Number positive	Number examined	Number positive	Male	Female	Male	Female	Male	Female	Male	Female		
W	2–5	31	16	31	16	8	8	5	5	0	2	3	1	57	59
N	2–5	27	5	41	8	2	2	1	1	0	3	2	2	16	28
W	6–12	73	18	74	19	12	10	1	5	3	2	2	2	74	72
N	6–12	63	13			9		2		2		0		25	25
Total:															
W	2–12	104	34	105	35	20	18	6	10	3	4	5	3	131	131
N	2–12	90	18	41	8	11	2	3	1	2	3	2	2	41	53

the ratio of positive to negative swabs was 1: 1.035 in the case of white children, and 1: 1.75 in the Negro children. In the older age group, however, this was not true; in both races there were the same or approximately the same number of negative and positive swabs.

DISCUSSION

A study which may be considered comparable to the present one, with NIH swab examination of persons of the white and Negro races representing the general population and unselected as regards any suspicion of contact with *Enterobius* infections, was one conducted at the National Training School for Boys, Washington, D. C. (Cram and Folan, 1939). Boys 12 to 19 years of age were examined at the time of their commitment to the School from 31 States, the District of Columbia and Puerto Rico; single swab examinations were made on 303 boys and multiple swab examinations on 303 boys. *Enterobius vermicularis* was found as follows: Of 364 white boys, positive 61, or 17 per cent, and of 242 Negroes, positive 14, or 6 per cent. In a group comparable to that of the present study, in which 4 swabs were made, pinworms were found in 36 of 180 white boys, an incidence of 20 per cent, and in 6 of 85 Negroes, an approximate incidence of 7 per cent, as compared with 25.2 per cent and 20.6 per cent, respectively, in boys 6 to 12 years old in the present investigation.

As regards the general population of Washington, D. C., the incidence of 19.8 per cent of *Enterobius* here found in Negro children 2 to 12 years old is slightly higher than the incidence of 15.5 per cent which we have found in the total of 789 Negroes examined, most of whom aside from the present group were examined as the result of an initial contact with a member of the family who had come to a "parasite clinic." Relative to white persons, examinations have previously been made on groups comparable to those included here. As regards children of preschool age, of 106 children in a private nursery school, 55 per cent were positive for *Enterobius* (Cram and Nolan, 1939). That incidence is almost identical with the present findings; however, in the private nursery school, the examinations covered an 18-month period, with an average of 9.2 swabs per child. As regards children of school age, Bozicevich (1937) from a single-swab examination found 31.3 per cent of 230 boys positive for *Enterobius* at the Washington Metropolitan Police Boys' Camp, and the following year Bozicevich and Brady (1938) from 2 to 4 swab examinations obtained an incidence of 57.3 per cent in 504 boys at the same camp. The ages ranged from 6 to 16 years and an analysis of the results indicated that the incidence of pinworms began to decline after 14 years. Compared to those findings, the 25.2 per cent incidence of pinworms found from 4 swabbings in the present investigation in Jewish children 6 to 12 years old is extremely low.

Concerning the relative incidence of pinworm infection in the white and Negro races, the present findings conform to previous findings in the general population of Washington, D. C., the incidence found in Negroes being lower than that in white persons.

SUMMARY

NIH anal swab examinations for *Enterobius vermicularis* were made on children of the white and Negro races in nursery schools and summer camps. Four swabbings per child showed an incidence of 33 per cent in 209 white children and of 19.8 per cent in 131 Negroes. In children of school age, 6 to 12 years old, examined in camp, there was only a slight difference in the incidence in the two races, 25.2 per cent in whites as compared with 20.6 per cent in Negroes; however, this sample of white children, exclusively Jewish, showed a much lower rate of infection than have other comparable samples previously reported. In nursery school children, 2 to 5 years old, there was found an incidence of 51.6 per cent in whites compared to

19.1 per cent in Negroes. The findings are analyzed as regards the incidence in the two sexes and the relation of positive and negative findings. Addition of these persons to others examined from the general population of Washington, D. C., brings the total to 2582 white persons, with the finding of pinworms in 1081 or 41.9 per cent, and 789 Negroes, with the finding of pinworms in 122 or 15.5 per cent.

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***Rotylenchulus reniformis*, nov. gen., n. sp., a nematode parasite of roots.**[1]

M. B. LINFORD and JULIETTE M. OLIVEIRA.

During 1935, enlarged female nematodes remotely suggestive of *Tylenchulus semi-penetrans* Cobb, 1913, were observed by Francis Yap of this Station on roots of cowpeas grown in soil from a pineapple field on the Island of Oahu. These females were usually only partly embedded in the roots, with their enlarged bodies lying exposed on the root surface or covered with egg masses and adherent soil. From soil about infested roots were obtained slender larvae, young females and somewhat degenerate males, all of similar size and form, with many characteristics of *Rotylenchus* Filipjev, 1936. What probably was this same form was observed during 1931 by H. R. Hagan and Francis Yap on cowpea roots, but, as the field source of that infestation was not determined and as the parasite appeared to be of minor importance, no detailed study was made. In 1936, however, when additional field infestations were found, investigations of this nematode were begun. Only taxonomy and life history are dealt with here.

The common name "reniform nematode" is proposed for this parasite, a name descriptive of the usual kidney shape of the adult female body.

In important morphological features this nematode is remote from *Tylenchulus* Cobb, 1913, despite somewhat similar metamorphosis of females and degeneration of males. From *Pratylenchus aberrans* (Thorne, 1935) Filipjev, 1936, it differs in number of ovaries as well as in life history. More closely allied to *Rotylenchus*, as shown by morphological resemblances, this new form differs strikingly in (a) metamorphosis of the female, (b) varied degenerate tendencies in the male, and (c) development from larva to male or young female without feeding and with no size increase. From all allied forms it differs in a more posterior placement of the dorsal duct opening into the lumen of the esophagus. A new genus is proposed, with a name suggesting its close affinity to *Rotylenchus* and its life history resemblance to *Tylenchulus*.

Rotylenchulus, nov. gen.

Diagnosis.—Tylenchidae with characters of *Rotylenchus* Filipjev, 1936, except: dorsal duct opening into lumen of esophagus approximately one stylet-length

[1] Published with the approval of the Director as Technical Paper No. 130 of the Pineapple Experiment Station, University of Hawaii.

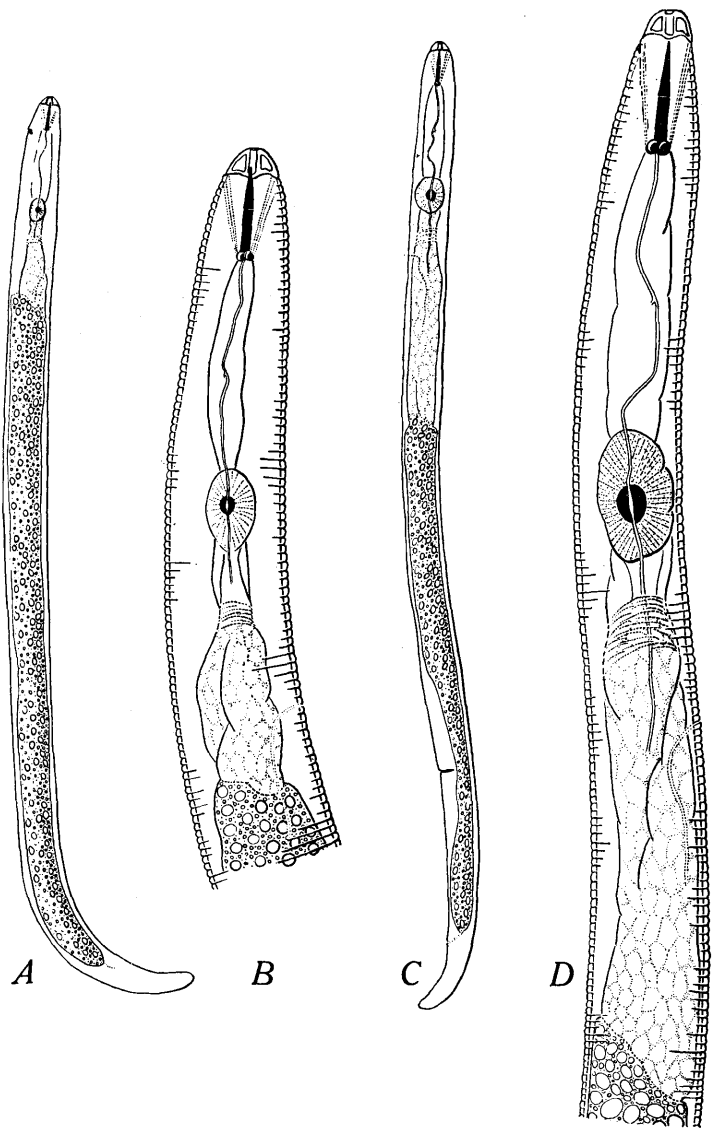

FIG. 1. *Rotylenchulus reniformis.* A—Young larva; ×330. B—Anterior region of young larva; ×1000. C—Young female before penetration and enlargement; ×330. D—Anterior end of young female; ×1000.

behind stylet; male no larger than larva, with weak stylet and esophagus; young female similar to larva in size and shape, with paired, undeveloped ovaries; mature female much enlarged especially in posterior part which becomes saccate, and with greatly thickened cuticle. Obligate plant parasites.

Type species.—*Rotylenchulus reniformis,* n. sp.

Rotylenchulus reniformis, n. sp. (figs. 1 & 2)

Measurements.—Larva: length = 316 to 441 μ, mean 394 μ; α = 20.5; β = 4.0;

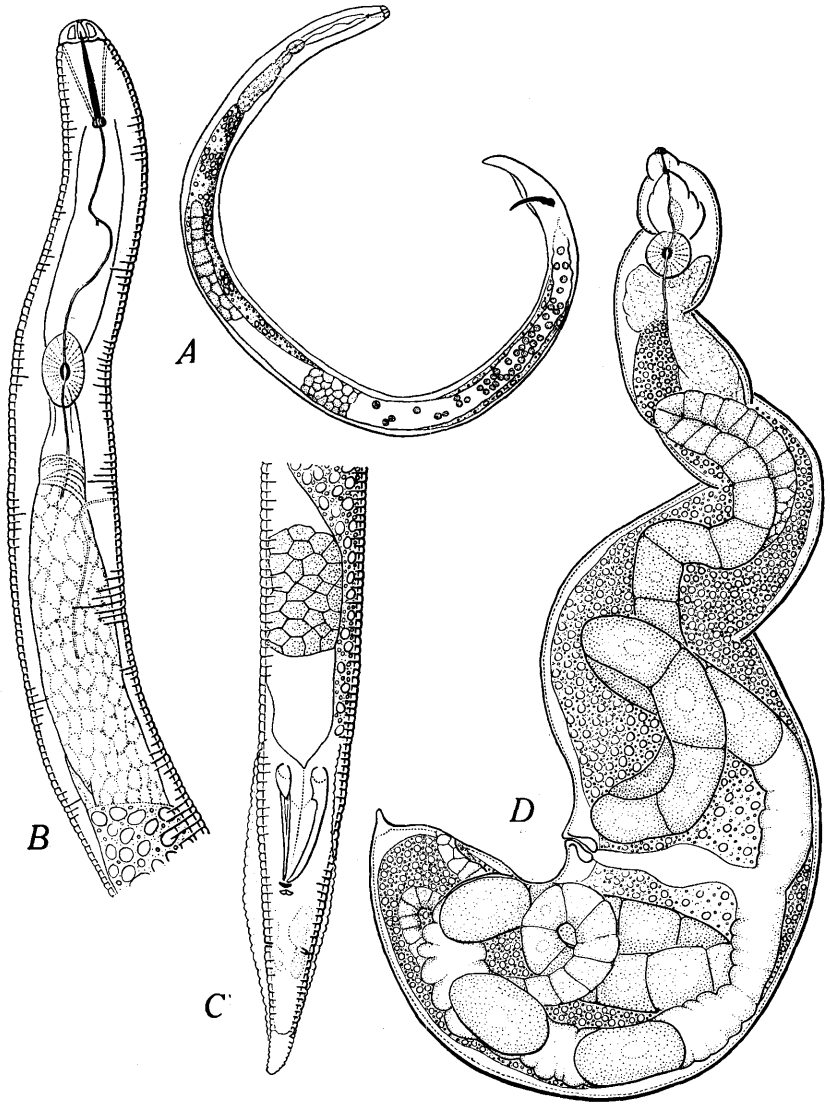

FIG. 2. *Rotylenchulus reniformis.* A—Male; ×330. B—Anterior portion of male; ×1000. C—Posterior region of male; ×1000. D—Mature reniform female; ×230 (This is semi-diagrammatic in that the ovaries are represented as superimposed on the intestine).

$\gamma = 13.0$. Male: length = 332 to 497 μ, mean 401 μ; $\alpha = 24.4$; $\beta = 4.2$; $\gamma = 11.8$. Young female: length = 321 to 432 μ, mean 376 μ; $\alpha = 19.3$; $\beta = 3.1$; $\gamma = 15.0$; $v = 72\%$. Mature female: reniform part of body, length = 324 to 483 μ, mean 404 μ; diameter = 121 to 168 μ, mean 139 μ. Egg: 70 to 118 by 34 to 49 μ, mean 94 μ by 42 μ.

Diagnosis.—With annulated cuticle and lateral fields bearing 4 longitudinal striae. Head with cuticularized framework and 6 radial ridges; buccal stylet tylenchoid with knobbed base, weak but evident in male. Outlet of dorsal eso-

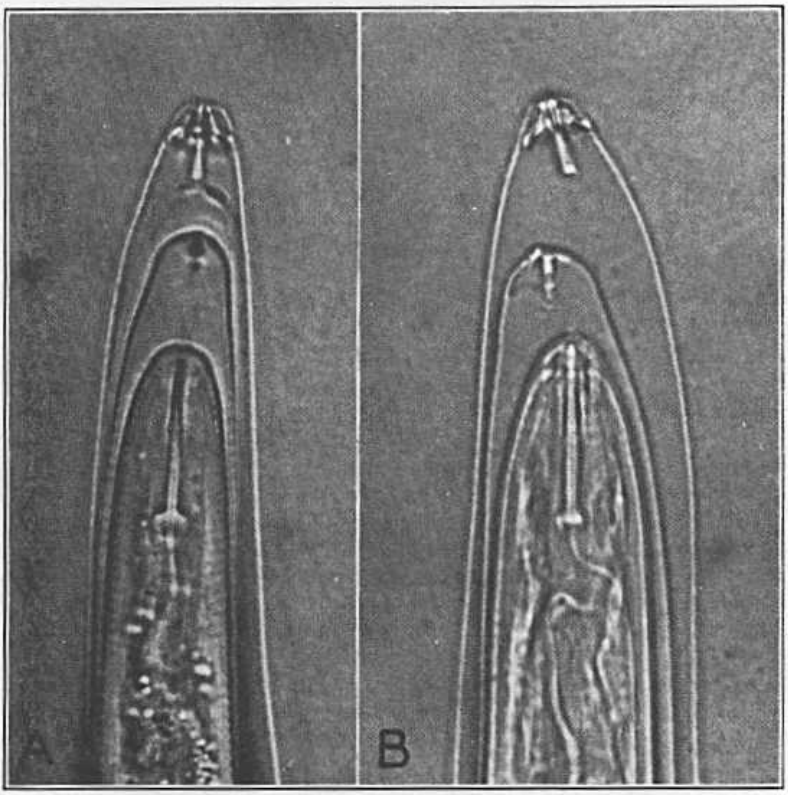

FIG. 3. Anterior end of female *Rotylenchulus reniformis*, late in the last molt; ×1400. A—Three loose cuticles visible: (1) the larval cuticle with cuticularized labial framework and stylet cast; (2) very thin, possibly multiple, with 2 lightly cuticularized rings over head; and (3) somewhat thicker, with short stylet cast. B—Another specimen, slightly flattened by coverslip, showing only cuticles 1 and 3, the thin second cuticle apparently not separated from the third.

phageal gland one stylet-length behind stylet. Excretory pore close behind median esophageal bulb. Posterior part of mature female enlarged, variably reniform, terminating in narrow, cylindrical spicate process; vulva prominent, central in flat or concave side of reniform body; vagina large, transverse; female apparatus amphidelphic. Male tail short, 1 to 1½ times length of spicula, with narrow bursa not enveloping terminus, and with one pair of lateral caudal papillae.

Description.—Larva: Cuticular annulations interrupted by lateral fields about ¼ body diameter in width, bearing 4 faint longitudinal striae. Head broadly conoid-convex, not set off by constriction, with well cuticularized framework and 6 radial ridges; buccal stylet typically tylenchoid, strong, 14 to 18 μ, mean 15.3 μ long, with large basal knobs. Esophagus tylenchoid; median bulb ellipsoidal with long cuticularized thickenings; esophageal glands situated in long asymmetrical posterior enlargement, with dorsal duct emptying into lumen of esophagus about one stylet-length behind stylet. Nerve ring just behind median bulb. Excretory pore 1 to 1.5 bulb lengths behind bulb. Anus inconspicuous; tail terminus variably rounded. Body well filled with reserves.

Male: Somewhat more slender than the larva, becoming almost transparent with age and exhaustion of reserves. Stylet, 14 to 18 μ, mean 15.4 μ long, weaker

than in larva but always evident; median bulb reduced in size and valves almost absent; posterior part of esophagus poorly defined; otherwise agreeing, in anterior part, with larva. One pair of lateral caudal papillae; bursa narrow, extending almost to terminus but not enveloping it; spicula slender, slightly arcuate and cephalated, 19 to 21 μ, mean 19.8 μ long; gubernaculum simple, about $\frac{2}{3}$ as long as spicula; testis single, outstretched; tail tapering uniformly to the slender but variable terminus, 1 to 1.5 as long as the spicula.

Young female: Before entering a root the female resembles the larva in size and general form. Stylet stronger and slightly longer, 16 to 20 μ, mean 18.8 μ long; median bulb larger with much larger and heavier valves; lining of esophagus anterior to bulb highly refractive. Vulva not prominent; ovaries paired, undeveloped.

Adult female: During feeding the immobile female enlarges until the posterior part of the body, usually on the root surface, becomes reniform, measuring 324 to 483 μ, mean 404 μ long by 121 to 168 μ, mean 139 μ wide at the vulva which is situated centrally on the flat or concave ventral side. The anterior part of the body, encased in the root cortex, becomes irregular in conformation varying with local resistance to expansion afforded by cortical cells. Cuticle very thick; striated where least distended. Head much as in larva but sharply set off from the expanded neck region; stylet as in young female. Median esophageal bulb much enlarged, spheroidal, fibrous, with heavy valves; anterior part of esophagus broadly conoid with base at bulb, narrowing anteriad to base of stylet; esophagus posterior to bulb containing large irregularly placed glands. Vulva large, with prominent lips, opening into a transverse vagina that extends $\frac{2}{3}$ to $\frac{3}{4}$ across body, funnel-shaped at inner end; female apparatus amphidelphic, ovaries long, irregularly coiled. Anus evident, situated near short spicate cylindrical or conical tail process.

Eggs: Laid unsegmented or sometimes in 2-cell stage in a gelatinous matrix, accumulating in rounded masses usually 0.5 to 0.8 mm (rarely over 1.0 mm) in diameter which usually cover the enlarged body of the female and become coated with adherent soil particles.

Type locality.—Island of Oahu, Territory of Hawaii.

Type host.—Cowpea roots (*Vigna sinensis* Endl.).

Known to parasitize roots of plants belonging to 30 families (Linford and Yap, 1940); produces no gall-like swellings nor other major symptoms.

LIFE HISTORY

Rotylenchulus reniformis is an obligate plant parasite with a highly specialized life history and with only the females parasitic. In varied types of observations males have never been seen feeding. Even the larvae do not feed but, when held in water or in soil devoid of roots, they develop relatively rapidly into mature males and infective young females.

These females embed themselves partly or, sometimes, entirely in the root cortex and begin feeding. Observations through the glass sides of miniature root-observation boxes (Linford, 1940) showed enlargement of the posterior part of the body on the third day and first oviposition on the ninth day after penetration into Whippoorwill cowpea roots. Eight days later, well-developed embryos were visible in eggs against the glass and hatching began promptly upon transfer to water.

Rate and duration of oviposition have not been determined precisely but both appear to differ with individual nematodes and especially with host plants. Limited study of egg masses from the favorable host, Whippoorwill cowpea, has given counts of as high as 78 eggs per mass with a mean of 54. When eggs plus empty shells were counted, the maximum figure was 196 per mass with a mean of 121.

To study molting, freshly-hatched larvae were placed singly into hollow ground slides of tap water and observed daily with a water-immersion objective. When bacterial contamination became heavy, nematodes were picked with a steel needle to fresh slides of water. Under these conditions males and females developed normally through a series of at least 3 superimposed molts, except that, lacking a firm substratum they remained encased within their multiple cast cuticles (Fig. 3, A & B).

First evidences of metamorphosis consist of gradual cessation of body movement and progressive dissolution of the cylindrical shaft and basal swellings of the buccal stylet. The nematode finally comes to rest in a crescentic posture and shows no more than very sluggish movement during the entire molting period, until it is fully differentiated as a male or female. The basal part of the stylet disappears at the same time that the crescentic valves in the esophageal bulb and other internal cuticularized structures become indistinct. Sluggish retractive movements of the nematode then disengage the head from the anterior conical part of the stylet which remains attached to the cuticularized head framework that is cast with the larval cuticle. At about this time, loosening and enlargement of the cuticle may be evidenced by both transverse and longitudinal folds, after which considerable space may appear between the nematode and this first cuticle.

When the head is first retracted from the larval cuticle, the anterior part of the nematode appears essentially naked. A very thin second cuticle is then formed and may promptly loosen, especially at the head. This cuticle carries neither labial framework nor any indication of the conical part of a stylet. Instead, however, there are one or sometimes two small and delicate rings of cuticularization, apparently corresponding with the buccal orifice. Despite the thinness of this second cuticle, this indication of two buccal orifices, which may be superimposed when only one is recognizable, suggests that this may actually represent two molts rather than one.

The third and last cuticle is then formed and loosened, less heavy than the first but much heavier than the second. It carries little cuticularized thickening over the head but does include, as an inward projection from the anterior end, a short conical cast of the anterior part of the stylet.

By the time this third cuticle is loosening, internal cuticularized structures are forming, including stylet, lining of the esophagus, valves in the median bulb, and spicula and gubernaculum of the male, and also the vulva is differentiating. Sex of the molting individual is indicated earlier, however, by enlargement and clearing of the tail region of the male and by clearing of the vulva region of the female. Not until the nematode appears fully developed does it become sufficiently active that, in soil, it could escape from its superimposed cuticles, and collections of nematodes from soil frequently include individuals exhibiting 2 loose cuticles and sometimes 3.

Four molts appear to be the rule among nematodes parasitic in animals, one of which may occur in the egg. With this species, however, no evidence has been obtained of a molt before hatching. Consequently, it appears either that there are only three molts or that one of the 3 recognized cuticles represents two separate molts. The frequent presence of 2 delicate cuticularized rings at the anterior end suggests that the thin second cuticle is actually double. In the majority of individuals only two loose cuticles are to be seen, the second and thinnest being apparently in intimate contact with the third. The second is well separated from the first by the inward projection of the stylet cast, but lacking a stylet cast, there is nothing to prevent the second from lying in contact with the third. Thus it is not unreasonable to assume the presence of one cuticle that has not been demonstrated.

A few larvae become sluggish and exhibit first stages of dissolution of the stylet base within 24 hours after hatching, but 5 days of larval activity appear about average in water. At the other extreme, some larvae have remained active in water 11 days. Seven to 9 days after the initial changes, however, both males and females appear fully formed and resume activity, giving a minimum period from hatching to the completion of molting of 8 days and an average period of approximately 13 days. This is short in comparison with estimates obtained by using black sand in miniature root-observation boxes where, in one test, a period of 22 days was observed between hatching and penetration.

The minimum life cycle thus appears to be a summation of 8 days for hatching, 8 days until the completion of molting, and 9 days until first eggs are laid, or a total of 25 days. This makes no allowance for the young female to locate a root and penetrate it, and probably is considerably shorter than the average length of a life cycle in soil under favorable conditions.

In collections of nematodes washed from soil associated with roots of infested plants, males of the reniform nematode are sometimes more numerous than females. Examination of a large number of individuals that have molted in water indicates, however, that under these conditions, the sex ratio approximates 1: 1. In one series there were 227 males and 250 females with a number of individuals that apparently died before sexual differentiation. The difference between these two figures is considered insignificant.

Enlarging females, before eggs are laid, strongly attract males which coil about the reniform body, moving sluggishly. Copulation has not been observed, probably because of technical difficulties. Males may persist and become enveloped by the pale amber colored secretion in which the eggs are deposited. In one series of observations, 12 well developed egg masses from cowpea roots contained an average of 5.9 males each. The only males seen inside of roots were associated with females that had completely embedded themselves in the cortex.

DISCUSSION

Rotylenchulus reniformis appears to have evolved from a *Rotylenchus*-like ancestor, becoming highly specialized for a sedentary parasitic mode of life with the acquisition of a superimposed series of molts without growth intervals, degeneration of males, and transformation of adult females to a reniform shape. Approaching the degree of body enlargement exhibited by species of *Heterodera* Schmidt, 1871, these females become efficient egg producers and provide their eggs with reserves so ample that the larvae may, without feeding, develop into mature males and young females. Development of males of *R. reniformis* exhibits none of the complexity of *Heterodera*, in agreement with other evidences of lesser specialization.

In comparison with *Pratylenchus aberrans*, *Rotylenchulus* appears the more highly specialized in that larvae of *P. aberrans* apparently feed, while those of Rotylenchulus do not.

It appears that *P. aberrans* bears, to a lesser degree, the evolutionary relationship to *Pratylenchus* that *R. reniformis* bears to *Rotylenchus*. The differences, however, between *P. aberrans* and *R. reniformis* are too great to make these species congeneric.

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Some host plants of the reniform nematode in Hawaii.[1] M. B. LINFORD and FRANCIS YAP.

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, 1940, first observed on roots of cowpea in the Hawaiian Islands, was soon found also attacking pineapple and several weeds representing a number of plant families. This indication of generalized host adaptability led to further observations and experimental tests with results that show this root parasite to have a vast potential host range and that help to define some factors of resistance or immunity.

Examination of plants collected from infested field and garden areas has been supplemented, as time permitted, by several small-scale glasshouse tests of crop plants and ornamentals chosen to include members of diverse families. When any mature females with eggs were observed, the plant was considered a host.

HOST-PARASITE RELATIONSHIPS

Among the host plants listed here, several compare favorably with the Whippoorwill cowpea in intensity of root infestation, rate of development of the parasite, and size of egg masses. Such plants are: artichoke, bean, beloperone, calendula, coleus, *Crotalaria spectabilis*, cucumber, eggplant, lettuce, nothopanax, okra, papaya, pineapple, purslane, richardsonia, sea-grape, stagger-weed, wild pea-bean, wooden-rose, and *Zinnia*. Many others may belong in this class, but limited observations suggest that root infestations tend to be more sparse.

Certain other hosts, however, are distinctly unfavorable to the parasite as shown by slow development of the females, maturation of few of them, and the laying of very few eggs. Some such plants have been compared closely in miniature root-observation boxes. In both French Breakfast and Japanese All Seasons radishes, only a small proportion of the females matured, and first eggs were laid after 13 days compared with 9 days on cowpea. Wild indigo carried many immature, almost transparent females after 3 weeks, but only one female with 2 eggs and another with 5. Two of these 5 eggs were well embryonated—several days old—demonstrating a very slow rate of egg laying. Bush lima bean also belongs in this group, as does feterita except that, in addition, its roots appear to be entered by very few of the parasites. On roots of turnip, *Brassica Rapa* L., and daikon, *Raphanus sativus* L. var. *longipinnatus* Bailey, many immature females have been seen, but none with eggs. These 2 plants are not listed as hosts.

Roots of still other plants, not listed as hosts, are penetrated by the reniform nematode in only small numbers if at all. Various plants grown in infested soil have been examined for exposed females and egg masses with negative results but some of these may have been escapes, or light infestations may have been overlooked, because some such plants, in a second test, have proved somewhat susceptible. Consequently no list of these plants can advisably be presented pending more critical trials. Three species, however, that appear resistant to the entry of *R. reniformis* are *Leucaena glauca* Benth., *Stenotaphrum secundatum* Kuntze, and Bermuda grass, *Cynodon Dactylon* (L.) Pers.

LIST OF HOSTS

Inclusion in the following list signifies that a plant has been seen by the writers bearing mature females and eggs of *R. reniformis*. The nomenclature is that of

[1] Published with the approval of the Director as Technical Paper No. 131 of the Pineapple Experiment Station, University of Hawaii.

Bailey, 1924, for plants included in his Manual. Capital letters following names indicate the sources of plants examined: A, from naturally infested field or garden; B, from glasshouse tests.

Ageratum conyzoides L. Ageratum ..... A
Ananas comosus Merr. Pineapple ..... A, B
Argyreia nervosa (Burm. f.) Bojer ..... B
Begonia semperflorens Link & Otto ..... B
Beloperone guttata Brandegee ..... B
Beta vulgaris L. Beet, Early Wonder ..... B
Beta vulgaris L. var. *Cicla* L. Swiss Chard ..... B
Bixa Orellana L. Annatto ..... B
Brassica oleracea L. var. *acephala* DC. Kale, Jersey ..... B
Brassica oleracea L. var. *botrytis* L. Cauliflower ..... B
Brassica oleracea L. var. *capitata* L. Cabbage, Flat Dutch ..... B
Brassica pekinensis Rupr. Pe-tsi, Chinese Cabbage ..... B
Buddleia asiatica Lour. ..... B
Cajanus Cajan Millsp. Pigeon Pea ..... B
Calendula officinalis L. Pot Marigold, Lemon Queen ..... B
Callistephus chinensis Nees. China Aster ..... B
Carica Papaya L. Papaya ..... B
Centella asiatica (L.) Urban. Asiatic Pennywort ..... A
Coccoloba uvifera (L.) Jacquin. Sea Grape ..... B
Coleus Blumei Benth. Coleus ..... B
Crepis japonica (L.) Benth. Asiatic Hawksbeard ..... A
Crotalaria spectabilis Roth ..... B
Cucumis sativus L. Cucumber, Long Green ..... B
Cucurbita Pepo L. Squash, Zucchini ..... B
Cynara Scolymus L. Artichoke, Green Globe ..... B
Daucus Carota L. var. *sativa* DC. Carrot, Danvers ..... B
Echeveria sp. ..... B
Eleusine indica (L.) Gaertn. Wire-grass ..... A
Emilia sonchifolia DC. Flora's Paint Brush ..... A
Erechtites valerianifolia (L.) Raf. Fireweed ..... A
Erigeron albidus (Willd.) Gray. Horseweed ..... A
Eugenia malaccensis L. Mountain Apple ..... B
Euphorbia hypericifolia L. Graceful Spurge ..... A
Euphorbia hirta L. Garden Spurge ..... A
Euphorbia pulcherrima Willd. Poinsettia ..... B
Hedychium coronarium Koenig. White Ginger ..... A
Hibiscus esculentus L. Okra, Long Green ..... B
Holchus Sorghum L. var. *caudatus* Bailey. Feterita ..... B
Impatiens Balsamina L. Garden Balsam ..... B
Indigofera suffruticosa Mill. Wild Indigo ..... B
Ipomoea tuberosa L. Wooden-Rose ..... B
Kalanchoe sp. ..... B
Lactuca sativa L. Lettuce, Mignonette ..... B
Lycopersicon esculentum Mill. Tomato, Marglobe ..... B
Murraea exotica L. Orange-Jessamine ..... B
Passiflora Seemanni Griseb. Passion-Flower ..... B
Phaseolus lathyroides L. Wild Pea-Bean ..... B
Phaseolus limensis Macf. Bush Lima Bean ..... B
Phaseolus vulgaris L. Bean, Stringless Green Pod ..... B
Phlox Drummondii Hook. ..... B
Pisum sativum L. Pea, Perfection ..... B
Polyscias Guilfoylei Bailey. Nothopanax ..... B
Portulaca oleracea L. Purslane ..... A, B
Prosopis chilensis (Molina) Stuntz. Algaroba ..... B
Raphanus sativus L. Radish, French Breakfast and Japanese All Seasons ..... B
Richardia scabra L. Richardsonia, False Ipecac ..... A
Schinus terebinthifolius Raddi. Christmas-Berry-Tree ..... A, B
Solanum Melongena L. Eggplant ..... B
Solanum nigrum L. Black Nightshade ..... A
Solanum tuberosum L. Potato, Bliss Triumph ..... B
Sonchus oleraceus L. Annual Sow Thistle ..... A
Stachys arvensis L. Stagger Weed, Hedge Nettle ..... B
Tagetes erecta L. African Marigold ..... B

Tagetes patula L. French Marigold, Dwarf Mixed ..... B
Vernonia cinerea (L.) Less. Iron-weed ..... A
Vigna sinensis Endl. Cowpea, Whippoorwill and Iron ..... A, B
Zea Mays L. Maize, Stowell's Evergreen ..... B
Zinnia elegans Jacq. Zinnia ..... B

These 65 host plant species represent 30 families, distributed widely among the Angiospermae. These families, together with numbers of host species observed in Hawaii are: Acanthaceae 1, Anacardiaceae 1, Araliaceae 1, Balsaminaceae 1, Begoniaceae 1, Bixaceae 1, Bromeliaceae 1, Caricaceae 1, Chenopodiaceae 1, Compositae 14, Convolvulaceae 2, Crassulaceae 2, Cruciferae 3, Cucurbitaceae 2, Euphorbiaceae 3, Graminae 3, Labiatae 2, Leguminosae 9, Loganiaceae 1, Malvaceae 1, Myrtaceae 1, Passifloraceae 1, Polemoniaceae 1, Polygonaceae 1, Portulaceae 1, Rubiaceae 1, Rutaceae 1, Solanaceae 4, Umbelliferae 2, and Zingiberaceae 1.

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Onion bloat or eelworm rot, a disease caused by the bulb or stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev. B. G. CHITWOOD, U. S. Bureau of Plant Industry, A. G. NEWHALL, Dept. of Plant Pathology, Cornell University, and R. L. CLEMENT, New York State Dept. of Agriculture and Markets.

HISTORICAL RÉSUMÉ

An eelworm disease of onions has been known in Europe since 1877 under the names "Kroefziekte," eel-disease, and onion bloat. The causal organism was named *Tylenchus putrefaciens* by Kühn (1877, 1879). It was later named *Tylenchus allii* by Beyerinck (1883) and still later (1886, 1887, 1888) shown by Ritzema Bos to be the same species as that described from teasel, narcissus, hyacinth, and rye. This species is now known as *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936. Ritzema Bos (1888) transferred the stains from rye and hyacinth to onions, and transferred the onion strain to rye, while Ramsbottom (1919) transferred the narcissus strain to onion. More recently Godfrey and Scott (1934) found the strain in garlic to be infective for salsify, parsley, and celery, while Walton (1937) reported a mass transfer from onions to parsnips. Later (1938) the same author reported that the nemas would persist in the soil 4 to 5 years.

Knowledge concerning the effects of the disease is due to Beyerinck, Chatin and Ritzema Bos. Germinating seedlings become twisted, enlarged, and often die. If they survive through the first few weeks or become infested later, according to Ritzema Bos, the disease is manifested by an enlargement of the inner bulb scales due to hypertrophy and multiplication of parenchyma cells. This causes a break in the outer scale resulting in a split onion. Diseased onions seldom flower (vide Chatin) but cases of as high as 1 to 2 per cent infested seed have been reported by Ritzema Bos.

Thus far onion bloat is known in this country only in New York State but this apparently restricted distribution is probably because of failure to recognize the disease elsewhere. The first recorded infestation of onions with *D. dipsaci* occurred at Canastota, New York, and the report was published by Steiner (1931). That infestation was first noted by the grower in 1929. The New York State Department of Agriculture financed, and one of us (A. G. N.) supervised, the steam sterilization of the area, consisting of $\frac{1}{3}$ acre of muckland. No further evidence of the disease has been found in that field.

Newhall (1939) and Newhall *et al.* (1939) reported on the finding of several infestations near Canastota, N. Y., in 1938, and near Florida, N. Y., in 1939. They concluded that the disease was not general in the onion-producing areas and that the spots located had been infested for several years. Despite reports by Godfrey (1924) that *D. dipsaci* is present in 17 counties of New York (only Wayne County being specifically mentioned), attempts by two of us (B. G. C. and R. L. C.) to locate infested dandelions and other weeds such as teasel in the onion-producing areas did not meet with success in 1939. An attempted transfer from onion to dandelion was unsuccessful. That, of course, does not signify that such a transfer cannot occur but it seems more probable that the disease on onions in New York

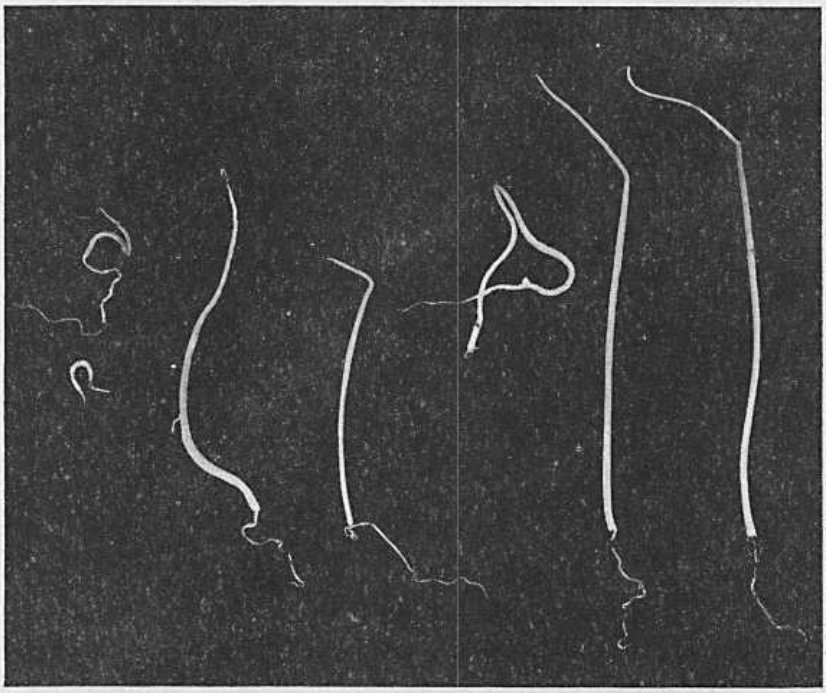

FIG. 1. Onion seedlings (variety Southport Globe) planted on soil infested with *Ditylenchus dipsaci* from chopped onions. Planted Sept. 21, 1939; photographed Nov. 1, 1939, by F. S. Blanton. The 2 seedlings at the right are normal. Note twisting and decolorization of infected seedlings.

originated with the introduction of a few diseased sets from an area in which the disease was endemic some years ago.

SYMPTOMS

Onions, germinated from seed in soil infested with *D. dipsaci* (from chopped onions) emerged from the soil 10 days after planting, were twisted and abnormally white, and had enlarged areas; the epidermis of the cotyledon was sometimes broken. This condition may be distinguished from smut injury (blackened enlargements) by the decolorization, twisting, and irregularity of the affected areas in seedlings infested with *D. dipsaci*. Many of the infected seedlings were dead within 3 weeks of the day the seeds were planted.

Onion sets, raised on soil similarly infested, first exhibited symptoms 21 days after planting. These included stunting and the yellowish spots and lesions gen-

erally termed eelworm spots or "spikkles."* At this time nemas were present in both the leaves and the onion itself.

Field inspection of onions has thus far been made only rather late in the season (mid-July) at which time infested areas were clearly demarcated by the prostrate position of the foliage. Such areas were generally termed "lightning struck." Only when the area persists from year to year does the grower become suspicious that it may be a disease. No foliar symptoms such as spikkles, yellow areas or

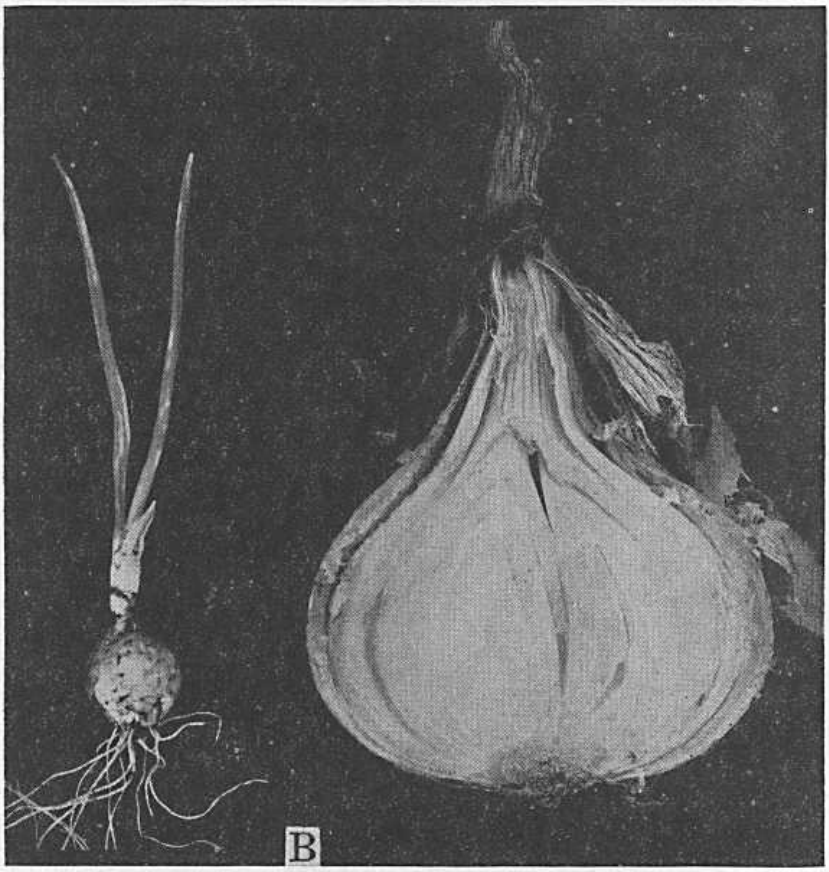

FIG. 2. A—Onion set (yellow type) planted on soil infested with *Ditylenchus dipsaci* from chopped onions. Planted Sept. 21, 1939; photographed Nov. 1, 1939, by F. S. Blanton. Note areas of decolorization, swellings, and open lesion. B—Longitudinal section of mature onion (variety Yellow Globe) naturally infected with *Ditylenchus dipsaci*. Origin: Canastota, N. Y. Note broken down parenchyma on inner side of first and second scales. Photographed Sept. 1, 1939, by F. S. Blanton.

open lesions have been noted in such nearly mature onions but when one passes from bulb to bulb, feeling of each, one finds that the skin often slips, taking with it the first scale. There is a definite increase in number of split and double onions in infested fields. Onion decay due to other causes such as bacteria, fungi, and onion maggot also appears to be increased in *D. dipsaci*-infested fields.

* The term spikkle is the word used in Holland for spot and has attained the special meaning of eelworm spot only by its restricted use in the bulb industry.

Many of the diseased onions are rotten at the base and contain varied types of secondary invaders. The mere finding of nemas in diseased onions is of no significance since such saprozoic and fungiphagous forms as *Aphelenchus avenae* Bastian, *Rhabditis* spp., *Pristionchus* sp., *Panagrolaimus subelongatus* (Cobb) Thorne, and *Aphelenchoides parietinus* (Bastian) Steiner are of common occurrence in onions affected by either *D. dipsaci* or other onion pests such as bacteria, maggots, onion thrips larvae, bulb mites, and numerous fungi, chief of which are several species of *Botrytis* and *Fusarium*.

In an area infested with *D. dipsaci* it is always possible to find bulbs which have not as yet begun to decay but which are clearly diseased. The diagnostic symptom at this stage of the disease is a white mealiness of the inner surface of

FIG. 3. Entire onion (variety Yellow Globe) naturally infected with *Ditylenchus dipsaci*. Origin: Canastota, N. Y. Same onion as that shown in Fig. 2, B. Note broken skin and depressed area on the right side indicating lack of resilience. Photographed Sept. 1, 1939, by F. S. Blanton.

the first scale. This is due to the breaking down of the parenchyma adjacent to the inner scale membrane, the cells coming loose from the vessels so that the latter may give a lacy appearance. It is this looseness of the parenchyma which causes the outer scale to slip off. Not infrequently the larvae of thrips (*Thrips tabaci* Lindeman) and mites (*Rhizoglyphus hyacinthi* Boisduval) invade the tissue between the outer scales and when accompanied by bacteria, as they often are, cause the outer skin or scale to slip easily. A more or less slimy malodorous condition accompanies these insects as in the case of maggots. *Botrytis* neck-rot and *Fusarium* dry-rot which usually enter through the neck and basal plate, respectively, penetrate evenly and deeply in all directions. The dull brownish-grey color of the tissue infected by either fungus is, likewise, easily distinguished from the lacy or

mealy white tissue in which *D. dipsaci* is found. Unless secondary organisms are present, onions infested with *D. dipsaci* are not particularly moist, in fact they tend to lose moisture, and they have no special odor. Splitting due to enlargement of the inner scales is usually most pronounced at the base but this symptom is not necessarily diagnostic of the disease. Many onions are superficially perfect. As

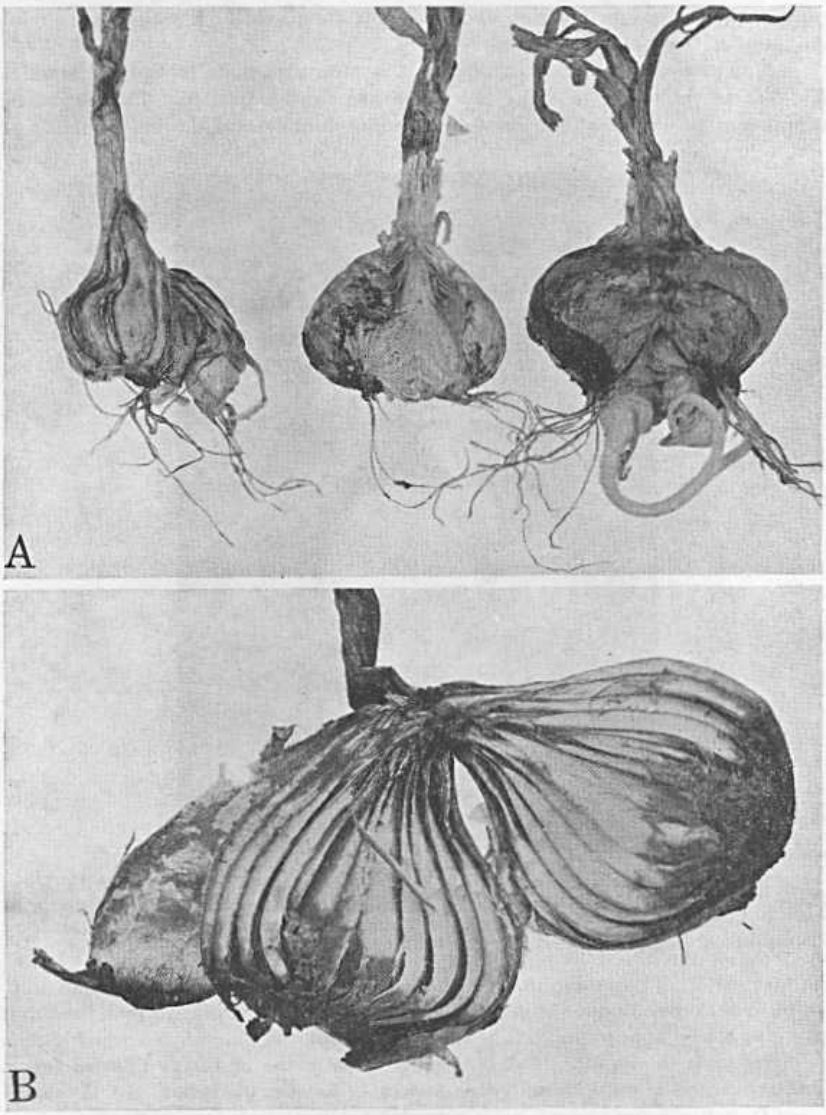

FIG. 4. A—Split and double onions (variety Yellow Globe) naturally infected with *Ditylenchus dipsaci*. Origin: Canastota, N. Y. Photographed by W. R. Fisher. B—Longitudinal section of onion (variety Yellow Globe) naturally infected with *Ditylenchus dipsaci*. Origin: Canastota, N. Y. Photographed August 1, 1939, by F. S. Blanton. The loose parenchyma giving a mealy appearance is diagnostic. The blackened condition of the base is due to secondary invasion.

the season progresses more and more onions decay. During storage, breakdown of the parenchyma continues and the outer scale often sloughs off, leaving exposed a second scale which is puffy in appearance, soft and yielding to the touch. It is only at this time (October) that the term "bloat" is an apt description of infested onions raised from sets. The characteristic white mealiness of the inside of the scales (sometimes several are involved) persists, but with drying the inside of the outer scale may appear stringy.

FIG. 5. A—Normal onions (variety Yellow Globe). Locality: Oniontown, N. Y. Photographed August 13, 1939, by R. L. Clement. B—Onions (variety Yellow Globe) infected with *Ditylenchus dipsaci;* same field as that shown in Fig. 5, A. Photographed August 13, 1939, by R. L. Clement.

Dry, cured onions have been artificially infested with *D. dipsaci* from both onion and narcissus. This was done by inserting small quantities of diseased tissue between the second and third scales in the neck region. In such onions the only change produced was the typical breaking apart of the parenchyma near the inner surface of the affected scales. After 30 days, the disease had sometimes progressed to the base of the bulb and in one instance had involved every scale.

GENERAL CONTROL CONSIDERATIONS

Examination of muck soil from severely diseased areas of onions failed to disclose *D. dipsaci* free in the soil. In each of three tests, seedlings grown on such soil did not become infected. It would appear, therefore, that nemas free in the soil are not necessarily the most important factor in the persistence of the disease from year to year. While not denying that they contribute in some measure, the writers are under the impression that diseased onions left in the soil are of more importance. Many growers sort their onions in the field leaving heaps of discards or culls. Others discard the non-saleable onions as they are gathered. In either instance, if *D. dipsaci* is present, many of the diseased onions are turned under in the fall. These onions provide an exceptionally favorable place for the propagation, distribution and perpetuation of the disease. When *D. dipsaci* is known to be present, all cull onions should be burned, including those not harvested because of their small size.

Since the onion strain of *D. dipsaci* is reported to attack parsnips, celery, and salsify, these crops should not be grown on land known to be infested. Since strains from narcissus and hyacinth are reported to attack onions, it would not be wise to plant onions on soil previously grown to narcissus or hyacinth, both of which are frequently diseased. Since the strain from rye is said to attack onions, it appears probable that rye might act as a reservoir host when used as a cover crop. The same may be said for barley and related plants. Their use in any rotation scheme therefore is discouraged.

Crop rotation is the most practical control measure but care should be taken that the other crops resorted to are not hosts of *D. dipsaci*. For the onion-producing areas of New York State, lettuce, spinach and carrots are suggested.

When crop rotation is not possible, soil sterilization may be resorted to. No satisfactory experimental data on such sterilization have as yet been obtained for the mucklands in which onions are usually grown in New York. Field treatments (Newhall *et al.*) indicate that sulphur at the rate of 1½ tons per acre may be satisfactory in eradicating the nema but this may also reduce the crop of onions considerably. In a single trial, chloropicrin, at the rate of 350 pounds per acre, applied in doses of 4 cc, spaced in rows with holes 15 inches apart and 8 inches deep, appeared to be satisfactory as an eradicant, with no damage to the crop. More data must be obtained before any control measure can be confidently recommended.

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Duboscqia penetrans, n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasite of the nematode _Pratylenchus pratensis_ (de Man) Filipjev. GERALD THORNE, U. S. Bureau of Plant Industry, Salt Lake City, Utah.

A collection of 131 specimens of *Pratylenchus pratensis* from soil about the roots of cotton plants included 87 individuals (66.4 per cent) which were parasitized by a new species of sporozoan. This material was collected at the Pee Dee Experiment Station, Florence, South Carolina, and kindly forwarded to the writer by Dr. George M. Armstrong, August 10, 1938. At the time of collection the nemas had left the maturing cotton roots and were living free in the soil where they had been attacked by the sporozoans which, in that locality, must be of some importance as a control agent.

Another collection made at Perry, Georgia, September 8, 1939, by C. W. McBeth, from soil about corn roots, contained 82 specimens of *P. pratensis*, 23 of which (28 per cent) harbored this same sporozoan. These two instances suggest that the parasite is widely distributed in the South Atlantic States though no case of such parasitism has been found among the writer's extensive collections of *P. pratensis* from Arizona, California, Colorado, Oregon, Texas and Utah or in foreign collections made near Toronto, Ontario, Canada; Beckingham, Kent, England; and Nhill, Victoria, Australia. The form of *P. pratensis* herein discussed is that in which males rarely occur.

Duboscqia penetrans, n. sp.

Diagnosis.—Spores oval, 2.5 to 3 μ in length; polar filament probably coiled within the spore. A parasite of *Pratylenchus pratensis*. Distinguished from the other two described species parasitic in nematodes, *D. trilobicola* Micoletzky and *D. de-mani* Micoletzky, by the size and form of the spore, that of *D. trilobicola* being elongate, 9.5 by 4 μ, while the sporonts of *D. de-mani* average 8 μ in length (Micoletzky, 1925, Mém. Acad. Roy. Sci. et Lett. Danemark, Copenhague, Sect. Sci., 8. s., **10** (2): 58–310). The type species of the genus, *D. legeri* Pérez, is a

parasite in the body cavity of a termite, *Reticulitermes lucifugus* (Rossi), and has spores 2.5 by 5 μ (Kudo, 1939, Protozoology).

Assignment of this species to *Duboscqia* is tentative. The sporont appears to produce 16 sporoblasts and finally 16 spores which constitute the chief diagnostic character of the genus. But the great difference in hosts suggests that careful comparisons of all stages of the parasite will reveal important differences which will justify establishing a new genus.

Life cycle of Duboscqia penetrans.—From the preserved material at hand it was possible to reconstruct the probable life cycle of the parasite. Possibly errors have been made which must later be rectified when living material is available for study but at present it appears to be as follows:

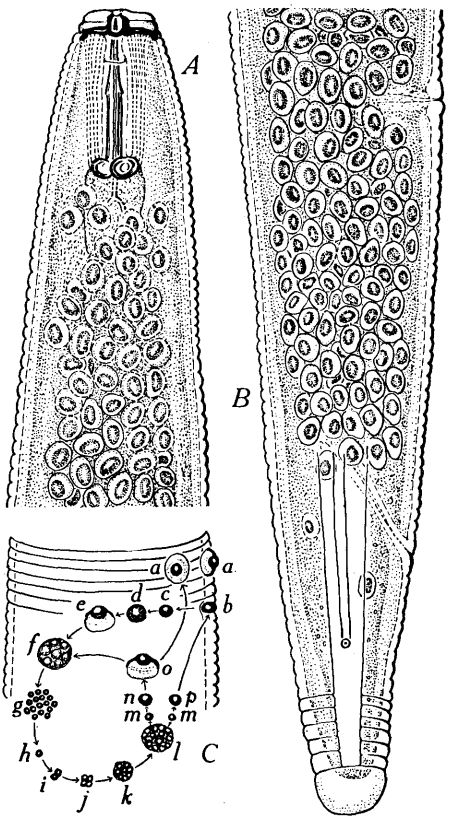

FIG. 1. A—Anterior portion of *Pratylenchus pratensis* parasitized by *Duboscqia penetrans.* B—Posterior portion of same. C—Probable life cycle of *Duboscqia penetrans; a,* spores attached to cuticle; *b,* sporoblast passing through cuticle; *c, d,* maturing sporoblast; *e,* mature spore; *f,* schizogony of spore; *g,* 16 sporonts formed during schizogony; *h, i, j, k, l,* one of the 16 sporonts undergoing repeated binary fission to form 16 sporoblasts; *m,* 2 of the 16 small sporoblasts; *n, o,* development of sporoblast into spore from which the life cycle is repeated within the nematode body but if liberated into soil it attaches to body as in *a; p,* developing sporoblast liberated into soil where it will attack and enter another nematode as shown by *b;* ×1200.

Spores and developing sporoblasts from the bodies of infested dead nematodes remain in the soil and there attack other nematodes with which they come in contact. There are two types of parasitism, internal and external.

External parasitism is by the adult spores only which attach themselves to the body of the nematode and, in some unknown manner, draw their sustenance from the nemic body which results in a breaking down and exhaustion of the tissues (Fig. 1, *a*). In one instance 29 spores were observed attached to the cuticle and in this individual the reproductive system had practically disappeared, the granules of the intestine were greatly reduced and general degeneration of the body contents had set in. Only a small number of parasites were within the body. A similar condition was noted in another individual on which 42 spores were attached.

Internal parasitism is a more complicated affair. The developing sporoblast attaches itself to the nematode and, probably by amoeboid movements, penetrates the cuticle and enters the body cavity (Fig. 1, *b*). Here it continues development and becomes a mature spore which passes through the process of schizogony, producing 16 schizonts (Fig. 1, *g*). Each schizont then becomes a sporont which, apparently by

simple binary fission, produces 16 sporoblasts, the group being held together by only a thin membrane which ruptures and releases them when sporogony is completed (Fig. 1, *l*). These sporoblasts develop within the body cavity, mature, pass through schizogony and repeat the process time after time until the body of the host is filled with spores. When the nematode dies and the body disintegrates the mature spores attach themselves and the sporoblasts invade other nematodes as outlined above.

Internal parasitism ranged from a few spore in some individuals to complete filling of the body cavity in others. Frequently the body of the nema was distended by the thousands of spores until it was not only wider but, in some instances, longer than the bodies of the largest unparasitized nemas, in one instance reaching a length of 0.66 mm while the largest unparasitized gravid female was only 0.56 mm long. Heavy parasitism appeared to destroy the reproductive system thereby preventing the production of eggs. Individuals with only a few parasites were observed producing eggs but doubtless oviposition would cease as the parasites increased. It was sometimes possible to see the minute scars in the cuticle where sporoblasts had entered.

Both spores and sporoblasts attack the nematode most frequently on the head and neck. Doubtless this is due to the anterior end of the nematode coming in contact with the spores as it forces its way through the soil, the pressure aiding them to become attached. However they may be found in small numbers at any point on the body, even at the tip of the tail.

The extremely small size of the spores of *Duboscqia penetrans* have made it impossible to observe the polar filament which is normally present in this group of organisms. At all stages of its development the sporoplasm remains densely concentrated and obscures details of the processes of schizogony and sporogony.

Host specificity. Duboscqia penetrans appears to be a specific parasite of *Pratylenchus pratensis* for although 21 other species of dorylaims, tylenchus, cephalobs and other common forms were found associated with it none were observed infested. The intestine of one specimen of *Dorylaimus obscurus* Thorne was gorged with thousands of spores but not one had penetrated the intestinal cells or entered the body cavity. Apparently they had been picked up in feeding and remained within the intestine but were unable to assume their parasitic role in the body cavity.

That one of our most serious plant-infesting nematodes is attacked by a parasite to an extent suggesting economic importance is most encouraging from the standpoint of developing natural control. Hitherto plant parasitic nematodes have been found especially free from attack by disease-producing organisms but now it does not seem too much to expect that in some localities forms other than *P. pratensis* also may have their natural enemies and that at some future time these organisms will be utilized as control agents.

Methods of clearing screen residues in separating nematodes from soil. GERALD THORNE, U. S. Bureau of Plant Industry, Salt Lake City, Utah.

Residues from the finer screens used in separating nemas from soil invariably contain suspended material that leaves them more or less murky and difficult to examine under the microscope. This is especially true of residues from bolting silk and wire screens with 75 or more meshes per inch. After experimenting with various clearing agents, the writer has found two that are very satisfactory under almost all conditions and a third which is successful in treating certain types of very fine suspended material.

CACTUS, OPUNTIA SP.

Species of *Opuntia* possess a thick, viscid sap which quickly collects and precipitates minute soil particles in jelly-like masses, a thin slice of the cactus an inch long being sufficient to settle 250 cc of screenings. The piece should be held in the solution with a pair of tweezers and the solution stirred with a whirling motion. As the sediment begins to collect in stringy masses, the cactus is removed and as soon as the material settles, the clear liquid containing the nemas is decanted. Two or three rinsings of the settled mass will remove most of the few nematodes caught in it, but it should be examined under the binocular before discarding. Meantime the nemas in the decanted portion have settled and the greater portion of the water can be poured off and discarded, leaving only a comparatively small amount of material to be examined. In this manner the labor of soil examination can be reduced to 1/5 or 1/4 of the usual time required.

Certain species of *Ferocactus* give fair results but none is comparable to the opuntias. A supply of cacti can be kept in the laboratory for long periods if collected with the attached roots and a small quantity of soil.

Is it not possible that the bitter waters of Marrah (Exodus **15**: 23–25) were sweetened by a method similar to this? Cacti do not occur in the region of the Sinai Desert but it seems quite probable that some other desert plant has similar properties and was used in this manner by Moses.

SALIVA

Saliva is just as effective, although perhaps not so sanitary, as the opuntias. A small amount placed in a beaker of muddy solution will readily collect and settle the soil particles. The same whirling motion during stirring should be used.

MILK

Certain types of exceedingly fine clay sediments can be settled by adding a small amount of either fresh or condensed milk and stirring with the whirling motion. However, milk leaves the solution slightly clouded and material treated in this manner must be allowed to settle, the supernatant portion decanted off and clear water added. In general, milk cannot be used successfully except in rare cases of extremely fine suspended particles.

Opuscula miscellanea nematologica. VIII. G. STEINER, U. S. Bureau of Plant Industry.

(1) THE ROOTING OF CUTTINGS IN RELATION TO SOIL NEMATODES

Casual observations have repeatedly shown that the cut surfaces of cuttings and similar kinds of propagative plant material (tubers, rhizomes, corms, etc.) attract a variety of nematodes from the surrounding soil. Such open wounds apparently lure these animals by offering an easy access to food, either because contents of cells are open or little protected, or because various kinds of organisms, already feeding on cell contents, may furnish food to the nematodes. If an invasion takes place, more or less complete failure of root formation and growth is then observed; decay sets in later on. The question of the significance of these nematodes in regard to such failures is then raised: Are these nematodes a primary cause of failure by attacking or destroying the wound tissues, or by preventing the proper formation of a callus and of root buds, or are they merely attracted by the intensive bacterial life or the fungi that soon develop? Because of lack of experimental work no definite answer to these questions is at present possible. Work on the matter, however, is highly desirable. Recent observations

point decidedly to a harmful effect by such "soil-nematode" invasions on cuttings to be rooted. The following cases are rather significant.

In experimental work with root-forming substances, cuttings of the Chinese magnolia (probably a *Magnolia soulangeana* hybrid)[1] failed to grow; they had found to contain considerable numbers of a nematode identified as *Plectus rhizophilus* de Man, 1880. This is a widely distributed soil species but rarely found in large numbers. The present cuttings harbored them in pure culture; adults as well as all larval stages and eggs were present. Males are not known to occur. Obviously this *Plectus* found suitable if not optimal life conditions on the tissues opened by the cut. It was not possible to determine the nature of the food of these plecti; the intestinal contents showed no identifiable particles. Bacteria appear to be excluded as food. It remains an open question whether outflowing sap or the contents of the cells exposed by the cut are fed on. It seems certain that these specimens of *P. rhizophilus* prevented the present cuttings from rooting properly; however the exact mode by which this was accomplished remains unknown.

In a second case, cuttings of snapdragons submitted to the writer[3] revealed an entirely different situation. No special precautions by sterilization had been taken. Accordingly the association of nematodes and other life forms on these cuttings, which also failed to root, was very different from that found on the Chinese magnolia cuttings. Fungus growth and bacteria were observed; small oligochaetes of the Naididae were present. The nematodes observed included numerous specimens of *Diplogaster aerivora* Cobb, 1916, *Rhabditis strongyloides* Schneider, 1860, *Aphelenchus avenae* Bastian, 1865, *Aphelenchoides tenuicaudatus* (de Man, 1895) Goodey, 1933, and a new species of *Chiloplacus, Ch. trilineatus,* n. sp., described below. *D. aerivora* is generally considered to have carnivorous and necrophagous life habits, *Rh. strongyloides* is bacteriophagous, *A. avenae* lives on fungi and plant tissues, *A. tenuicaudatus* is a predatory form, living on other nematodes, while it is assumed that *Ch. trilineatus* has a saprophytic mode of life. Thus the members of the present association of nemic forms complement each other and balance the other organisms and the primary food material of the "milieu" very well. The possibility that *Rh. strongyloides* is a primary agent in the failure of these cuttings by acting as carrier of decay-producing bacteria may also be mentioned.

Chiloplacus trilineatus, n. sp. (Fig. 1)

This new species of *Chiloplacus* may easily be recognized by the presence of 3 lateral wings and the arrangement of the copulatory papillae in the male.

Description.—Chiloplacus resembling *Ch. symmetricus* (Thorne, 1925) Thorne, 1937. Female tail conical, broad-obtuse, male tail convex conoid, narrow-obtuse, with fine terminal mucro. Annulation of cuticle plain, laterally interrupted by 3 wings bordering 2 narrow lateral fields (combined width in middle region of body 3.5 μ with a corresponding body width of 23 μ). Cervical papillae and phasmids as figured. Head somewhat truncate; ventrosubmedial labial probolae asymmetrical with ventral prongs longer than lateral ones, but somewhat variable. Cephalic probolae separated by narrow, rounded axils, with setose corners and very slightly

[1] Identification by Paul Russell of the Bureau of Plant Industry, U. S. Dept. of Agriculture.

been sterilized and planted in sterile sand.[2] Upon examination, the cuts were

[2] This material was collected by F. W. Went of the Calif. Inst. of Technology and submitted for examination through G. L. Stout, of the Calif. Dept. of Agriculture.

[3] By D. Folsom of the Maine Agricultural Experiment Station.

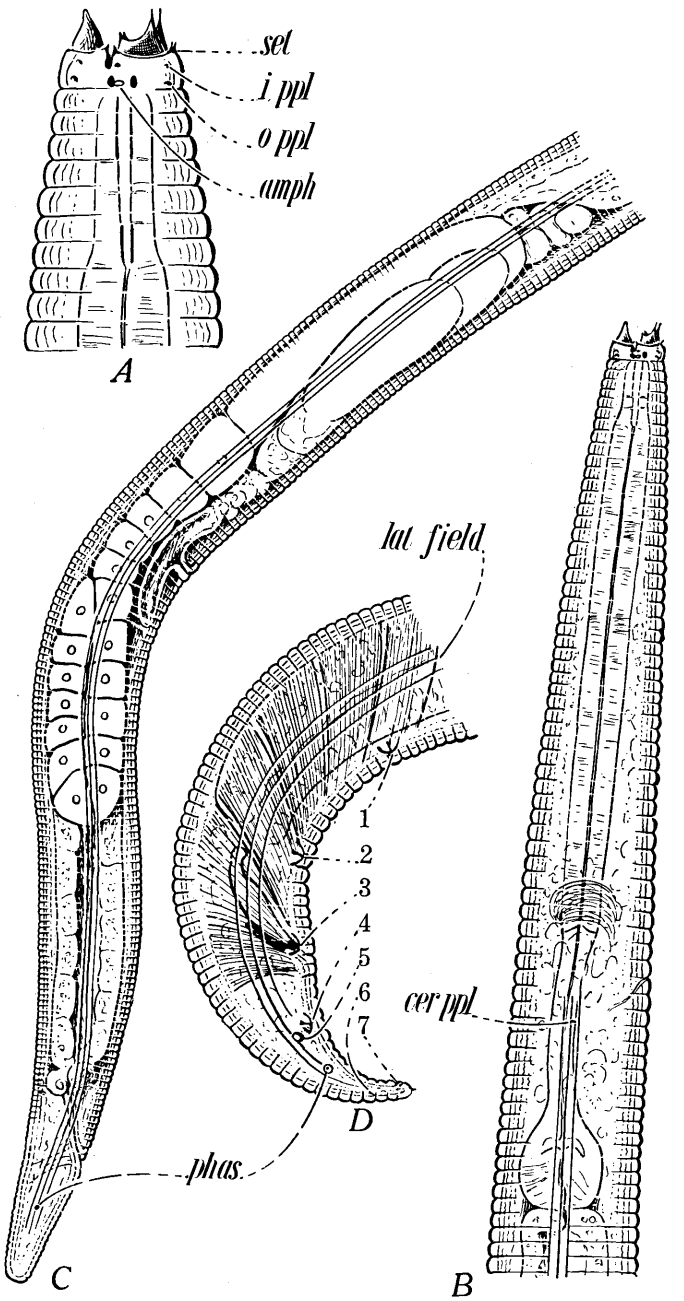

FIG. 1. *Chiloplacus trilineatus.* A—Head end; *amph,* amphid; *i ppl,* inner circle of papillae; *o ppl,* outer circle of papillae; *set,* setose endings of axil borders; about × 2100. B—Esophageal region; *cer ppl,* cervical papilla; × 800. C—Posterior part of female showing female sexual apparatus; *phas,* phasmid; × 520. D—Tail end of male; *lat field,* lateral field; *phas,* phasmid; *1–7,* copulatory papillae; × 800.

concave anterior borders. Anterior circle of 6 and posterior circle of 4 cephalic papillae, amphids level with the latter. Buccal cavity of typical form; pro- and telorhabdions most distinct; no toothlet seen. Esophagus with cylindrical, narrow, undifferentiated corpus, faintly set off from slightly thinner isthmus; terminal bulb almost spherical. Nerve ring encircles posterior end of corpus. Vulva not prominent; vesiculate receptaculum seminis at anterior bend of female apparatus; end of ovary reflexed forward, almost reaching region of vulva, but proper S-shaped bend absent. Testis to right of intestine, end reflexed backward. Spicula as figured; distal part broad, proximal part handle-shaped, faintly capitate. Gubernaculum about ¼ length of spiculum, lineate. Copulatory papillae as figured: (1) a series of 4 ventrosubmedian, broadly convex papillae, one at latitude of anus, one level with inner end of spicula, one a little over twice length of spicula in front of anus, and one at first third of tail; (2) one lateral, much smaller than the 4 just mentioned, at beginning of 2nd third of tail, and a very minute one, lateral, near tail end at latitude of those marked 7 in fig. 1, D; (3) a small subdorsal papilla a short distance in front of tail end; (4) two fine papillae, close together, slightly ventrosubmedian near tail end. The phasmid as indicated in figure 1, D.

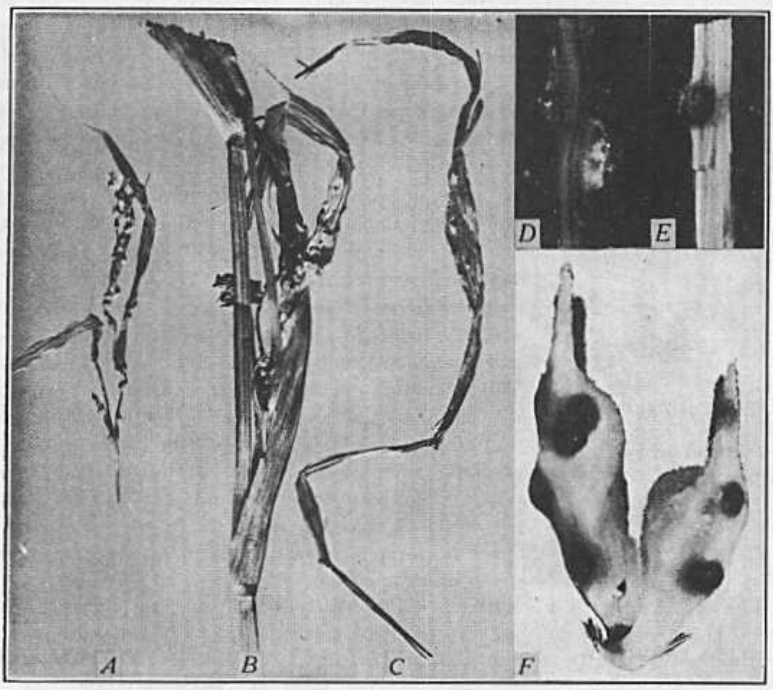

FIG. 2. *Ehrharta longiflora* with galls caused by the nematode *Anguina australis*. A, B & C—Inflorescence, stem, and leaf galls, respectively; slightly magnified. D & E—Stem galls; ×10. F—Glumes with galls; ×10.

Measurements.—♀: total length = 0.428 to 0.62 mm; α = 18 to 20.6; β = 3.4 to 4; γ = 13 to 16.3; v = 65%. ♂: total length = 0.54 to 0.616 mm; α = 20.8 to 22.6; β = 3.8 to 3.85; γ = 19 to 19.4.

Diagnosis.—*Chiloplacus* resembling *Ch. symmetricus* but differing from it by presence of 3 lateral wings and cephalic probolae ending in a setose point at each

axil; male tail differing from that of *Ch. symmetricus* by presence of terminal mucro, by presence of 2 minute, ventrosubmedian, subterminal papillae and by absence of one of the lateral papillae.

Type locality.—Orono, Maine, U.S.A.

Type host.—Snapdragons (diseased cuttings).

(2) A NEW GRASS NEMATODE, ANGUINA AUSTRALIS, N. SP. (FIGS. 2–4)

In recent years several new and old species of the plant-parasitic nematode genus *Anguina* have been discovered in the U.S.A. The proper identification and classification of these forms makes desirable a restudy of the known species with regard to certain characters which were ignored or neglected by earlier authors. In checking for this purpose all available material the present new species was discovered in the collections of the Division of Nematology, U. S. Bureau of Plant Industry.

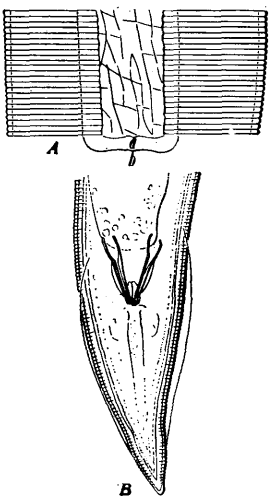

FIG. 3. *Anguina australis.* A—Annulation and lateral field in middle region of body; *a*, width of lateral field; *b*, width of lateral chord; ×515.

From a taxonomic viewpoint, the genus *Anguina* is a very perplexing one. The differentiating morphological characters of the various species are often difficult to observe and in some instances are incompletely known. Experimental work as to life cycle and host specificity is lacking, yet it is felt that such data would be of great help in differentiating the various species.

In January 1927, W. A. Carne, Botanist and Plant Pathologist of the Department of Agriculture, Perth, W. Australia, submitted to N. A. Cobb, then in charge of the Division of Nematology, U. S. Bureau of Plant Industry, galled specimens of the grass *Ehrharta longiflora* Sm. Cobb, with some hesitation, referred the nematode causing these galls to *Tylenchus phlei* Horn, 1888 and reported it as such to W. A. Carne. Apparently no record of the finding was prepared for publication. However, some portions of the material originally submitted and preserved dry were still at hand. This offered an opportunity for a restudy of the material and a comparison of the *Ehrharta* parasite with the other newly discovered species from Australia, *Anguina microlaenae* (S. G. M. Fawcett) n. comb., which infests *Microlaena stipoides* R. Br., another member of the Gramineae. Since the present material was dry and the nematodes could not be revived, certain features of the new species need to be rechecked by a study on living specimens.

Ehrharta longiflora is a native grass of South Africa and was introduced into Australia in 1878; it belongs to the Phalarideae. It is of moderate pasture value and prefers moist soil in need of liming.[1] The galls occur on the stems, leaves and inflorescences; the seeds however seem not to be attacked. If the glumes of the spikelets are galled, seed development is more or less completely stopped. Figure 2 shows such galls on a dried herbarium specimen of Ehrharta. To the left is a heavily galled inflorescence (Fig. 2, A). The galls are of brownish color and form broad, oval to round elevations, the base being flattened out and not narrowed or pediculate (Figs. 2, D–F). Their size varies from 0.7 to 1.27 mm by 0.6 to 1.2 mm. Each gall usually harbors from 1 to 3 large females and 1 or 2 males of smaller and more slender form than the female.

[1] For this information we are indebted to Mrs. Frances C. Weintraub of the Bureau of Plant Industry, U. S. Dept. Agriculture.

Anguina australis, n. sp.

Description.—The adult. Fully grown females of *A. australis* in mature galls usually coiled into a circle or a spiral as shown in figure 4, A; males mostly

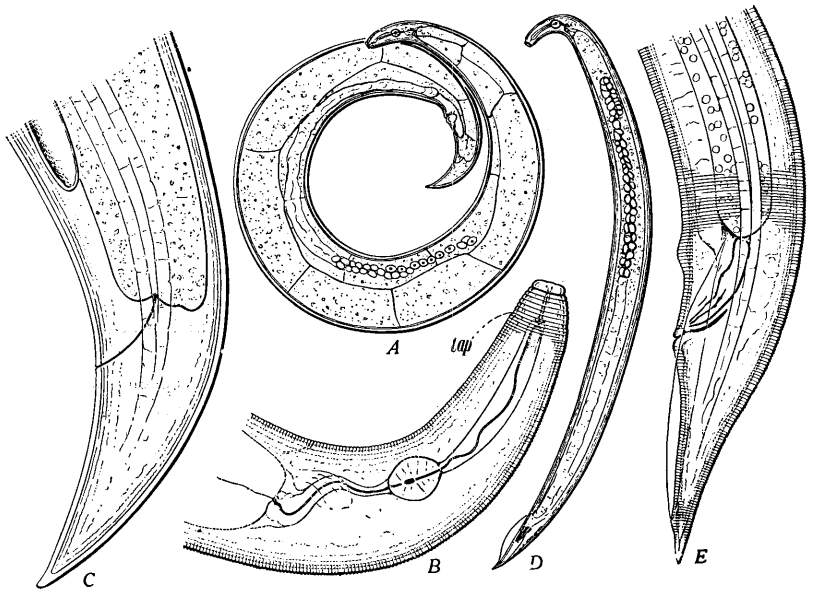

FIG. 4. *Anguina australis.* A—Young female; ×60. B—Anterior end of male; *tap*, sudden tapering of body; ×460. C—Tail end of female; ×280. D—Male; ×60. E—Tail end of male; ×460.

stretched out or of circumflexed shape (Fig. 4, D) but always bent ventrally, never dorsally. Females of much larger size than males. Body tapering toward both ends, tail conical with terminus obtusely pointed, head end broadly rounded. Cuticle finely annulated; surface annules about 1 μ wide; subcuticular ones only half that width; annulation interrupted by lateral fields. These latter in middle region of body in male about 10 to 18 μ wide with corresponding body-width of 48 to 72 μ, in female about 22 μ wide with corresponding body-width of 92 μ. Lateral fields with irregular longitudinal striae and also some irregular-transverse or oblique-transverse striae (Fig. 3, A). Lateral chords wider than lateral fields, as shown in figure 3, A and rapidly tapering anteriad from intestine and posteriad from anus. Head end slightly set off from rest of body by a sudden tapering at height of anterior end of esophagus, this in some specimens more pronounced than in others (Fig. 4, A); annulation here also more distinct. Head broad, low-convex, not annulated, with faint indication of 6 lips. Cheilorhabdions small, rather faint; stylet 8 to 9 μ long, with distinct spherical basal knobs. Procorpus of esophagus cylindroid, rather narrow, well set off from spherical metacorpus bulb; latter with distinct valves. Terminal portion of esophagus not distinct, apparently somewhat spread out over anterior end of intestine; form and size of esophageal glands obscure but outlet of dorsal gland close behind buccal stylet. Intestine with large cells; these numbering possibly 19. Rectum and anus faint, reduced. Excretory pore ventral to anterior end of intestine. Female with prominent vulvar lips, a short postvulvar, saccate, uterine branch and an ovary extending forward to the nerve ring, the end portion eventually being twisted around, figure 4, A, representing an early developmental stage. Male with bursa beginning at

level of proximal ends of spicula, reaching close to tail end, annulated, of moderate width. Spicula 31 μ long, shaped as shown in figures 3, B and 4, E; gubernaculum 10 μ long, forming gliding plate for the spicula. Testis with end reflexed.

Measurements.—♀: length = 2.163 to 2.5 mm; α = 19 to 20, β = 17 to 20, γ = 28 to 31; v = 88 to 93%. ♂: length = 1.386 to 1.557 mm; α = 19 to 20; β = 11 to 12; γ = 14 to 20.

Eggs and larvae.—Eggs oblong, 29 to 38 μ by 85 to 100 μ, deposited before segmentation begins. No description of larvae possible because only shrunken specimens were seen; total length about 0.9 mm with 0.015 mm body width.

Diagnosis.—*Anguina* with head region set off by slight sudden tapering of body opposite proximal end of buccal stylet; with spherical or slightly subspherical metacorpus bulb. Male not more than $\frac{3}{5}$ size of female, when fixed more or less curved ventrad but never spirally wound like female; bursa narrow, extending from region of proximal end of spicula to about $\frac{1}{5}$ length of tail in front of tail end. Postvulvar uterine branch saccate, slightly shorter than half distance vulva to anus; causing nonpedate galls on stems, leaves, racemes and glumes of grass *Ehrharta longiflora* Sm. in West Australia.

Remarks.—As mentioned above, the only other *Anguina* species at present reported from the Australian continent is *A. microlaenae*. This form produces galls on stems, leaves, and inflorescences of *Microlaena stipoides* R. Br., a grass also of the Phalarideae. These galls, unlike the ones caused by the present form, are pediculate, *i.e.*, in a fully grown condition they are attached to their substratum by a narrowed base. Young developing galls, according to Miss Fawcett, rest on the substratum with a broad base. Assuming that there could be a mutual exchange of nematodes and hosts, it would be of much interest to know whether the type of gall is determined by the host or by the nematode. At present, however, we can only assume that the two species differ in the type of galls they produce, those caused by *A. microlaenae* being pediculate but not those caused by *A. australis*. There may be other differences in the galls of these two forms, particularly histological differences. This, however, cannot be ascertained because the material of galled *Ehrharta* on hand is not considered satisfactory for such comparative studies.

(3) FURTHER NOTES ON APHELENCHOIDES LIMBERI STEINER 1936 (FIG. 5)

This species, hitherto reported only from The Netherlands, was discovered in 1938 in a lesion on a dahlia tuber which originated in Germany (Bad Kostritz), and in 1939 in elm roots from California. As in the original material, only female specimens were observed.

Measurements.—♀ (3): length = 0.67 to 0.82 mm; α = 30.6 to 32.6; β = 9.5 to 11.7; γ = 17.8 to 19.6; v = 68 to 70%. Eggs in utero = 44 to 48 μ by 21 to 27 μ. The lateral fields resemble those of other *Aphelenchoides* species, being 4 μ wide with a corresponding body width of 26 μ, and consisting of 3 longitudinal bands, a wider central and 2 narrower marginal ones.

The form of the ovary varies greatly; in some specimens it was outstretched straight forward (Fig. 5, D), in others it formed an S-shaped flexure (Fig. 5, C), and in still others the end was reflexed (Fig. 5, B). A terminal cap-cell was always present and in numerous specimens the ovary extended cephalad to the nerve ring or its neighborhood. The vulvar lips are slightly prominent. As the vagina leads inward and forward the vulvo-vaginal dilatator muscles are arranged as shown in figures 5, E and F. The transverse position of the posterior dilatator muscles in regard to the longitudinal body axis is particularly interesting. There are 2 pairs of anterior *dilatatores vulvae et vaginae* in serial arrangement and in the usual inclined position (Fig. 5, E). The transverse vulva is about $\frac{3}{5}$ as long

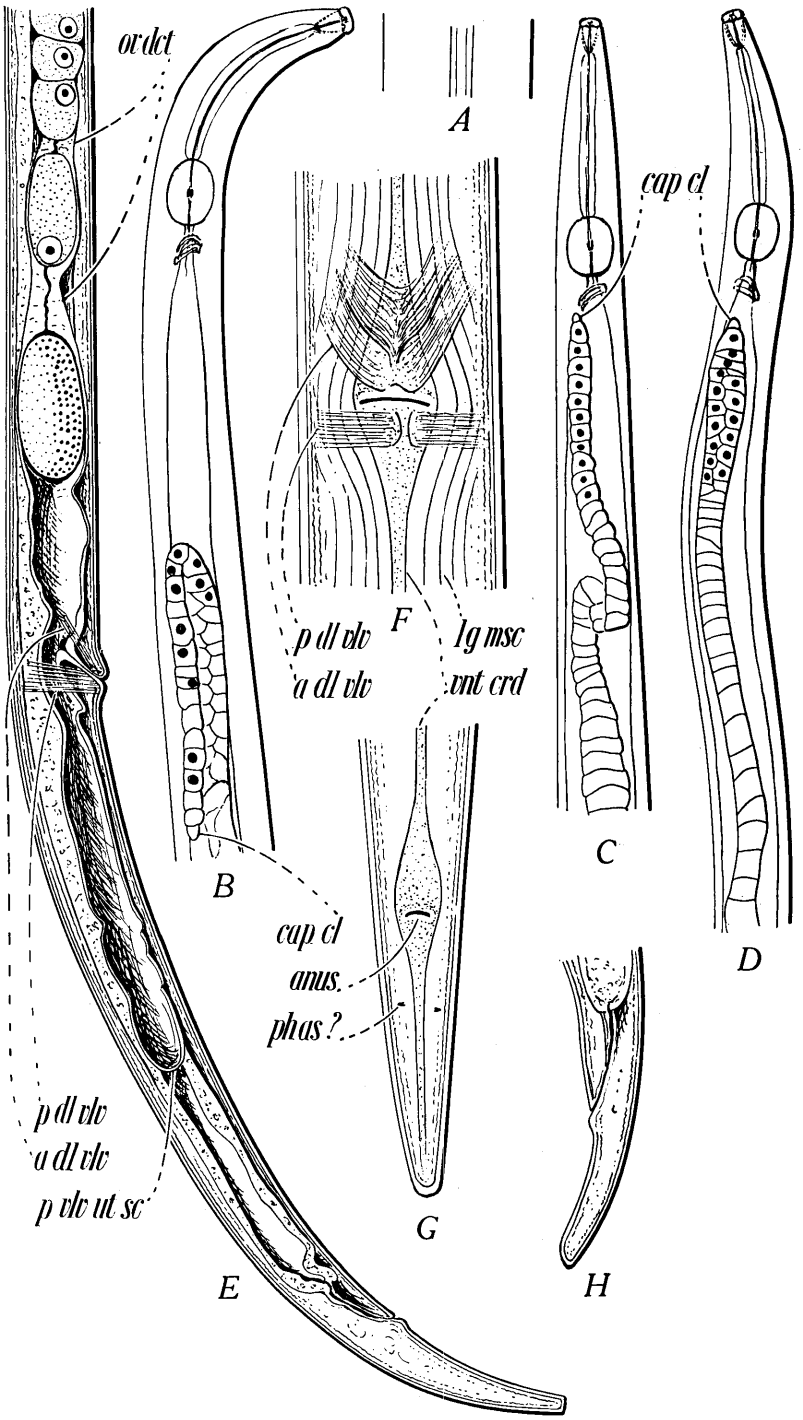

FIG. 5. *Aphelenchoides limberi.* A—Width and striation of lateral fields in relation to body width; ×520. B—Specimen with reflexed ovary; *cap cl,* cap cell; ×520. C—Specimen with S-shaped flexure in ovary; *cap cl,* cap cell; ×520. D—Specimen with straight ovary and oöcytes partly arranged in double series; *cap cl,* cap cell; ×520. E—Vulvar and anal region of female; *a dl vlv,* anterior vulvo-vaginal dilatator muscle; *ovdct,* oviduct; *p dl vlv,* posterior vulvo-vaginal dilatator muscle; *p vlv ut sc,* postvulvar uterine sac; ×520. F—Ventral view of vulvar region; *a dl vlv,* anterior vulvo-vaginal dilatator muscle; *lg msc,* longitudinal body muscles; *p dl vlv,* posterior vulvo-vaginal dilatator muscle; *vnt crd,* ventral chord; ×800. G—Obtusely rounded tail end; *anus,* anus; *phas?,* vestigial marks of phasmids; *vnt crd,* ventral chord; ×800. H—Obtuse-truncate tail; ×520.

as the body diameter. A posterior branch of the uterus extends about ½ the distance to the anus. A ventral view of the latter is presented in figure 5, G; the thickening of the ventral chord in the anal region is noticeable. The tail terminus exhibits much variation, transversely or obliquely truncate types being most numerous. What appear to be vestigial phasmids were seen in one instance (Fig. 5, G).

The inefficacy of methyl bromide fumigation against the chrysanthemum foliar nematode. J. R. CHRISTIE and GRACE SHERMAN COBB, U. S. Bureau of Plant Industry.

In late autumn the chrysanthemum foliar nematode, *Aphelenchoides ritzemabosi* (Schwartz, 1911), is to a considerable extent on the surface of the plant. Many specimens are in the growing points at the ends of stolons where they are located between the young, partly formed leaves. Some of these specimens will have entered the young leaves, when these are present, and be in the leaf parenchyma. Other specimens are lodged in crevices and depressions on the surface of stolons, old stems, and other parts of the plant, near or slightly below soil level. Therefore it seemed possible that fumigation with methyl bromide might kill these nematodes and be less injurious to the plants than the hot-water treatment now being employed.

The 3 chrysanthemum plants used in this test were dug from a field in Connecticut early in November and shipped to the writers. On arrival the plants were washed to remove from the roots all adhering soil, after which they were wrapped and kept moist until treatment. Washing removed some but not all of the nematodes, for an examination of the plants after washing showed that many adults and larvae in all stages of development were still present.

The plants were fumigated with methyl bromide at the rate of 3 pounds to 1,000 cu. ft. for 1½ hours at a temperature of 70° F. and under 15 inches of sustained vacuum. This was selected as probably the most severe treatment that chrysanthemum plants are likely to tolerate without appreciable injury. The supposition was apparently correct, as the treatment used resulted in noticeable injury where stolons terminated in leaf growth.

A critical examination of the treated plants was begun about 18 hours after they came out of the treating chamber; during this interval the plants had been kept wrapped to prevent drying.

Young, recently hatched larvae apparently had been killed. Specimens of this age were moderately numerous prior to treatment, but living ones could not be found after treatment and a few dead ones were seen. Except for these very young individuals, the treatment had no apparent effect whatever on the nematodes. All other stages were as numerous and as active after treating as before; in fact they seemed ever more numerous, probably because quiescent specimens had been revived by moistening. The plants were kept wrapped at room temperature and examined again after 2 days, but the nematodes were still numerous and active.

This test seems to eliminate methyl bromide fumigation as a method for treating chrysanthemum or other plants infested with the chrysanthemum foliar nematode. Nor is it likely, in the opinion of the writers, that such fumigation will be effective against the strawberry bud nematode, *Aphelenchoides fragariae* (Ritzema Bos, 1891), or any of the other related plant-parasitic *Aphelenchoides*.

The writers wish to thank Mr. A. D. McDonnell, of the Connecticut Agricultural Experiment Station, who provided the infested plants, and Mr. H. S. Dean and his associates, of the U. S. Bureau of Entomology and Plant Quarantine, who conducted the treatment.

Report of the Brayton H. Ransom Memorial Trust Fund

DECEMBER 31, 1939

The trustees met on January 12, 1939, and approved use of $1350.00 from the Fund as a loan at 4 per cent interest, the note to be covered by life insurance policies as collateral, with interest payable semi-annually.

The status of the Fund, since the previous statement in the Proceedings of the Helminthological Society, January, 1939, is as follows:

BALANCE ON HAND, December 31, 1938	$1387.37
RECEIPTS:	
Bank interest to Jan. 1, 1939	13.80
" " " July 1, 1939	.51
Semi-annual interest on loan	27.00
TOTAL RECEIPTS	$1428.68
DISBURSEMENTS:	
As a loan at 4 per cent interest	$1350.00
Award to Proceedings of the Helminthological Society	25.00
Toward rent of safe deposit box	2.00
TOTAL DISBURSEMENTS	$1377.00
BALANCE ON HAND, December 31, 1939	51.68
	$1428.68

ELOISE B. CRAM,
Secretary-Treasurer

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