TAXONOMY AND BIOLOGY OF *Verutus volvingentis* N. GEN. N. SP. (TYLENCHIDA-NEMATA)

BY

ROBERT PAUL ESSER

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
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JUNE, 1980

Chairman: Armen C. Tarjan
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A new subfamily Verutinae is proposed. Females differ from all other subfamilies in the Heteroderidae in possessing a sausage shaped body with an uncommonly large subequatorial vulva.

The genus and species named Verutus volvingentis is described. Larval ecdysis was not noted within the egg. Eggs are not deposited in a gelatinous matrix. Anatomical features include a phasmid that appears only on tail of cast cuticles of the first stage larvae. A previously undescribed muscle, the "median dilator vulvae," was named. A description of the rectal musculature and nervous system is given. A detailed account of male and female development is presented. Male development is completed in 6-15 days; female development took 17 days. Larvae entered the root by pushing through the middle lamella between 2

epidermal cells. Tissue discoloration occurred 3-4 days after feeding. Nuclei of invaded cells enlarged and exudate production by the host was incited. Larvae and eggs survived at least 3 years in the absence of food. Eggs are the dominant survival stage.

The nematode is widely distributed in Florida in moist habitats. Testing of selected economic crops as hosts proved negative. Host plants inoculated with a minimal number of nematodes died in $13\frac{1}{2}$ months. Control plants were maintained in a healthy vigorous condition.

Catenaria anguillulae killed males but not larvae or eggs in biological control tests.

INTRODUCTION

The family Heteroderidae contains a large number of highly pathogenic species included in 17 genera, 14 of which have been erected since 1956. Pathogenicity has not been proved for the following genera: Atalodera Wouts & Sher, 1971; Meloidoderita Poghossian, 1966; Meloidodorella Khan, 1972; Meloinema Choi & Geraert, 1973; Punctodera Mulvey & Stone, 1976; Sarisodera Wouts & Sher, 1971; Sherodera Wouts, 1973; and Thecavermiculatus Robbins, 1978.

The principal objectives of this research were to establish the systematic position of the new genus of nematodes described in this work and to investigate the pathogenic potential of the new taxon.

Secondary objectives included: host testing, life cycle and developmental studies, longevity, host-parasite relationships and anatomical studies.

HISTORY

In March, 1969, Mr. Wayne W. Smith, "Agricultural Products Specialist," with the Florida Department of Agriculture submitted 14 samples from a field near Apopka, Florida, for regulatory analysis. Four of the samples were infested with larvae that resembled Heterodera sp. A search of the sample material for Heterodera cysts revealed females that did not fit the generic concept of any known nematode phytoparasitic genus described at that time.

In May, 1969, the site from which the samples originated was surveyed in an attempt to isolate and identify the host plant of the undescribed nematode. The host was found to be buttonweed (Diodia virginiana L.) and was subsequently infected with the nematode in greenhouse culture.

SECTION I
TAXONOMY AND SYSTEMATICS

Taxonomic Position of the New Genus

Verutinae n. subf.

Diagnosis: Heteroderidae (Filipjev, 1934) Skarbilovitch, 1947.

Female: Mature female saccate, sausage to reniform-shaped (Fig. 12), vulva uncommonly large, subequatorial in position, vulval lips strongly protuberant (Fig. 11), ovaries reflexed, anus subterminal, cyst stage absent, body striae present, phasmid obscure, strong sexual dimorphism present.

Male: (Fig. 15) Body vermiform, caudal alae absent, tail rounded flatly to truncate, body untwisted, one testis present.

Type genus Verutus n. gen. (from the Latin "armed with a dart").

Affinities with the Family Heteroderidae

Females. Table 1 shows comparative female distinguishing characteristics of genera contained in the subfamilies of Heteroderidae.

Verutus n. gen. differs from all other members of the Heteroderinae in lacking a cyst stage and a terminal vulva. It differs from all members of the Ataloderinae, and

Meloidogyninae in lacking a terminal or subterminal vulva, and a spheroid body (Fig. 1). Verutus is most closely related to Meloidoderinae, one member of which, Meloidodera floridensis Chitwood, Hannon, & Esser, 1956, possesses a subequatorial vulva (Fig. 2) and a spheroid or subspheroid body shape. Verutus eggs are deposited as they mature (Fig. 3), and not retained in large numbers within the female body as in females of Meloidoderinae (Fig. 4). The body is completely annulated in the subfamilies Meloidoderinae, Meloidogyninae, and Verutinae n. subfam. Members of Ataloderinae and Heteroderinae possess an irregular body pattern, or lack body markings. In some members of these 2 subfamilies, annulation may be present on the cervical area or about the vulva, but not on the body. The vulva is widely separated from the anus in the Verutinae (Fig. 11) and in the genus Meloidodera (Fig. 2) in the Meloidoderinae. In all other subfamilies the vulva is located in the perineal area or near the anus (Fig. 4, Table 1). In some members of Ataloderinae, Heteroderinae, and Meloidoderinae the vulva is situated on a papule (Fig. 5). Neither Meloidoderinae or Verutinae are so equipped. A labial disc (Fig. 14) is described only in Ataloderinae, Meloidoderinae, and Verutinae. An attempt was made to utilize the presence of a gelatinous matrix, or a sub-crystalline layer (Fig. 34) in the diagnosis. This was not possible due to lack of data concerning these criteria in many species descriptions. Genera of the Heteroderidae were also compared to the new

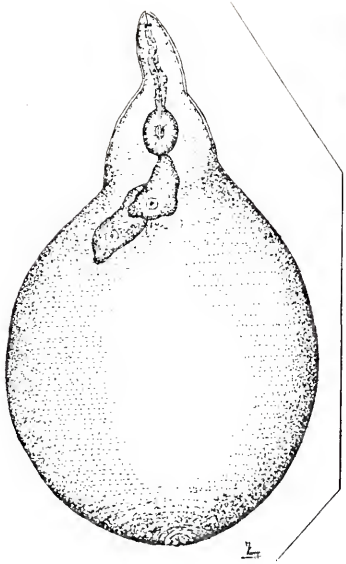

Figure 1. Meloidogyne sp., showing spheroid shape and terminal vulva of a mature female.

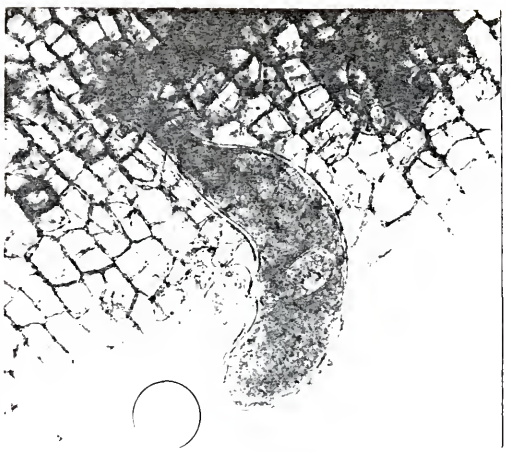

Figure 3. Verutus n. gen. female in root tissue with a single deposited egg.

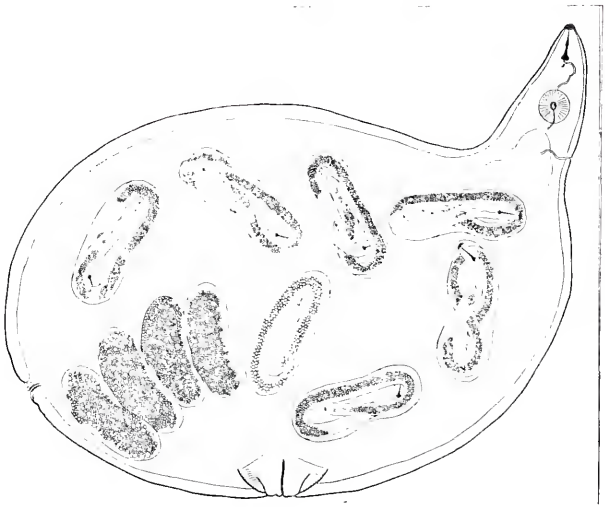

Figure 2. Meloidodera floridensis showing spheroid shape and equatorial vulva position.

genus on the basis of the measurement (length/greatest body width) alpha. This was found to be infeasible due to omitted data and differences in the measurement criteria used by some authors. Some measure the total body length, others exclude the neck and head from the measurement (Mulvey & Stone, 1976).

A key to the subfamilies of Heteroderidae based on mature females is as follows.

Key to subfamilies of the Heteroderidae

1. Female forms a cyst----------Heteroderinae
   Female does not form a cyst----------2

2. Annulation absent or limited only to cervical or vulva area----------Ataloderinae
   Annulation present on body (may be sparse)----------3

3. Body sausage or reniform-shaped vulva uncommonly large----------Verutinae n. gen.
   Body ovoid or pear-shaped, vulva not uncommonly large----------4

4. Eggs retained in large numbers in female body, labial disc present----------Meloidoderinae
   Eggs not retained in body in large numbers (exception Meloidoderita), labial disc present----------Meloidogyninae

Males. Table 2 compares selected male characteristics of genera included in the subfamilies of the Heteroderidae. It can be seen that few definitive differences exist between males. Only the subfamilies Ataloderinae and Verutinae contain genera without a twist in the male body (Fig. 6-C). Males of both subfamilies also possess a truncate tail terminus, similar spicules and gubernaculum, and a tubus (Fig. 6-A,B). Stylet and body length

Table 1. Comparative distinguishing characteristics of females in genera of Heteroderidae.

Subfamily Genus	Cyst Stage	Cuticle Markings	Vulva Anus Gap	Vulva on a Papule	Lip Disc	Egg Retention	HPR*	Galls on Host	Egg Sac	CL**	Vulva Position
1/70	no	head & vulva striae	very close	yes	yes	yes	?	?	?	?	terminal
1/89	no	none	" "	"	"	"	"	"	"	"	" "
1/121	"	head & vulva striae	" "	no	"	"	semi-endo	?	no	yes	" "
2/118	yes	pattern	close	"	no	"	" "	no	"	no	" "
2/13	"	" "	" "	no & yes	"	"	" "	"	yes	yes	" "
2/99	"	pattern & punctations	" "	no	"	"	endo	yes	"	?	" "
2/102	"	" "	" "	"	"	"	semi-endo	no	"	"	" "
2/71	"	lace-like	very close	"	"	"	?	?	?	?	terminal recessed
3/63	no	striae	not close	"	yes	"	semi-endo	no	no	yes	terminal
3/16	"	" "	far apart	"	"	"	" "	"	"	"	mid-body
3/95	"	" "	not close	"	"	"	?	?	?	?	terminal
4/41	"	" "	very close	yes	"	no	endo	yes	yes	yes	" "
4/56	"▽	" "	?	"	?	yes	semi-endo	"	"	no	" "
4/17	no	" "		no & yes	no	no	endo	"	"	"	" "
4/92	"	striae sparse	close	no	"	?	?	?	?	?	" "
5/130	"	striae	far apart	"	yes	no	semi-endo	rare	no	yes	mid-body

*HPR = Host parasite relationship, Semi-endo=semi-endoparasite, Endo=endoparasite.
**CL = Crystalline layer.

Legend Subfamily & Genus
1=Ataloderinae; 70=Atalodera, 89=Sherodera, 121=Thecavermiculatus
2=Heteroderinae; 118=Globodera, 13=Heterodera, 99=Meloidodorella, 102=Punctodera
71=Sarisodera
3=Meloidoderinae; 63=Cryphodera, 16=Meloidodera, 95=Zeylandodera
4=Meloidogyninae; 41=Hypsoperine, 56=Meloidoderita, 17=Meloidogyne, 92=Meloinema
5=Verutinae; 130=Verutus

▽Egg filled cyst develops. Golden, 1976; Andrews et al, 1977.

Table 2. Comparative distinguishing characteristics of males contained in the Heteroderidae.

Subfamilies & Genera	Twisted Body	Terminus Shape	Body Length µm	Spear Length µm	Oral Disc Present	Position Hemizonad to ex. Pore	Tubus*
ATALODERINAE							
Atalodera	no	angular truncate	1036-1420	24-29	yes	anterior adjacent	yes
Sherodera	"	" "	850-1160	26-31	no	?	"
Thecavermiculatus			male unknown				
HETERODERINAE							
Globodera	yes	bluntly rounded	1198	26-28	"	anterior adjacent	no
Heterodera	"	" "	variable	variable	"	" "	yes & no
Meloidodorella	?	" "	980	21	"	?	no
Punctodora	no	" "	900-1500	variable	yes	?	"
Sarisodera	"	angular truncate	597-1405	38-46	"?	anterior adjacent	yes
MELOIDODERINAE							
Cryphodera	yes	bluntly rounded	596-894	29-37	yes	" "	no
Meloidodera	"	" "	457-505	20-24	?	" "	"
Zelandodera	?	" "	764-973	31-38	yes	?	"
MELOIDOGYNIDAE							
Hypsoperine	yes	" "	1200-1700	18-19	no	anterior adjacent	"
Meloidoderita	no	sharp conoid	350-432	15-19	?	" "	"
Meloidogyne	yes	bluntly rounded	810-2000	13-33	no	anterior & posterior	"
Meloinema	"	conoid	1700-2420	36-40	"	posterior (60µm)	slight
VERUTINAE							
Verutus	no	angular truncate	650-1020	21-27	yes	posterior adjacent	yes

*Protuberant cloacal cuticle. DiEdwardo & Perry, 1964.

measurements also overlap in both subfamilies. Except for the striated gubernaculum of the genus Sherodera it would be difficult to differentiate between males of Ataloderidae and Verutinae. The only other genus with a truncate tail terminus and tubus is Sarisodera in the subfamily Heteroderinae. The Sarisodera male can be separated from males of Ataloderinae and Verutinae by its long stylet (38-46 µm). Meloidoderita possesses a sharp conoid tail and is the only male in the Heteroderidae with caudal alae. All other genera, not included in the aforementioned, have rounded or bluntly conoid tails (Fig. 6-C) with or without a twist. The only small males have been described in Meloidoderita (350-432 µm), and Meloidodera (457-505 µm). Males in the genus Meloinema stand apart from all other males in possessing a distinct subacutely conoid tail (Fig. 6-D).

Larvae. The larvae of the new genus (Fig. 8) closely resemble larvae in the subfamilies Atalodorinae, Meloidoderinae, and all of the larvae in the genera of Heteroderinae (except Meloidodorella, which has a short stylet [11-16 µm and reduced telorhabdions]). Larvae of the Meloidogyninae differ from the new genus, for the most part, in having a small stylet, small telorhabdions, and fine body striae. The principal character peculiar to first-stage larvae of the new genus is the absence of a detectable phasmid.

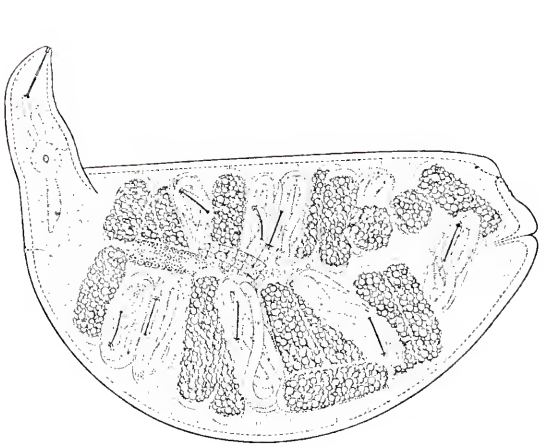

Figure 4. Cryphodera eucalypti (after Colbran), showing egg retention in the mature female, and separation of the vulva and anus.

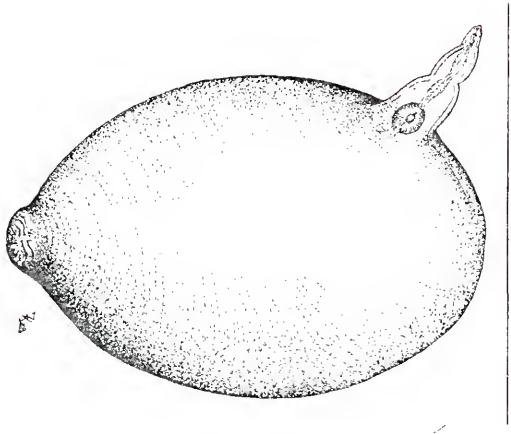

Figure 5. Hypsoperine graminus Sledge and Golden, 1964. A mature female showing a vulva situated on a papule.

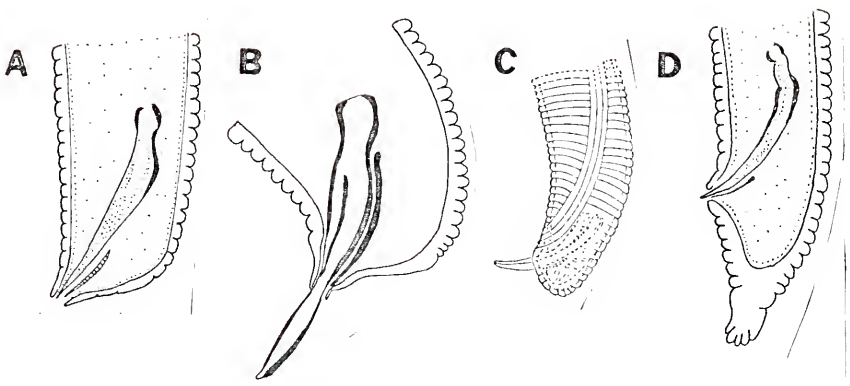

Figure 6. Heteroderidae male tail types; A,B - Truncate with tubus. A - Sherodera (redrawn from Wouts & Sher), B - Verutus, C - Rounded twisted type. Meloidodera (after Hopper), D - Blunt conoid. Meloinema (redrawn from Choi & Geraert).

Comparisons were made of esophageal gland structures in the 16 genera of the Heteroderidae to determine if the glands could be used in generic or subfamily diagnosis. Esophageal glands with two lobes, the anterior lobe overlapping the posterior lobe, were found in the Verutinae, Meloidoderinae, and Heteroderinae (Fig. 9 A,D,F,H,J). Considerable variation in esophageal gland structure was noted in the 27 species descriptions examined in 4 genera of the Heteroderinae (Fig. 9 F-K). Esophageal glands in 29 species of Meloidogynidae all consisted of a single lobe. Meloinema differed from all other genera in having an extremely long esophagus (300 µm). However, in the description the esophagus length range was listed at 125-130 µm, which contradicts the illustration. The genera

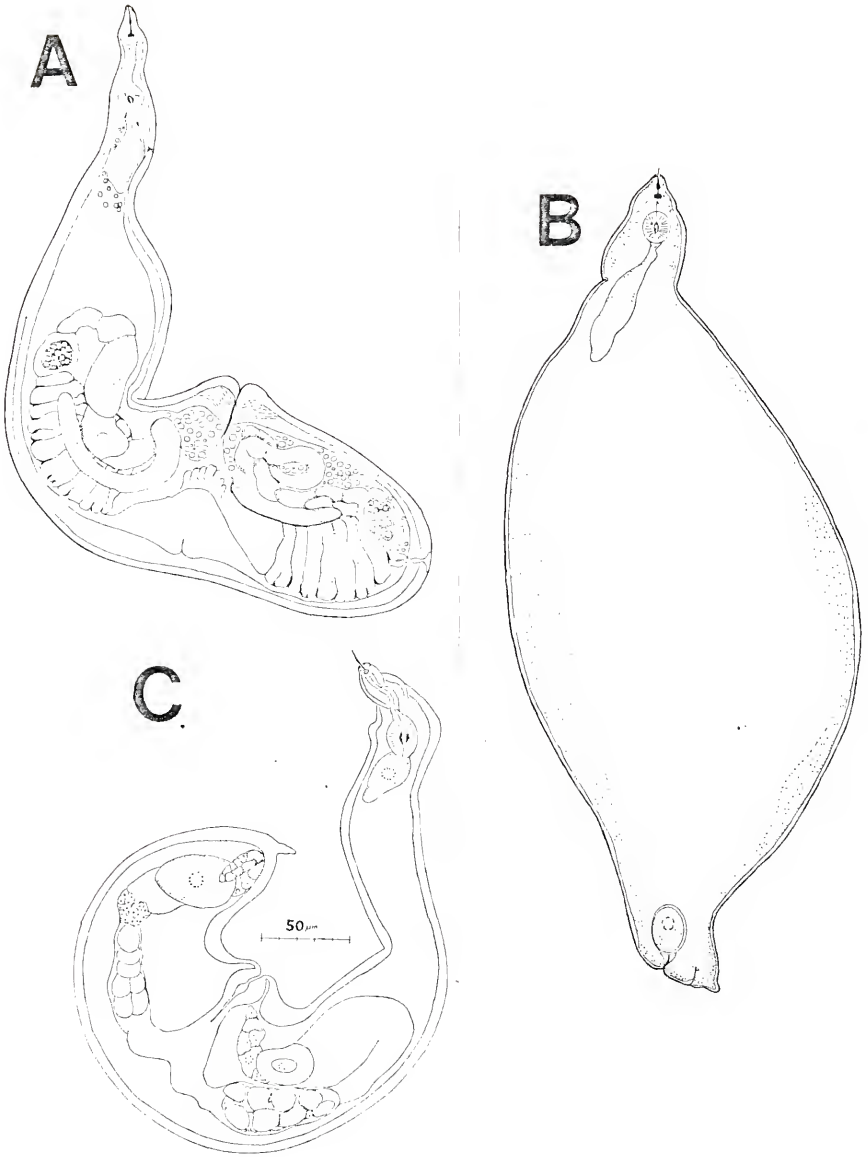

Figure 7. A comparison of female appearances in three subfamilies. A) Verutinae, B) Nacobbinae, C) Rotylenchulinae.

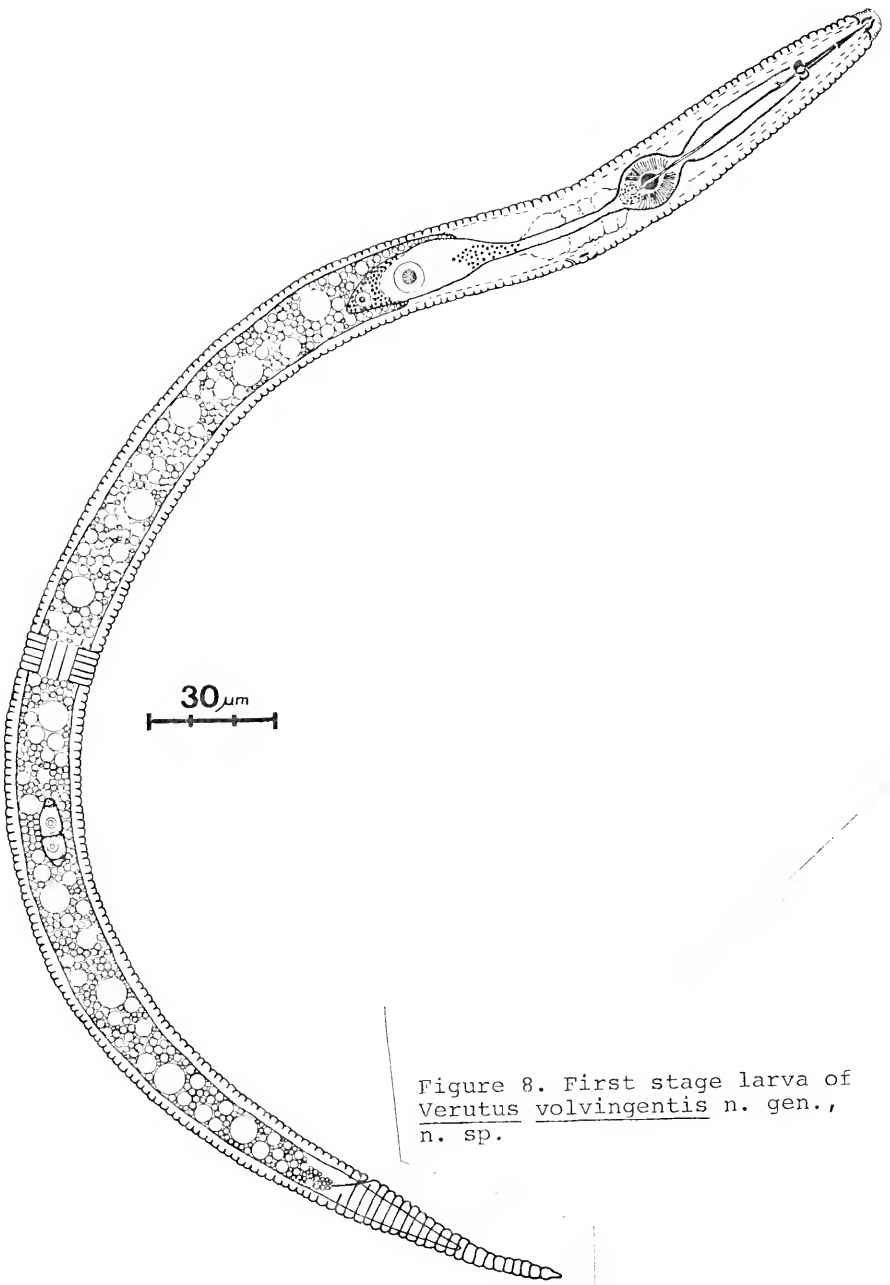

Figure 8. First stage larva of Verutus volvingentis n. gen., n. sp.

Sherodera and Zelandodera, Wouts, 1973, are not included in Figure 9 since neither genus is represented by an illustration of the larval esophageal glands in the literature. The Verutinae also differ from the Ataloderinae and Meloidogyninae in possessing two esophageal gland lobes.

Affinities with the Subfamily Rotylenchulinae, Husain & Khan, 1967

The females of Verutus (Fig. 7-A) closely resemble females in Rotylenchulinae (Fig. 7-C). Verutus females differ in the absence of a well-defined tail tip, a dorsal gland orifice that originates less than one stylet length from the base of the telorhabdions, possession of a large muscular uterus, and, principally, by the absence of a vermiform, vulvate juvenile female stage.

Verutinae males differ markedly in general appearance from Rotylenchulinae males which have a tapering conoid tail, are usually less than 500 µm long, and assume a C-shaped body position. Rotylenchulinae males possess an elongate, truncate cephalic framework in contrast to the slightly convex, shallow cephalic framework of Verutinae males.

Affinities with the Subfamily Nacobbinae, Chitwood & Chitwood, 1937

Nacobbinae females differ from females of Verutinae in body shape (Fig. 7-B), position of a posteriorly situated vulva, and in having a single gonad. Nacobbinae

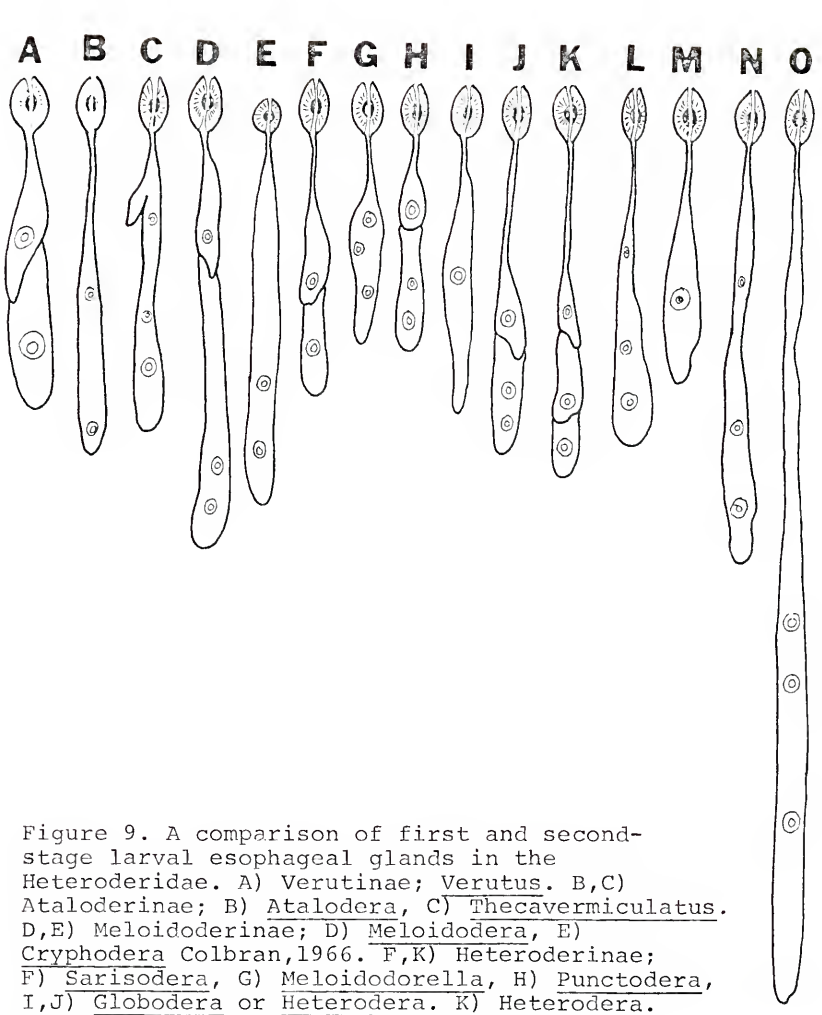

Figure 9. A comparison of first and second-stage larval esophageal glands in the Heteroderidae. A) Verutinae; Verutus. B,C) Ataloderinae; B) Atalodera, C) Thecavermiculatus. D,E) Meloidoderinae; D) Meloidodera, E) Cryphodera Colbran,1966. F,K) Heteroderinae; F) Sarisodera, G) Meloidodorella, H) Punctodera, I,J) Globodera or Heterodera. K) Heterodera. L-O Meloidogynidae; L) Meloidoderita, M) Meloidogyne, N) Hypsoperine, O) Meloinema. (Esophageal glands are directly proportional to the size of the metacorpus shown.)

females form prominent root galls while Verutinae females do not.

Nacobbinae males possess conoid tails and caudal alae, both of which are absent in Verutinae males.

Nacobbinae larvae differ from Verutinae larvae in having a bluntly rounded tail tip.

Discussion. The new subfamily Verutinae does not fit within the concepts of the subfamilies in the family Heteroderinae, or the subfamilies, Nacobbinae or Rotylenchulinae. It is therefore proposed as a new subfamily. Subfamilies proposed by Husain, 1976, but not included in the analysis are Meloineminae and Meloidoderellinae.

The position of the Verutinae in the Animal Kingdom is shown in the following scheme:

Kingdom-Animalia
Subkingdom-Metazoa
Branch-Enterozoa
Division-Bilateria
Section-Pseudocoelomata
Phylum-Nemata (Rudolphi,1808), Cobb,1919
Class-Secernentia (von Linstow,1905)Chitwood,1958
Order-Tylenchida Thorne,1949
Suborder-Tylenchina Pearse,1942
Superfamily-Heteroderoidea (Filipjev,1934)Golden, 1971
Family-Heteroderidae (Filipjev,1934)Skarbilovitch, 1947

Subfamily-Verutinae

Genus-Verutus

Species-volvingentis

In the above scheme categories above Phylum are based on Storer and Usinger, 1957. Classifications below Section are based on schemes proposed by Golden, 1971, Wouts, 1972, Andrassy, 1976, Husain, 1976, and Stone, 1977.

Taxonomy of the New Genus

Methods; Measurement Preparation

Specimens to be measured were placed in water within a "Zut" ring on a glass microscope slide (Esser, 1973-b), and a cover slip placed on the zut. The nematodes ceased moving in 3 to 5 min., and measurements were taken according to the method proposed by Esser, 1971. While the nematodes are in the quiescent state one has 20 to 30 min. to make observations and measurements before deterioration, swelling and/or shrinkage occur.

Permanent Fixation

When specimens on slides are to be fixed permanently the cover slip is removed and 2 or 3 drops of 2% formalin are added to the exposed zut well. The specimens are then transferred to a BPI watch glass for permanent fixation in lactophenol (Esser, 1973-a).

En face Preparation

A new method was devised to study en face preparations. Live immobile females or males, or freshly killed nematodes in 2% formalin were used as subjects.

Procedure. A 12 X 12 X 3 mm square of 1.7% water agar is cut very evenly with a razor blade (Fig. 10-A) and placed on a microscope slide. A 3- to 4-mm piece is precisely cut from the square (Fig. 10-A) and laid with the outer face down (Fig. 10-B). Nematodes are placed on the upper side of the cut piece with the longitudinal axis of the head parallel with the outer edge of the cut piece (Fig. 10-B). The cut piece is then placed back into the same position it occupied in Fig. 10-A, then gently pushed into its original position against the parent block (Fig. 10-C). A small (4-mm) drop of water is applied to a 15-mm cover slip that is then placed waterside down over the cut line (Fig. 10-C). A drop of immersion oil is applied to the center of the cover slip at the junction of the cut pieces. When the body of the nematode is properly aligned the en face appears as in Fig. 10-D . If the en face is off-center or below the field of focus, the cover slip is removed, the cut piece placed backside down, and the specimens reoriented. Water must be added to the cover slip each time it is placed on the agar block. It takes 10 to 15 min. to prepare an en face ready for viewing using this technique. Locating the en face is rather easy since it lies within the cut line.

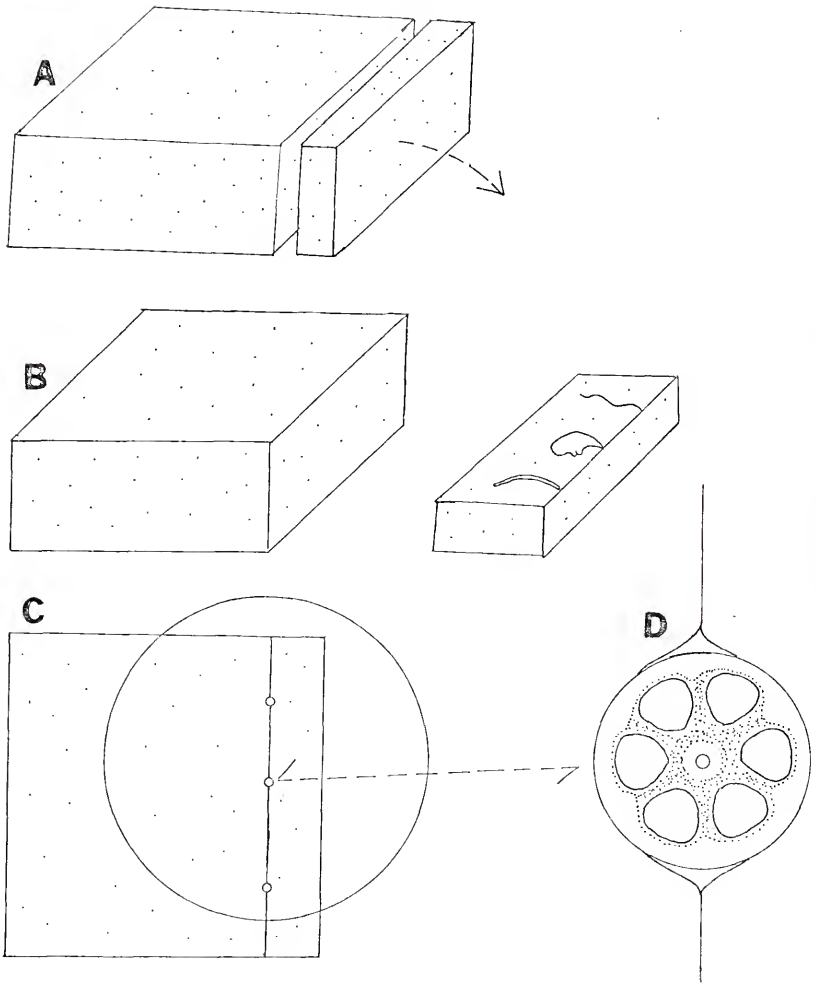

Figure 10. Water agar en face method; A) 12 X 12 X 3 mm square of water agar with a 3- to 4-mm piece cut off, B) Specimens aligned on outer edge of the inner face of the cut piece, C) Re-alignment of the separated agar pieces, with cover slip in place, D) Closeup of en face in junction line of re-aligned agar.

External Cuticle Preparation

Lateral lines and phasmids were not well-defined in live or fixed specimens. Several stains were tested to bring out the lateral lines including: methyl blue, acid fuchsin, merthiolate, iodine, chlorazol black-E, and propionic carmine, none of which enhanced cuticular incisures or phasmids. Lateral incisures were brought out clearly by making squash mounts. Cut or uncut females, males, and larvae were placed in a 4-mm drop of water, and a 18-mm cover slip applied. A needle point was pressed against the cover slip until the body contents gushed out. Examination for the phasmid was made of each squash mount specimen.

Nervous System Preparation

Chlorazol black-E in lactophenol was used with the 4-min. fixation method (Esser, 1973-a).

Verutus n. gen.

Diagnosis: Verutinae, with characters of the subfamily. Mature female (Fig. 11): Body swollen, reniform or sausage-shaped (Fig. 12). Cephalic framework moderately sclerotized, lips striated, set off, amphids obscure, oral disc hexagonal (Fig. 14). Body striated, lateral lines irregular, sometimes indistinct. Crystalline layer present (Fig. 34). Stylet tylenchoid, dorsal gland orifice near telorhabdion base. Uncommonly large protuberant post-equatorial vulva.

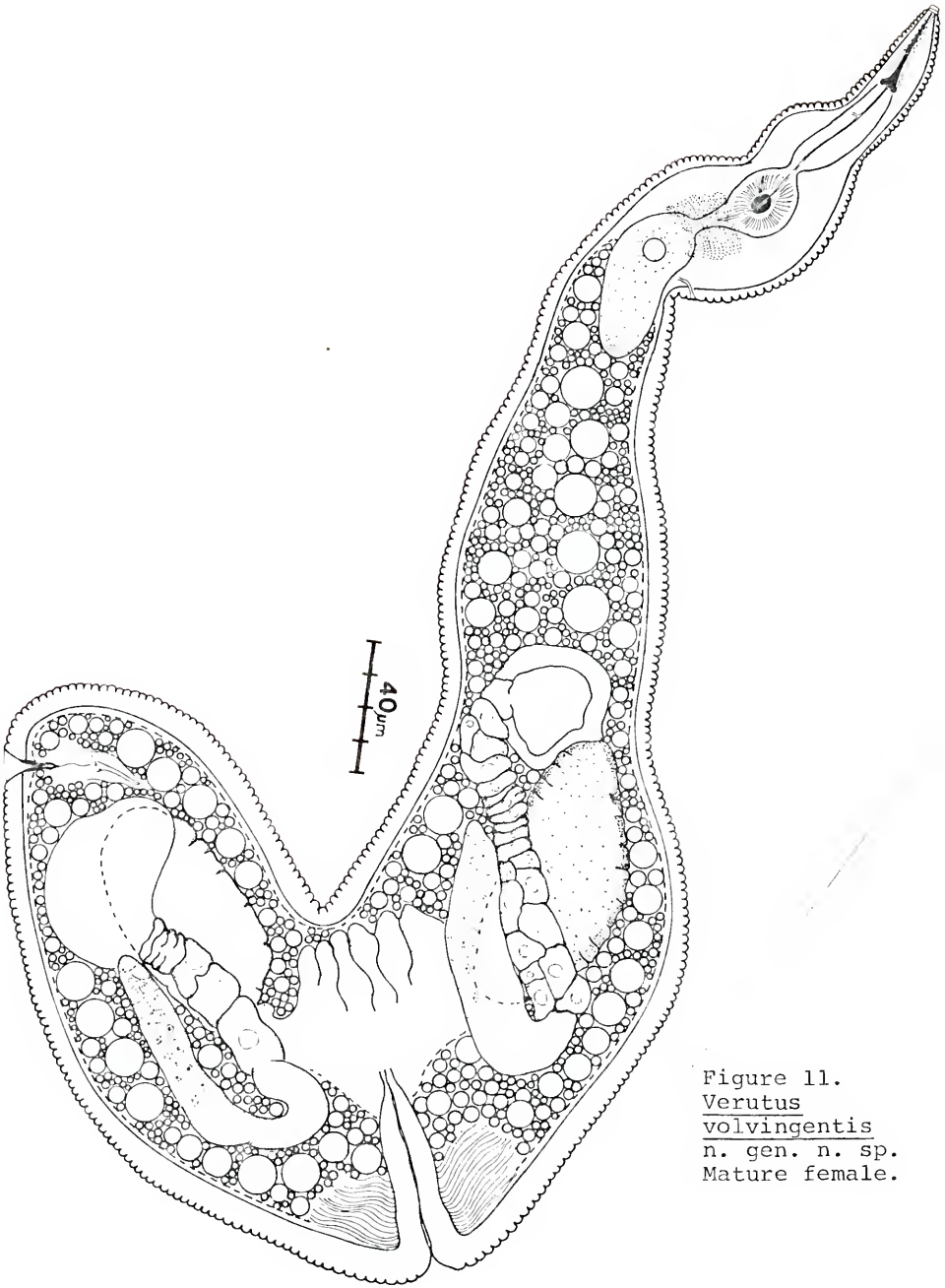

Figure 11. Verutus volvingentis n. gen. n. sp. Mature female.

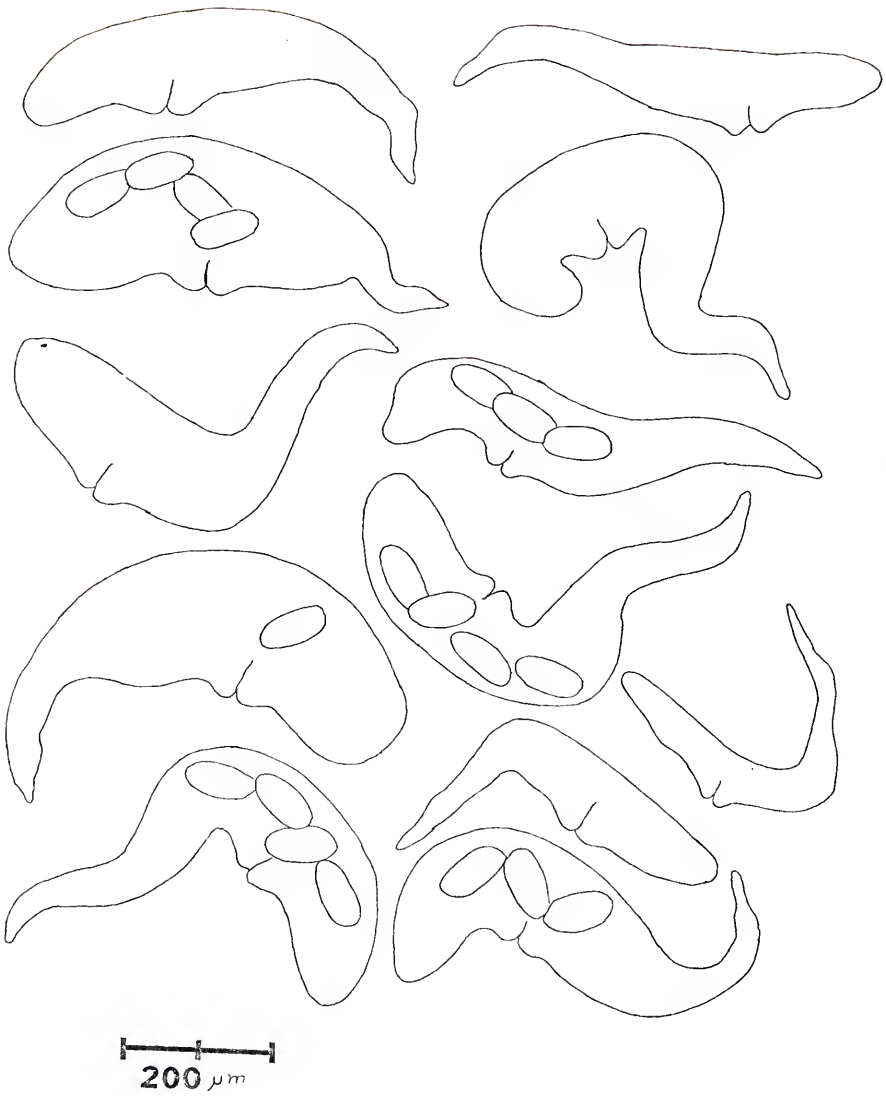

Figure 12. Verutus volvingentis n. gen. n. sp., female body shapes.

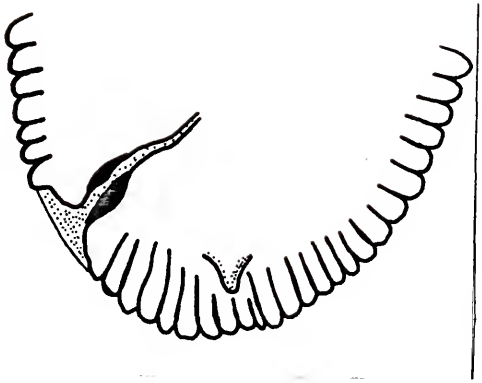

Figure 13. Vestigial larval tail tip on posterior area of a mature female.

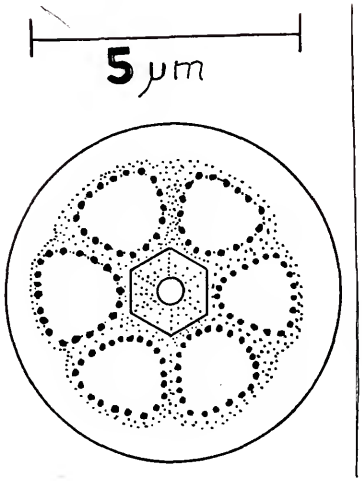

Figure 14. En face view of a mature female.

Gonads didelphic and amphidelphic. Ovaries reflexed. Anus subterminal forming small depression (Fig. 13). Tail vestigial (Fig. 13) or absent.

Female. (Table 1) Females differ from all other females in the Heteroderidae in possessing a reniform or sausage-shaped body, with an uncommonly large vulva in a post equatorial position with strongly protuberant lips. Esophagus typically tylenchoid, procorpus moderately expanded, metacorpus moderate in size. Isthmus narrower than procorpus, esophageal gland a single lobe moderately overlapping the intestine. Deirids and phasmids not observed.

Male. (Fig. 15) Body vermiform, monodelphic, lips striated not set off, oral disc circular (Fig. 16), amphidial openings elliptical on lateral lips. Body untwisted. Spicules and gubernaculum tylenchoid. Caudal alae absent, tail terminus angular truncate (Fig. 6-B). Phasmids or deirids not detected.

Verutus volvingentis[1] n. sp.

Female. (35 specimens) Total length = 662.7 (500-930) µm; width = 141.4 (94-207) µm; tail = 10.2 (3.9-15.7) µm; esophagus = 188.3 (150-290) µm; a = 4.7 (3.0-6.5); c = 69.4 (34-155); total stylet length = 26.1 (23.5-29.4) µm; vulva % = 67.5 (50-75); excretory pore 139.8 (122-183) µm.

Female holotype. Total length = 540 µm; width = 118 µm; tail = 15 µm; esophagus = 148 µm; a = 4.6; b = 3.6; c = 36; stylet = 27.2 µm; vulva % = 71.2; excretory pore = 114 µm from anterior end.

Female description. (Fig. 11) Body pearly white, reniform or sausage-shaped (Fig. 12), anterior part of body sometimes twisted upward lying in a different plane than the posterior swollen portion. Head and neck occasionally reflexed across the posterior body. Six equidistant lips surround a hexagon-shaped oral disc (Fig. 14). Amphid apertures or lip papillae not observed. Lips set off, comprised of 2 annules. Cuticle 9-10 µm thick, evenly striated, striae about 2.5 µm apart. The occurrence and

[1]volv = vulva, ingen = remarkable size.

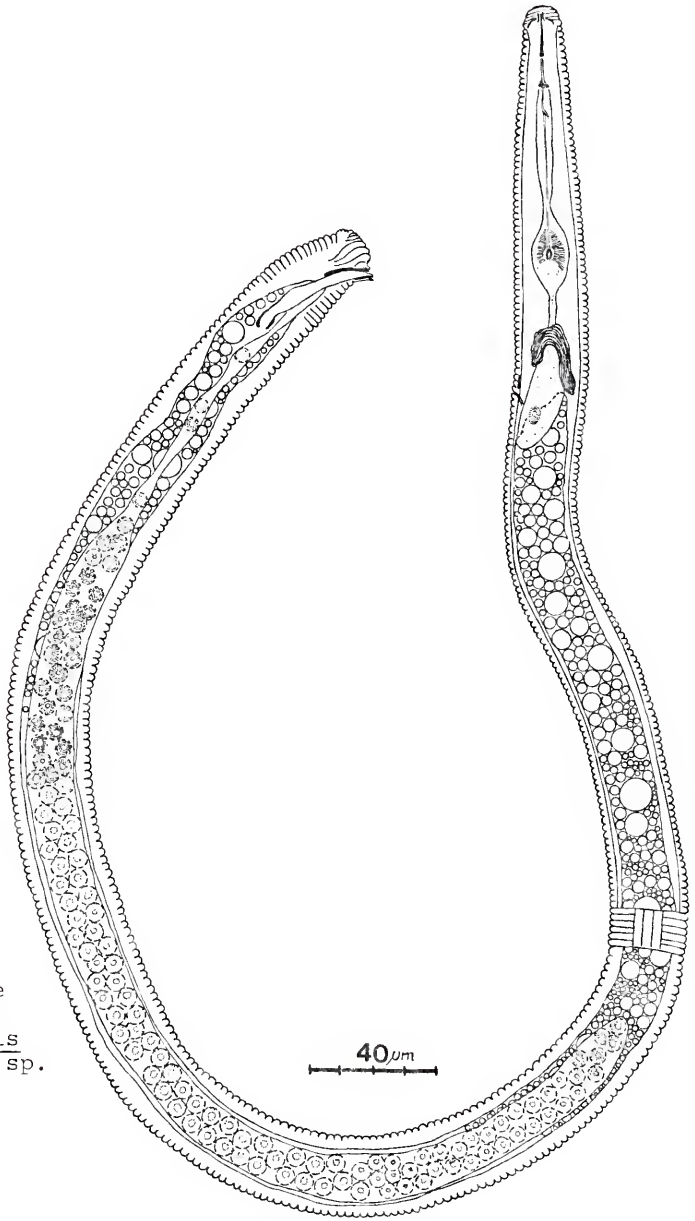

Figure 15. Mature male of Verutus volvingentis n. gen. n. sp.

appearance of lateral lines are variable: lines may proceed for a short distance beyond anus and fade out, or appear as midline cuticular interruptions or irregularities extending slightly past the vulva area. They appear as 1 or 2 lines of irregular blocks in the cervical area. In the tail area they appear as a mass of irregularities in the tail tip area with sometimes a wide separation (5 µm) of the striae (Fig. 17). In a few females lateral lines were not observed. Stylet tylenchoid, telorhabdions (Fig. 11, 18), 4-5 wide by 1-2 µm long, directed posteriad. Prorhabdions 14 µm. Two stylets with tips protruding from the body measured 25.5 and 25.6 µm, respectively. The dorsal gland orifice appears 7-11 µm posterior to the telorhabdion base. A moderately swollen procorpus narrows prior to the well-developed metacorpus. A clearly defined metacorpal valve is present. A short narrow isthmus leads from the metacorpus followed by a single distinct esophageal gland laying ventrally over the intestine. The intestine extends from beneath the mid-area of the esophageal gland to the rectal intestinal valve. The sclerotized portion of the rectum is 12 µm long in a lateral view. The rectum dilates anteriorly extending 30 µm beyond the sclerotized portion (Fig. 19) as a finely sclerotized tube (15 µm long) which joins the intestine. The oval anus (Fig. 20) lies in a depression (Fig. 19). Tail is usually absent, occasionally vestigial (Fig. 19). Differences in orientation of the body

do not permit an accurate tail annule count, or anal body diameter measurement. One female was noted (Fig. 21) with a granular mass over the anus, assumed to be excreta. The excretory pore lies at the level of the esophageal gland 134.4 (113-183) µm from the oral opening. The nerve ring appears as a mass of tissue surrounding the isthmus.

Gonad amphidelphic, anterior branch 136-147 µm long, posterior branch 117-130 µm long. The vulva appears as a transverse slit (Fig. 22) about 62 m wide. In some females the vulva lips protrude markedly. The vulva striae do not form a distinctive pattern, but surround the vulva rather uniformly (Fig. 22). In some females prolapse of the vaginal walls causes the vulva to widen, and the vaginal lining prolapses externally (Fig. 23). Wide muscle bands, the dilator vulvae appear at either end of the vulva underlying the cuticle (Fig. 22). Vulva epitygma were not observed. The vagina extends 42 to 70.5 µm into the body where it joins the well-developed vagina uterina (58-70 µm; Fig. 11). A constriction appears at the junction of the vagina uterina and the uterus. The uterus is a large muscular sac (70 X 35 µm) that joins directly with the spermatheca (Fig. 24). It comprises 4 to 5 rows of large cells with a furrow in the center for expansion. The spermatheca is a roughly circular thick walled chamber 35 to 40 µm in diameter. The oviduct, a thickened area comprised of small cells, lies between the spermatheca and the maturation zone of the ovaries. The ovary is reflexed at the spermatheca,

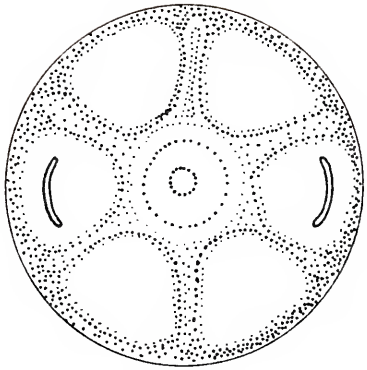

Figure 16. Verutus volvingentis male en face view.

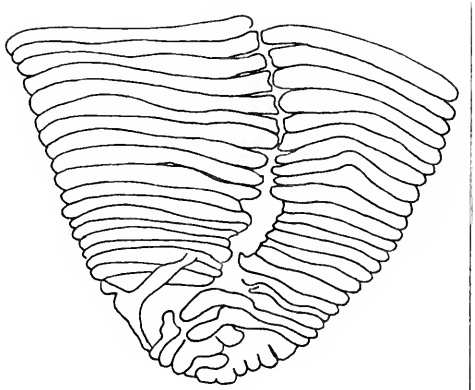

Figure 17. Female tail area showing lateral field irregularities.

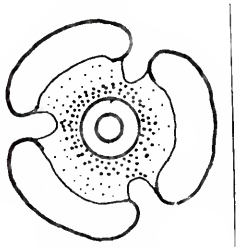

Figure 18. Telorhabdions of a mature female posterior view.

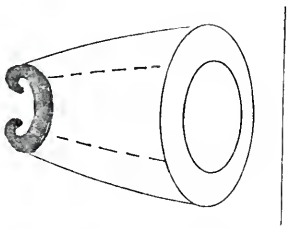

Figure 20. Anus (left) and rectum of a mature female.

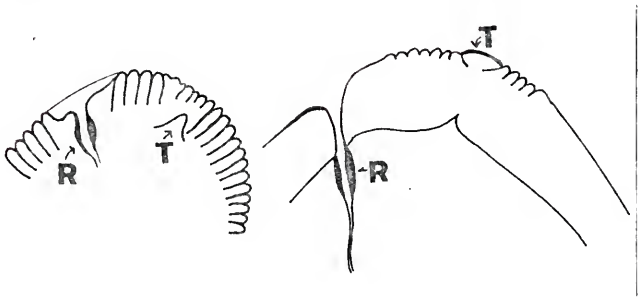

Figure 19. Posterior region of mature females, showing vestigial larval tail tips (T), and rectum (R).

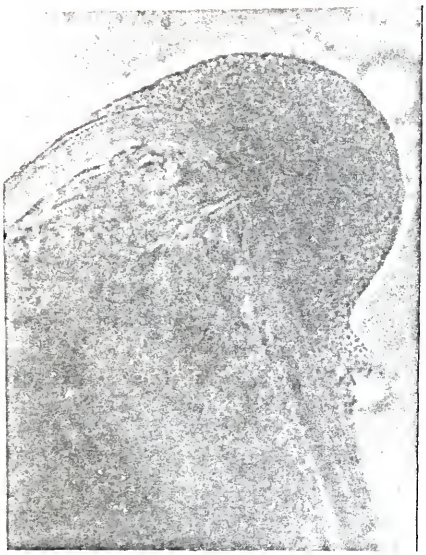

Figure 21. Excreta exuded from the anus of a mature female.

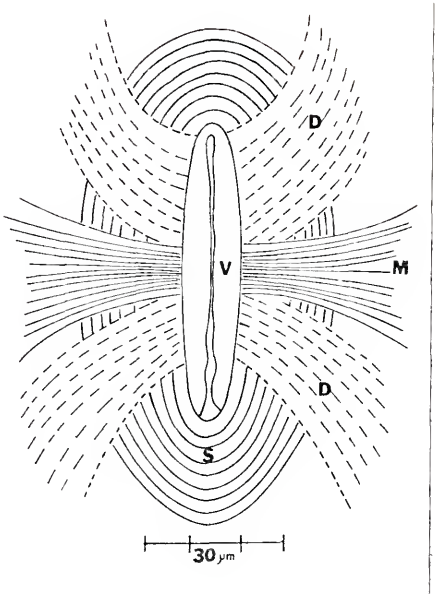

Figure 22. Vulva and vulva muscles of a mature female. V= vulva lips, S=striae surrounding vulva, D=dilator vulvae muscles, M-median dilator vulvae muscles (striae cutaway to show underlying muscles).

and 1 or 2 times in the maturation zone area. The cap cell and germinal zone cells are rarely delineated in live or fixed specimens.

Males. (24 specimens, Fig. 15) Body length = 830.8 (650-1020) µm; width = 28.9 (25.5-35.5) µm; tail = 9.5 (5-12.7) µm; esophagus = 153.5 (122-188) µm; a = 28.7 (24.3-32.8) µm; b = 5.4 (4.5-6.6) µm; c = 99.2 (59.1-178.6) µm; total stylet length = 25.3 (21.5-27.4) µm; dorsal gland orifice = 4.6 (2-6.8) µm behind the base of the telorhabdions;

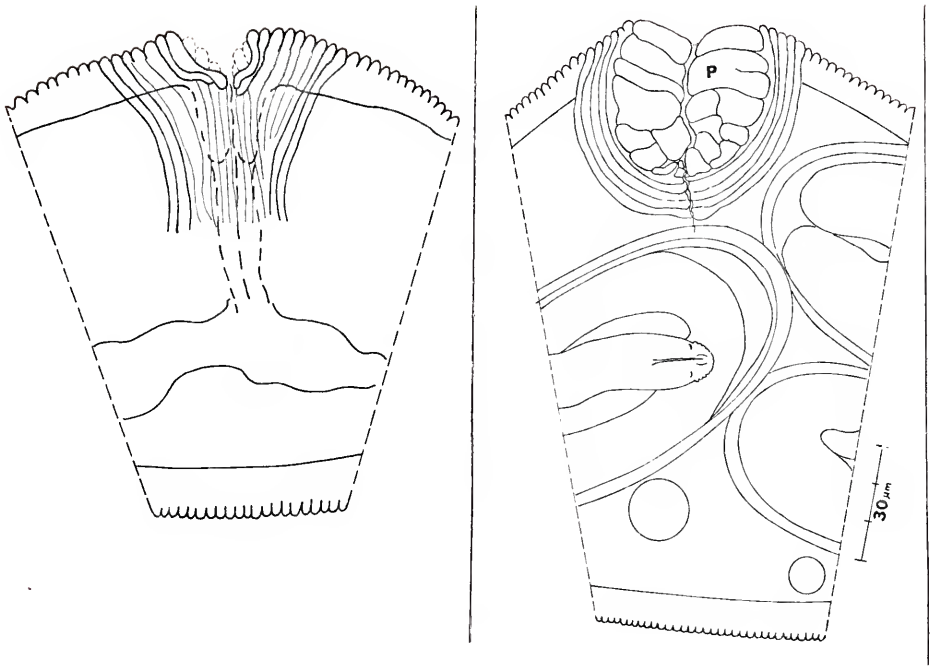

Figure 23. Lateral views of an unprolapsed (left) and prolapsed (right) vagina of mature females P=prolapsed vaginal tissue.

spicules = 40.4 (36.2-46.6) µm; gubernaculum = 16.2 (14.7-18.6) µm.

Allotype. Total body length 790 µm; width = 25 µm; tail = 6 µm; esophagus = 140 µm; a = 31.6; b = 5.6; c = 131.7; total stylet length = 22 µm; dorsal gland orifice = 6 µm; spicules = 40 µm; gubernaculum = 15 µm.

Male description. Body vermiform, untwisted (Fig. 15), 6 equidistant lips (Fig. 16) surround a circular oral disc that stands out clearly in profile. Crescent-shaped amphids appear indistinctly on posterior margin of lateral

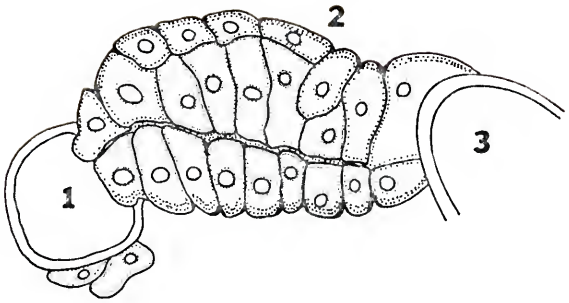

Figure 24. Uterus of a mature female: 1) spermatheca, 2) uterus, 3) egg.

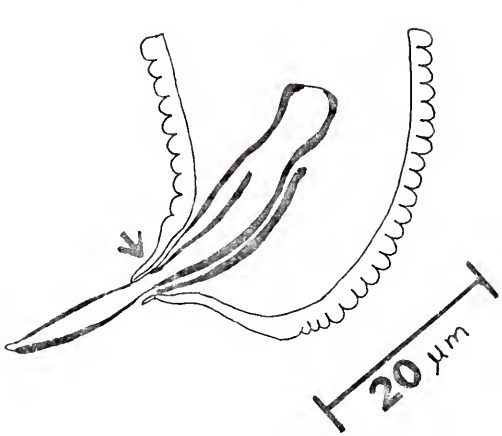

Figure 25. Male tail showing tubus (arrow).

lips. Labium moderately sclerotized, comprising 4 to 7 labial annules, counting from first reduced annule at onset of cephalic sclerotization. Labium rounded, not set off, papillae not observed. Body striae about 2 µm wide, sometimes ending irregularly at the terminus (Fig. 15). Four unareolated lateral fields present, extending from region of corpus to cloacal area. Phasmid not observed. Excretory pore lying in posterior esophageal gland area, 103-146 µm from oral disc (mean = 124 µm). Hemizonid 4 µm long, located just posterior to excretory pore. Stylet typically tylenchoid. Telorhabdions sloping posteriorly. Cheilorhabdions extending through lip annules 2 to 4. Esophagus comprising a moderately swollen procorpus that dilates just prior to oval, distinct metacorpus containing a valve slightly smaller than that of female. A narrow isthmus precedes a single ventral esophageal gland with single nucleus. Cardia not observed. Nerve ring appearing as an irregular band of tissue overlapping isthmus, and extending past the esophageal gland about 1/3 of its length (Fig. 15). Intestine overlapping about 1/2 of esophageal gland and extending uniformly to cloaca. Tail bluntly hemispherical to truncate; tail terminus annulated. Anal lips in form of tubus (Fig. 25). Caudal alae absent. Spicules equal and slightly arcuate when seen in lateral view. Capitulum moderately swollen, followed by slight constriction, and moderately swollen calomus. Lamina wide at the center tapering at both

extremities. Sclerotized piece arising at junction of lamina and calomus and projecting along ventral wall of calomus. The gubernaculum with teeth on lateral sides of cuneus seen in ventral view when cuneus is situated between spicules (Fig. 26). Male gonaduct originating from ventral face of the cloaca. Narrow vas deferens (Fig. 15) about 90 µm long is followed by rather long seminal vesicle, usually filled with sperm. Germinal and growth zones sometimes indistinguishable. Testes have been observed in which entire tube was filled with sperm and an observable germinal and growth zones were not present. Cephalids and deirids not observed.

Larval description. (49 first-stage larvae; Fig. 8) Length = 492 (430-540) µm; width = 18.5 (16-20.2) µm; tail = 53.6 (46-64) µm; esophagus = 163 (132-190) µm; a = 26.5 (22-30); b = 3.2 (2.6-3.7); c = 9.1 (6.7-10.5); anal body diameter = 4.5 (4.0-5.2) µm; stylet = 23.1 (21.5-24.5) µm; dorsal gland orifice 6.4 (4-8.9) µm posterior to telorhabdions; excretory pore 93.8 (79-103) µm from oral disc.

Body vermiform, labium rounded, cephalic framework consisting of 16, C-shaped sclerotized pieces lying 4 µm below oral disc. Head bearing 6 annules. Four lateral incisures beginning as single line, 45 µm posterior to oral disc forming 4 lines at median procorpus. The 4 lines resolve into a single line just posterior to anus (30 µm from the tail tip). Width of lateral incisures at mid-body is 5-7 µm. Excretory pore located in mid-isthmus

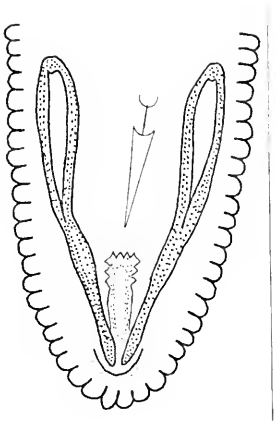

Figure 26. Male tail in a ventral view, showing toothed cuneus of gubernaculum (arrow)

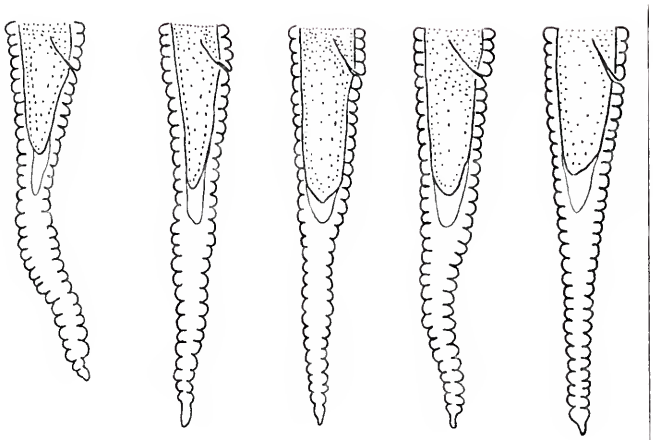

Figure 27. Larval tail shapes.

area. Hemizonid located 1 annule anterior to excretory pore. Stylet well-developed, prorhabdion 10.8-12 µm long. Rounded telorhabdions usually laying in an even plane, occasionally sloping posteriorly. Procorpus (38 µm long by 6-7 µm wide), moderately swollen, narrowing just prior to the well-developed metacorpus (15 µm long by 12 µm wide). Metacorpus valve a wide oval shape. Isthmus narrow, 25 µm long. Esophageal glands about 35 µm long, lying on ventral side of body. Posterior lobe sometimes filled with coarse granules (digestive fluid). Coarse granules also appearing in anterior end of anterior lobe, in isthmus, and in a large vesicle in posterior part of metacorpus (Fig. 8). A single large nucleus present in the posterior esophageal gland lobe. Anterior esophageal gland lobe not strongly set-off. It is delineated by a weak line of demarcation on posterior lobe, and has a very large nucleus surrounded by a large clear area (Fig. 8). Esophageal glands overlapping intestine by about 1/2 of their length, extending as a straight tube to undilated rectum. Anus oval, 1 to 2 µm wide. Nerve ring appearing either as group of nerve cells (Fig. 31), or as fine band of tissue surrounding isthmus (Fig. 8). Genital primordia appearing about 160 µm anterior to tail tip (Fig. 8). Tail conoid (Fig. 27), with 26-29 annules. Tail tip usually awl-shaped. Hyaline area of tail 25.4 (23.5-31.3) µm long. Deirids, phasmids and cardia not observed.

Ova. (Fig. 28) Eggs broadly oval 50 X 100 µm. No markings observed on shell. An en-utero egg was 52 X 103 µm.

Third-stage larvae. (10 specimens) Length = 572 (500-677) µm; width = 31.3 (27.4-34.3) µm; tail = 11.1 (9.8-13.7) µm; a = 19.5 (17.3-22.6); c = 54.5 (46.7-63.2); dorsal gland orifice = 3.9 (2.9-6.8) µm; excretory pore = 115.9 (109-122) µm.

Body slightly swollen, tail rounded, head and esophagus similar to that of first-stage larvae (Fig. 29).

Type specimens. Holotype collected May, 1969 by Wayne W. Smith. Collection number B-5018; Allotype same data as holotype. Type slides in Bureau of Nematology nematode collection, Division of Plant Industry, Florida Department of Agriculture.

Type habitat. Soil about roots, and roots of Diodia virginiana growing near bodies of water.

Type locality. Irrigation ditch bank bordering Hwy 50, 4 miles west of Hwy 27 near Clermont, Florida. (Original site now commercially developed.

Anatomy

Lateral Incisures

These structures are very difficult to see in live or fixed specimens even when various stains were used. It was possible to see them, however, by squeezing out the body contents and examining the lateral sides of the integument

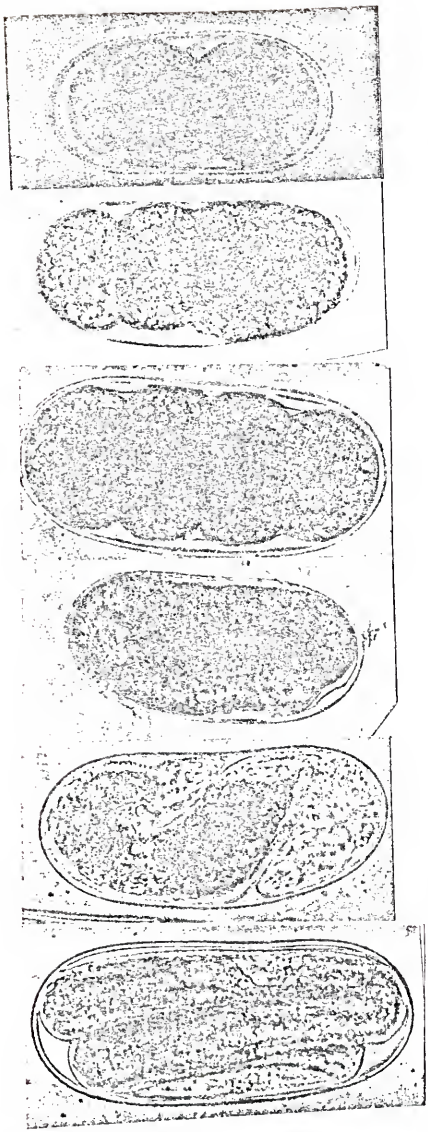

Figure 28. Ova. Top to bottom: 2-cell, 3-cell, 4-cell, 5-cell, tadpole stage, first-stage larva.

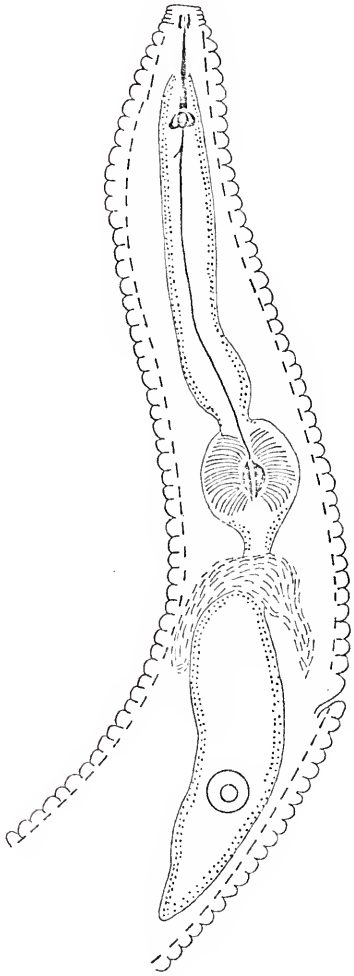

Figure 29. Anterior portion of third-stage larval male.

using an oil immersion objective. This procedure was not necessary for males and females.

Phasmid. Over a hundred each of males, females, and first-stage larvae were examined for phasmids with negative results in both ventral and lateral views. The phasmid and its lining were only detected on the cast integument of first-stage larvae early in the first molt. The phasmid was located between the 14th and 18th annule from the tail tip. When its location was known, fixed and living first-stage larvae were examined to see if the phasmid was detectable; in no case was it observed.

Muscles

Vulva. The dilator vulvae musculature are well developed in broad bands in mature females, extending from vaginal epithelium to a ventrolateral insertion in the hydodermis (Fig. 22). A band of muscle also attaches to vagina on either side of median part of vulva, herein called "median dilator vulvae" (Fig. 22).

Rectal muscles. Rectal musculature was observed in a third-stage female (Fig. 30). The H-shaped muscle surrounds the rectum or rectal intestinal valve. The depressor ani extends into the dorsal hypodermis, and the dilator ani is inserted in ventral hypodermis. A sarcoplasm band nucleus as described by Chitwood & Chitwood, 1937 was not observed.

Procorpus and Metacorpus

In third-stage larvae the procorpus is short and stout while metacorpus is a well-developed, wide oval. The esophagus of mature females is very similar to that of third-stage larvae. Geraert, 1978, found that the metacorpus enlarges in saccate females (Heterodera carotae Jones, 1950), as was the case in V. volvingentis.

In males the esophagus is shorter, more slender and the metacorpus is smaller and more elongate.

Nervous System

In males and females stained with chlorazol black-E the circum-esophageal commissure appears as a flat band of tissue that surrounds the posterior part of isthmus (Fig. 11, 15) and proceeds posteriorly a short distance past the anterior part of the basal bulb as 2 ventral ganglion. Anterior and posterior nerve cords were not seen. In first-stage larvae stained with chlorazol black-E, the circum-esophageal commissure appears looped around the isthmus, either as a flat band of tissue (Fig. 8) or as an accumulation of nerve cells (Fig. 31). The ventral ganglion proceeds posteriorly a short distance, branching dorsally and ventrally. The dorsal nerve arises from the dorsal portion of the ventral ganglion, and becomes indistinguishable a short distance posterior to the esophageal gland. The ventral nerve arises from the ventral portion of the ventral gnaglion, and proceeds as a chain of ganglia (92 in 1 specimen) in the

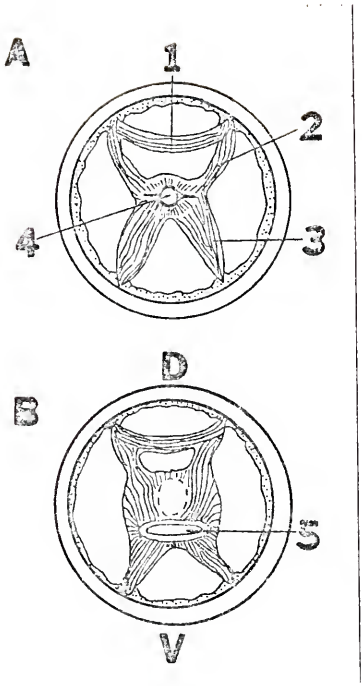

Figure 30. Rectal musculature.
A) Rectal-intestinal valve area: (1) sarcoplasm, (2) depressor ani, (3) dilator ani, (4) rectal intestinal valve.
B) Rectal area: (5) mid-rectum.
D) Dorsal side.
V) Ventral side.

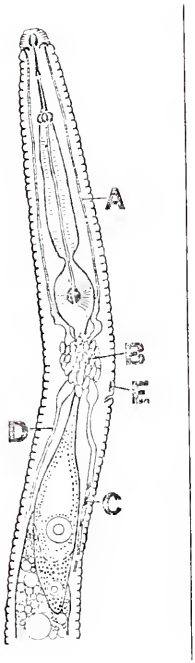

Figure 31. Anterior nervous system in a first-stage larva.
A) Anterior ventral nerve cord; b) Circum-oral commissure; C) Ventral nerve; D) Dorsal nerve; E) Hemizonad.

hypodermis. The ganglial chain forms rectal commissures that surround the rectum with 3 dorsal and 5 ventral ganglia (Fig. 33). A dorsal rectal ganglion (Fig. 33) is present where rectal commissures rejoin post-rectally in the dorsal position. Three ganglia are present in the medial caudal nerve (Fig. 33). Anteriorly, a large ganglion arises from the dorsal portion of the nerve ring, and one from the ventral side (Fig. 31). The 2 nerve cords extend around either side of the metacorpus forming a small mass of nerve cells just anterior to the metacorpus. Dorsal and ventral nerves proceed from this ganglion to sclerotized area of the labium. Cephalic nerves appear as elongate, spindle-shaped processes. Lateral and papillary nerves were not detected.

Crystalline Layer

Brown et al, 1971, reported the subcrystalline layer is a complex of long-chain fatty acids. It was hypothesized that sugar exudates from the integument of Heterodera spp. are converted to long chain fatty acids by soil fungi thereby producing the crystalline layer. A crystalline layer was observed on the integument of about 10% of the females of the new genus. This layer assumes the form of the striae and other designs and modifications present in the parent integument (Fig. 34-B,C). The subcrystalline layer is usually fragmented and sloughs off the female body (Fig. 34-A, D).

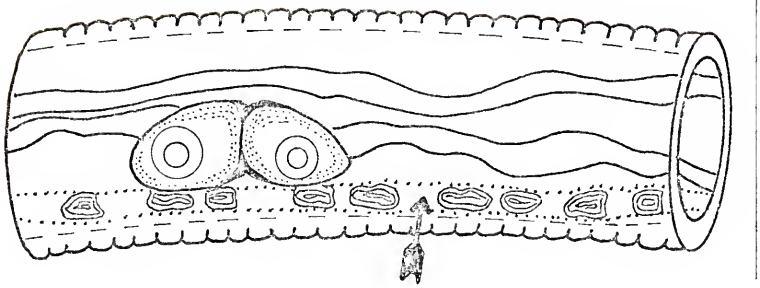

Figure 32. Ventral nerve cord (arrow) in the area of the genital primordia.

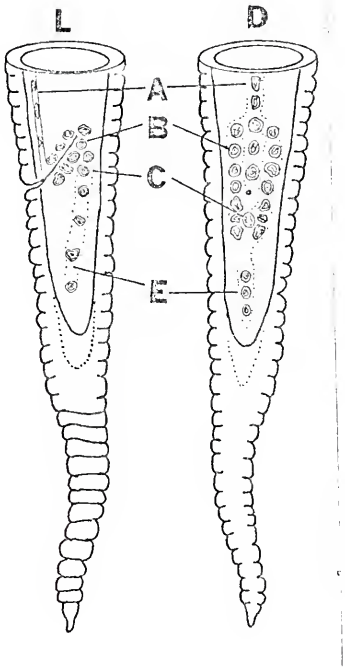

Figure 33. Nerves in the tail of a first-stage larva. A) ventral ganglion; b) rectal commissure; C) dorsal rectal ganglion; D) dorsal view; E) medial caudal nerve; L) lateral view.

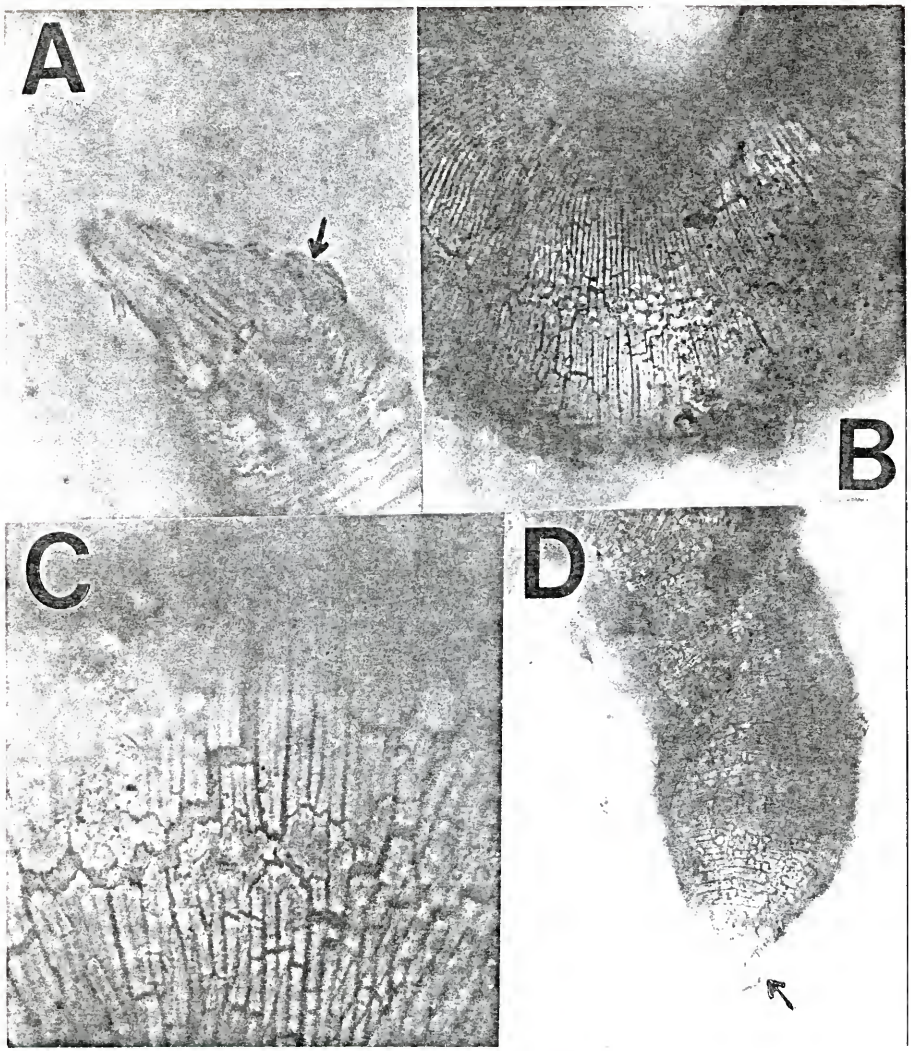

Figure 34. Crystalline layer. A) Separation of the layer from the anterior end; B,C) Layer at mid-body; D) Layer fragmenting from tail area.

Gubernaculum

A gubernaculum was isolated from the surrounding tissue for observation of the dorsal and ventral faces (Fig. 35). The dorsal face is longer, measuring 16 µm, and shows serrated margins on the cuneus (Fig. 35-D). The ventral face is shorter (11 µm), ventrally grooved, and serrations were not observed in the focal plane (Fig. 35-V).

SECTION II
REPRODUCTIVE DEVELOPMENT

Early Development

The female deposits a naked undivided egg in the environment (gelatinous matrix absent).

Four-hundred eggs in lots of 50 were examined under the oil immersion lens to determine if a molt occurred in the egg as described for Heterodera rostochiensis Wollenweber by Hagemeyer, 1951, and in Meloidogyne sp. by Christie and Cobb, 1941. In no case was evidence of ecdysis present. After examination, the larvae were expelled from the eggs (Fig. 36) by exerting a gentle pressure with a fine needle tip on the cover slip. None of the larvae expelled from the 400 eggs showed evidence of ecdysis. It is concluded based on these data that a molt does not occur in the egg.

First-stage larvae possess binucleate genital primordia with posterior and anterior cap cells (Fig. 32, 38-A). Shortly after the first molt, determination of sex is possible by examination of the rectal area. If spicular primordia cells are present (Fig. 37), a male is developing. Absence of spicular primordia cells indicate a female is developing.

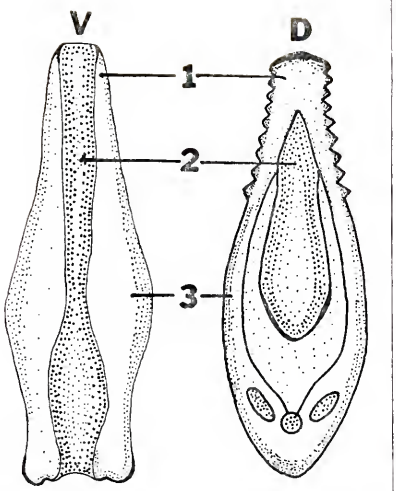

Figure 35. Gubernaculum (top is anterior). V) ventral face (4 µm wide by 11 µm long); D) dorsal face (5 µm wide by 16 m long); 1=cuneus, 2= corpus, 3=crura.

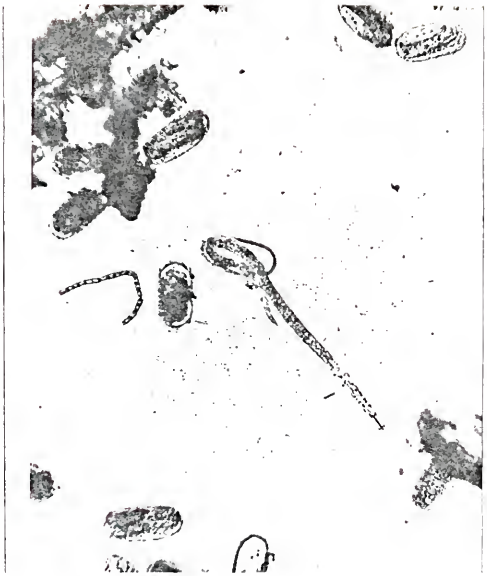

Figure 36. Larvae forced from egg by applying cover slip pressure.

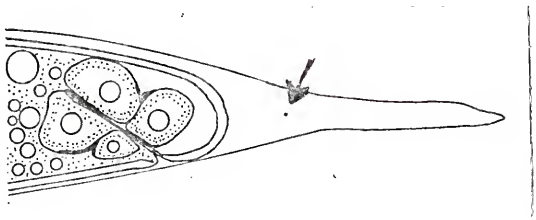

Figure 37. Spicular primordia cells in the cloacal area of an early second-stage male. Phasmid is shown (arrow) on first-stage exuviae.

Male Development

Shortly after the first molt, and before the first-stage integument is cast off, the genital primordium begins to divide and proliferate posteriorly (Fig. 38 B,C). The body widens, and the testes join the spicular primordia shortly before, and after, the first molted integument is lost (Fig. 38-D). Sperm cells are large and angular at this stage. Following the second molt, little development is evident in the testes and spicular primordia. The esophagus is not clearly differentiated at this stage of development. The gubernaculum is the anlage of sclerotization, followed by the lamina of the spicules. The calomus and capitulum are the last to become sclerotized. Development proceeds to completion after the second exuviae is cast. In 2 cases observed, the male left the third-stage exuviae embedded in the root. Empty exuviae are not uncommon in infected roots. Table 3 shows the length of time required for development of males. Male development from penetration of the first-stage larva until a fully developed male was observed took place in a minimum of 6 days and a maximum of 15 days in roots growing in water agar. Three first-stage larvae that entered a root about the same time all molted to the second-stage in 48 hours. Twenty-four hours later, all 3 molted to the third-stage. Three days later the final molt occurred for all 3 males within $9\frac{1}{2}$ hours. Total average time required was 10 days and 20 hours. Other periods

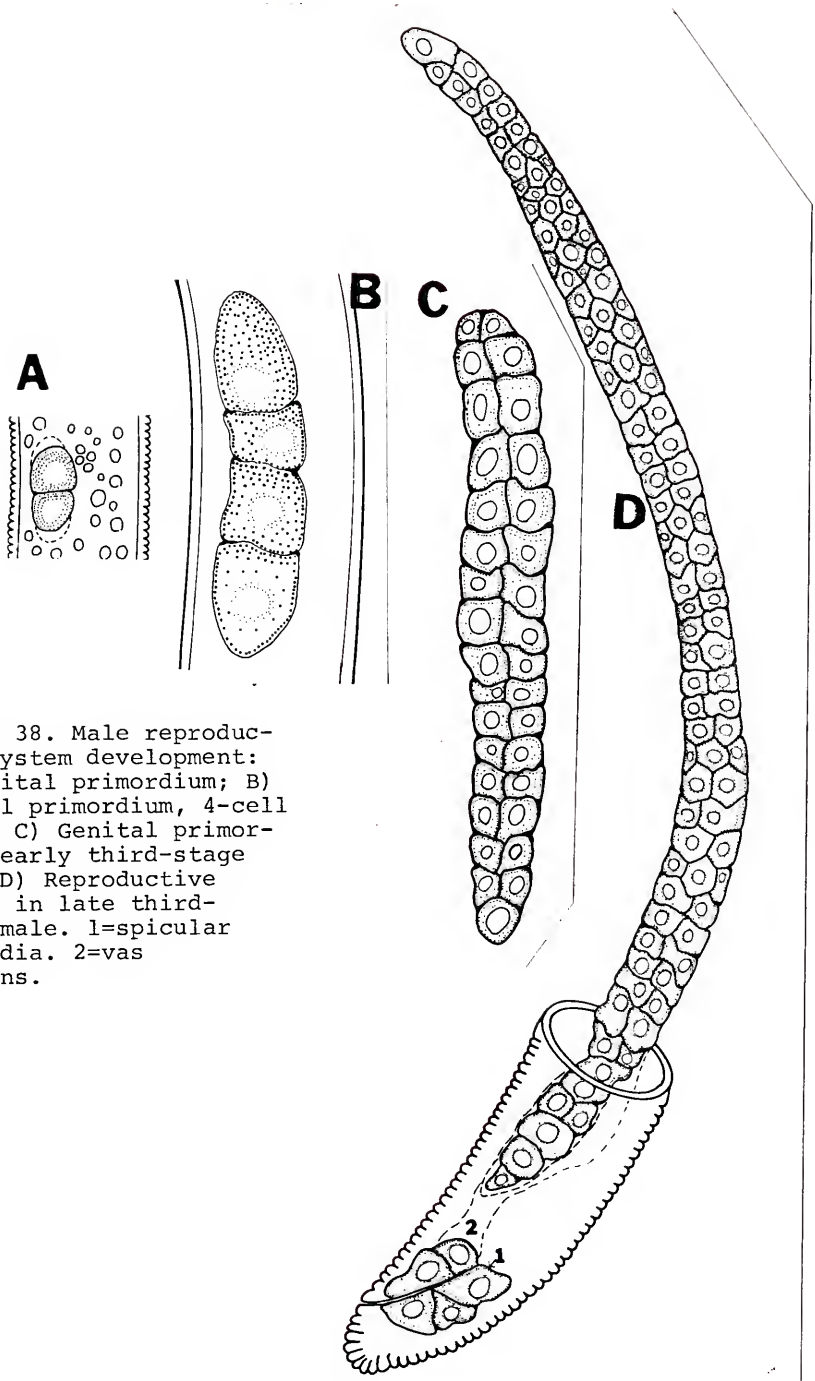

Figure 38. Male reproductive system development: A) genital primordium; B) Genital primordium, 4-cell stage; C) Genital primordium, early third-stage male; D) Reproductive system in late third-stage male. 1=spicular primordia. 2=vas deferens.

of male development observed were: a 15-day cycle; a 10-day, 4-hour cycle (Table 3); and a 6-day cycle.

Table 3. Male Development

Time (hours)	Activity	Width (μm)
0	penetration	28.2
24	feeding	32.9
$32\frac{1}{2}$	" "	32.9
48	" "	42.3
72	ecdysis (first)	37.6
81	" "	37.6
96	" "	37.6
105	feeding	42.3
122	" "	49.3
145	shedding integument	49.3
168	feeding	47.0
$176\frac{1}{2}$	" "	51.7
192	ecdysis (second)	42.3
195	Third-stage larva emerged from exuviae which remained in root.	42.3
197	Migration along root (length 700 μm), stylet 24 μm.	37.6
202	Migration to a new root, no physical change.	38.6
212	no change	38.6
236	ecdysis (third & fourth)	38.6
244	development complete	33.3

Total time = 10 days, 4 hours.

It is shown in Table 3 that the width of the feeding larva increases with time until $176\frac{1}{2}$ hours have elapsed when a maximum width of 51.7 μm is attained. After the second ecdysis the width decreases until the male is fully developed with a width of 33.3 μm. Feeding has ceased at this time and it is postulated that the decrease in width is due to energy expended during ecdysis and migration.

Female Development

Shortly after the first molt the body swells and the genital primordium proliferates anteriorly, and posteriorly (Fig. 38-A,B). The cells in the center bulge toward the body wall forming the vaginal primordium, after which the anterior and posterior branches elongate and develop (Fig. 39). The vagina first appears as a large opening with very large vaginal primordia cells on either side (Fig. 40). After the second molt the body swells and the gonad completes its development (Fig. 41). The reproductive system is complete when the third-stage exuviae is cast (Fig. 42). Mature females (Fig. 11,43) are usually swollen more than virgin females, possess convoluted ovaries, and contain sperm in the spermatheca (Fig. 43). The vagina uterina was very narrow in a few females (Fig. 44-C). In older females the vagina uterina is well-developed, with thick folds capable of containing several eggs. In several females a severely prolapsed vagina was noted (Fig. 23-right).

Female Life History

Only 1 female developed to maturity in life history tests. The onset of ecdysis was never observed. Vulva development was seen 4 days after root penetration by the first-stage larva. The ovaries were defined 7 days after penetration, a fully developed female was evident 17 days after penetration. Seventeen eggs were deposited on the

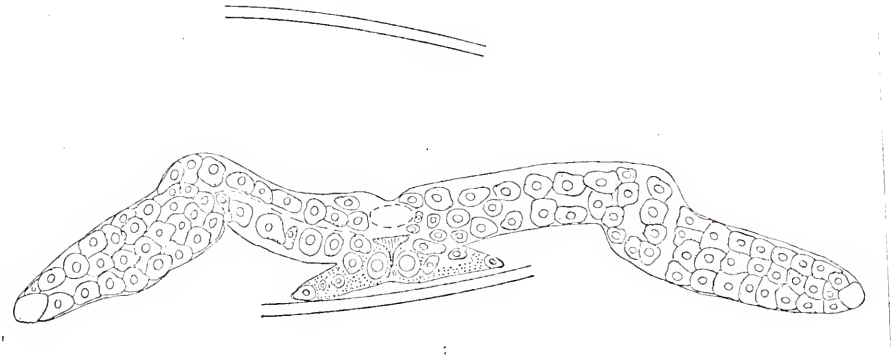

Figure 39. Early third-stage female gonad.

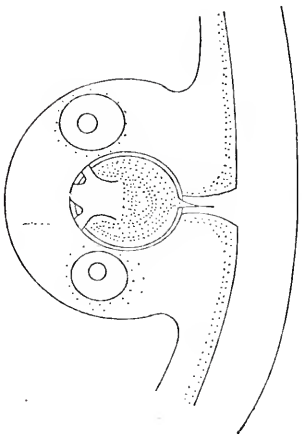

Figure 40. Vaginal development of a late third-stage female.

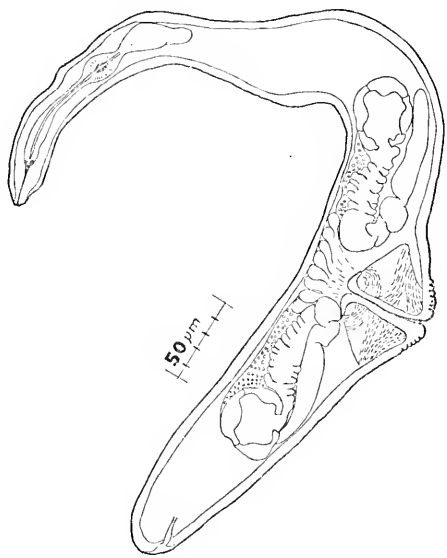

Figure 41. Virgin female with gonad development complete.

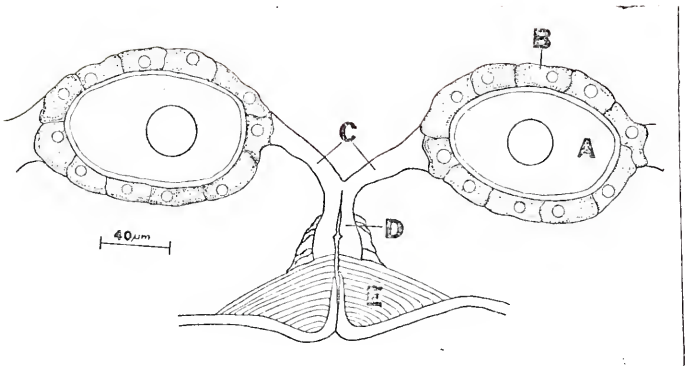

Figure 44. Anatomy of the female reproductive system from vulva to uteri: A=Egg in uterus; B=uterus cells; C=Narrow vagina uterina; D= Vagina; and E=Vaginal muscles.

Figure 42. Final female ecdysis.

Figure 43. Mature female.

same day development was considered complete. Males were not observed near the female prior to oviposition.

Conclusions

Critical examination failed to reveal ecdysis in the egg. Early development proceeded as known in most phytoparasitic nematodes. The distinct spicular primordia that appeared early in male development was not noted in other similar studies (Chitwood & Buhrer, 1946; Christie and Cobb, 1941; Hirschmann & Triantaphyllou, 1971; and Raski, 1950).

Another unique feature of male development occurred when the male abandoned the third-stage larval integument, leaving it embedded in the root following final ecdysis.

Male growth measured by body widths during development has not been reported in other developmental reports examined by the author. A loss in width of 18 µm was shown from a maximum of 52 µm. Time for male development varied from 6 to 15 days.

The unique feature of female development was the occurrence of huge vaginal primordia cells.

SECTION III
HOST-PARASITE RELATIONSHIPS

Methods

A variety of devices and ideas were tested to study the host-parasite relationships and life history of the new nematode species, all but one of which were unsuccessful.

Macro-Observation Boxes

Wood chambers were patterned after rearing chambers used by Dean, 1929, and by Minton, 1962. The box is 23-cm square with a 2.3-cm chamber enclosed by glass and removable wood sides. Buttonweed plants, well established in white sand in the chamber, were inoculated with groups of 100 larvae at the site of healthy root flushes under the glass. Development of the nematode either failed to occur or took place in areas away from visible root sites. This method was abandoned after a number of failures. In the next trial plastic boxes, 17.5 cm long by 9.3 cm wide by 3 cm deep, were filled with either white, or with black volcanic sand, planted with buttonweed and then inoculated with 100 larvae of the new species. In this system the activities of the nematodes were obscured by the substrate. The macro-observation boxes were used to observe nematodes on roots growing in soil. Limitations of magnification and depth

of focus severely handicapped close observation by this method.

Micro-Observation Units

Trials were conducted utilizing small plastic boxes of various sizes, and plastic petri dishes containing a poured 4-mm layer of water agar. Success was assured using the following procedure: A 4-mm layer of 1% sterile water agar is poured into a 9.2-cm plastic petri dish lid. A 5-mm ring of water agar is removed from the outside perimeter of the agar ring after hardening. A 5-mm glass rod is heated over a glass flame until slightly red, then used to burn a hole into the side of the closed petri dish. The burn area should be sanded so the dish can be easily separated. A stem cutting of buttonweed with 1 or 2 small leaves is inserted through the hole and into the agar with the leaves external to the dish. When primary roots emerge and grow into the agar, a 5-mm well is cut into the agar 1-cm lateral to a primary root. Twenty-five first-stage larvae, and 5 mature males in a small drop of sterile water were inoculated into the agar well. A root map was drawn (Fig. 45) when larvae made contact with the root. Each larvae that situated itself at a particular site on the root was assigned an alphabetical letter which was placed at the approximate site on the root map. For each observation, the date, time, dish number, and larva letter was recorded. Observations and measurements were made using the oil

immersion lens by placing a small drop of water on a cover slip, which was inverted and placed over the root site where larvae were attached. Basic data taken when possible included: time elapsed from the inoculation to the time larvae penetrated the root, time elapsed between penetration of the larva to the appearance of root discoloration, if and when a larva left its feeding site, body width measurements, and ecdysis observations.

Behavior Studies

Eighteen plates containing plants in agar were inoculated. Life history activities were observed in 5 plates; the remainder were abandoned due to plant death, visibility problems, or severe bacterial contamination.

As soon as the water in the inoculation well dried the males and larvae migrated into the agar.

Male Behavior

Males migrated at random in the agar. A proclivity to the root by males was not noted. Several males were seen with lips in contact with the epidermis of a primary root. In no case was stylet movement or metacorpus valve pulsation noted in such contacts. One male lay quiescent very close to a primary root the duration of the trial. Most males migrated slowly through the agar after which they became quiescent.

Larval Behavior

A total of 49 larvae of 450 inoculated was observed penetrating roots. Data were taken until they departed, ceased activity, or completed development.

Root penetration. Larvae migrated to the root following pathways peculiar to most nematodes in agar (Wallace, 1964). One group of 4 larvae reached the root in 19, 23, 30, and 36 min., respectively, following inoculation. Stylet movement was initiated about 4 min. after lip contact with the root. Stylet thrusts were recorded at 92 per min., and 112 per min. by 2 larvae shortly after contact with the root. Klinkenberg, 1963, recorded 69 thrusts per min. for Pratylenchus crenatus Loof, 1960. Pressure was exerted on the epidermal surface by thrusts of the nematode head and stylet. The head slid over the cell surface as it thrust until the stylet tip was over the middle lamella between 2 epidermal cells (Fig. 46-A). At this point the metacorpus valve moved intermittently indicating digestive enzymes were extruded into the attack site (Fig. 47). The lamella between the 2 cells separated and the nematode slipped laterally into the opening (Fig. 46-B). After penetration, the larvae migrated obliquely 1-3 cells and 1-3 cells deep (Fig. 46-C). One root with a 100 µm diameter was penetrated 61 µm laterally and 50 µm deep.

Feeding. Once the larvae were situated in the root, feeding began immediately. In very small roots, feeding

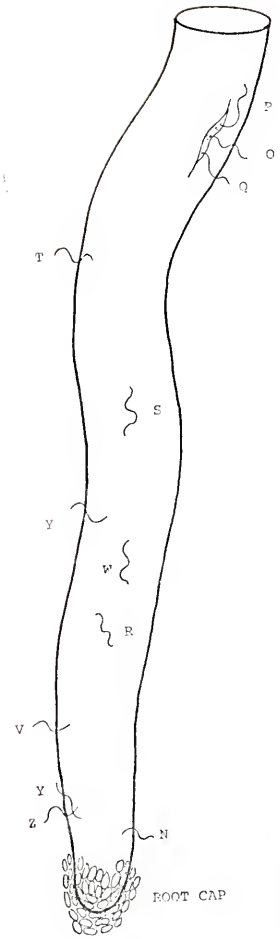

Figure 45. A root map charting the progress of each nematode that occupied a feeding site in one of the inoculated petri dishes. Each letter represents a larva at a feeding site.

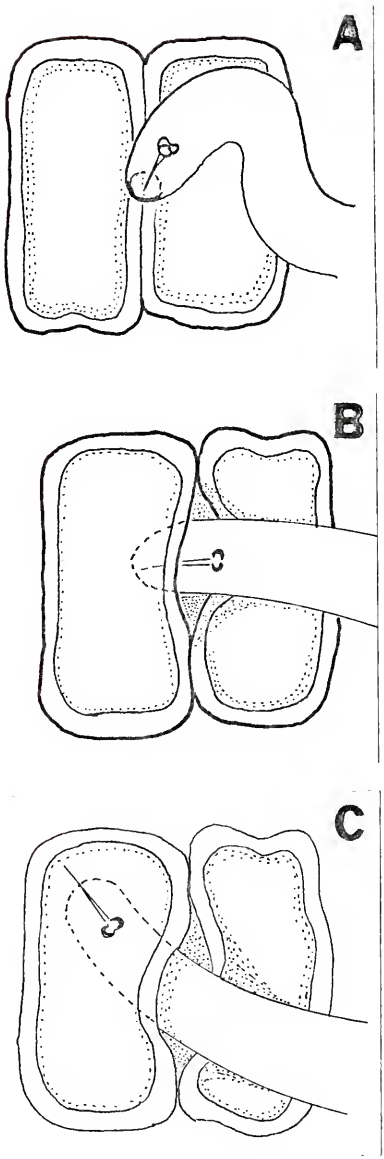

Figure 46. Mode of root entry by larvae. A=larva with head centered on middle lamella between two cells.
B=Penetration. C=Feeding site.

Figure 47. Larval head in contact with middle lamella. Note small area of discoloration in front of oral aperture.

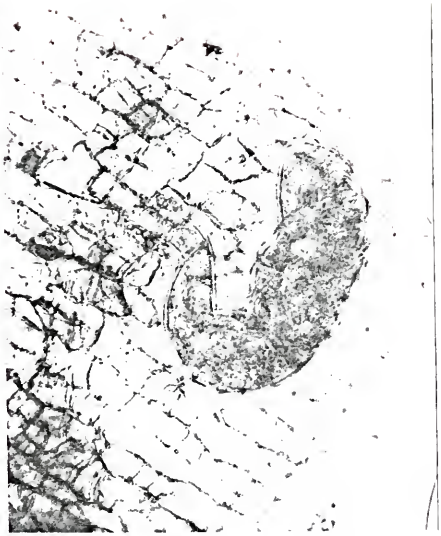

Figure 48. A mature female in a shallow, hand-cut epidermal section.

occurred in the cortex or pericycle. One larva fed in a cortical cell occupied by another larva feeding in the pericycle. Feeding sites were rather shallow (1-4 epidermal cells deep) in mature roots. A very shallow hand cut longitudinal section (Fig. 48) underneath a feeding female rarely cuts the female.

Tissue discoloration. Yellowing of the tissue (Fig. 49-A) appeared initially 3, 4, and $4\frac{1}{2}$ hours following penetration. In some cases (Fig. 49-B) the discoloration was confined to the cell wall. Larvae were also noted with the stylet inserted in the cell wall (Fig. 49-B). Nuclei of

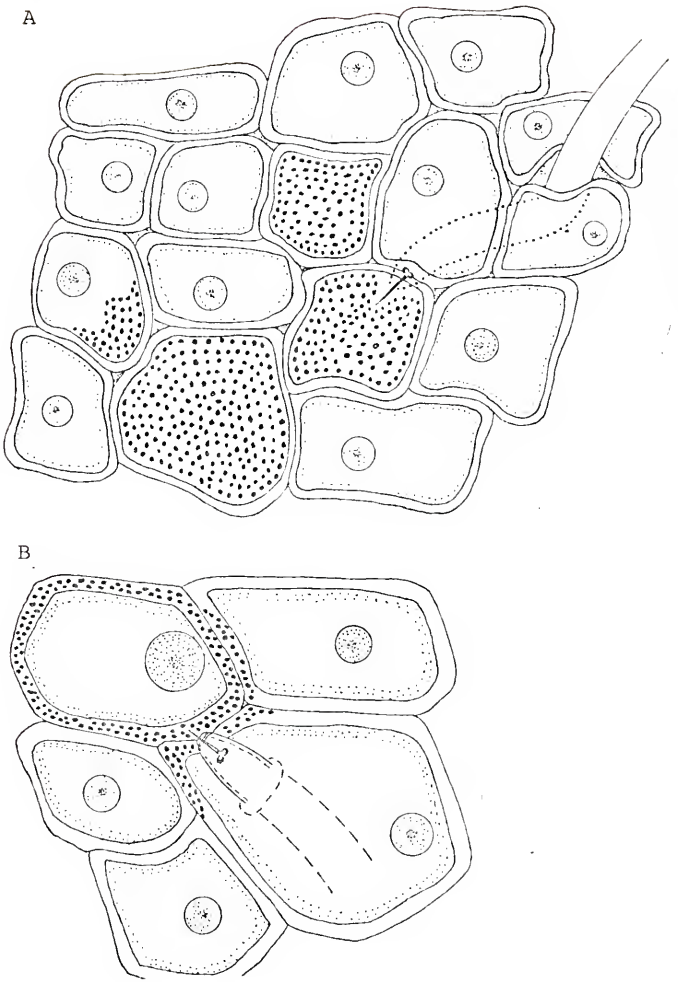

Figure 49. Tissue discoloration. A) Heavy stippled area indicates yellow discoloration in cells $4\frac{1}{2}$ hours after larval entry. B) Stippled area indicates yellowing in cell walls. Note difference in nuclei size between healthy and attacked cell.

cells with discolored walls were consistently enlarged (11-13 µm) in comparison to healthy cells containing nuclei (5-7 µm, Fig. 49-B).

Feeding migration. A few larvae touched the root and departed without entry. Eleven larvae entered the root, fed, and after a few hours or several days departed. Some of the departing nematodes took up a new feeding position on the same root or entered a different root and resumed feeding. Some larvae were never seen again after leaving a feeding site. Entry of an epidermal site predisposes the site for entry of searching larvae. Nine larvae were seen feeding together in a single, large longitudinal lesion (Fig. 50). Such lesions are usually abandoned by feeding larvae. One assumes the excess of enzymes and metabolites in such a large open lesion renders the site unfavorable for the development of the nematode.

Attack sites. Larvae have been detected feeding at root tips (Fig. 51-A), root scales (Fig. 51-B), along feeder roots, and rhizomes, singly, or in groups (Fig. 51-C,D). In mature rhizomes, females are commonly seen either singly or in groups (Fig. 51-E). Females can almost always be found at the junction of secondary roots emerging from the rhizome (Fig. 51-F). Larvae and third-stage males have also been observed on chlorophyll-bearing stem tissue at the soil line. Larvae and mature females have been detected in root leaf scales just below chlorophyll-bearing aerial leaf scales (Fig. 51-B). Many females resembling

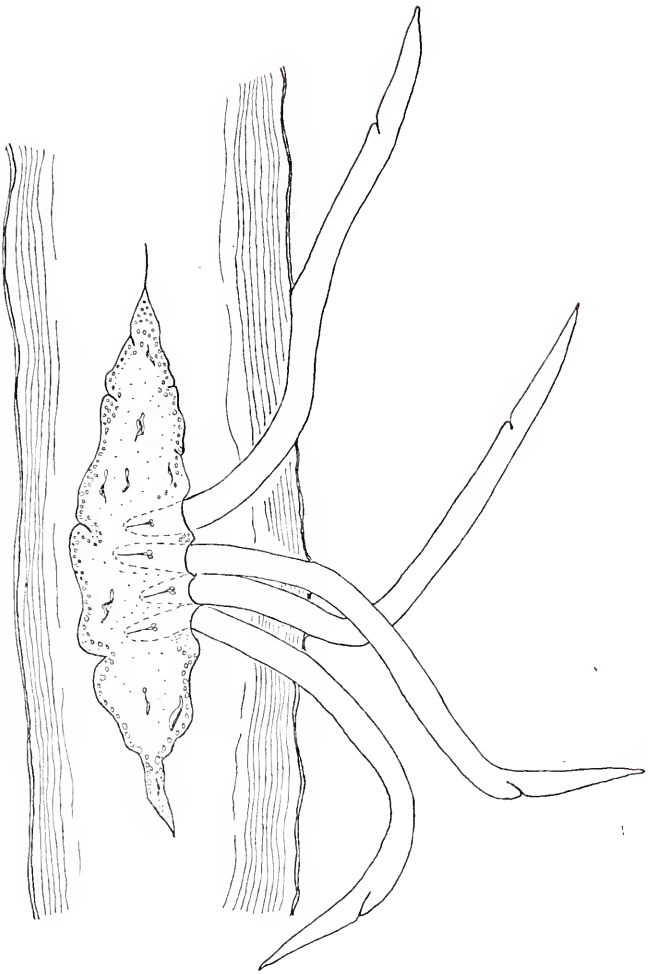

Figure 50. Large lesion occupied by 4 larvae.

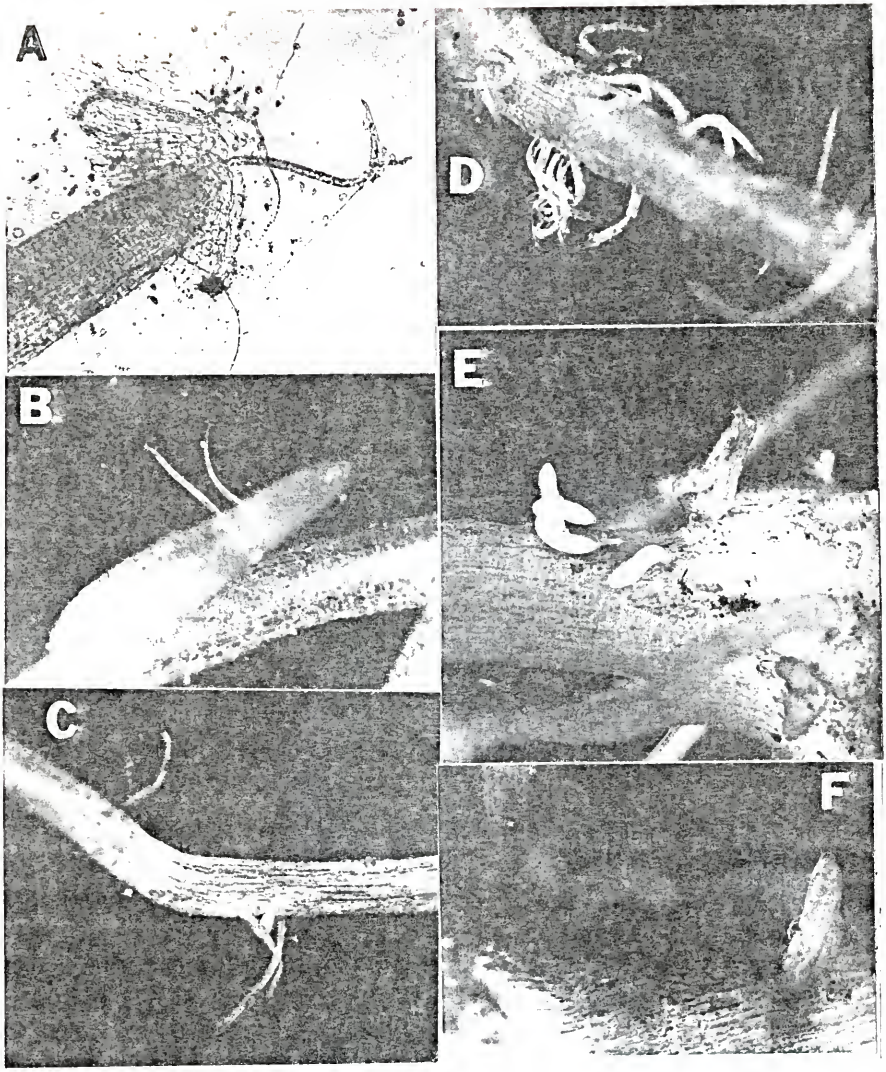

Figure 51. Attack sites. A) Larvae feeding at root tip; B) Larvae feeding on root-leaf scale; C) Larvae feeding on feeder root; D) Large group of larvae in rhizome; E) Group of mature females in mature rhizome; F) Females at secondary root juncture.

small white sausages lie appressed to large rhizome pieces (Fig. 51-E).

Host exudates. About 5% of the females examined possessed an accumulation of spheroid objects around the cervical region (Fig. 52). The exudates were closely associated with the integument but did not adhere as do the cement bodies adhering to the integument of cyst nematodes described by Shepherd and Clark, 1978. The cement bodies are depicted as brown hardened exudates originating from the integument and grossly resemble the spheroid bodies of V. volvingentis. Exudates of the new species differ in appearing to have a crystalline composition (Fig. 53).

The spheroid bodies appear to be exudates originating from the host in response to feeding activities of the new genus. Exudates have also been noted in Meloidodera floridensis (Fig. 54).

Oviposition and fecundity. Eggs are deposited naked in the substrate. Five to 25 eggs usually lie inside the ventral space formed by the body coil, or they are scattered about the female body near the vulva. One large egg mass contained 185 eggs in various states of development. Standard soil washing procedures usually wash the eggs from the female so the number of eggs deposited is difficult to ascertain. To determine the number of eggs contained in mature females, 123 female specimens were examined (Table 4).

Figure 52. Spheroid bodies attached to anterior region of a mature female.

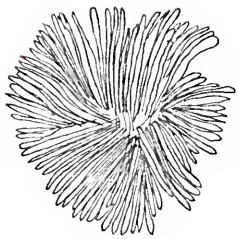

Figure 53. Appearance of a single spheroid body.

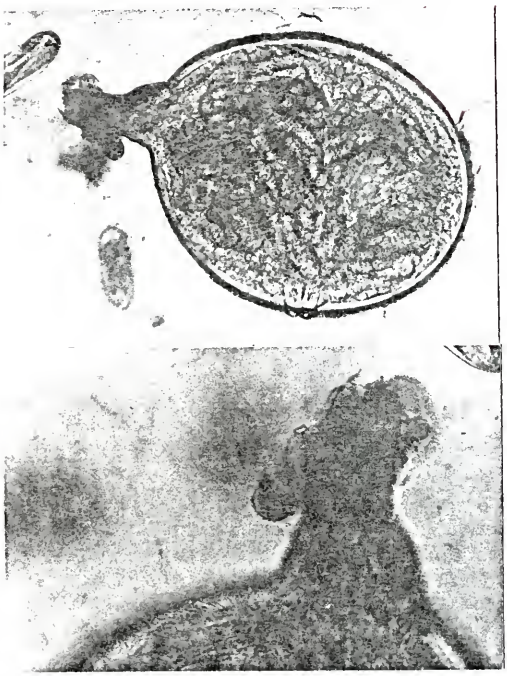

Figure 54. Exudates on the anterior end of Meloidodera floridensis.

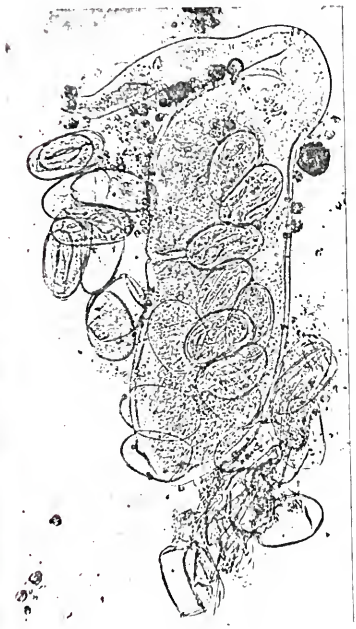

Figure 55. Female containing 7 eggs.

Table 4. Number of fully developed eggs detected in 123 mature females.

Egg number	Females
0	47
1	28
2	22
3	8
4	12
5	5
6	0
7	1

Most females contained 1 or 2 eggs with a maximum of 7 eggs in 1 female (Fig. 55). The largest egg measured was 128 X 70.5 µm, inside a female.

Longevity. All plants which had been inoculated with V. volvingentis for pathogenicity trials had died by September, 1973. Five 1.9 X 20.3 cm soil plugs were removed from each of 7 pots and the nematode population counted (Table 5). Most pots contained large numbers of eggs. The pots were maintained in the greenhouse in a fallow condition until October, 1976, when a sample similar to the soil sample in September, 1973, was taken and the nematode population counted (Table 5).

No females survived the longevity test while male and larval survival was minimal. It appears feasible that eggs are the prime survival stage in this species.

Egg viability. A 15-cm clay pot in which buttonweed was root-bound, but nematode free, was saturated with sterile water. The pot was then placed in a beaker and an additional 100 ml of sterile water added. The effluent was

Table 5. Survival stage and numbers of nematodes recovered before and after a longevity test.

Pot	Females		Males		Larvae		Eggs	
	Sept. 1973	Oct. 1976	Sept. 1973	Oct. 1976	Sept. 1973	Oct. 1976	Sept. 1973	Oct. 1976
1	2	0	0	0	18	6	552	257
2	0	0	18	0	8	0	1282	5
3	1	0	0	0	0	0	198	6
4	0	0	1	0	3	0	41	287
5	0	0	0	0	0	0	1	2
6	1	0	1	0	6	0	811	2
7	0	0	0	2	8	6	336	129
Total	4	0	20	2	43	12	3221	688
Mean	.57	0	2.85	.28	6.1	1.7	460	98.2

collected, and added back to the pot. The collection and addition procedure was repeated 9 times, after which the final effluent was filtered and the leachate placed in an Erlenmeyer flask in the refrigerator.

To test egg viability, 10 eggs from 3-year-old fallowed soil were placed in a drop of sterile water in each of 4 dishes. Ten drops of stock leachate was added to the water containing eggs in each of the 4 dishes. Four similar dishes containing eggs in sterile water but without leachate served as controls. Results of the test are shown in Table 6.

Table 6. Larval hatch from eggs kept in fallow soil 3 years.

Examination date	Larvae emerged	
	Sterile water and leachate	Sterile water only
	Dish no.	Dish no.
	1-2-3-4	1-2-3-4
10/26/76	0-0-0-0	0-0-0-0
10/27/76	0-0-0-0	0-0-0-0
10/28/76	1-0-2-0	0-0-0-0
10/29/76	0-0-1-0	0-0-0-0
11/ 1/76	1-0-0-0	0-0-0-0
11/ 4/76	0-0-1-0	0-0-0-0
11/ 8/76	1-3-4-2	0-0-0-0
Total	3-3-8-2	0-0-0-0

Results. Larvae emerged from eggs only to which leachate was added. Eggs placed in sterile water failed to hatch. The test demonstrated that eggs can survive 3 years in fallow soil. Attempts to inoculate buttonweed with larvae hatched from 3-year-old eggs from fallow soil were

unsuccessful. Failure is attributed to low inoculum levels and reduced nematode viability.

Conclusions

Larvae entered roots by penetrating the middle lamella between 2 epidermal cells. Tissue discoloration became evident 3-4 hours after entry of the nematode into the root. A number of larvae abandoned the site after actively feeding. Nuclei in invaded cells were distinctly larger than nuclei in cells not entered by the nematode.

Host exudates were noticeably extruded at attack sites and these exudates adhered to the cervical area of female feeding at the site.

In longevity tests, eggs and larvae survived 3 years in the absence of a host. Results indicate that ova are the survival stage of this nematode.

SECTION IV
HOST PLANT INVESTIGATIONS

Host Plant

Diodia virginiana L. (buttonweed) in the family Rubiaceae is a perennial of no known economic importance. Buttonweed (Fig. 56) is comprised of smooth, weedy stems bearing lanceolate leaves. The plant creeps across the soil as it grows forming a dense mat when abundant (Fig. 57). It usually is found in a mixture of herbaceous plants peculiar to vegetation growing near bodies of water. The vegetative form is found from Florida West to Texas, and North to New England and Missouri (Small, 1933; Rickett, 1967).

Host Habitat

Buttonweed is found growing on moist soil adjacent to bodies of water such as lakes, ponds, water-bearing ditches, swamps, and prairies (Fig. 58). Maximum growth appears from 1 to 40 meters from the water's edge. As soil becomes less moist and elevation increases, buttonweed decreases until none are found. When collecting buttonweed a body of water will almost always be in sight.

Norton, 1978,lists 15 genera of phytoparasitic nematodes comprising 30 species in aquatic habitats. Eight genera and 2 species listed by Norton were found associated

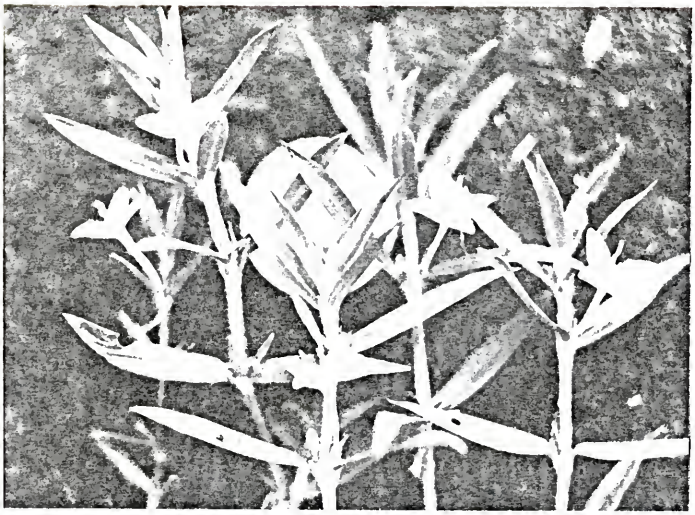

Figure 56. Diodia virginiana in flower. (A Susan Anthony dollar is in the background for comparison.)

Figure 57. A mat of buttonweed mixed with herbaceous plants.

Figure 58. Paynes Prairie, Gainesville, Florida. A prime habitat for buttonweed and Verutus volvingentis.

with buttonweed in an aquatic habitat. Ten genera and 23 species of phytoparasitic nematodes not included in Norton's list were detected (Table 7).

Host Testing

A primary consideration, when a previously undescribed phytoparasite is encountered, is the determination of its potential as a parasite of economic crop plants. In the original site where the new genus was collected corn and soybean plantings were established adjacent to buttonweed plants. The 2 aforementioned plants, in addition to 19 other plants, were placed in soil infested with the new genus. Results of the host testing are shown in Table 8.

Table 7. Phytoparasitic nematodes found in soil associated with roots of buttonweed.

Phytoparasite	Occurrence
Belonolaimus sp.	infrequent
Cacopaurus sp.	moderate
Criconema sp.	infrequent
Criconemoides curvatum Raski, 1952	frequent
" " mutabile Taylor, 1936	infrequent
" " xenoplax Raski, 1952	" "
Dolichodorus heterocephalus Cobb, 1914	moderate
Helicotylenchus crenicauda Sher, 1966	frequent
" " dihystera (Cobb, 1893) Sher, 1961	infrequent
" " erythrinae (Zimmermann, 1904) Golden, 1961	" "
" " longicaudatus Sher, 1966	" "
" " paxilli Yuen, 1964	" "
Hemicriconemoides wessoni Chitwood & Birchfield, 1957	" "
Hemicycliophora zuckermani Brzeski, 1965	" "
Heterodera sp.	" "
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949	" "
Pratylenchus brachyurus (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941	" "
Trichodorus christiei Allen, 1957	frequent
" " proximus " " " "	infrequent
Trophonema arenarium (Raski, 1956) Raski, 1957	moderate
Tylenchorhynchus irregularis Wu, 1969	" "
Xiphinema americanum Cobb, 1913	infrequent

Table 8. *Verutus volvingentis* host testing results.

Test plant	Weeks tested	Nematodes found Roots	Soil
Amaranthus spinosus L. (spiny amaranth)	12	0	box
Ardesia crenata Sims (spiceberry)	13	0	1-L
Beta vulgaris L. (beet)	18	0	2-L, 28-E
Chrysanthemum sp. (mum)	12	0	2-E
Commelina sp.	4	0	box
Cucumus sativus L. (cucumber)	18	0	2-E
Daucus carota L. (carrot)	22	0	3-E
Digitaria sanguinalis (L.) Scop. (hairy crabgrass)	4	0	box
Glycines max L. Merr. (soybean)	68	0	2-L, 42-E
Gossypium hirsutum L. (upland cotton)	12	0	1-L, 43-E
Hibiscus esculentus L. (okra)	78	0	0
Ludwigia peruviana (L.) Hara (primrose willow)	4	6-F	box
Lycopersicon esculentum Mill. (tomato)	10	0	0
Nephrolepis exaltata (L.) Schott (Boston fern)	60	0	1-L, 9-E
Nicotiana tabacum L. (tobacco)	20	0	2-E
Phaseolus vulgaris L. (common bean)	10	0	3-L, 60-E
Philodendron scandens C. Koch & H. Sello subsp. *oxycardium* Bunt (parlor ivy)	12	0	2-L, 4-E
Pteris vittata L. (ladder braken fern)	4	0	box
Richardia braziliense (Mog.) Gomez	8	0	2-L, 3-E
Rumohra adiantiformis (G. Forst.) Ching (leather-leaf fern)	12	0	21-L, 3-E
Zea mays L. (corn) 'silver beauty'	12	0	1-L, 9-E

L = larvae; E = eggs; F = females; box = plants grown in the population maintainence box.

Methods

Plants to be tested were seeded into or propagated by cuttings in 20-cm clay pots filled with steamed soil. When a healthy state of growth was evident, the pot was inoculated with 50 cc of soil and roots taken from a soil box heavily infested with V. volvingentis. Plants were harvested in a minimum of 4 weeks. Roots were removed, washed, and examined using a dissecting microscope to see if any stage of the new nematode was in the root system. Five 1.9 X 20.3 cm soil plugs were removed from each inoculated plant and processed for the nematode (Table 8). A few plants were placed directly in a population maintainence box.

Results

Females of the new taxon developed only in roots of Ludwigia peruviana (Onagraceae), a weed of no economic importance. Larvae were not detected in any of the other host plants examined.

Results of this host test and because this nematode has not been reported in numerous surveys of economic crop plants indicate the nematode is doubtful as a threat to economic crops, and very likely has a limited host range. In the original site, the infested buttonweed was found growing into plantings of both corn and soybeans, neither of which proved to be hosts in field samples or host tests. Since the nematode habitat includes irrigation

ditch banks serving as borders for numerous crops, the nematode has ample opportunity to infest a variety of crops, should they prove susceptible.

Host Symptoms

Aboveground symptoms of nematode injury were not noted in any site examined. Symptoms were noted only in inoculated population maintainence boxes, inoculated pots, and in plants grown in water agar.

Generalized aboveground symptoms include stunting, chlorosis, seed pod reduction, and death. Healthy green stolons and leaves gradually turn chlorotic until the entire plant is a pale yellow-green color. Plants begin to decline and die 6 months after inoculation until no living plants remain. In the final stages of decline, germinating seeds produce small plants with 2-4 leaves that rarely assume a healthy green color. Such plants rarely survive very long, and their roots are almost always infected with females and larvae of V. volvingentis.

Root symptoms are expressed as shallow epidermal lesions of a pale yellow to brown or dark brown color. Lesions comprise 3-15 cells and are present at feeding sites. Lesions enlarge and darken with time (Fig. 59). In a few cases, epidermal swelling in the form of a small rounded protuberance appeared at the feeding site. In very small roots lesions sometimes encircle the root resulting in a necrotic constriction that causes blocking of

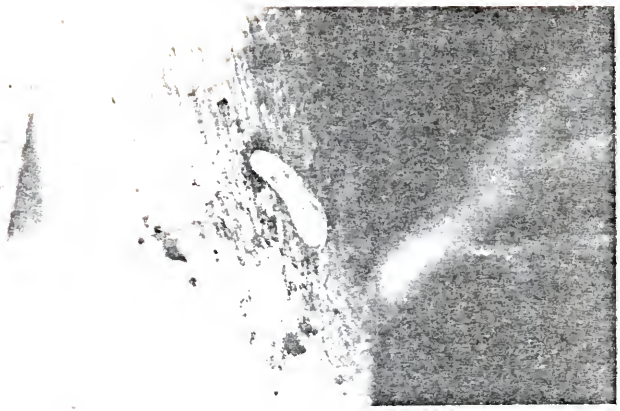

Figure 59. Young female in root, showing large dark lesion at feeding site.

conductive elements in the root and subsequent detachment of the root at the lesion site.

Feeder roots of buttonweed are very succulent and detach quite easily. Thus, it is not practical to compare root systems of infected and nematode-free plants under experimental conditions.

Pathogenicity

Twenty cuttings of buttonweed were taken from plants grown in steamed soil and transplanted to steamed soil in 20-cm clay pots. Four months later all foliage extending outside the pot perimeters were clipped off, and 25 larvae of V. volvingentis were inoculated into each of 10 pots. Ten pots were left as uninoculated checks. Pots were arranged in a randomized block design using "Tippets Random Number Table" (Le Clerg, Leonard & Clark, 1966).

Pathogenicity was evaluated by clipping foliage extending beyond the pot perimeter, and calculating the dry weights. Clippings were dried in a heat chamber for 3 weeks at 57°C. The total number of seed pods produced were counted on each plant just prior to taking perimeter clippings.

Eight 20 cm deep by 2 cm wide soil plugs were removed from each pot to evaluate the nematode population. Soil from the 8 plugs was thoroughly mixed and 150 cc subsamples

were removed from each of the mixtures. Samples were processed using the sugar-centrifuge technique.

Results

Table 9 shows the severe effect of the nematodes on the plant after 1 year. Thirteen days after final evaluation data were taken, 4 chlorotic, severely stunted plants still survived the initial inoculation (Fig. 60-A). Leaves on the surviving plants were very chlorotic (Fig. 61) and seed production was reduced considerably (Fig. 62). Inoculated plants yielded 2.9 grams of seed pods, while check plants yielded 15.7 grams. Seeds sprouting in infested soil in pots inoculated with the nematode were attacked at a very early stage of development and rarely produced more than 1 or 2 yellowed leaves before dying.

Within 60 days of the final leaf evaluation, the 4 surviving inoculated plants were dead. All control plants were in a healthystate of growth.

Table 10 shows the developmental stage and numbers of individuals in each stage in the 150 cc of soil examined from each replication. This table indicated that the egg is the survival stage of the nematode.

Discussion

The severe parasitism by the nematode resulted in eventual death of all inoculated plants. This occurred under greenhouse conditions when the plants were maintained

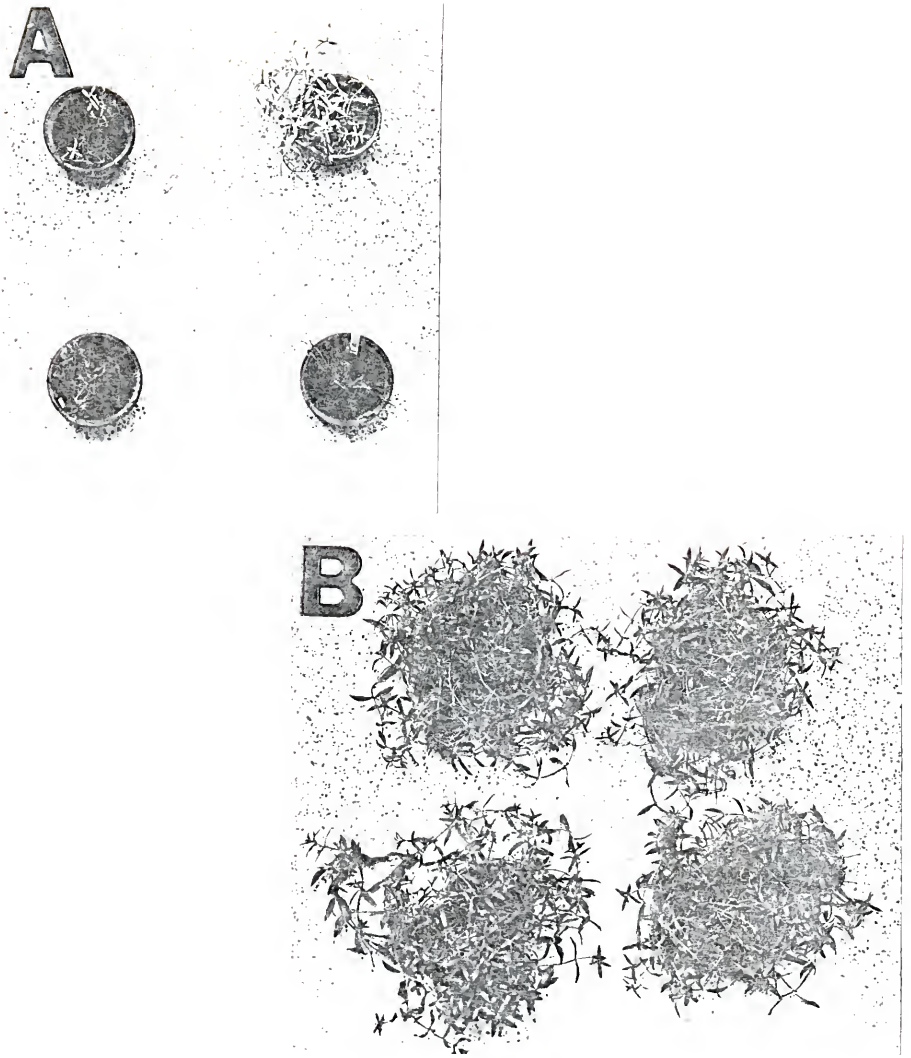

Figure 60. Appearance of inoculated (A) and uninoculated (B) plants at the conclusion of the pathogenicity trial.

Table 9. Effect of Verutus volvingentis on foliage and seed pod production of inoculated plants.

Days after inoculation	Mean dry weight of pot clippings per plant in grams		Mean number of seed pods per plant	
	untreated	treated	untreated	treated
95	11.54	11.40	320.2	324.0
217	4.9	2.6*	0	0
398	24.0	7.2**	512.0	168.3

* 2 inoculated plants died.

** 5 inoculated plants died.

Table 10. Nematode population density in treated and untreated soil.

Days after inoculation	Mean numbers of eggs or nematodes from inoculated plants				Uninoculated plants
	eggs	larvae	males	females	
406	1144	26	7	3.0	0
504	728	9	2	0.4	0

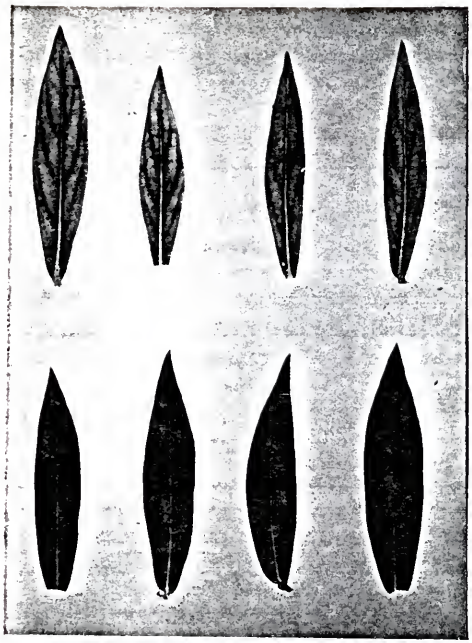

Figure 61. Comparison of leaves from inoculated (top), and uninoculated plants (bottom).

Figure 62. A comparison of seeds from inoculated (left) and uninoculated (right).

several months past their normal annual cycle. Symptoms produced in the greenhouse were never observed in the natural habitat. It is believed that the nematode population is in ecological balance with the environment and is not large enough to severely damage its host when buttonweed dies out after about 6 months of growth, following the first frost. During the next 6 months enough nematode eggs and seeds of buttonweed survive to establish a well balanced association between the host and its parasite. In the greenhouse there is no intervening period to retard nematode population

growth. As a consequence the parasite increases unchecked, severely damaging or killing its host. Factors in the natural habitat such as biological control agents and population-limiting physical changes are also absent from the controlled environment of the greenhouse.

Distribution of V. volvingentis in Florida

A total of 266 soil samples, and an almost corresponding number of root samples, were examined from collection sites in 13 Florida counties (Table 11).

Ninety-eight samples were positive and 168 samples were negative. Plants examined in the survey are shown in Table 12. The nematode was detected in soil from 13 hosts in the survey, but in roots of buttonweed only. It is doubtful if the 12 plants surveyed are true hosts of the new nematode since most of them were growing close to buttonweed.

The nematode was detected in Alachua, Lake, Orange, and Sumter counties. The largest population of the nematode occurred in Alachua county within Paynes prairie (Fig. 57), which is a relatively undisturbed, natural, ecological unit in Florida. The prairie supports large plantings of buttonweed. Samples taken at the waterline or from high dry sites in the prairie are almost always free of the nematode and buttonweed. About 90% of the negative sample results originate from plants other than buttonweed in

Table 11. Areas in Florida samples for Verutus volvingentis.

County	Site	Sample results	
		Positive	Negative
Alachua	Gainesville-Terwilliger school	3	9
	Newmans lake	1	2
	Paynes prairie	65	29
	Earleton	0	3
Baker	roadside	0	1
Bradford	" "	0	1
Broward	Pompano Beach	0	1
Gadsden	Chattahoochee	0	1
Lake	Clermont	27	91
	Grand Island	0	5
	Howey-in-the-Hills	0	1
	Leesburg	0	2
	Minneola	1	0
Marion	Ocala	0	1
Orange	Plymouth*	0	1
	Zellwood	0	2
Putnam	Florahome*	0	6
Seminole	Sanford	0	3
Sumter	Oxford	0	5
	Rutland	0	1
	Tarrytown	1	1
Union	roadside	0	1
Volusia	Barberville	0	1

*Buttonweed was not sampled at this site.

Table 12. Plants examined for Verutus volvingentis in the Florida Survey.

Scientific name	Vernacular name	Result
Alternanthera sp.		negative
Andropogon glomeratus (Walt)B.S.P.	bushy beardgrass	" "
Baccaris halimifolia L.	saltbush	positive
Bacopa caroliniana (Walt) G.L. Robinson	water hyssop	negative
Bidens laevis (L.)B.S.P.	beggars tick	positive
Brassica oleraceae L. (acephala group)	collards	negative
Cassia obtusifolia L.	coffee-weed	" "
Cephalanthus occidentalis L.	buttonbush	" "
Circium sp.	thistle	" "
Cuphea carthagensis (Jacq) Macbride		positive
Cyperus odoratus L.		negative
Diodia teres Walt	poor Joe	" "
" " virginiana L.	buttonweed	positive
Eupatorium sp.		positive
Geranium carolinianum L.	cranebill	negative
Clottidium vesicarium (Jacq) Mohr	bladderpod	positive
Glycines max (L.) Merr	soybean	" "
Hypericum mutilum L.	dwarf St. Johns wort	negative
Juncus effusus L.	soft rush	positive
Lachnanthes caroliniana (Lam.) Dandy	redroot	negative
Linaria sp.	toadflax	positive
Ludwigia arcuata Walt	false loosestrife	" "
" " leptocarpa (Nutt.) Hara		negative
Mikania scandens (L.) Willd.	climbing hempweed	" "
Nelumbo lutea (Willd.) Pers.	American lotus	" "
Paronychia baldwinii Fenzl		" "
Paspalum urvillei Steud.	vasey grass	" "
Polygonum hydropiperoides Michx.	smartweed	" "
" " persicaria L.	heartweed	" "
" " punctatum Ell.	water smartweed	positive
Ptilimnium capillaceum (Michx.) Raf.	mock bishop weed	negative
Rhexia mariana L.	meadow beauty	" "
Sabal palmetto (Walt.) Todd ex. Schult & Schult f.	cabbage palmetto	positive
Salix sp.	willow	negative
Scirpus sp.	bulrush	" "
Scoparia dulcis L.	sweet broom	" "
Stenotaphrum secundatum (Walt.) O. Kuntze	St. Augustine grass	" "
Typha sp.	cattail	" "
Zantedeschia sp.	calla lily	" "
Zea mays L.	corn	positive

higher, less moist areas, or from areas in water or at the water's edge.

Conclusions

V. volvingentis occurs in Florida in moist habitats. Paynes Prairie contains large populations of the nematode and its host. The host range appears to be very limited. None of the economic host plants tested were susceptible to the nematode. Three months following a low level inoculation of the nematode on its host, little or no effect was evident. Severe symptoms occurred 7 months after inoculation. Fourteen months after inoculation all inoculated plants were dead. All uninoculated plants in the test were in a healthy vigorous condition. It is believed that death of the inoculated plants was a result of growing an annual plant in the greehouse, devoid of natural inimical ecological factors present in its natural habitat, and past its time of normal growth.

SECTION V
BIOLOGICAL CONTROL INTERACTIONS

Males and larvae were exposed to zoospores of Catenaria anguillulae Sorokin. Within 10 min., zoospores were attached to the cephalic region of larvae (Fig. 63-A), and males (Fig. 63-B), and to the male cloacal area (Fig. 63-C). Development of the fungus was only completed in males. Zoospores were released from an infected male 78 hours after the initial infection.

Two-hundred eggs were exposed to a culture of zoospores to test the susceptibility of ova to the fungus. Zoospores became attached to 25% of the eggs in culture but no thalli developed subsequently.

Eggs were also exposed to a culture of an aquatic Phycomycete, Rhizophidium sp., a member of the Chytridiales, known to attack eggs of invertebrates. Sporangia of the fungus was observed attached to a number of eggs (Fig. 64); however, further development of the fungus was not noted.

A natural population of the nematode was found infested with endospores of Pasteuria ramosa Metchnikoff (Fig. 63-D). A single larvae hatching from an egg in the population was also noted with endospores attached.

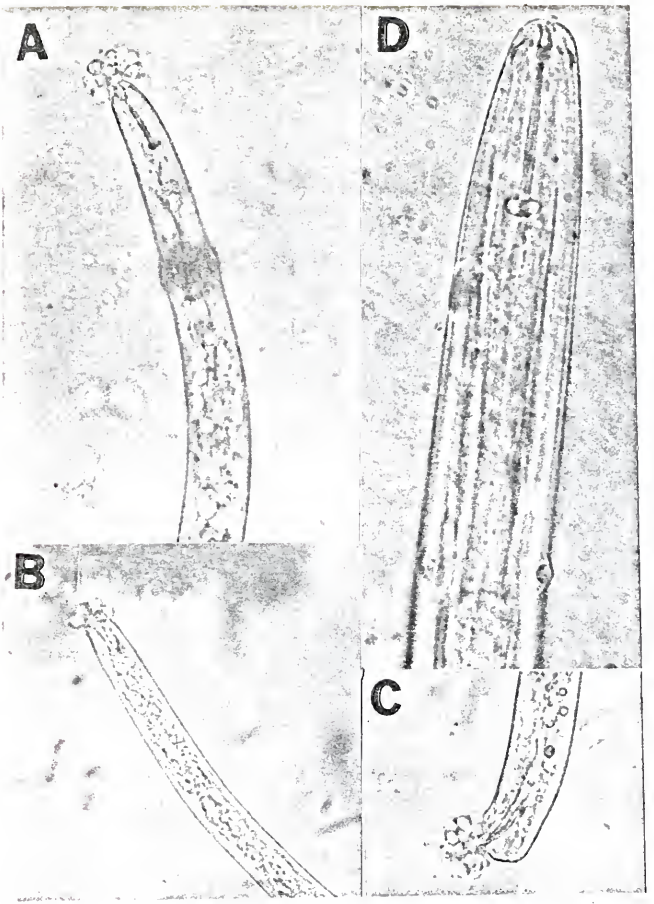

Figure 63. Biological control interactions. Catenaria anguillulae zoospores attached to the cephalic region of a larva (A), a male (B), male cloacal region (C). Endospores of Pasteuria ramosa on a larva (D).

Figure 64. Sporangium of Rhizophidium sp. attached to an egg.

Females were not noted in biological control interactions. Biological control agents attached to ova but failed to penetrate the shell and complete development.

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BIOGRAPHICAL SKETCH

Robert Paul Esser was born November 8, 1924, in Hackensack, New Jersey. He graduated from Hillsborough High School in Tampa, Florida, served 4 years in the United States Coast Guard during World War II, and worked as a watchmaker prior to entering the University of Florida in 1951. He graduated from the University of Florida in 1955 with a B.S.in Agriculture. In 1955 he was employed by the "State Plant Board of Florida" (now "Florida Department of Agriculture and Consumer Services "). In 1955 he joined the Nematology Department as a Laboratory Technician. In 1956 he was appointed Nematologist. From 1956 to 1980 he has published 70 articles dealing with nematodes. He has lectured in Invertebrate Zoology and Soil Microbiology every year for the past 15 years. He was co-editor of the "Nematology Newsletter" for 3 years, Associate Editor of the "Society of Nematologists" (SON) for 2 years, and served on the Executive Committee of SON for 3 years. He was also chairman of the SON Data Retrieval Committee, and organized and chaired the first SON regulatory colloquium at Ottawa, Canada. He served 2 years on the survey committee of the "Intersociety Consortium for Plant Protection" and is presently on a special data retrieval analysis committee of the same organization. He is a member of FNF, ESN, SON,

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He is married to Hannelore Esser, and has 3 daughters, Lorelei, Victoria, and Robin.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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