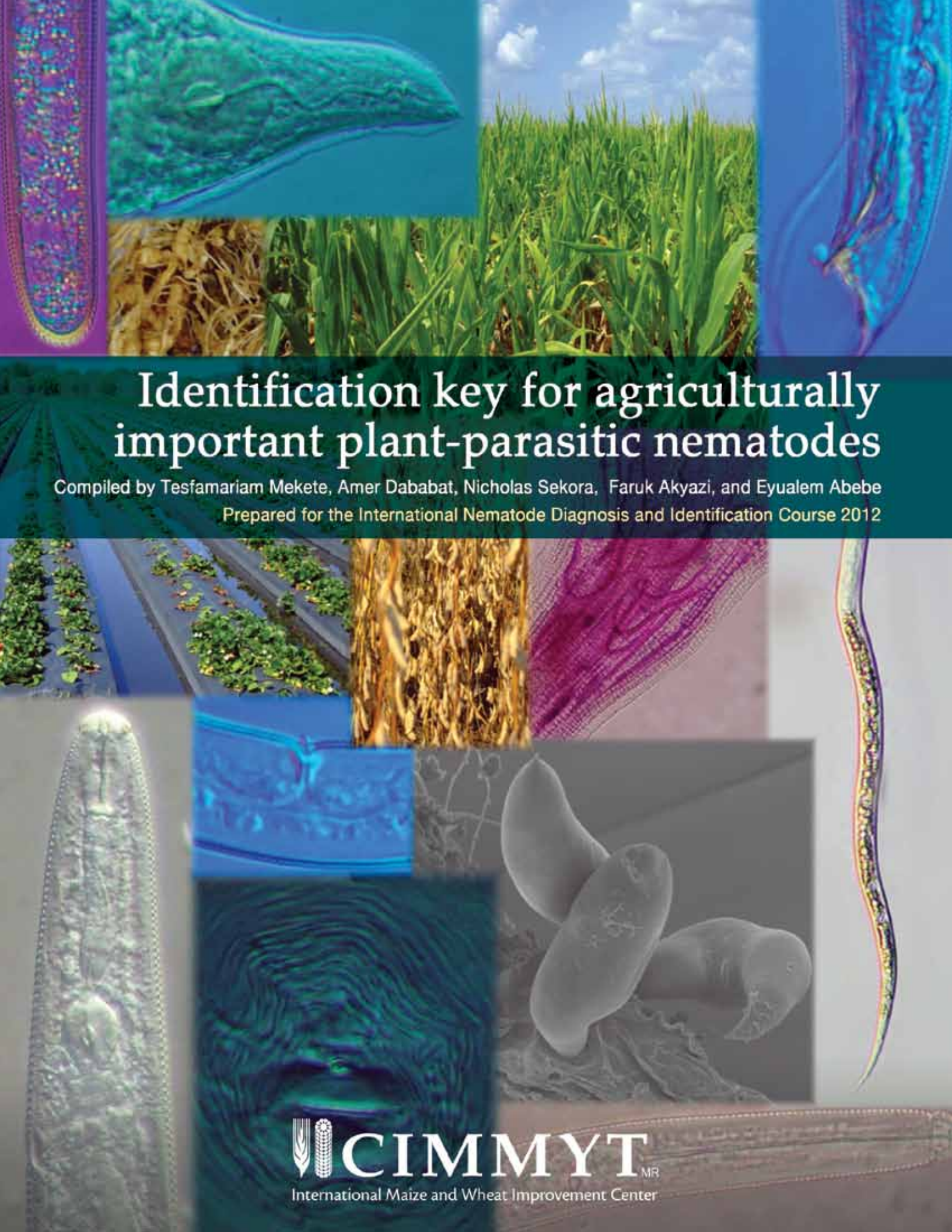

Identification key for agriculturally important plant-parasitic nematodes

Compiled by Tesfamariam Mekete, Amer Dababat, Nicholas Sekora, Faruk Akyazi, and Eyualem Abebe

Prepared for the International Nematode Diagnosis and Identification Course 2012

CIMMYT MR
International Maize and Wheat Improvement Center

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A manual for nematology

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The course is intended to provide basic training in the identification of major plant and soil nematodes with special emphasis on cereal cyst and lesion nematodes. The course also covers training in sampling techniques for nematodes, their extraction, and subsequent data analysis. The course is for agricultural experts, plant pathologists and researchers in other disciplines working with plant parasitic nematodes.

We are extremely grateful to Dr. Amer Dababat for organizing this valuable training for scientists from different parts of the world. We are also grateful to all the scientists who gave their time to review the draft of this manual and offered additions and changes. Prof. Halil Elekcioğlu (Cukurova University, Adana, Turkey), Dr. Halil Toktay (Biological Control Research Station, Adana, Turkey), and Dr. Deliang Peng (Institute of Plant Protection, Chinese Academy of Agricultural Sciences) are gratefully acknowledged for their contribution to the development of the scientific program and evaluating abstracts from potential contributors. Dr. Gul Erginbas Orakci is thanked for her assistance and technical preparation in the lab in Eskisehir. Dr. Alexei Morgounov and Ms. Bahar Erdemel (CIMMYT Turkey) are thanked for their valuable support.

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CIMMYT (International Maize and Wheat Improvement Center) and ICARDA (International Center for Agricultural Research in the Dry Areas) are non-profit International Research Centers with the mandate to improve agriculture in developing countries as part of the Consultative Group of International Agriculture Research. CIMMYT's headquarters are in Mexico, with 15 outreach offices around the world, one of which is in Turkey. CIMMYT's mandate is to improve the productivity of wheat and maize systems through sustainable management. The geographic focus of the Turkey office is West Asia, North Africa and Central Asia. ICARDA is based in Syria and works regionally in dryland areas on cereal, legume and animal production systems, to improve the productivity of these crops through sound management practices. ICWIP (ICARDA-CIMMYT Wheat Improvement Program) is the collective effort of both centers to address food security for cereals in West Asia, North Africa and Central Asia. CIMMYT is gratefully acknowledged for its scientific leadership in the research of Cereal Cyst Nematodes (CCN), technical input, and capacity building. For further information visit **www.cimmyt.org** and **www.icarda.org**.

TAGEM is the headquarters of the Turkish national agricultural research system in Ankara. Their key objectives are is to develop a research strategy, determine the priorities and coordinate research programs. TAGEM is thanked for its coordination of the joint CIMMYT-Turkey collaboration on soil borne pathogens of wheat and the leadership and oversight of the joint research program on cereal cyst nematode with a number of national programs and CIMMYT.

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Contents

I. Introduction

Nematodes are diverse metazoans with an estimated total number of a million species (Lambshead, 2004). They are arguably the most numerous metazoans in soil and aquatic sediments. From an environmental point of view, nematodes are part of nearly all ecosystems in their roles as bacterivores, herbivores, parasites of animals and plants, and consumers of dissolved as well as particulate organic matter. Considering their impact on crops, McCarter (2009) estimated a global total loss of $118 billion for 2001, of which nearly half was related to only two crops; rice and maize. This being so, it is remarkable that they are among the least studied, with close to only 26,000 (estimated < 3%) species described to date (Hugot et al., 2001; Hallan, 2007). Accuracy of identification is, therefore, fundamental to our understanding and communication of the ecological role of any organism. Traditionally, nematology has its strength in agricultural applications because of the economic implications. As a result, nematode species delimitation methods in the context of agricultural and health-related applications are more refined at the species and below species level than methods employed in nematode biodiversity studies.

This workshop plans to discuss the latest in systematic and evolutionary research on various plant parasitic nematodes with special emphasis on cereal cyst nematodes. Hands-on training in morphology and molecular phylogenetic analyses will be provided during the workshop.

This manual is a compilation from published references and full credit is given at the end of each section for the illustrations and pictures produced. This manual will have limited distribution and is prepared for non-profit educational purposes.

II. Methods: Extraction, identification, and quantification of plant-parasitic nematodes

1. Applicability

These methods can be used to extract plant-parasitic nematodes from both soil and root samples and also to prepare inoculum for lab, greenhouse, or field experiments. Identification and quantification should be conducted using the appropriate method for the nematode of interest.

2. Summary of Methods

A. Sampling

Nematode sampling has become increasingly important for many reasons. Diagnostic sampling may include root samples or visual assessments. The objectives of sampling are for diagnosis of nematode problems, detection, and to provide advice for management programs and for research purposes. Without confirmation through proper sampling, poor plant growth because of nematodes may be misinterpreted as nutrient deficiencies or other factors. The quarantine regulations of many countries also require that planting materials should be produced on land certified free from nematodes, which requires accurate detection of the regulated nematode species in question.

Making the proper management decision for a nematode problem depends on the correct diagnosis, which also depends on proper sample collection and handling. Population densities of plant parasitic nematodes vary greatly in time and space. Generally, nematodes are distributed in patches. Therefore, proper sampling techniques should be considered as a major component of sampling for diagnosis. Sampling can be carried out at random or systematically. The best approach in soil sampling is to bulk cores in a bucket, mix thoroughly, and process 100 cc or more depending on the purpose of the work. Equipment for collecting soil samples for nematode assays includes shovels, soil augers or tubes, and motorized samplers. Details of sampling procedures can be found at http://nematology.ifas.ufl.edu/assaylab/.

B. Extraction

Vermiform (free-living nematodes) and ectoparasitic nematodes can be extracted from soil using a combination of screening, centrifugation and flotation to separate the nematodes from the surrounding debris. Modified Baermann trays can be used to extract both ectoparasites and endoparasites from soil and plant tissue, respectively. Live endoparasites can also be extracted by macerating root tissue and freeing up the enclosed nematodes.

C. Identification

Nematodes can be identified using several methods, including light microscopy, fatty acid analysis, and PCR analysis. More specific methods (esterase and malate dehydrogenase staining, host differentials, morphological studies, and fatty acid analysis) are used for identifying *Meloidogyne* species and races.

3. Interference

Some of the more sensitive identification techniques (PCR analysis, esterase and malate dehydrogenase staining, and, at this point, fatty acid analysis) require individual nematodes or pure populations of nematodes for accurate identification.

4. Safety

Both the PCR analysis and esterase-malate dehydrogenase methods require the use of certain chemicals that can be/are toxic to humans and the environment. Proper safety precautions should be taken to prevent unnecessary exposure to the chemicals.

5. Extraction

A. From Soil

The classic method of extraction of nematodes from soil is conducted following the method of Jenkins (1964). The soil sample is mixed thoroughly, but gently when tumbling, to homogenize the nematodes within the soil. A measured volume of soil (either 100 cm^3 or 250 cm^3) is rinsed through a 864 μm (20 mesh)

sieve into a large pitcher. The filtrate is mixed with a pressurized water spray to fill the pitcher. After allowing the water and soil in the pitcher to settle for 20 seconds, the suspension is poured over a 38 μm (400 mesh) sieve held at a 45° angle (Figure I). Material captured on the sieve is rinsed into a 100 mL centrifuge tube and centrifuged for 3 minutes at 1,700 rpm. The supernatant is poured off and the pellet is resuspended in a 1.328 M sucrose solution (specific gravity = 1.10) before a repeated centrifugation at 1,700 rpm for 3 minutes. Following centrifugation, the supernatant is poured over a 25 μm (500 mesh) sieve and rinsed with water to remove any traces of sucrose. The resulting material captured on the sieve can be examined under a light microscope for identification and quantification.

Figure I. Rinsing material collected on a 38 μm sieve (A) and collecting nematodes after centrifugation with a 25 μm sieve (B).

An alternative method to centrifugation of the soil sample is a modified Baermann tray or funnel. In this case, the required volume of soil is rinsed through a 864 μm sieve and over a 38 μm sieve, just as with the centrifugation method. The captured material is rinsed into a coffee filter placed within a plastic bowl or funnel and supported by a screen (Figure II). The water level is brought up to at least 1.0 cm above the coffee filter and allowed to incubate for 24 hours. Following incubation, the filter and screen are removed from the bowl and the water left in the bowl or funnel base is poured over a 25 μm sieve. The material contains only live, mobile nematodes and can be observed under a light microscope.

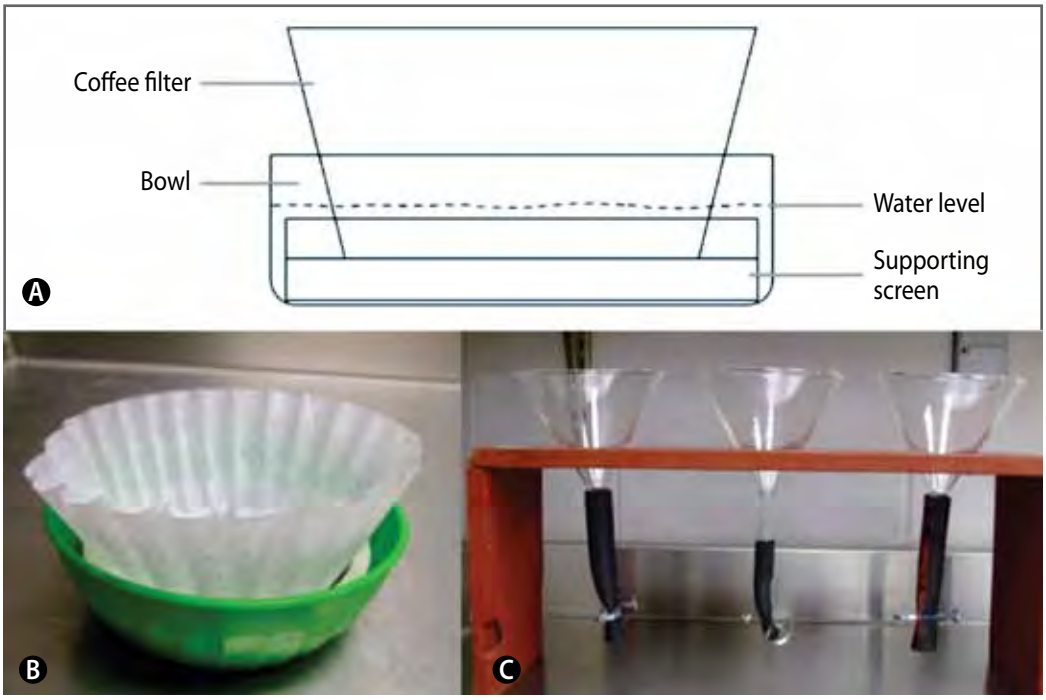


Figure II. Setup for a basic Baermann extraction method (A), example of a Baermann tray (B) and funnel (C).

B. From roots

Endoparasites within root tissue can be extracted using a modification of the Baermann method used for soil extraction. Rinsed root tissue is cut into small pieces about 2.5 cm in length. A total of 10.0 grams of cut root tissue is placed into a blender with 150 mL of water. Roots are macerated using a pulsing action on the blender for 10 seconds. The blended material is poured onto a coffee filter supported by a mesh screen within a plastic bowl and incubated for 24 hours. The water in the bowl is poured over a 25 μm sieve to collect the nematodes and can be observed under a light microscope.

C. *Meloidogyne* egg extraction

Eggs of *Meloidogyne* females are extracted using bleach solution (NaOCl) as described by Hussey and Barker (1973). Rinsed root tissue containing females and egg masses are chopped and placed into a container with 400 mL of a 0.6% NaOCl solution. The roots are agitated in the solution for four minutes before being rinsed over nested 74 μm and 25 μm sieves. While rinsing the material to remove any traces of bleach, the root tissue is rubbed thoroughly to break free any remaining egg masses. Eggs collected on the 25 μm sieve can be enumerated under a light microscope and used for inoculation.

D. Cyst extraction from soil

Mature cysts and females of Heteroderidae can be extracted by either rinsing soil over a 250 μm sieve or through a Fenwick Can (Figure III). The Fenwick Can floats cysts out of soil and debris by using flowing water to mix the soil which allows the cysts to be caught on an attached sieve. Upon collection, cysts can be enumerated per volume of soil and the number of eggs per cyst can be determined by crushing the enumerated cysts.

6. Identification

A. Light microscopy

Light microscopy is the classic method for identification and enumeration of nematodes. However, a typical compound microscope is less effective than an inverted compound microscope. There are several methods used in enumeration, but most utilize a Petri dish with scored lines to prevent repeated counting of individuals.

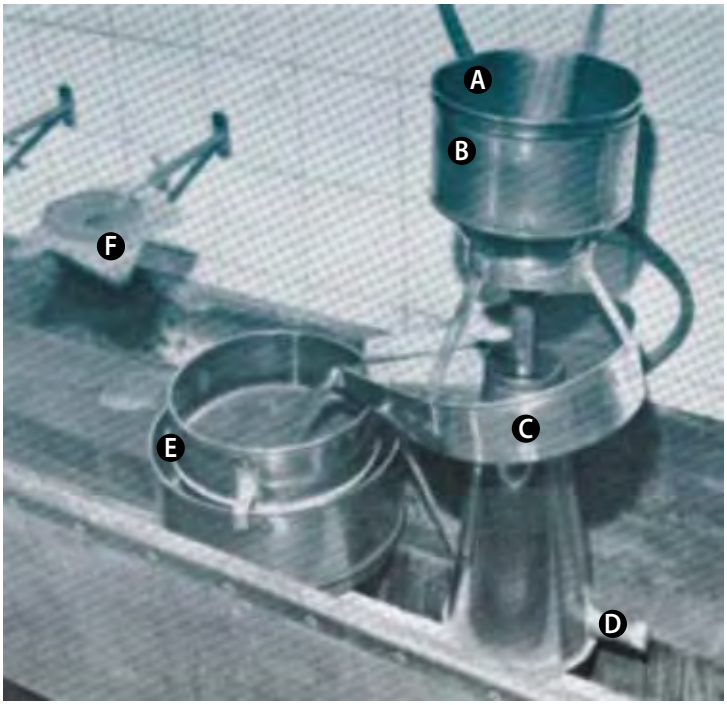


Figure III. Setup for a basic Fenwick extraction method.
A= Sieve; B= Water can; C=Over flow collar: D=Outlet; E: Sieve; F: Cyst collector.

B. Fatty acid analysis

Recently, fatty acid analysis has been found to be useful in the identification of plant-parasitic nematodes (Sekora et al., 2009). This technique utilizes fatty acid methyl ester (FAME) analysis used to identify bacteria and fungi (Kunitzky et al., 2005) and shows great promise for rapid identification of several economically-important nematodes, most notably those within the *Meloidogyne* genus.

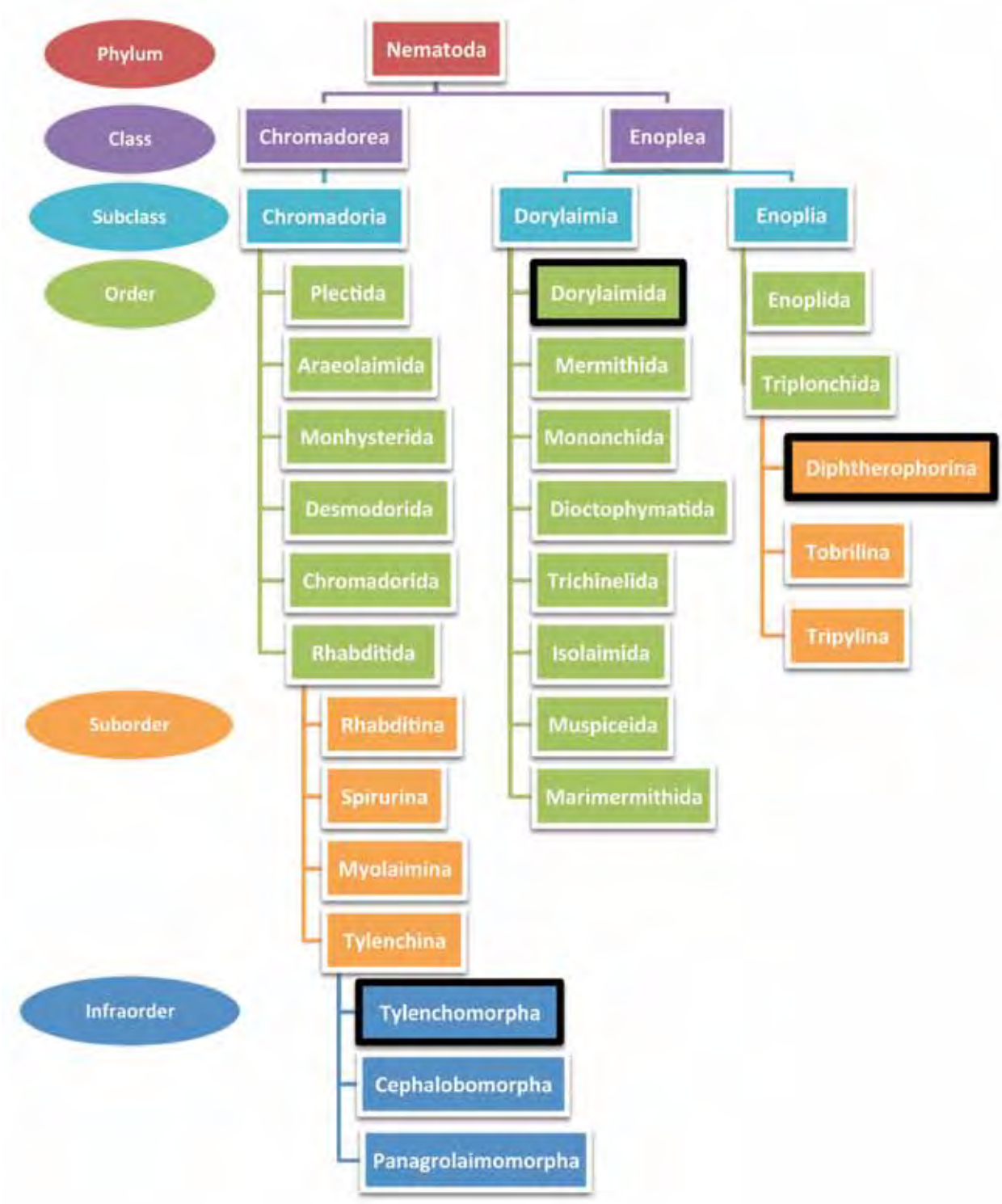
Fatty acids from the selected nematodes are extracted following the procedure established by Sasser (1990). FAME extractions are analyzed using an Agilent 6890N automated gas chromatography system equipped with an Ultra 2 Cross-linked 5% Phenyl Methyl Siloxane column (Agilent Part# 19091B-102) and a flame ionization detector (FID). The resulting profiles are analyzed with the Sherlock Analysis Software (MIDI, Inc., Newark, DE). Profiles developed for a given nematode isolate are used in comparison to the unknown sample to match the corresponding nematode to the fatty acid profile observed.

C. *Meloidogyne* identification

Identification within the *Meloidogyne* genus can be difficult and may require several tests to determine the correct identification depending on whether you want to know the species or race. Both the esterase-malate dehydrogenase (Dickson et al., 1971; Brito 2008) and PCR methods (Harris et al., 1990; Powers and Harris 1993; Tigano et al., 2005) can be used for identification to the species level, but have not been shown to identify to races within species. Species identification can also be carried out using perineal pattern observation under a compound light microscope (Hartman and Sasser, 1985). Race identification within *M. arenaria, M. hapla, M. incognita,* and *M. javanica* can be verified using differential host tests (Hartman and Sasser, 1985), but this method requires 45 days to complete in the greenhouse and does not account for mixed populations. FAME analysis has also shown promise in identification to the race level (Sekora et al., 2009), but more work must be done to establish this method's true power. In our lab, we use a combination of perineal patterns, esterase-malate dehydrogenase staining, and FAME analysis to identify *Meloidogyne* samples.

III. Nematode classification

The taxonomic rank into which nematodes are placed varies with different authors. In 2002, De Ley and Blaxter provided a new classification system mainly based on molecular phylogenetic results and additional morphological analyses. The scheme for this classification would be:



The major nematode orders that plant-parasitic nematodes belong to are Rhabditida, Dorylaimida, and Triplonchida.

Order Rhabditida

Infraorder Tylenchomorpha

Superfamily Aphelenchoidea

Family: Aphelenchidae
Family: Aphelenchoididae
Family: Paraphelenchidae

Superfamily Criconematoidae

Family: Criconematidae
Family: Hemicycliophoridae
Family: Tylenchulidae

Superfamily Myenchoidea

Family: Myenchidae

Superfamily Sphaerularioidea

Family: Allantonematidae
Family: Anguinidae
Family: Iotonchiidae
Family: Neotylenchidae
Family: Parasitylenchidae
Family: Sphaerulariidae
Family: Sychnotylenchidae

Superfamily Tylenchoidea

Family: Atylenchidae
Family: Belonolaimidae
Family: Dolichodoridae
Family: Ecphyadophoridae
Family: Heteroderidae
Family: Hoplolaimidae
Family: Meloidogynidae
Family: Pratylenchidae
Family: Psilenchidae
Family: Telotylenchidae
Family: Tylenchidae
Family: Tylodoridae

Order Triplonchida

Suborder Diphtherophorina

Superfamily Diphtherophoroidea

Family: Diphtherophoridae
Family: Trichodoridae

Suborder Tobrilina

Superfamily Prismatolaimoidea

Family: Prismatolaimidae

Superfamily Tobriloidea

Family: Pandolaimidae
Family: Rhabdodemaniidae
Family: Tobrilidae
Family: Triodontolaimidae

Suborder Triplonchida

Family: Bastianiidae
Family: Odontolaimidae

Suborder Tripylina

Superfamily Tripyloidea

Family: Onchulidae
Family: Tripylidae

IV. Identification key for major plant-parasitic nematodes of the infraorder Tylenchomorpha

1. Esophagus 1 or 2 parts ..... non plant parasite
   Esophagus 3 or 4 parts 2

2. Stoma with stylet (Fig. 1) 3
   Stoma without stylet ..... Section II

3. Lip region without setae 4
   Lip region with setae 29

4. Esophagus 4 part, median bulb present (Fig. 2) 5
   Esophagus 3 part, median bulb absent ..... Neotylenchoidea

5. Female nematode body cylindrical, mobile (Fig. 3) 6
   Female nematode body swollen, globose or saccate 26

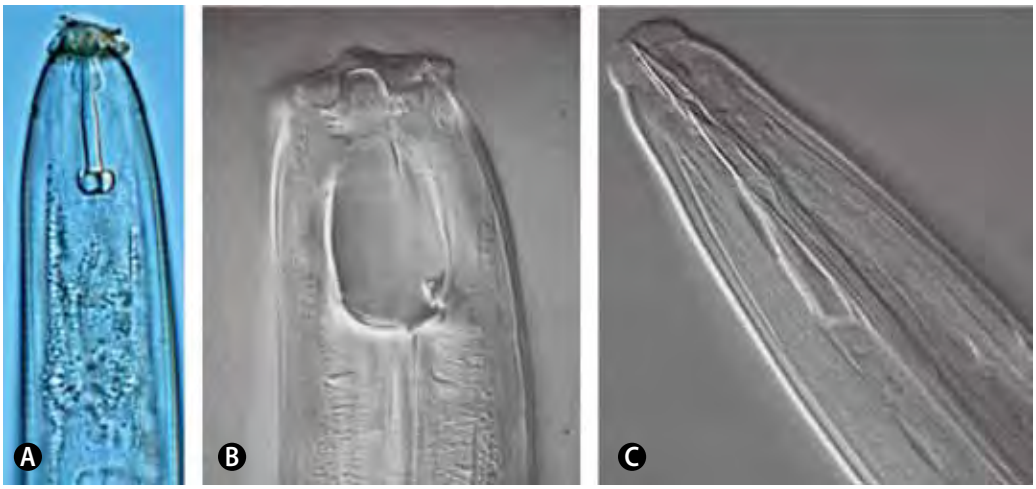


Figure 1. Stoma with stylet (A) and without (B,C).

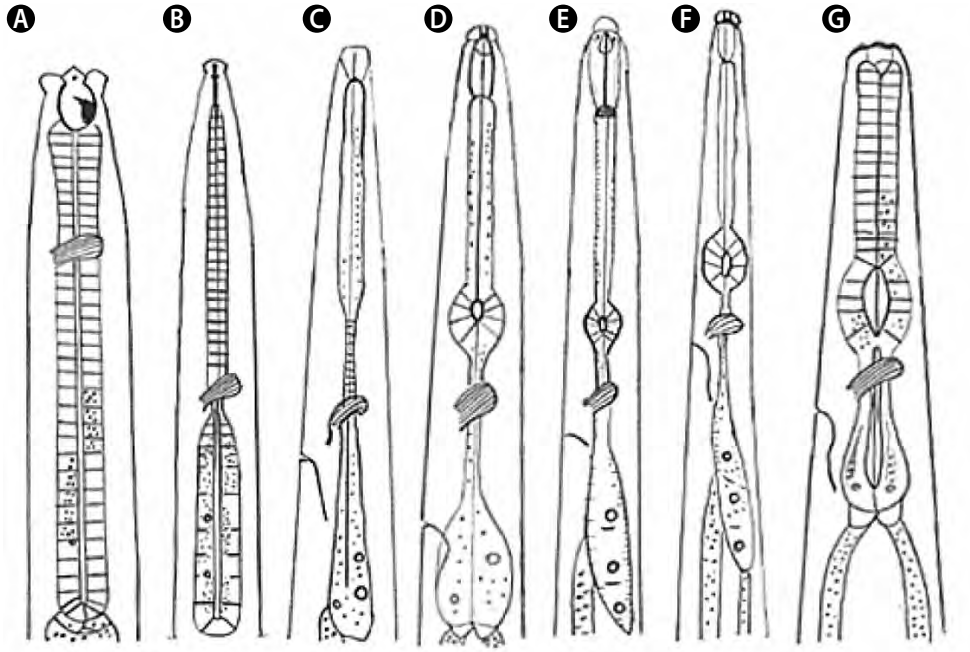


Figure 2. Different types of esophagi: one part (A), two part Dorylaimoid (B), three part (C), four part Tylenchoid (D, E, F) and four part Rhabditoid (G).

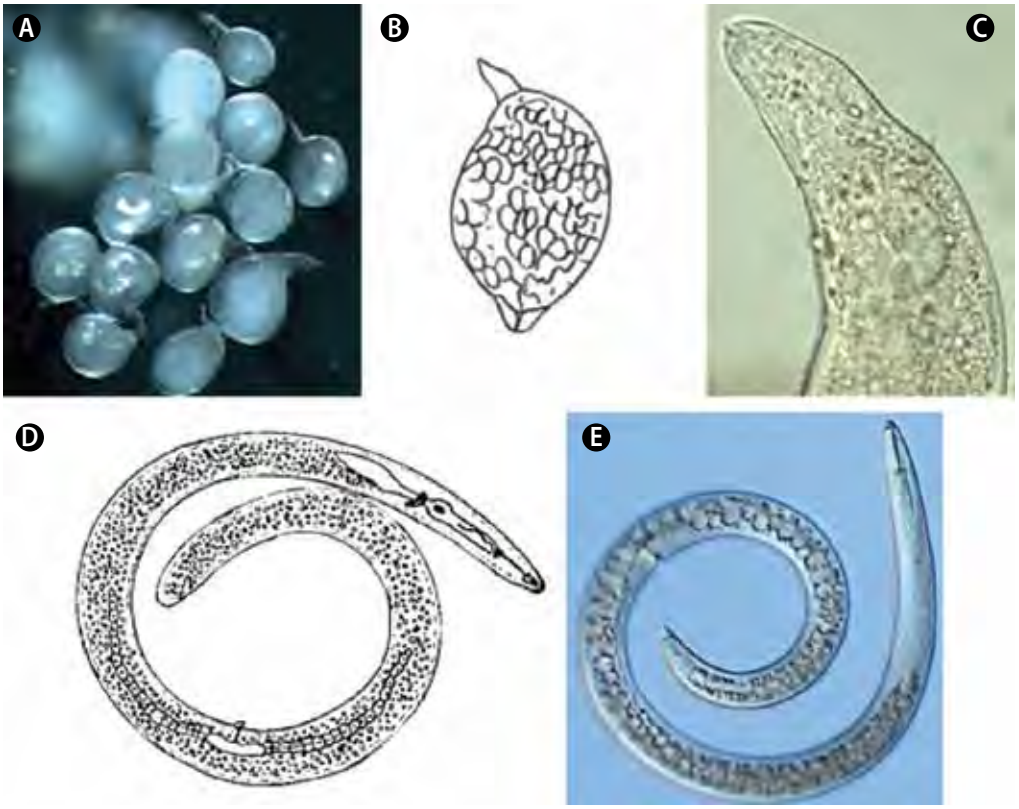


Figure 3. Female nematode body types, swollen (A, B, C) and cylindrical (D, E)

6. Vulva located near middle of the body (Fig. 4) 7
   Vulva located in posterior third of the body 17

7. Basal bulb not overlapping intestine (Fig. 4) 8
   Basal bulb overlapping intestine 11

8. Stylet long about 3X body width at stylet base (Fig. 5) ..... *Dolichodorus*
   (Rounded, striated lip region, tail rounded with spike)
   Stylet short about 2X or less body width at stylet base 9

9. Tail terminus pointed (Fig. 6) ..... *Merlinius*
   (Lateral field with six lines; female tail acute or subactute, Esophageal glands usually enclosed within a bulb; if not enclosed, then of about equal length, and therefore considered as not overlapping the intestine)
   Tail terminus not pointed 10

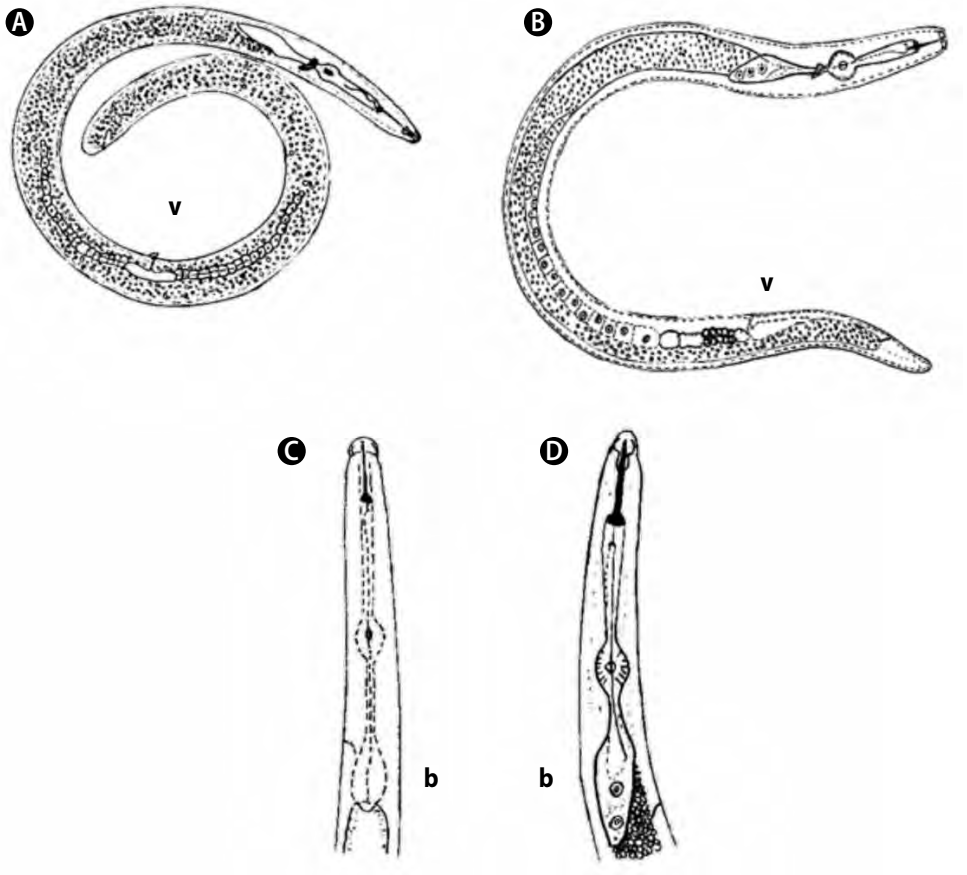


Figure 4. (A) Vulva [v] location near mid body, (B) posteriorly toward tail, (C) basal bulb [b] without overlap, and (D) intestines with overlap.

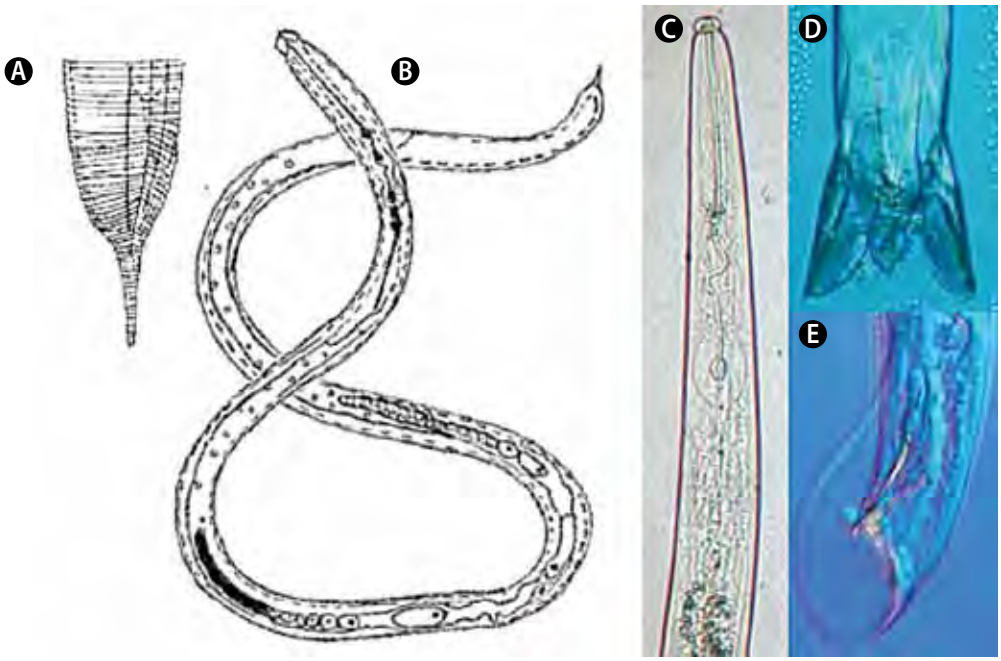


Figure 5. *Dolichodorus* female and juvenile tail shape (A), female full body (B), head region (C), male tail dorsal view (D), and male tail lateral view (E).

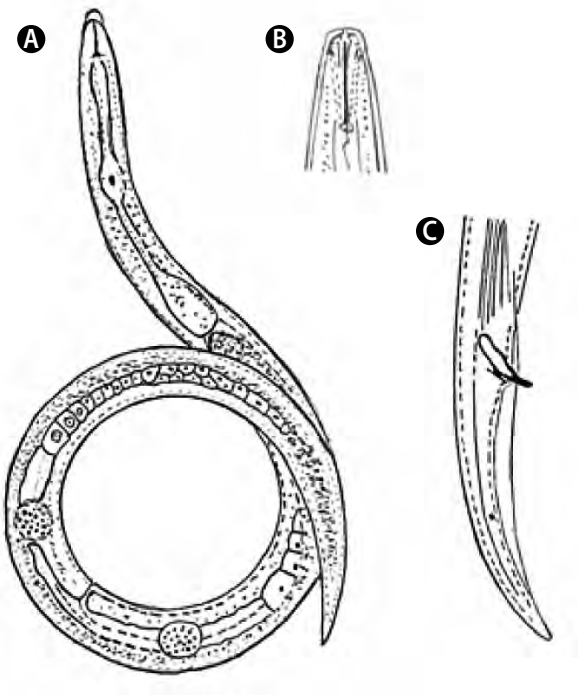


Figure 6. *Merlinius* female (A), stylet region (B), and male tail (C).

10. Tail filiform, terminus may be clavate (Fig. 7) ..... *Psilenchus*
(Stylet without basal knobs, metacorpus prominent, cephalic sclerotization absent; filiform tail, usually with clavate or non-clavate terminus, amphids oblique slits, distinct phasmids on tail, elongate spermatheca, paired ovaries.)

Tail not filiform, terminus not clavate (Fig. 8) ..... *Tylenchorhynchus*
(Short stylet, tail tip rounded, 2,3,4, or 5 lateral lines (usually 4), body medium sized, tail conoid to subcylindroid (c'= 2-4), lip region continuous to slightly offset, stylet 15-30 μm long, cone about as long as shaft, sometimes needle-like)

11. Lip region offset by constriction from body, more than ½ higher than wide 12

Lip region not offset by constriction from body, or slightly offset; less than ½ as high as wide 16

12. Stylet massive and short, large stylet knobs 13

Stylet thin, very long (3 or more times the body width at stylet base), small stylet knobs (Fig. 9) ..... *Belonolaimus*
(Esophageal glands not enclosed within a bulb, mostly unequal in length, intestines overlap esophagus, body length usually greater or equal to 1.75 mm)

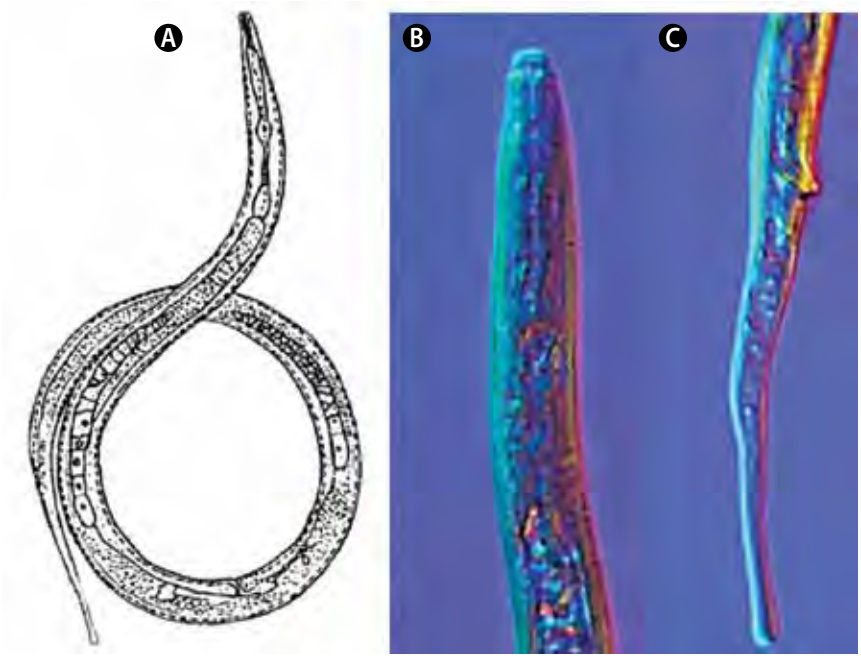


Figure 7. *Psilenchus* full body (A), head region (B), and male tail (C).

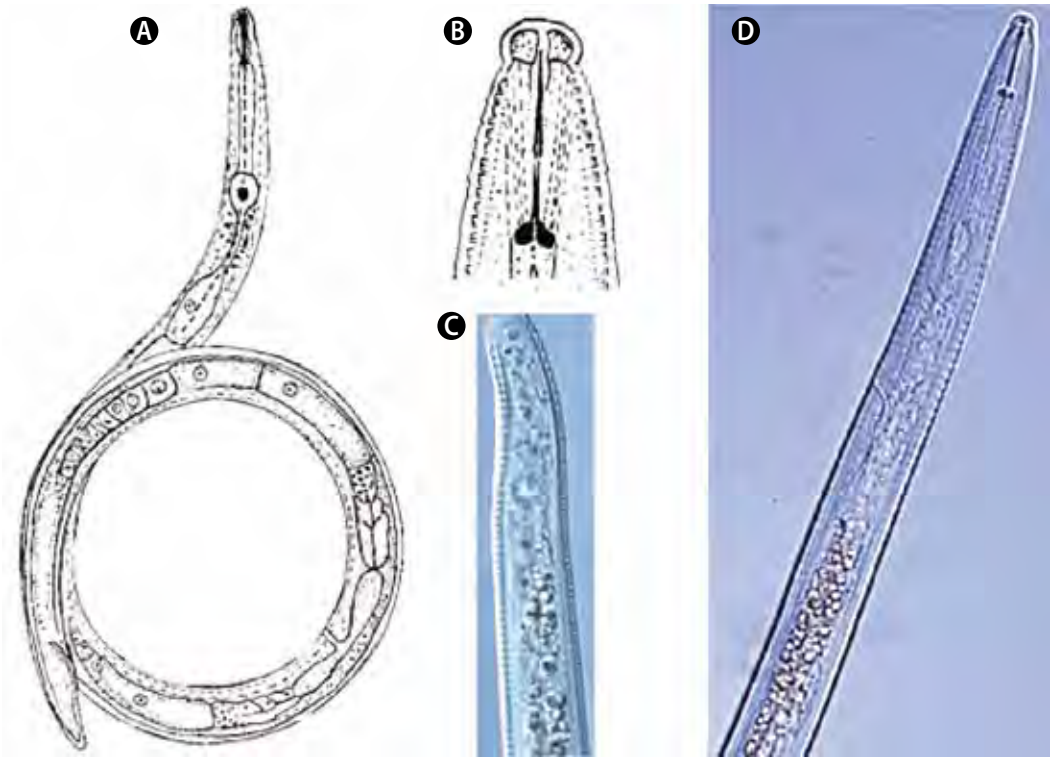


Figure 8. ***Tylenchorhynchus*** **full body (A), short stylet (B), round tail tip (C), basal bulb not overlapping intestine (D).**

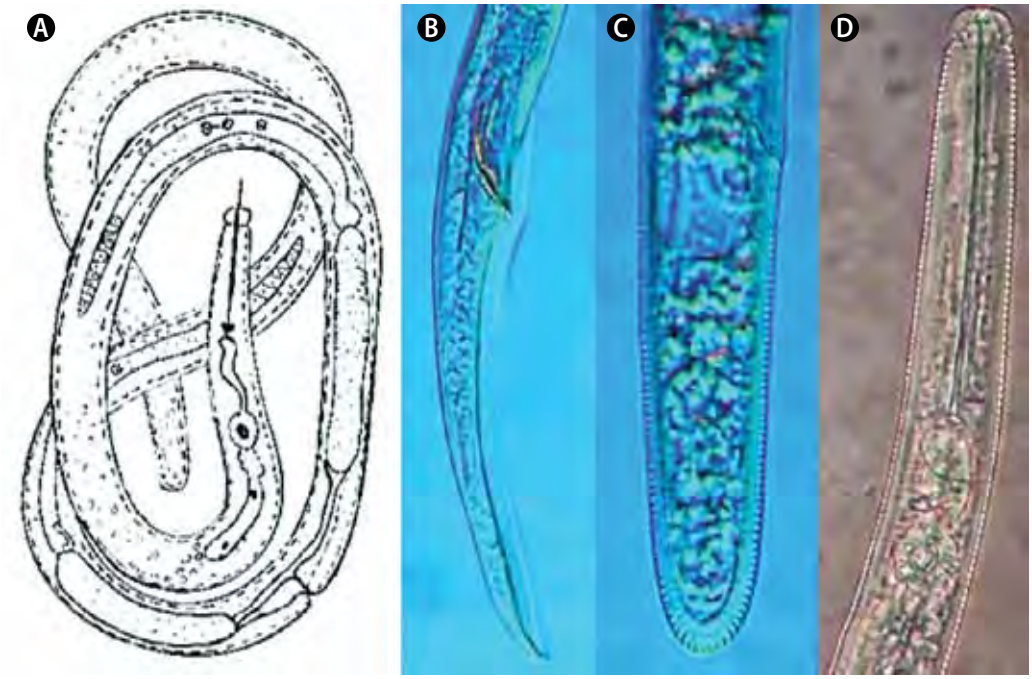


Figure 9. ***Belonolaimus*** **full body length (A), male tail region (B), female tail region (C), and head region (D).**

13. Body straight, stylet knob anteriorly projected (Fig. 10) ..... *Hoplolaimus*
(Gland bulb overlap intestine dorsal and lateral; tail short and rounded, DGO 3-10 μm from stylet, phasmids enlarged to scuttelum, eratically situated sometimes anterior to vulva level, not opposite to each other, labial framework and stylet massive, stylet knobs anchor or tulip- shaped, body straight and long, 4 or less lateral lines, generally aerolated at the level of phasmids, two genital branches)

Body C-shaped or spiral shaped, stylet knobs not anteriorly projected 14

14. Lip region without striation, epiptygma present (Fig. 11) ..... *Peltamigratus*
(Phasmids located anterior to anus and they are opposite each other)

Lip region with striation, epiptygma absent 15

15. Both scutella (phasmids) located from tail terminus to anal region (Fig. 12) ..... *Scutellonema*
(Gland bulb overlap intestine dorsal and lateral, Tail short and rounded, DGO 4-8 μm from stylet, Phasmids enlarged to scuttelum opposite to each other, Body spiral to C- shaped, 4 lateral lines, generally aerolated at the level of phasmids, sometimes transverse striae scattered over the whole field, two genital branches)

Scutella not so located (Fig. 11) ..... *Aorolaimus*

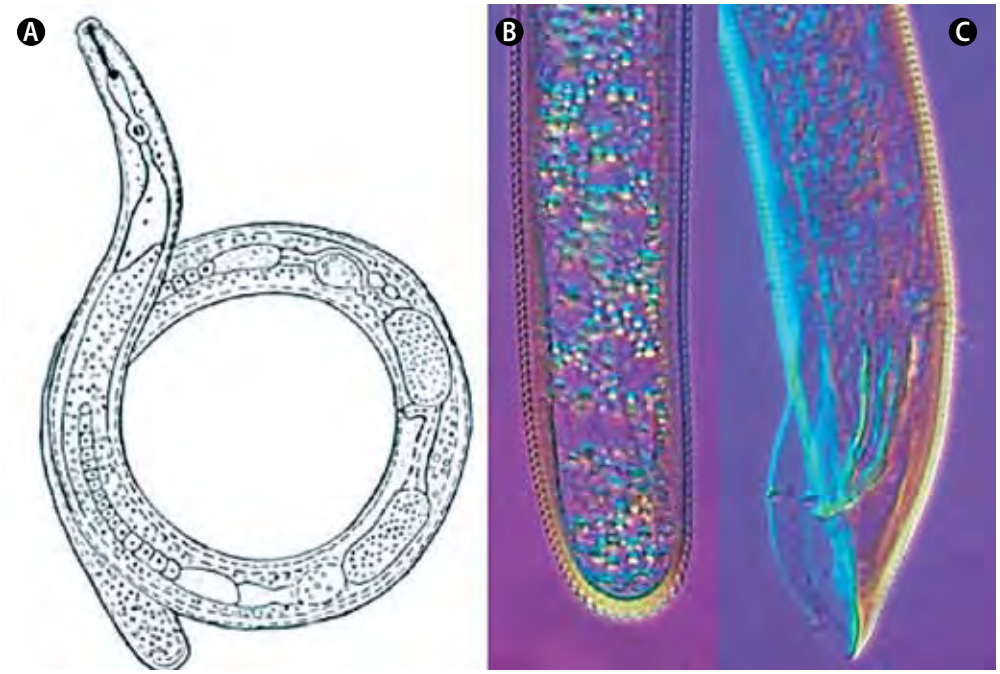


Figure 10. *Hoplolaimus* full body (A), female tail (B), and male tail (C).

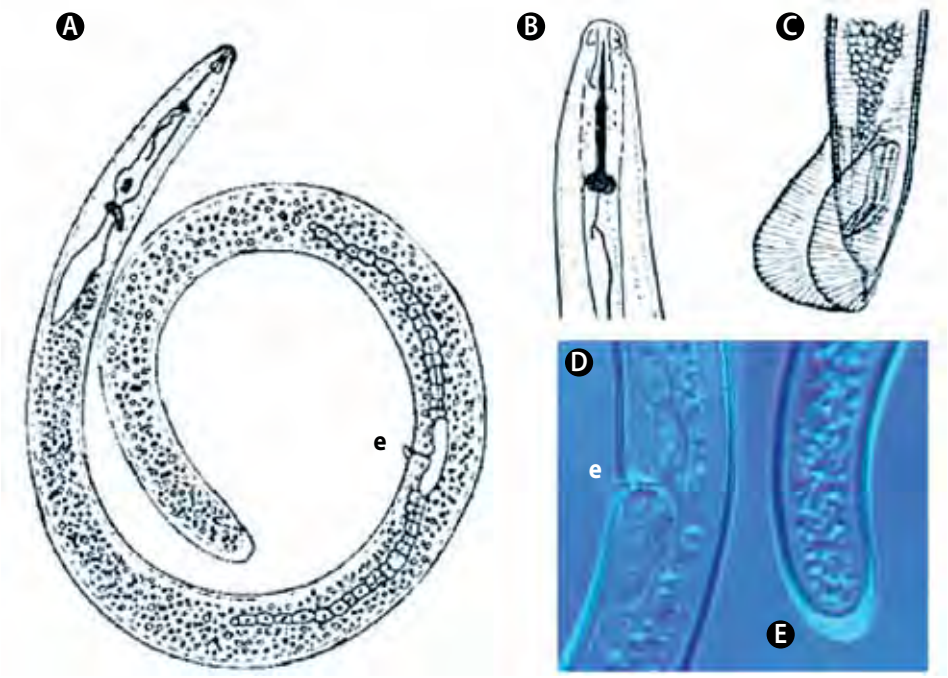


Figure 11. *Peltamigratus* female full body with epitygma [e] (A), stylet region (B), male tail (C), epiptygma (D), and female tail region (E).

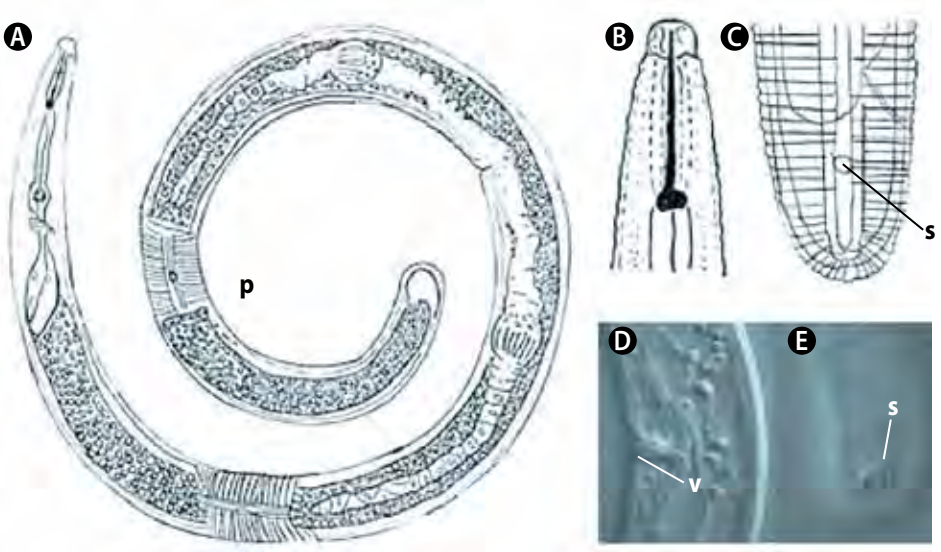


Figure 12. *Aorolaimus* female body with phasmid [p] midbody (A), stylet region of *Aorolaimus* and *Scutellonema* (B), tail region of *Scutellonema* with scutellum [s] (C), vulva [v] without epitygma (D), and scutellum [s] near tail tip of *Scutellonema* (E).

16. Body short, 0.5 to 0.8 mm (Fig. 13, 14).....*Radopholus*
(Secondary sexual dimorphism strongly marked, female genital branches equally developed, lip region low and not offset, long tail (c'=2-4 μm) and tail terminus rounded or almost pointed, phasmids at mid tail or slightly anterior; male characterized by high lips, rounded, not setoff, weak cephalic sclerotization, reduced stylet, basal knobs faint or absent, reduced oesophagus.)

Body long, 0.9 to 4.2 mm (Fig. 13, 14) ..... *Hirschmanniella*
(No sexual dimorphism, flattened lip area, not offset, strong labial sclerotization, esophagus overlapping intestine ventrally, equally developed two female genital tract, tail tip mucronate, female lip region low, rounded or flattened.)

17. Cuticle prominently annulated, base of stylet in or almost in median bulb 18
Cuticle not prominently annulated, base of stylet is not in median bulb 22

18. Cuticular sheath present..... 19
Cuticular sheath absent 20

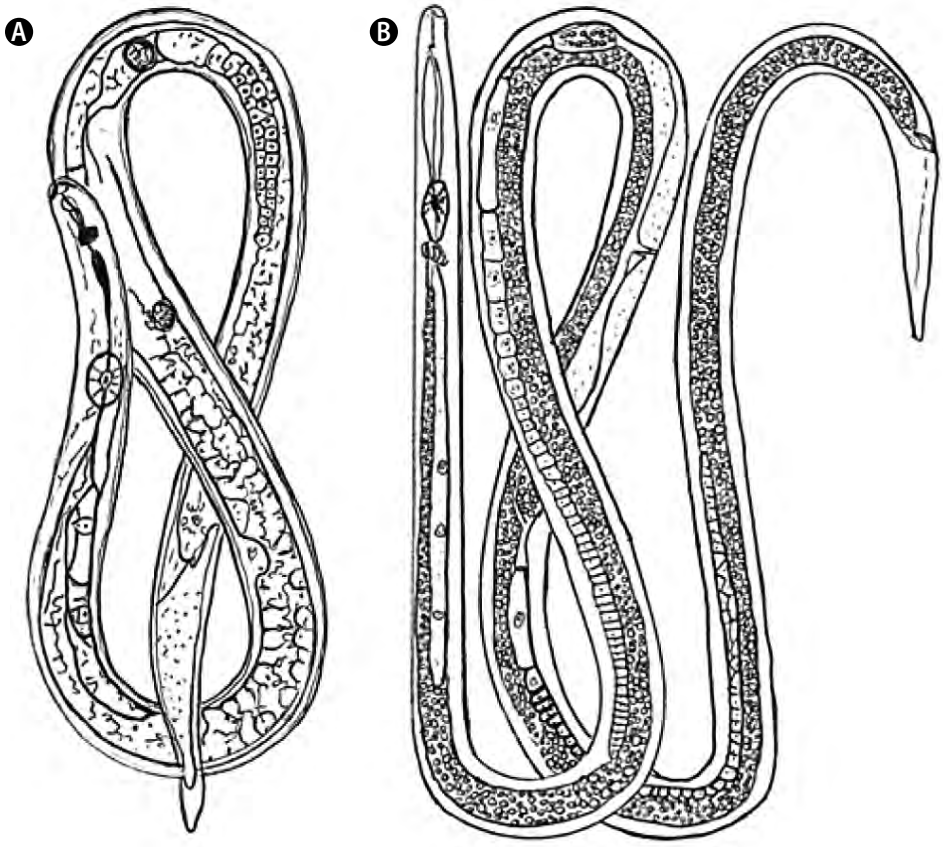


Figure 13. Female full body of *Radopholus* (A) and *Hirschmanniella* (B).

Figure 14. *Radopholus* mature female full body (A), male anterior head (B), male tail (C), female anterior end (D); *Hirshmanniella* anterior end (E) and tail region (F).

19. Stylet knobs anchor shaped, forward directed (Fig. 15) .....*Hemicriconemoides*
(Cuticle with 2 detached layers, body at short distance at both ends, vulva posterior, tail short, conoid to rounded, labial framework heavily sclerotized, strong stylet, basal knobs forward directed)

Stylet shape rounded, sloping backward (Fig. 16) .....*Hemicycliophora*
(Cuticle with 2 detached layers, labial annuli generally not modified or separated, vulva a transverse slit over half of body diameter long.)

20. Body elongate, cylindrical, tail elongate (Fig. 17) .....*Caloosia*
(Esophagus criconemoid type; cuticle thick and coarsely annulated.)

Body stout, usually fusiform.....21

21. Annules with spines or scale like extension (Fig. 15).....*Criconema*
(Annuli smooth or variously ornamented, annuli of labial region smooth, usually with one annulus wide and clearly set off from next body annulus, labial region usually with six pseudolips rounded and projecting from first annulus, vulva slit like or completely overhanging anterior lip, tail conoid-pointed to bluntly rounded, *Criconema* (*Nothocriconema*)

Annules plain without spines or scale like extensions (Fig. 15)..... *Criconemella*
(Annuli smooth or finely crenate, first annuli reduced, in some species first annulus more or less forward directed, submedian lobes generally well developed, vulva open or closed, anterior lip may be ornamented, *Criconemella* (*Macroposthonia, Criconemoides, Mesocriconema*)

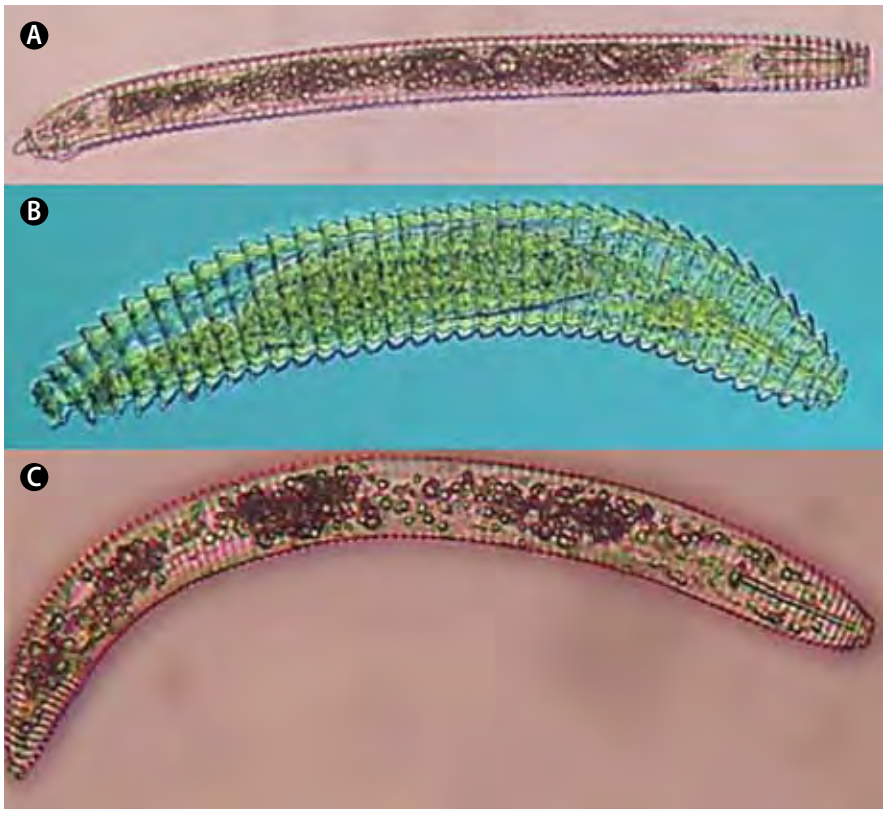


Figure 15. Entire body of *Hemicriconemoides* (A), *Criconema* (B), and *Criconemella* (C).

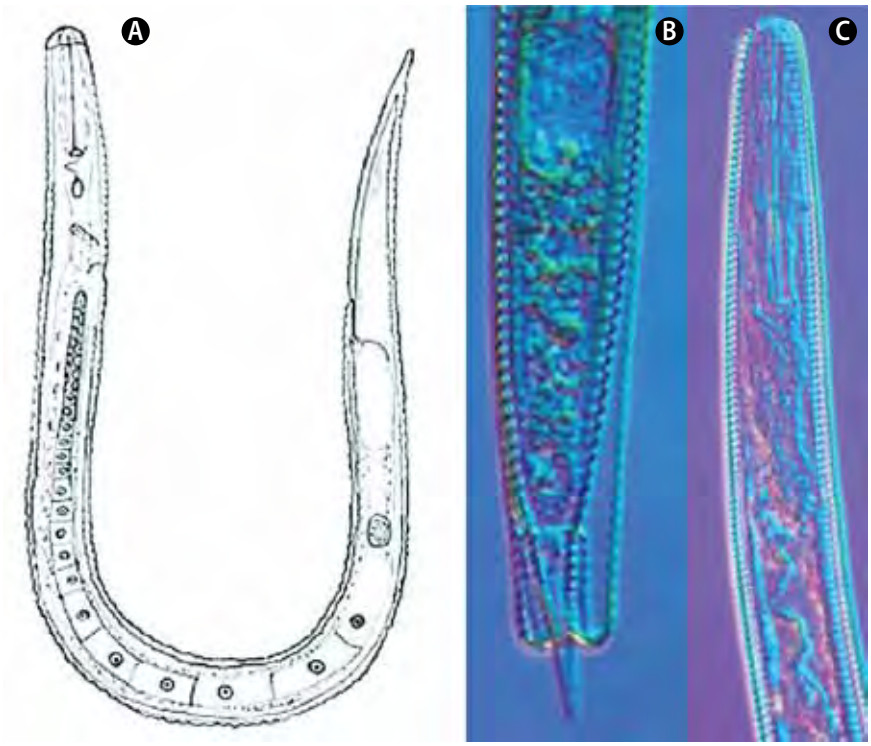


Figure 16. *Hemicycliophora* female full body (A), tail (B), and head (C).

Figure 17. *Caloosia* female body (A), tail (B), and head (C).

22. Body after death spiral (Fig. 19) ..... *Helicotylenchus*
(Gland bulb overlap intestine mostly ventrally, tail short, with or without ventral projection, tail more curved dorsally, DGO often far from the stylet (6-16 μm), pore-like small phasmid, body spiral to straight, labial region continuous to slightly offset, rounded or flattened, 4 lines in lateral fields, two genital branches, posterior one sometimes degenerate, stylet average size ~25 μm)

Body death position straight or slightly curved 23

23. Esophagus not overlapping intestine (Fig. 18) ..... *Tylenchus*
(Stylet conus half or more than the stylet length. Lateral fields with 4 incisures, phasmids dorso-sublateral behind the vulva, cephalic region continuous, annulated, framework with light or no sclerotization, tail generally ventrally arcuate, regularly tapering to a pointed or minute rounded terminus, excretory pore usually opposite to the basal bulb, postvulval uterine sac about a body width or less long, spermatheca round to oval and offset, arcuate spicules.)

Esophagus overlapping intestine 24

24. Median bulb, its valve and stylet well developed, lip region flattened short ventral overlap, monovarial, low flat lip (Fig. 20) ..... *Pratylenchus*
(Males rare, but with sexual dimorphism, body length under 0.8 mm, lip area low, flattened anteriorly, not or weakly offset, oesophageal glands overlap ventrally the intestine for a medium distance, posterior branch of female genital tract reduced to postvulval sac, female tail terminus rounded and rarely pointed.)

Median bulb and its valve small, stylet usually small, its length almost equal to body width at stylet base 25

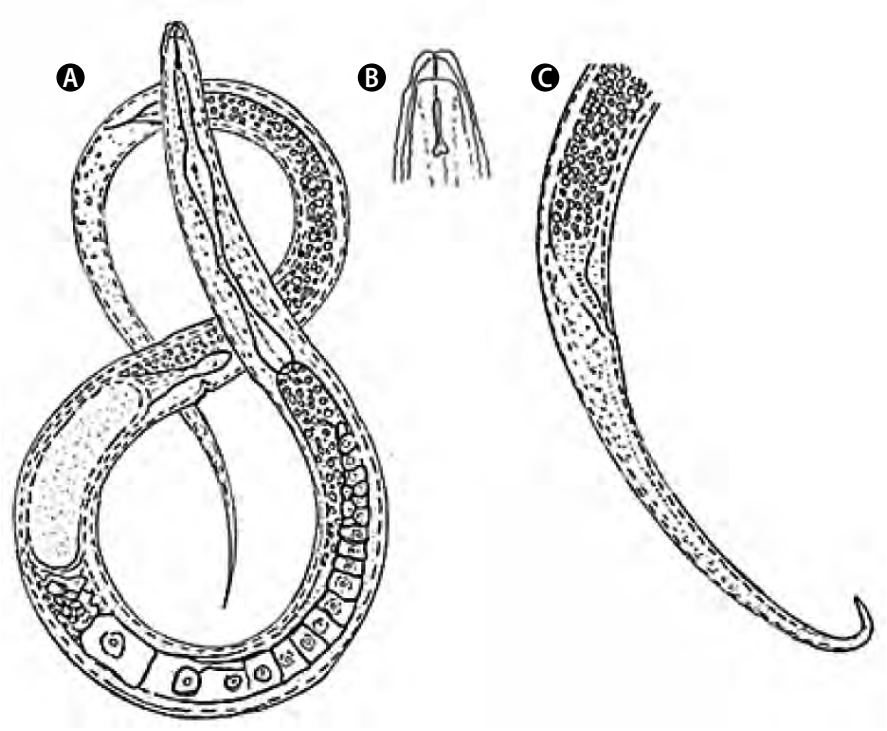


Figure 18. *Tylenchulus* full body view (A), stylet region (B), and tail (C).

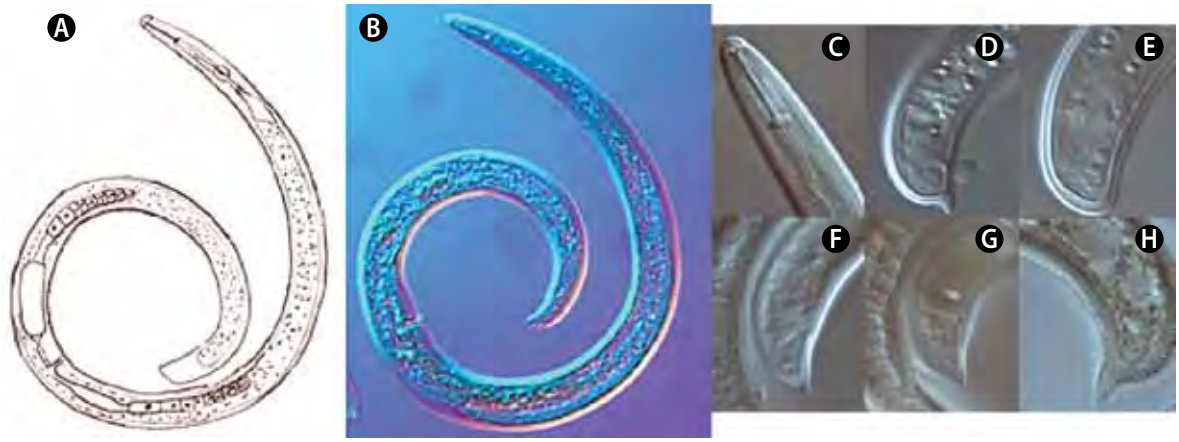


Figure 19. Full body spiral habitus of *Helicotylenchus* spp., (A, B), dorsal gland opening distance from stylet end (C), rounded tail with terminal projection (D), hemispherical annulated tail terminus (E), irregular tail projections (F, G), tail with non-annulated ventral projection (H).

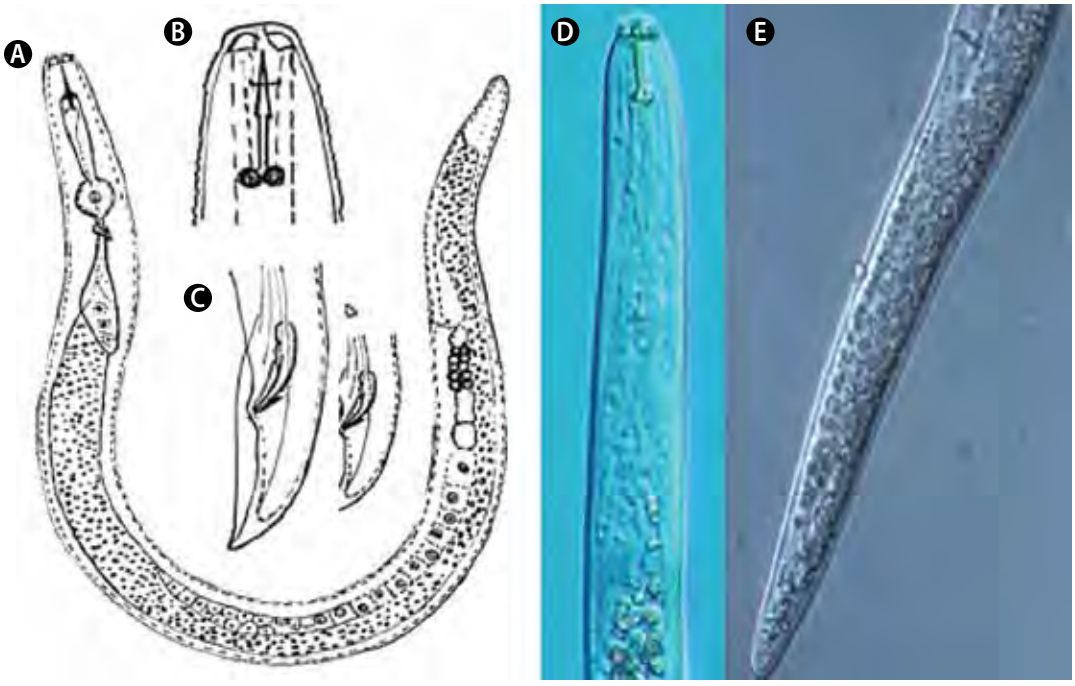


Figure 20. *Pratylenchus* entire body (A), head (B, D) and tail regions (C, E).

25. Mature female mostly obese (Fig. 21) ..... *Anguina*
(Procorpus separated from the median bulb by constriction, isthmus separated from the glandular bulb by a constriction, multiple rows of oocytes in the ovary, can be recovered in root galls, sometimes in leaves or flower parts, can form galls on plant roots.)

Mature female slender (Fig. 21) ..... *Ditylenchus*
(Mature female not swollen or slightly swollen, Isthmus not separated from the glandular bulb by a constriction, gland bulb overlap long or short, ovary outstretched; slender forms; uterus 4 or 5 rows of cells, found in bulbs, stems, leaves, and tubers.)

26. Swollen female with pointed tail 27

Swollen female without pointed tail 28

27. Mature female kidney shaped, with short pointed tail (Fig. 22) ..... *Rotylenchulus*
(Glandular overlap very long, mostly lateral, tail short and rounded, DGO 13-33 μm from stylet body vermiform, spiral to C-shaped, phasmids at the mid-tail, mature female: body obese, kidney shaped, thick cuticle, tail conical pointed with or without hyaline, male: vermiform, anterior end reduced, bursa difficult to see, not quite to the tail, 4 lateral lines, median bulb with strong valve, two genital branches)

Mature female not kidney shaped, with long pointed tail (Fig. 22) ..... *Tylenchulus*
(Mature female: Excretory pore near vulva, anus absent, posterior body exterior to root)

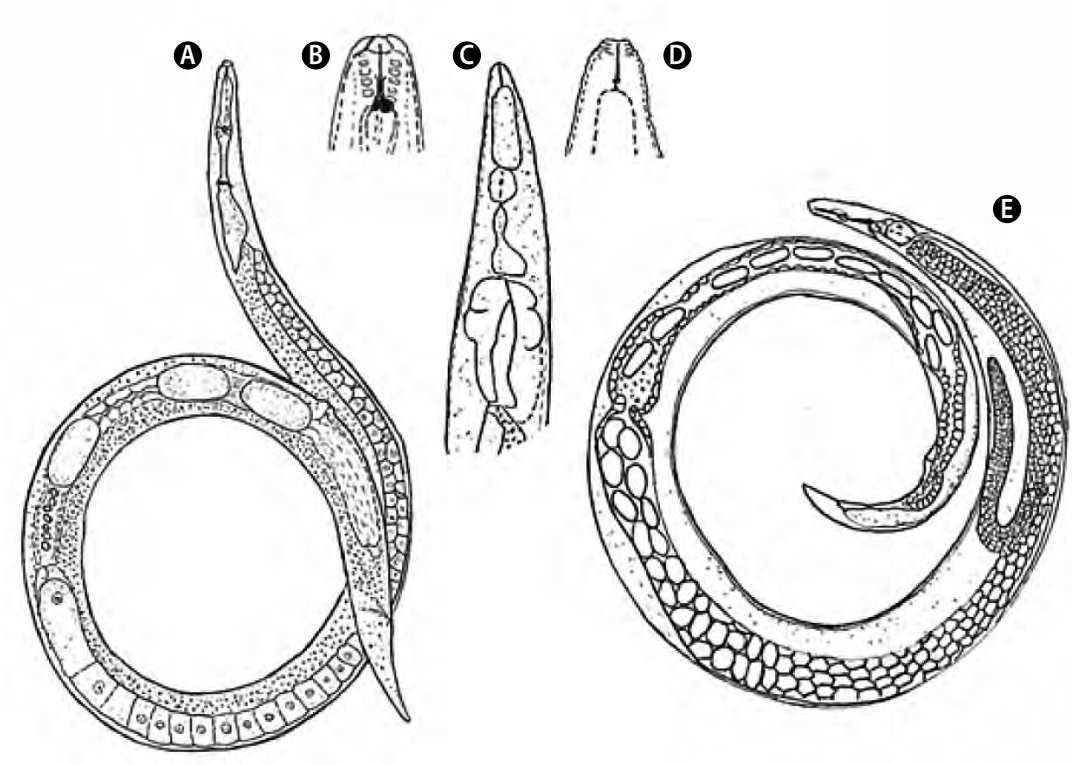


Figure 21. Entire female body of *Ditylenchus* (A), *Ditylenchus* stylet region (B), esophageal region of *Anguina* (C), *Anguina* stylet region (D), and *Anguina* female full body (E).

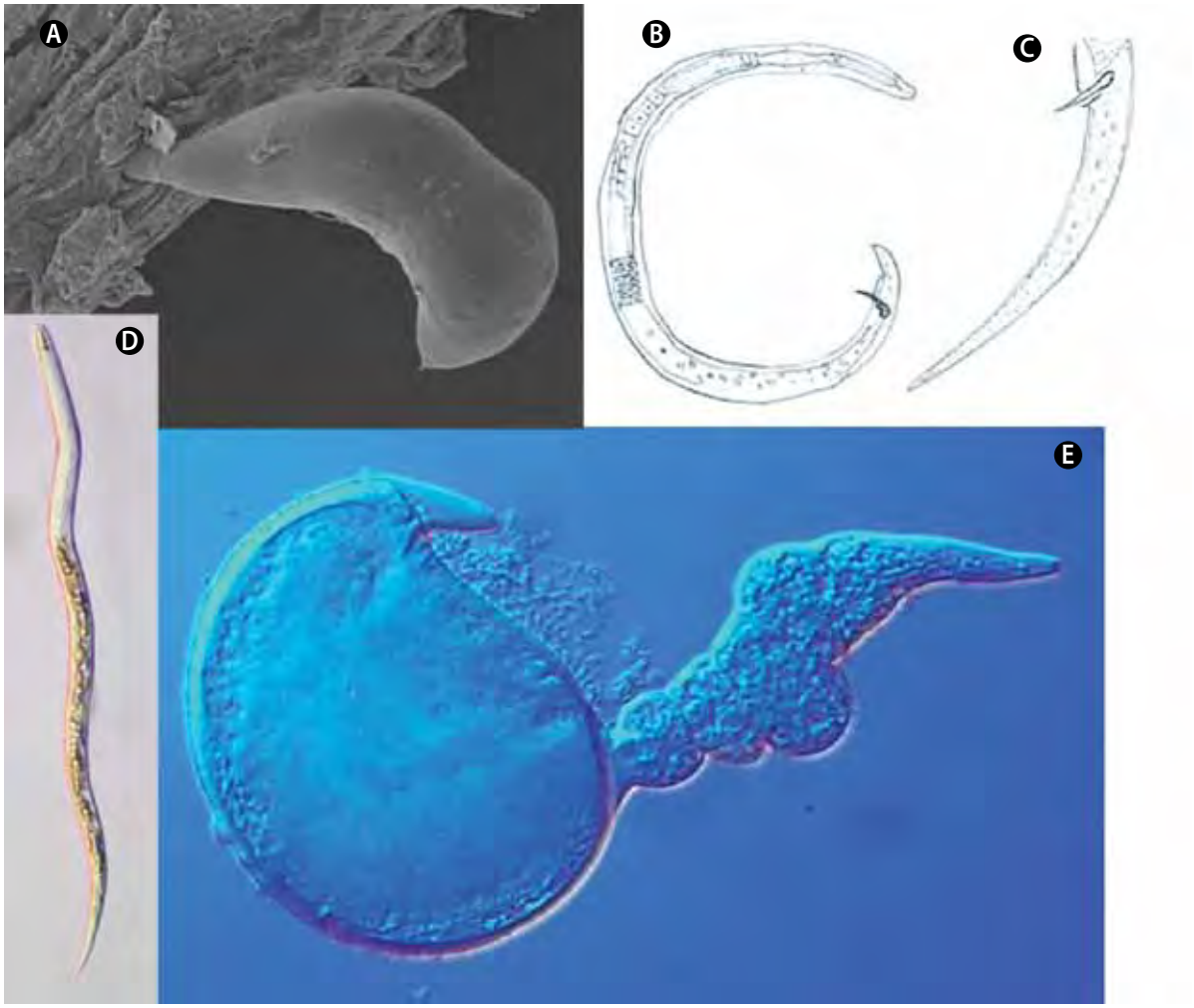


Figure 22. ***Rotylenchulus*** **female protruding from root tissue (A),** ***Rotylenchulus*** **male full body (B) and tail region (C),** ***Tylenchulus*** **juvenile full body (D) and female full body extracted from root tissue (E).**

28. Mature female white, without eggs inside body (Fig. 23).....*Meloidogyne*
(Females with irregular body annules around perineal pattern; excretory pore at level with stylet or close behind it; lip region with two lateral lips wider than four sublateral lips; second-stage juvenile stylet <20 μm; weakly labial framework.)

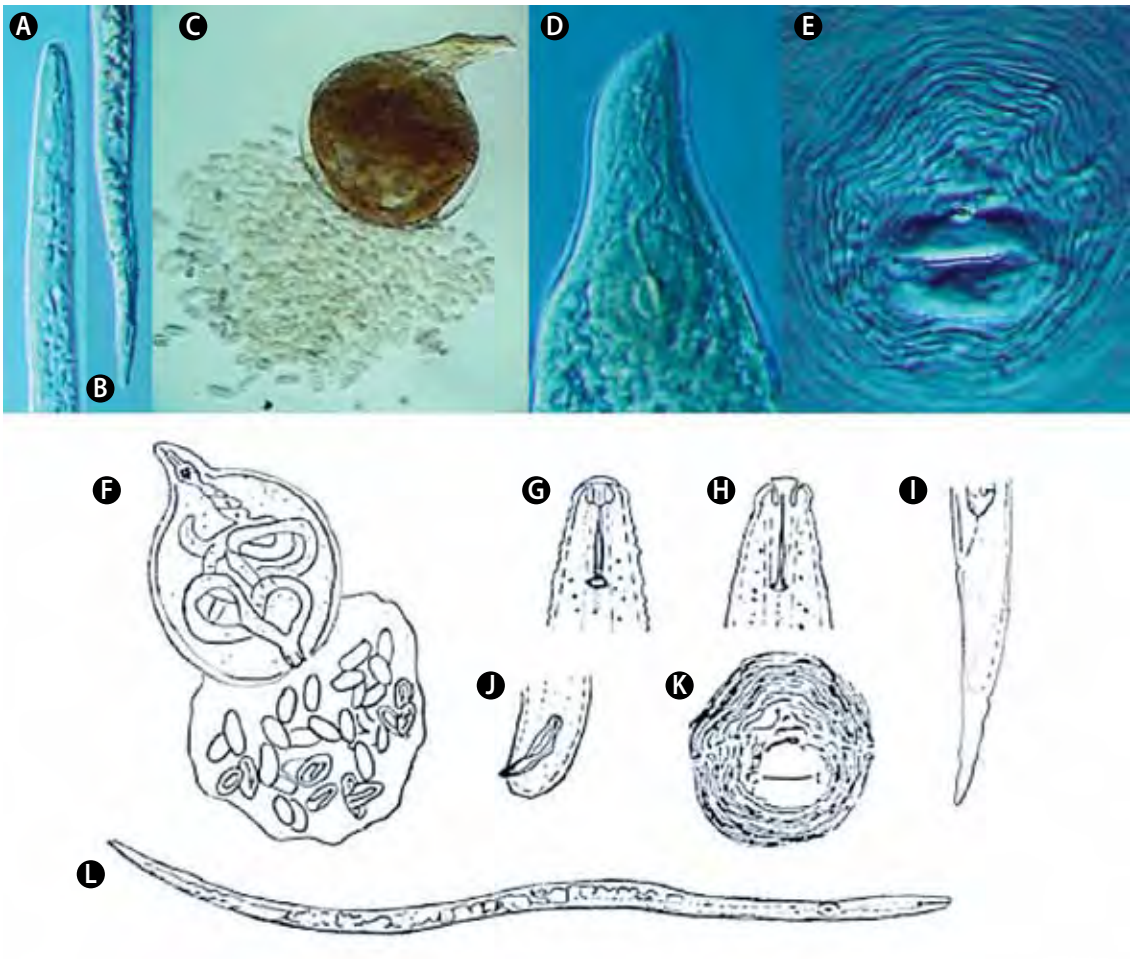


Figure 23. *Meloidogyne* juvenile anterior end (A), juvenile tail (B, I), mature female with eggs (C, F), mature female anterior end (D), female perineal pattern (E), male anterior end (G, H), male tail (J), perineal pattern (K), and juvenile full body (L).

Mature female creamy or brown with eggs inside body (Fig. 24) .....*Heterdodera*
(Cysts generally lemon-shaped, rarely spherical and then with protrusions; vulva on a terminal cone, with fenestra [circumfenestrate, bifenestrate, or ambifenestrate]; bullaae present or absent; stylet <30 µm.)

29. Lip region smooth and offset.....*Eutylenchus*
Lip region annulated and not offset.....*Atylenchus*

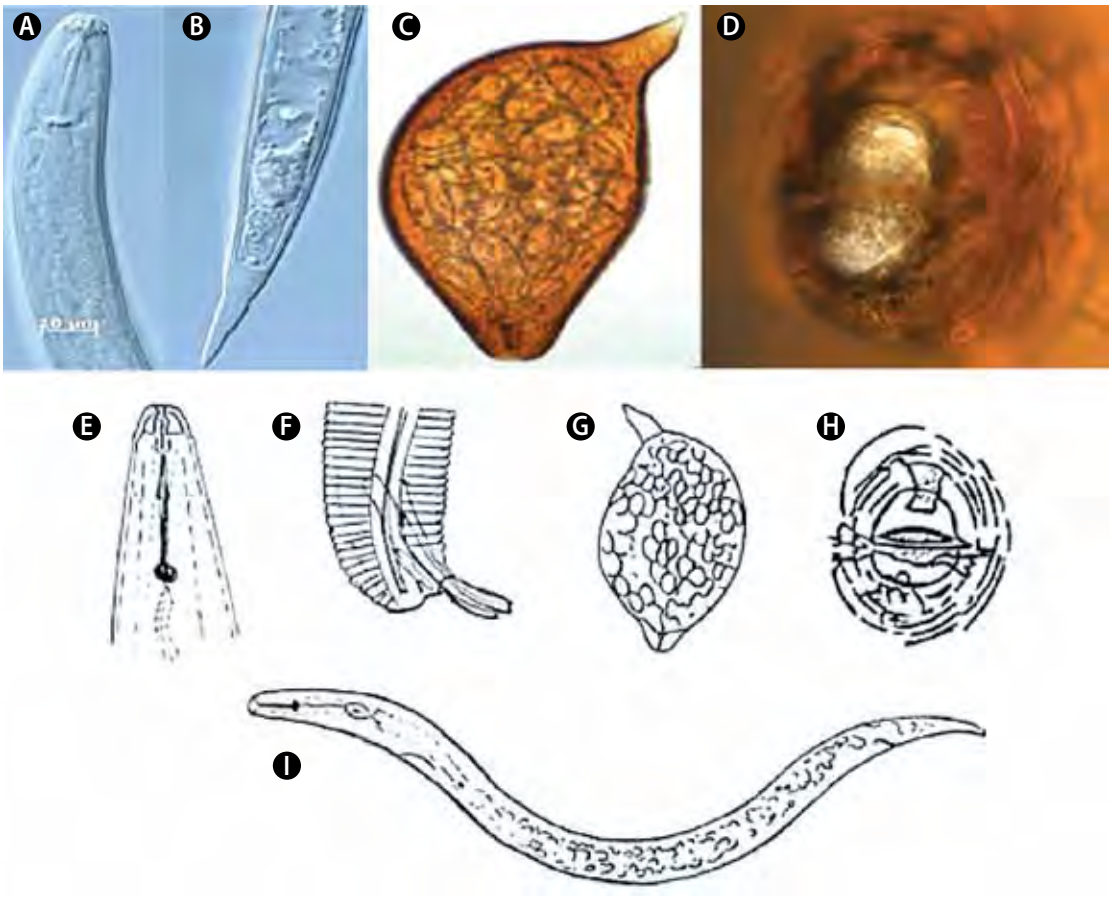


Figure 24. *Heterodera* juvenile anterior end (A), juvenile tail (B), mature cyst with vulval cone (C, G), vulval cone with fenestration (D, H), male anterior end (E), male tail (F), and juvenile full body (I).

V. Identification of family Heteroderidae to genus level after Subbotin et al., 2010

The family Heteroderidae consists of seven genera (Subbotin et al., 2010). These genera are separated mainly on the basis of the shape and fenestration of cysts and presence or absence of the vulval cone.

1. Cysts circumfenestrate (Fig. 25) 2
   Cysts ambifenestrate, bifenestrate, or without fenestration ..... *Heterodera*

2. Cysts with terminal cone (Fig. 26) 3
   Cysts without terminal cone 4

3. Vulva slit in cysts 12-18 µm long, J2 with four lateral incisures ..... *Cactodera*
   Vulva slit in cysts 5-8 µm long, J2 with three lateral incisures ..... *Betulodera*

4. Anal region with fenestration ..... *Punctodera*
   Anal region without fenestration 5

5. Mature female and cyst spheroidal, perineal tubercles usually present ..... *Globodera*
   Mature female and cyst elongate-oval shape, tubercles usually absent 6

6. Bullae absent in cysts (Fig. 26), J2 with DGO=11-15 µm, c= 9-11, stylet <20 ..... *Paradolichodera*
   Bullae present in cysts, J2 with DGO=7-8 µm, c= 5-6, stylet 22-24 ..... *Dolichodera*

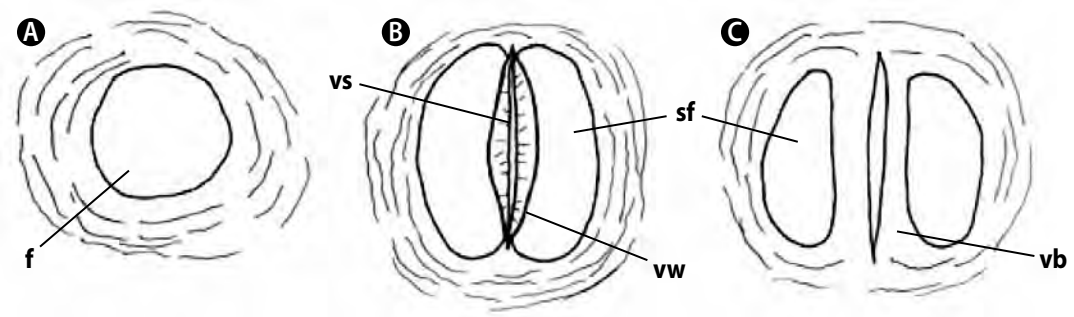


Figure 25. Heteroderidae fenestration with fenestra [f], semifenestra [sf], vulval slit [vs], vaginal wall [vw], and vulval bridge [vb]: circumfenestrate (A), ambifenestrate (B), and bifenestrate (C).

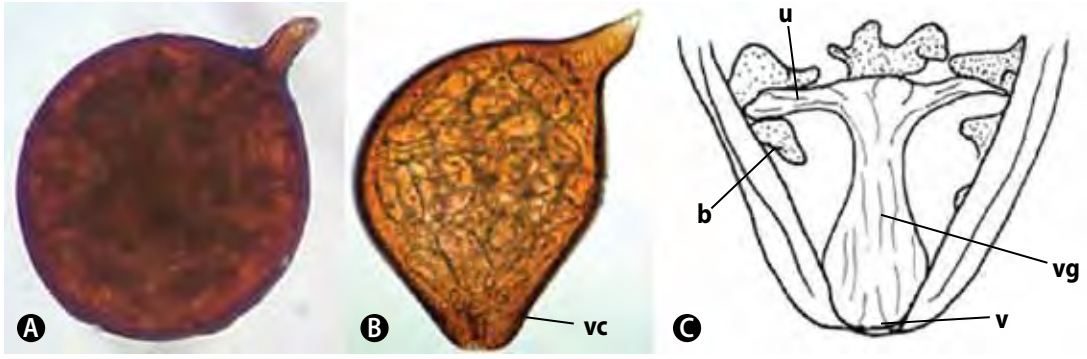


Figure 26. Heteroderidae round cyst (A), cyst with vulval cone [vc] (B), diagram of vulval cone with vulva [v], vagina [vg], bullae [b], and underbridge [u] (C).

VI. Identification key for major plant-parasitic nematodes of the orders Triplonchida and Dorylaimida

1. Stylet long; 3X or more than body width at stylet base 2
   Stylet short; 2X or less body width at stylet base 3

2. Stylet extension flanged, guiding ring near stylet base (Fig. 27, 28) ..... *Xiphinema*
   (Stylet long, straight, tapering to a long slender point with long extensions; body long, stylet extensions with sclerotized basal flanges; guiding ring near base of stylet, just anterior to junction of stylet and stylet extensions)
   Stylet extension not flanged, guiding ring near apex of stylet (Fig. 27, 29) ..... *Longidorus*
   (Stylet extensions without basal flanges; guiding ring near apex of stylet, amphid openings minute, slit-like; amphids consisting of large pouches that almost encircle the head)

3. Female genital branches monodelphic 4
   Female genital branches didelphic 5

4. With caudal alae (bursa) ..... *Allotrichodorus*
   (Cuticle swells after fixation, distinct spermatheca, post vulvar uterine sac present, prominent vaginal cuticularization, striated and bristles spicules)
   Without caudal alae ..... *Monotrichodorus*
   (Cuticle not swollen after fixation; prominent vaginal cuticularization)

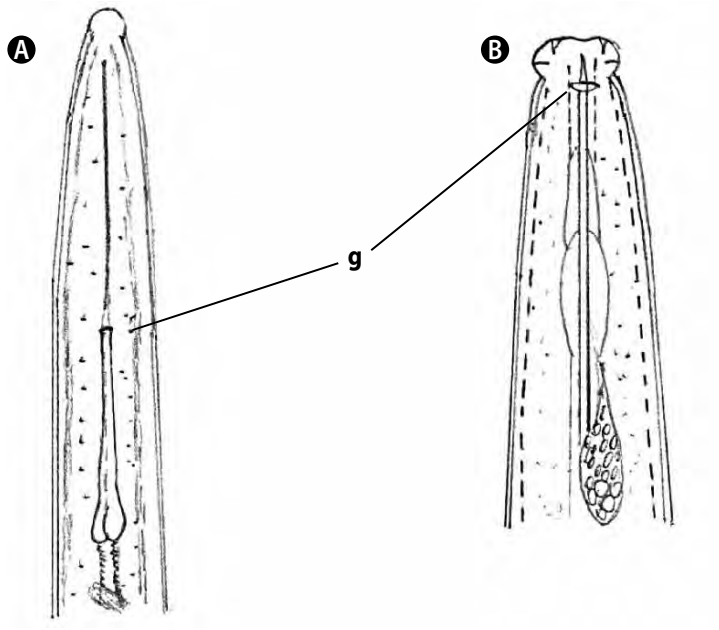


Figure 27. Stylet with guiding ring [g] near base (A) and near tip (B).

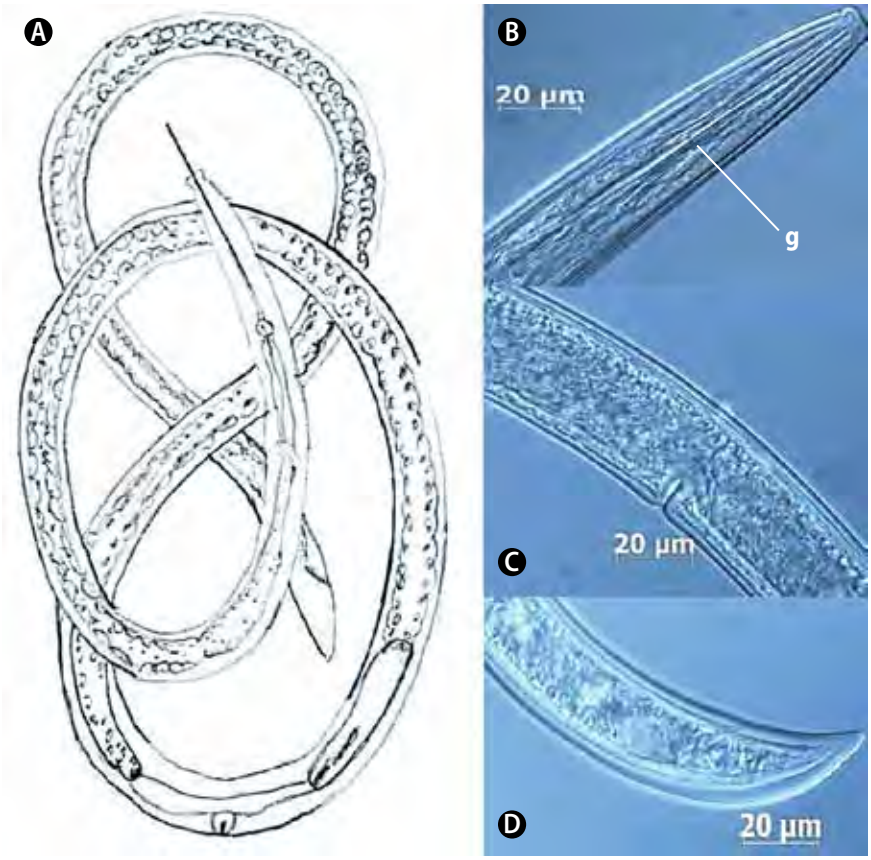


Figure 28. ***Xiphinema*** **full body (A), head with stylet and guiding ring [g] (B), vulva region (C), and tail region (D).**

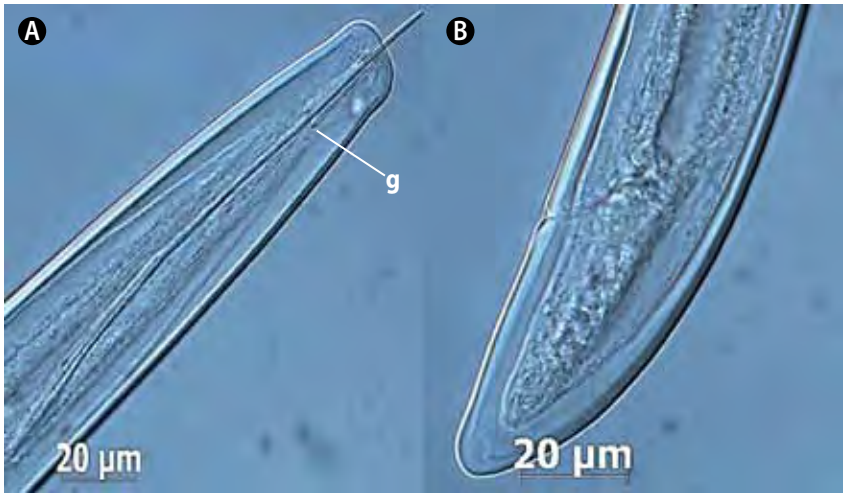


Figure 29. ***Longidorus*** **head with stylet and guiding ring [g] (A) and tail region (B).**

5. Length of vagina about half of body diameter, males common (Fig. 30) .....*Trichodorus*
(Well-developed vaginal sclerotizations; males without caudal alae (bursa), cuticle does not swell on fixation, didelphic, no bursa, striated or non-striated spicules, 3 equally spaced preanal ventro-median supplementary papillae present)

Length of vagina about 30% of the corresponding body diameter, males rare..... *Paratrichodorus*
(Stylet short, curved; body short and thick (0.45-1.5 mm long), cuticle swells post fixation, esophagus overlapping for some distance, didelphic, 1-2 pairs of lateral pores, vagina not pronounced, bursa present, striated spicules, 2-3 ventromedian supplementary papilla (2 in bursa region, 1 if present well separated from bursal region)

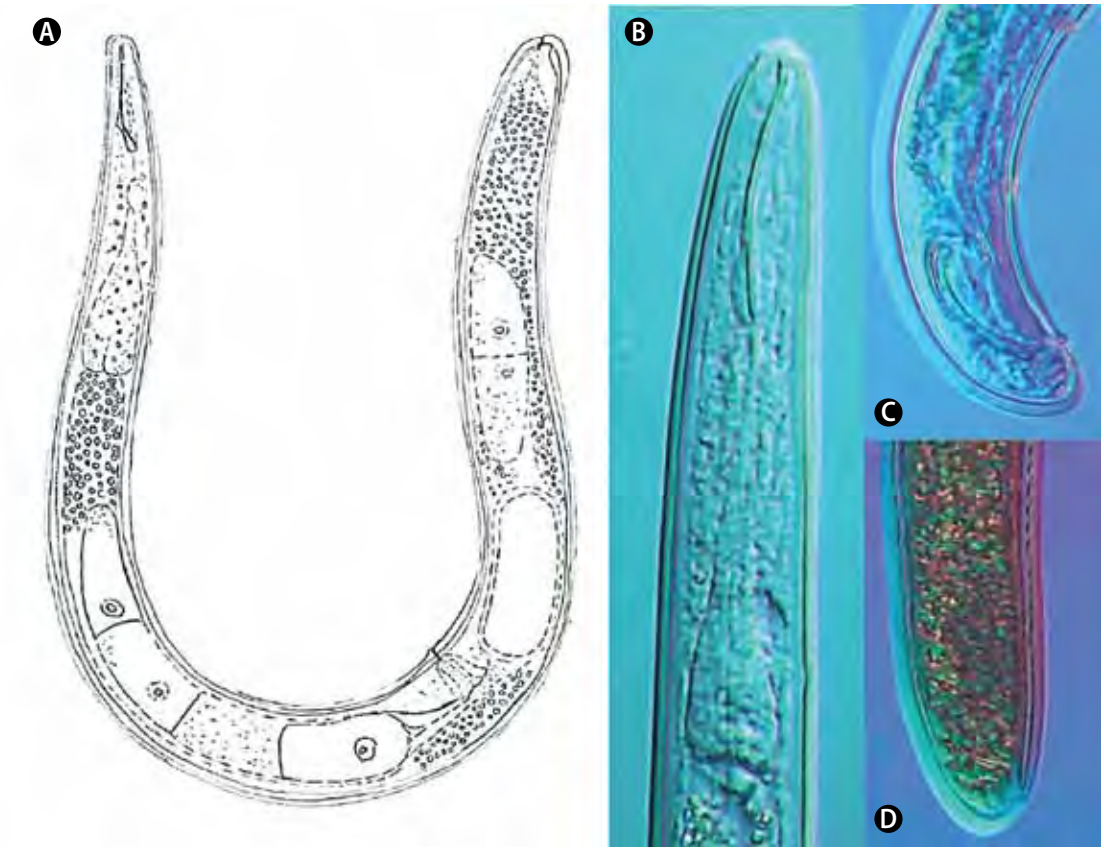


Figure 30. *Trichodorus* full body (A), lip region with stylet (B), male tail region (C) and female tail region (D).

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