10750 NEMATOLOGICAL EXAMINATION*

10750 A. Introduction

1. Occurrence and Impact

Nematodes are aquatic animals present in all parts of the world in fresh, brackish, and salt waters and in soil. A freshwater nematode has been defined as “any nematode species inhabiting either fresh water or non-brackish swampy soil below the water table; hence a species that will not drown in fresh water; a species fitted to utilize oxygen dissolved in fresh water.”1 Nematodes are a food source for other invertebrates, small fish, and fungi, and play a fundamental role in cycling carbon and nitrogen through the benthic ecosystem. This role is filled primarily by bacterivorous nematodes. Predaceous nematodes from families Aporcelaimidae, Diplogasteridae, Dorylaimidae, and Mononchidae abound in fresh water, devouring other nematodes, oligochaetes, and other small invertebrates. Their role in ingestion of algae and diatoms is less clear; however, dorylaimids occasionally are seen with bright amber, yellow, or green coloration as a consequence of algae in the gut.

Bacterivorous freshwater nematodes can ingest human enteric pathogens, and these bacteria and enteric viruses can survive chlorination inside nematode bodies. These nematodes often appear in large numbers in secondary wastewater effluents and have been used as bioindicators of water quality. 2

2. Nematode Characterization

Aquatic nematodes are well adapted to their habitat. Their long, slender bodies (see Figure 10750:1) are encased by a strong, protective, usually smooth cuticle (skin) inflated by a high-turgor pressure system. Some aquatic species have a long, filamentous, nonmuscular tail. Whipping of the tail, together with the undulating sinuous body movement, propels the nematode through the water at a very rapid rate. Many aquatic nematodes possess glands in the tail that produce a sticky secretion controlled by a spinneret at the tail tip. These secretions temporarily fasten the nematode to a substrate so that anchored nematodes can function without interference by water currents.

Oxygen requirements of most freshwater nematodes are low and the metabolism of some species may be nearly anaerobic.

The body is pierced by six to eight distinct openings and few to many minute apertures. First is the oral aperture at the apex of the anterior end, followed by two amphids on the head or neck region, the excretory pore (usually near the esophagus base), the vulva and anus in females, and the cloaca in males, and, on some nematodes, two small pores on the tail called phasmids. Cuticular ornamentation such as engravings, pores, spines, alae, or inflations also may be present.

Internally a stoma may be present, armed with teeth, or unarmored, uncollapsed or collapsed, or sometimes modified to form a hollow spear. An esophagus follows the stoma and terminates in esophageal glands. The intestine extends from the base of the esophageal glands to the rectum, which leads to the anus in females or cloaca in males.

The female gonad is single or paired and consists of an ovary, uterus, and vagina. It exits at the vulva. The male gonad consists of one or two testes, vas efferans, and vas deferens, and exits in the cloaca. Males possess spicules, which are the male copulatory organ, and their guide, the gubernaculum.

The nervous system comprises a nerve ring encircling the esophagus and connected ganglia and nerve cells. Appropriate muscle cell groups are present.

Although nematodes do not possess respiratory and circulatory organs, they tolerate large variations in the levels of salts and other environmental chemicals. Aerobic metabolism is dependent on the diffusion of oxygen into their tissues. Lacking a circulatory system, nematodes rely on diffusion through the tissues for translocation of nutrients, respiratory gases, and waste products.

3. References


4. Bibliography


NEMATOLOGICAL EXAMINATION (10750)/Collection and Processing Techniques for Nematodes

10750 B. Collection and Processing Techniques for Nematodes

1. Samples

Principal samples are: tap or well water, free-flowing or standing water without bottom sediment, bottom sediment, and aquatic plants and coarse detritus such as stones, twigs, or leaves.

a. Sample collection:

1) Tap water—Place a 20-cm, 325 mesh (45-µm pore size) sieve at a 45° angle under the discharge. Adjust water flow to a moderately slow rate, with no splashing, striking upper one-third of sieve. Run for 4 h.

2) Free-flowing or standing water—Take samples from sites where bottom sediments are absent or too deep to be collected. Collect five subsamples as follows: Hold 20-cm, 325 mesh sieve firmly at a 45° angle. Dip 3- to 4-L stainless steel pitcher in water and fill to 1-L mark. Pour contents slowly through top one-third of sieve. Repeat three more times. Collect additional 1 L to wash and concentrate detritus on sieve surface from top to bottom of sieve.

3) Bottom sediment sample—For bottom-to-surface depth less than 20 cm, stir bottom with hand garden rake. Scoop up stirred sediments in stainless steel pitcher. Add water to pitcher to within 5 cm of top. Stir, then wait 30 s. Pour contents of pitcher onto a 20 mesh (1-mm pore size) sieve nested on a 325 mesh sieve with the surface held at a 45° angle until dense detritus reaches pitcher lip. (Usually about 9/10 of the pitcher is poured off.)

For bottom-to-surface depth of 20 to 30 cm, collect duplicate samples by holding a 325 mesh sieve at a 90° angle near the bottom. Using hand rake, stir bottom sediments so that they roll up in a dense cloud in front of sieve. Let cloud settle about 10 s, then move sieve into cloud about 2.5 to 5 cm above the bottom. Bring sieve out of water while holding it at a 45° angle.

4) Aquatic plants, plant or inorganic debris—Randomly collect live floating or submerged plants of one species from target site and place in 1-L jars filled with collection site water. Do not fill more than half of jar with plant material. If several plant species are present, take two or more samples. Place plant and inorganic debris (sticks, leaves, pebbles, etc.) in 1-L jar to about half its volume.

b. Sample concentration: Concentrate detritus present on sieve face by washing tap water across sieve face from top to bottom. Place sieve on lip of a clean, empty 250-mL beaker, bring beaker forward until bottom side is up at a 45° angle. Wash detritus into beaker by flushing tap water from another beaker through the bottom one-third of the bottom side. Pour sample into jar.

c. Sample transport and storage: Regardless of collection mode keep sample jars cool. On very hot days use ice to cool them. Accurate taxonomic determination is most effective when nematodes are live and healthy. Because nematode mortality, deterioration, and obfuscation of diagnostic characters begins at time of collection, process samples for diagnosis within 24 h and complete diagnostic processing within 48 h.

Cold storage retards, but does not entirely halt, deterioration and rot. Plan survey so that samples can be processed on the same day they are taken. In an emergency, preserve entire sample indefinitely in 4% formalin (never use alcohol). Add equal volume of 8% formalin solution to sample. If sample jar is more than half full, decant excess water after a minimum 40-min settling period. Preserved specimens will shrink to some degree and body pores and lumens may be made obscure.

2. Sample Processing

a. Specialized apparatus:

1) Custom pipet, for clean-water samples—Take a 29-cm-long disposable pipet and place a piece of 12-cm-long rubber tube snuggly over about 3 cm of the conical pickup end. Add a wire baret clamp on the rubber tube. (Clean by removing clamp and flushing with a syringe.)

2) Baermann funnel, for samples containing debris—Use a glass funnel with a 15.5-cm top opening and 1.5-cm tube. Fit a rubber tube to the exit tube and close with a baret wire clamp. Place an 8- to 10-cm-diam coarse screen wire disk (3-mm pores) in the funnel opening. Add tap water until it lies just above the wire disk. Insert a facial tissue over the disk.

b. Procedure:

1) Clear or relatively clear water—Shake to obtain homogenous mix, then pour slowly onto the surface of a 7.6-cm, 325 mesh sieve. Concentrate as indicated in ¶ 1b, above. Pour concentrated residues into 50-mL conical-bottom centrifuge tube or tubes. Let nematodes settle for 40 min. Insert a custom pipet, ¶ a1), above, with rubber tube closed by finger pressure, to tube bottom. Depress rubber tube to take up the ball of nematodes on bottom of cone. Discharge about 0.05 mL (small drop) of pipet contents onto a microscope slide. Cover drop with a 22-mm cover slip. Diagnose nematodes using a compound microscope.

2) Samples with much debris—Pour concentrated samples very slowly onto the facial tissue in a Baermann funnel. After 24 h flush funnel into a 250-mL beaker. Process as directed in ¶ 2b1), above.

3) Samples containing live plants, plant debris, or inorganic material—Process samples immediately on return to laboratory. Shake vigorously and pour contents into beaker. Concentrate samples as directed in ¶ 1b, above, and, depending on clarity of sample, proceed according to ¶ 2b1) or 2), above. If the laboratory is equipped to process samples with an excess of debris using the centrifugal flotation technique,¶ preferably use this technique.

3. Reference

1. General Discussion

The following key was devised so that persons trained in biology, but not necessarily in nematology, could use it. The illustrations include original drawings, photocopies of published drawings, or photocopies on which figures were redrawn. The two most important references were Goodey\textsuperscript{1} and Chitwood & Chitwood.\textsuperscript{2} Other publications used as references and for illustrative material are listed in the bibliography.

Published literature indicates that several genera in this key contain species predominantly associated with terrestrial habitats. Presence of such nematodes suggests runoff from banks or higher ground in which various plant species (often food sources for these nematodes) are growing. These genera are indicated by an asterisk (*).

2. Key

Refer to Couplet No.

1. Cephalic setae indistinct or absent ........................................ 2

Cephalic setae absent but setae-like head appendages present ........................................ 64

Cephalic setae present ........................................ 69

2.(1) Stylet present ........................................ 3

Stylet absent ........................................ 38

3.(2) Base of stylet knobbed or flanged ........................................ 4

Stylet knobs or flanges absent ........................................ 29
4.(3) Valvate median esophageal bulb present ........................................ 5

Valvate median esophageal bulb absent ........................................ 22

5.(4) Females eel-like ................................................................. 6

Females swollen ............................................................................ 21

6.(5) Vulva at mid-body ............................................................... 7

Vulva on lower third of body ......................................................... 14

7.(6) Esophagus not overlapping intestine ...................................... 8

Esophagus overlapping intestine .................................................. 11

8.(7) Stylet length less than 50 μm. ................................................ 9

Stylet length greater than 80 μm ..................................................... 10

Dolichodorus

9.(8) Tail terminus pointed .......................................................... 11

Tetylenchus*

Tail terminus not pointed ................................................................ 10

10.(9) Tail terminus knobby .......................................................... 12

Psilenchus*

Tail terminus never knobby or pointed .......................................... 13

Tylenchorhynchus*
Refer to Couplet No.

11. (7) Labium offset. ......................................................... 12

Labium flattened, amalgamate or nearly so .............................. 13

12. (11) Stylet massive, 40–50 µm long ..................................  Hoploaimus*

Stylet long and thin, longer than 90 µm .................................. Belonolaimus*

13. (11) Body 0.5–1.0 mm long, tail tip not mucronate .............. Radopholus*

Body 2–3 mm long, tail tip usually mucronate ......................... Hirschmanniella

14. (6) Cuticle heavily annulated, stylet elongate ..................... 15

Cuticle not heavily annulated, stylet short .............................. 17

15. (14) Cuticular sheath absent .............................................. 16

Cuticular sheath present .................................................... Hemicycliophora

16. (15) Annules with cuticular spines or scales ....................... Criconema*

Annules plain without spines or scales ................................... Criconemoides*

17. (14) Body death position straight ...................................... 18

Body death position spiral .................................................. Helicotylenchus*
18. (17) Median esophageal bulb distinct but not pronounced...

Refer to Couplet No. 19

Median esophageal bulb well developed...

Aphelenchoides

19. (18) Esophagus overlapping intestine...

20. (19) Median bulb and valves small, stylet usually weak...

Ditylenchus*

Median bulb, valves and stylet well developed, labium flattened...

Pratylenchus*

21. (5) Female body soft, white, with few or no internal eggs...

Meloidogyne*

Female body a rigid brown cyst usually with many internal eggs...

Heterodera*

22. (4) Stylet short, less than 100 μm...

23. Stylet long, greater than 100 μm...

Xiphinema*

23. (22) Stylet complex...

24. Stylet simple...

25. Stylet with anterior arch-like portion...

Diptherophora*

Stylet with dorsal thickening piece...

Tylencholaimellus*

25. (23) Stylet knobs elongate, flange-like...

26. Stylet knobs round...

27.
Refer to Couplet No.

26.(25) Filiform tail .................................................  Aulolaimoides

Round tail ..........................................................  Enchodelus

27.(25) Tail rounded .................................................. 28

Tail pointed ..........................................................  Nothotylenchus

28.(27) Esophagus base elongate ..............................  Tylencholoaimus

Esophagus base oval ...............................................  Doryllium

29.(3) Valvate median esophageal bulb absent .............. 30

Valvate median esophageal bulb present ..................... 37

30.(29) Stomal walls not cuticularized .......................... 31

Stomal walls cuticularized
(Acinolaimus, Metactinolaimus, Paractinolaimus) .......  Actinolaiminae

31.(30) Esophagus with basal expansions .................... 32

Esophagus expanding uniformly ................................ Oionchus

32.(31) Terminal fifth or sixth of esophagus an ovoid bulb ........................................................................ 33

Posterior third of esophagus swollen ........................... 36
33.(32) Stylet axial, positioned centrally ................................................................. 34

Stylet not axial, originating from tooth in stoma wall .................................................. Campydoara*

34.(33) Gonads paired; vulva usually near mid-body ................................................. 35

Gonad single, posterior to vulva; vulva anterior to mid-body ........................................ Tyleptus*

35.(34) Stylet slender ................................................................................................. Leptonchus*

Stylet not slender ...................................................................................................... Dorylaimoides*

36.(32) Stylet axial, positioned centrally (Dorylaimus, Eudorylaimus, Labronema, Mesodorylaimus, Thormia, Laimydorus, Prodorylaimus) ................................................................................... Dorylaiminae

Stylet not axial, originating from tooth in stoma wall ................................................ Nygolaimus

37.(29) Tail pointed .................................................................................................. Seinura*

Tail rounded ............................................................................................................. Aphelenchus*

38.(2) Teeth present, prominent .............................................................................. 39

Teeth absent, minute, or indistinct ........................................................................... 50
39.(38) Esophagus without mid-region expansion

Esophagus expanded at mid-region

40.(39) Tail pointed or tapering

Tail rounded

41.(40) Male tail without setae

Male tail with setae

42.(41) Stoma with denticles

Stoma without denticles

43.(42) Denticles scattered or in longitudinal rows

Denticles in transverse rows

44.(43) Denticles situated on longitudinal rib of stoma

Denticles scattered on stoma wall

45.(42) Tooth anteriorly directed

Tooth retorse

Refer to Couplet No.

Oncholaimus

Mylonchulus

Prionchulus

Sporonchulus

Anatonchus
46.(45) Tooth in basal part of stoma .................................................. Iotonechus

Tooth in anterior part of stoma .................................................. Mononchus

47.(40) Stoma with prominent medial or apical tooth ........................................ 48

Stoma with small basal tooth .................................................. Bathyodontus

48.(47) Stoma with 3 teeth, without small basal tooth, caudal glands opening terminally ........................................ Enoplocheilus

Stoma with large anterior and small basal tooth, caudal glands opening ventrally ........................................ Mononchulus

49.(39) Lip region with rib-like armature ........................................ Mononchoides

Lip region without rib-like armature ........................................ Diplogaster

50.(38) Esophagus with basal expansions ........................................ 51

Esophagus uniformly cylindrical ........................................ 60

51.(50) Esophagus without mid-region expansion ........................................ 52

Esophagus expanded at mid-region ........................................ 55
52.(51) Amphids distinct .............................................. 53

Amphids indistinct ..................................................... 54

53.(52) Stoma walls anteriorly inflated with minute tooth ........................................... Microlaimus

Stoma walls without tooth and with straight, tapering sides ........................................ Leptolaimus

54.(52) Stoma with 3 rod-like thickenings ...................................................... Rhabdolaimus

Stoma without rod-like thickenings .............................................................. Monochromadora

55.(51) Gonads paired .......................................................... 56

Gonads single .................................................................. 58

56.(55) Stomal walls straight, amalgamated ..................................................... 57

Stomal walls separated, not straight .......................................................... Alloionema

57.(56) Moderately swollen metacorpus, stoma not excessively elongate ................. Rhabditis

Elongate, cylindrical metacorpus, stoma elongate ........................................ Cylindrocorpus

58.(55) Tail with sharp terminus ................................................................... 59

Tail bluntly conical ........................................................................... Cephalobus
59. (58) Anterior part of stoma a broad, open chamber

\[ \text{Panagrolaimus} \]

Stoma narrow, collapsed

\[ \text{Eucephalobus} \]

60. (50) Stoma absent or indistinct

\[ .61 \]

Stoma distinct

\[ .63 \]

61. (60) Lip region narrow, tooth absent

\[ .62 \]

Lip region broad, small denticle apparent in stomal area

\[ \text{Tripyla} \]

62. (61) Amphid aperture appearing as large slit

\[ \text{Amphidelus} \]

Amphid aperture appearing as minute pores

\[ \text{Alaimus} \]

63. (60) Stoma narrow and long

\[ \text{Cryptonchus} \]

Stoma wide and shallow

\[ \text{Bathyonchus} \]

64. (1) Body symmetrical

\[ .65 \]

Body asymmetrical, bearing series of protuberances on side

\[ \text{Bunonema}^* \]

65. (64) Lip appendages not elaborate

\[ .66 \]

Lip appendages elaborate

\[ .68 \]
66.(65) Lateral lip appendages thorn-like, directed laterally

Lateral lip appendages not thorn-like or directed laterally

--- Refer to Couplet No. 67 ---

67.(66) Papillae or setae horn-like

Lips flap-like and pointed anteriorly

--- Refer to Couplet No. 68 ---

68.(65) Lip appendages forked and elaborately fringed

Lip appendages membranous and wing-like

--- Refer to Couplet No. 69 ---

69.(1) Post-cephalic setae absent

Post-cephalic setae present (may be very faint ex. Tobrilus)

--- Refer to Couplet No. 70 ---

70.(69) Stylet absent

Stylet present

--- Refer to Couplet No. 71 ---

71.(70) Teeth absent, minute or indistinct

Teeth usually present, prominent

--- Refer to Couplet No. 72 ---

72.(71) Esophagus with basal expansions

Esophagus uniformly cylindrical

--- Refer to Couplet No. 73 ---

73.(72) Amphids oval, spiral, or stirrup-shaped

Amphids circular
74. (73) Amphids spiral

Refer to Couplet No. 75

Amphids not spiral

75. (74) Cuticular punctations absent

Cuticular punctations present

76. (75) Esophageal bulb without valves

Esophageal bulb valvate

Plectus & Anaplectus

77. (76) Esophageal-intestinal valve elongate

Paraplectonema

Esophageal-intestinal valve shortened

Paraphanolaimus

78. (75) Labial region characteristically flap-like

Euteratocephalus

Labial region not flap-like, lips bluntly rounded

Ethmolaimus

79. (74) Amphids oval

Greenenema

Amphids stirrup-shaped

Chronogaster

80. (73) Esophageal-intestinal valve shortened

Desmolaimus

Esophageal-intestinal valve elongate
81.(80) Excretory pore and large excretory gland present ................ Domorganus

Excretory pore and gland indistinct or absent ................ Monhystera

82.(72) Stoma wide and shallow, conspicuous, tail filiform ................ Prismatolaimus

Stoma narrow, elongate, collapsed or inconspicuous ................ 83

83.(82) Gonad single ........................................ Cylindrolaimus

Gonads paired .............................................. 84

84.(83) Amphids inconspicuous ................................ Tripyla

Amphids conspicuous ........................................ Aphanolaimus

85.(71) Terminal fifth or sixth of esophagus an ovoid bulb ................ 86

Esophagus uniformly cylindrical, stoma with massive teeth .......... Ironus

86.(85) Cuticular punctations present ................................ 87

Cuticular punctations absent ..................................... 89

87.(86) Amphids not spiral ........................................ 88

Amphids spiral .................................................. Achromadora
88.(87) Four longitudinal rows of cuticular markings present ......................................... Chromadora

No longitudinal rows of cuticular markings present .................................................. Prochromadorea

89.(86) Amphids distinct ................................................. 90

Amphids indistinct ...................................................... Buderius

90.(89) Female gonad double, amphids hook-shaped ........................................ Anonchus

Female gonad single, amphid circular ................................................................. Monhystrella

91.(70) Lip region annulated, not set off .............................................. Atylenchus

Lip region smooth, set off ............................................................. Eutylenchus

92.(69) Esophagus with basal expansion ............................................. 93

Esophagus uniformly cylindrical ................................................................. 98

93.(92) Cuticular punctation present, amphids not circular .................. 94

Cuticular punctation present, amphids circular ........................................ 97
Refer to
Couplet No.

94.(93) Ocelli (eye spots) present ........................................... 95

Ocelli absent ................................................................. 96

95.(94) Stoma with three equal-sized teeth .................................. Chromadorina

Stoma with at least one large tooth ......................................... Punctodora

96.(94) Cuticle with lateral longitudinal rows of punctuation ............... Hypodontolaimus

Cuticle without lateral differentiations ....................................... Chromadorita

97.(93) Esophageal bulb valvate .............................................. Prodesmodora

Esophageal bulb without valves ............................................... Odontolaimus

98.(92) Amphid anterior on body ............................................... 99

Amphid posteriorly located ....................................................... Bastiania

99.(98) Amphid spiral ............................................................. Paracyatholaimus

Amphid cup-shaped or obscure ................................................. 100
3. References


4. Bibliography


