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 bactivorous 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.

Bactivorous 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 unarmed, 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

1. Cobb, N.A. 1914. North American fresh-water nematodes. *Trans. Amer. Microscop. Soc.* 33:35.
2. Forget, G., P. Gagnon, W.A. Sanchez & B.J. Dutka. 2000. Overview of methods and results of the eight country international development research centre IDRC WaterTox project. *Environ. Toxicol.* 15:264.

* Approved by Standard Methods Committee, 1997. Formerly numbered 10550. Joint Task Group: Byron J. Adams (chair).

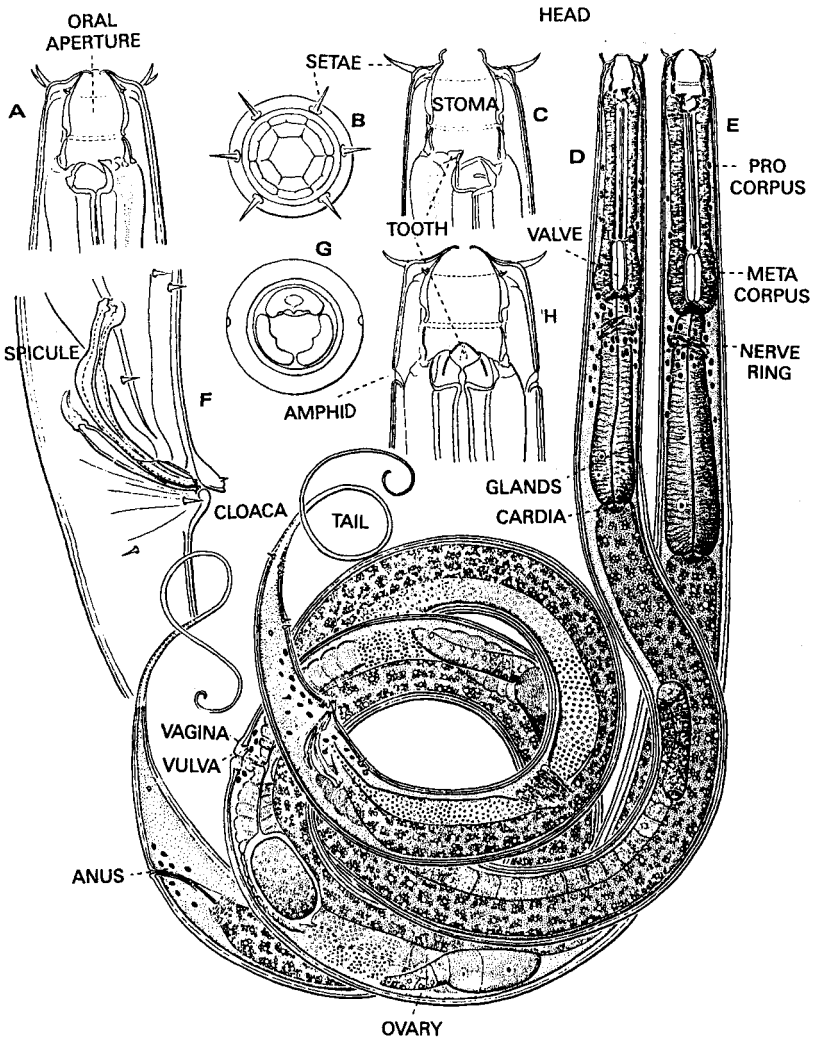

Figure 10750:1. *Butlerius* sp., a freshwater nematode. A. head; B. *en face* view of head showing six setae and a central oral aperture; C. head lateral view showing stoma and tooth; D. male; E. female; F. male tail portion showing spicules, gubernaculum, and cloaca; G. head section showing aphids; H. ventral view of head showing aphids and tooth. Russell, C.C., Department of Plant Pathology, Oklahoma State Univ., Stillwater.

4. Bibliography

PETERS, B.G. 1930. A biological investigation of sewage. *J. Helminth.* 8:133.

EDMONDSON, W.T., ed. 1959. Ward & Whipple's Fresh Water Biology, 2nd ed. John Wiley & Sons, New York, N.Y.

CHANG, S.L. 1960. Survival and protection against chlorination of human enteric pathogens in free-living nematodes isolated from water supplies. *Amer. J. Trop. Med. Hyg.* 9:136.

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CHANG, S.L. 1970. Interactions between animal viruses and higher forms of microbes. *J. San. Eng. Div., Proc. Amer. Soc. Civil Eng.* 96:151.

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TOMBES, A.S., A.R. ABERNATHY, D.M. WELCH & S.A. LEWIS. 1979. The relationship between rainfall and nematode density in drinking water. *Water Res.* 13:619.

MOTT, J.B., G. MULAMOOTTIL & A.D. HARRISON. 1981. A 13-month survey of nematodes at three water treatment plants in Southern Ontario, Canada. *Water Res.* 15:729.

ESSER, R.P, & G.R. BUCKINGHAM. 1987. Genera and species of free-living nematodes occupying fresh water habitats in North America. *In* J.A. Veech & D.W. Dickson, eds. Vistas on Nematology. E.O. Painter Printing Co., DeLeon Springs, Fla.

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 on to 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 roil 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 snugly over about 3 cm of the conical pickup end. Add a wire buret 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 buret 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 ¶ 1*b*, above. Pour concentrated residues into 50-mL conical-bottom centrifuge tube or tubes. Let nematodes settle for 40 min. Insert a custom pipet, ¶ *a*1), 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 on to 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 ¶ 2*b*1), 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 ¶ 1*b*, above, and, depending on clarity of sample, proceed according to ¶ 2*b*1) or 2), above. If the laboratory is equipped to process samples with an excess of debris using the centrifugal flotation technique,[1] preferably use this technique.

3. Reference

1. Caveness, F.E. & H.J. Jensen. 1955. Modification of the centrifugal technique for the concentration of nematodes and their eggs from soil and plant tissue. *Proc. Helminth. Soc. Wash.* 22:87.

10750 C. Illustrated Key to Freshwater Nematodes

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[1] and Chitwood & Chitwood.[2] Other publications used as references and for illustrative material are listed in the bibliography.

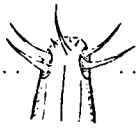
Published literature indicates that several genera in this key contain species predominantly associated with terrestrial habitats. Presence of such nematodes suggests runoff from banks or higher ground in which various plant species (often food sources for these nematodes) are growing. These genera are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

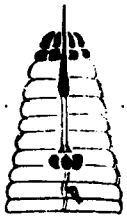
2. Key

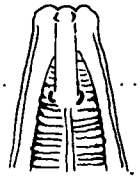
Refer to Couplet No.

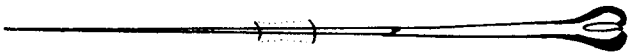
1. Cephalic setae indistinct or absent .....  2

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

   Cephalic setae present .....  69

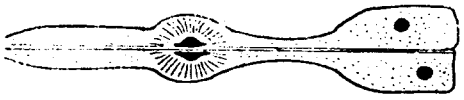
2.(1) Stylet present .....  3

   Stylet absent .....  38

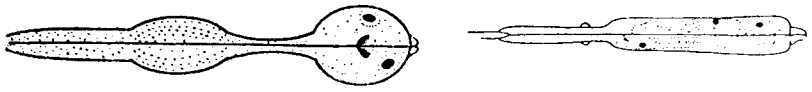
3.(2) Base of stylet knobbed or flanged ...  4

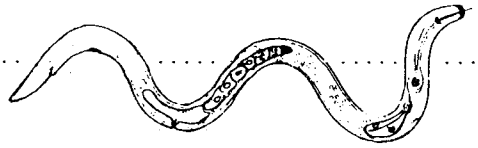
   Stylet knobs or flanges absent .....  29

Refer to Couplet No.

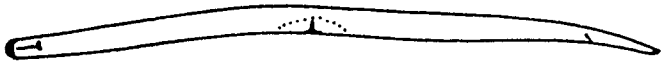
4.(3) Valvate median esophageal bulb present .....  5

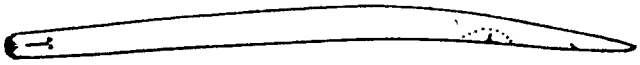
Valvate median esophageal bulb absent 22

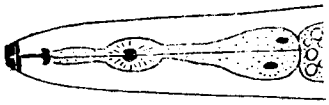


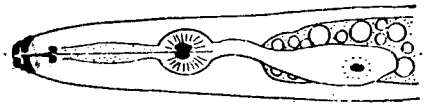
5.(4) Females eel-like .....  6

Females swollen .....  21

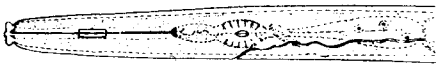
6.(5) Vulva at mid-body .....  ... 7

Vulva on lower third of body ....  14

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

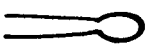
Esophagus overlapping intestine .....  11

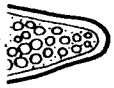
8.(7) Stylet length less than 50 µm 9

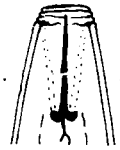
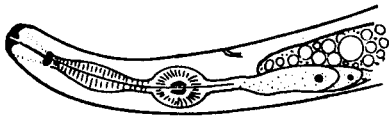
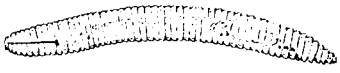
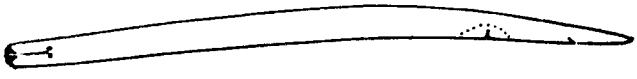
Stylet length
greater than 80 µm .....  ..... *Dolichodorus*

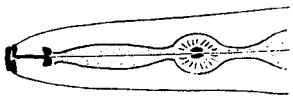
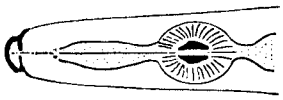
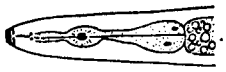
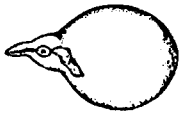
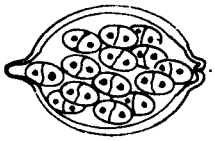
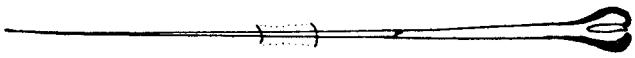
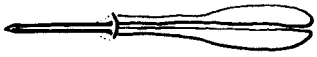
9.(8) Tail terminus pointed .....  ..... *Tetylenchus**

Tail terminus not pointed 10

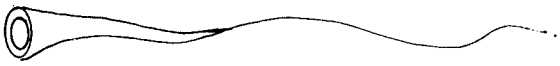
10.(9) Tail terminus knobbed .....  ..... *Psilenchus**

Tail terminus never knobbed or
pointed .....  ..... *Tylenchorhynchus**

		Refer to Couplet No.
11.(7)	Labium offset 	12
	Labium flattened, amalgamated 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**

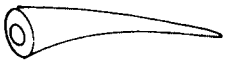
		Refer to Couplet No.
18.(17)	Median esophageal bulb distinct but not pronounced 	19
	Median esophageal bulb well developed 	*Aphelenchoides*
19.(18)	Esophagus overlapping intestine 	20
	Esophagus not overlapping intestine 	*Tetylenchus**
20.(19)	Median bulb and valves small, stylet usually weak 	*Ditylenchus**
	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
24.(23)	Stylet with anterior arch-like portion 	*Diphtherophora**
	Stylet with dorsal thickening piece 	*Tylencholaimellus**
25.(23)	Stylet knobs elongate, flange-like 	26
	Stylet knobs round 	27

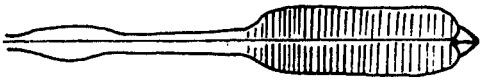
Refer to Couplet No.

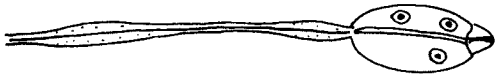
26.(25) Filiform tail .....  ..... *Aulolaimoides*

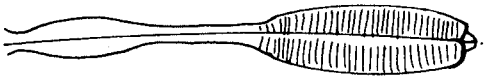
Round tail .....  ..... *Enchodelus*

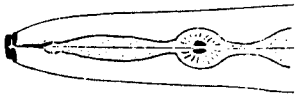
27.(25) Tail rounded .....  28

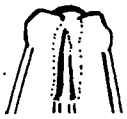
Tail pointed .....  ..... *Nothotylenchus*

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

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

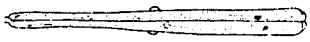
29.(3) Valvate median esophageal bulb absent .....  30

Valvate median esophageal bulb present.....  37

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

Stomal walls cuticularized (*Actinolaimus, Metactinolaimus, Paractinolaimus*) .....  .....*Actinolaiminae*

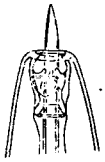
31.(30) Esophagus with basal expansions .....   32

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

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

Posterior third of esophagus swollen .....  36

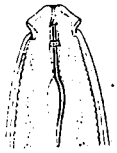
Refer to Couplet No.

33.(32) Stylet axial, positioned centrally .....  34

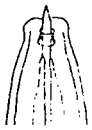
Stylet not axial, originating from tooth in stoma wall .....  ..... *Campydora**

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

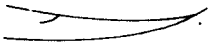
Gonad single, posterior to vulva; vulva anterior to mid-body ..... *Tyleptus**

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

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

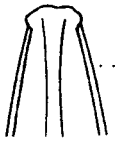
36.(32) Stylet axial, positioned centrally (*Dorylaimus, Eudorylaimus, Labronema, Mesodorylaimus, Thornia, Laimydorus, Prodorylaimus*) .....  ..... Dorylaiminae

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

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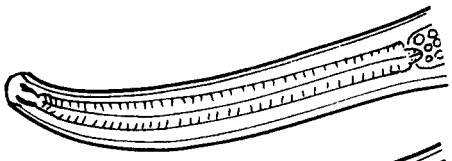
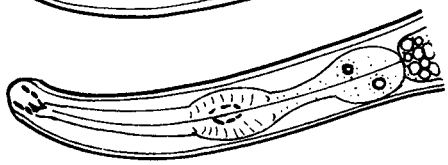
39.(38) Esophagus without mid-region expansion .....  40

Esophagus expanded at mid-region .....  49

40.(39) Tail pointed or tapering .....  41

Tail rounded .....  47

41.(40) Male tail without setae .....  42

Male tail with setae .....  ..... *Oncholaimus*

42.(41) Stoma with denticles .....  43

Stoma without denticles .....  45

43.(42) Denticles scattered or in longitudinal rows .....  44

Denticles in transverse rows .....  ..... *Mylonchulus*

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

Denticles scattered on stoma wall .....  ..... *Sporonchulus*

45.(42) Tooth anteriorly directed .....  46

Tooth retrorse .....  ..... *Anatonchus*

Refer to Couplet No.

46.(45) Tooth in basal part of stoma .....  ..... *Iotonchus*

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

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

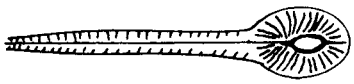
Stoma with small basal tooth .....  ..... *Bathyodontus*

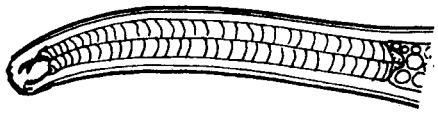
48.(47) Stoma with 3 teeth, without small basal tooth, caudal glands opening terminally .....  ..... *Enoplocheilus*

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

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

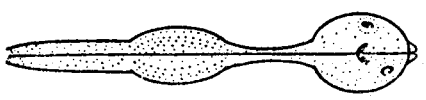
Lip region without rib-like armature .....  ..... *Diplogaster*

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

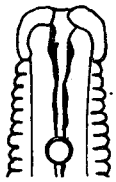
Esophagus uniformly cylindrical .....  60

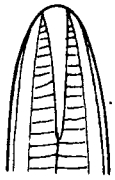
51.(50) Esophagus without mid-region expansion .....  52

Esophagus expanded at mid-region 55

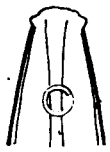


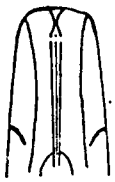
Refer to Couplet No.

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*

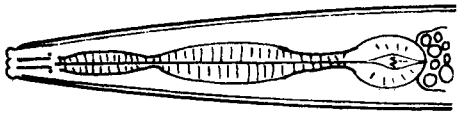
Stoma without rod-like thickenings  ..... *Monochromadora*

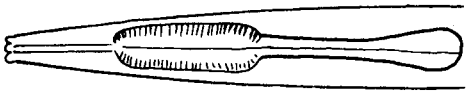
55.(51) Gonads paired  56

Gonads single  58

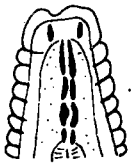
56.(55) Stomal walls straight, amalgamated  57

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

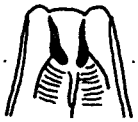
57.(56) Moderately swollen metacorpus, stoma not excessively elongate  ..... *Rhabditis*

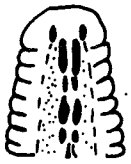
Elongate, cylindrical metacorpus, stoma elongate  ..... *Cylindrocorpus*

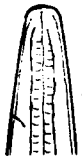
58.(55) Tail with sharp terminus  59

Tail bluntly conical   ..... *Cephalobus*

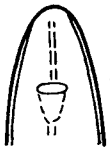
Refer to Couplet No.

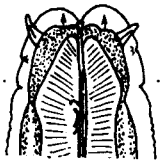
59.(58) Anterior part of stoma a broad, open chamber .....  ..... *Panagrolaimus*

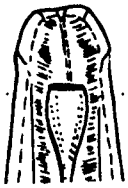
Stoma narrow, collapsed .....  ..... *Eucephalobus*

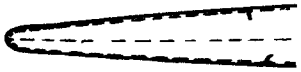
60.(50) Stoma absent or indistinct .....  61

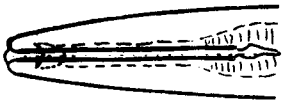
Stoma distinct .....  63

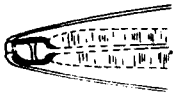
61.(60) Lip region narrow, tooth absent .....  62

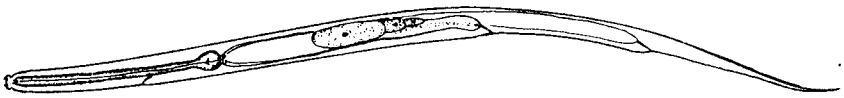
Lip region broad, small denticle apparent in stomal area .....  ..... *Tripyla*

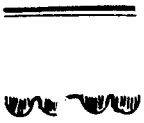
62.(61) Amphid aperture appearing as large slit .....  ..... *Amphidelus*

Amphid aperture appearing as minute pores .....  ..... *Alaimus*

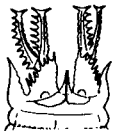
63.(60) Stoma narrow and long .....  ..... *Cryptonchus*

Stoma wide and shallow .....  ..... *Bathyonchus*

64.(1) Body symmetrical ....  65

Body asymmetrical, bearing series of protuberances on side .....   ..... *Bunonema**

65.(64) Lip appendages not elaborate .....  66

Lip appendages elaborate .....  68

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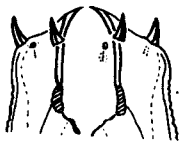
66.(65) Lateral lip appendages thorn-like, directed laterally  ..... *Diploscapter*

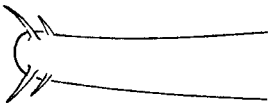
Lateral lip appendages not thorn-like or directed laterally 67

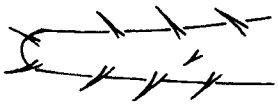
67.(66) Papillae or setae horn-like  ..... *Macrolaimus*

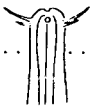
Lips flap-like and pointed anteriorly  ..... *Teratocephalus*

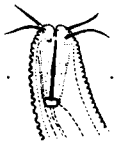
68.(65) Lip appendages forked and elaborately fringed  ..... *Acrobeles**

Lip appendages membranous and wing-like  ..... *Wilsonema**

69.(1) Post-cephalic setae absent  70

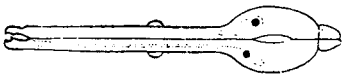
Post-cephalic setae present (may be very faint ex. *Tobrilus*)  92

70.(69) Stylet absent  71

Stylet present  91

71.(70) Teeth absent, minute or indistinct  72

Teeth usually present, prominent  85

72.(71) Esophagus with basal expansions  73

Esophagus uniformly cylindrical  82

73.(72) Amphids oval, spiral, or stirrup-shaped  74

Amphids circular 80

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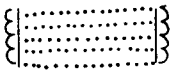
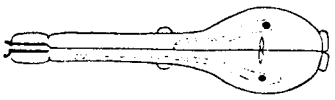
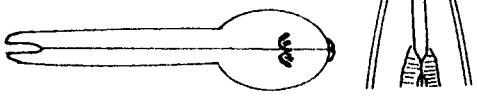
74.(73) Amphids spiral  75

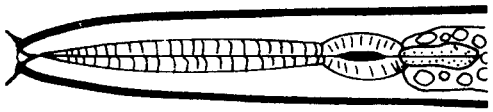
Amphids not spiral 79

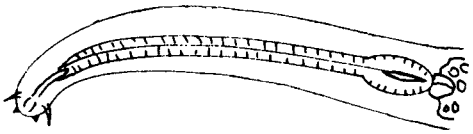
75.(74) Cuticular punctations absent  76

Cuticular punctations present  78

76.(75) Esophageal bulb without valves  77

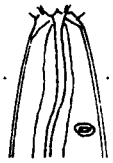
Esophageal bulb valvate  ..... *Plectus & Anaplectus*

77.(76) Esophageal-intestinal valve elongate  ..... *Paraplectonema*

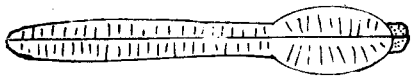
Esophageal-intestinal valve shortened  ..... *Paraphanolaimus*

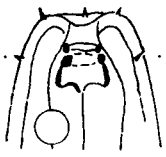
78.(75) Labial region characteristically flap-like  ..... *Euteratocephalus*

Labial region not flap-like, lips bluntly rounded  ..... *Ethmolaimus*

79.(74) Amphids oval   ..... *Greenenenema*

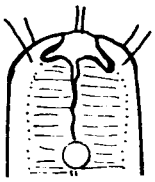
Amphids stirrup-shaped  ..... *Chronogaster*

80.(73) Esophageal-intestinal valve shortened  81

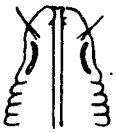
Esophageal-intestinal valve elongate   ..... *Desmolaimus*

Refer to Couplet No.

81.(80) Excretory pore and large excretory gland present .....  ..... *Domorganus*

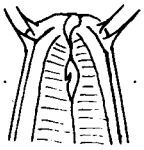
Excretory pore and gland indistinct or absent .....  ..... *Monhystera*

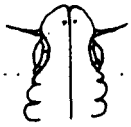
82.(72) Stoma wide and shallow, conspicuous, tail filiform .....  ..... *Prismatolaimus*

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

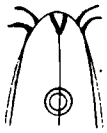
Amphids spiral .....  .....  ..... *Achromadora*

Refer to Couplet No.

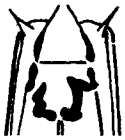
88.(87) Four longitudinal rows of cuticular markings present ..... *Chromadora*

No longitudinal rows of cuticular markings present ..... *Prochromadorella*

89.(86) Amphids distinct 90

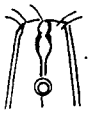


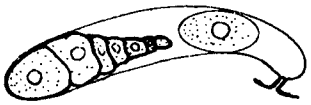
Amphids indistinct ..... *Butlerius*



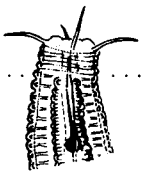
90.(89) Female gonad double, amphids hook-shaped ..... *Anonchus*

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

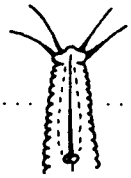




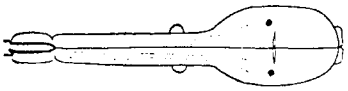
91.(70) Lip region annulated, not set off ..... *Atylenchus*



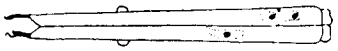
Lip region smooth, set off ..... *Eutylenchus*



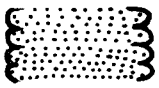
92.(69) Esophagus with basal expansion 93



Esophagus uniformly cylindrical 98



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

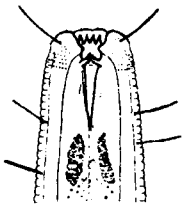


Cuticular punctation present, amphids circular 97

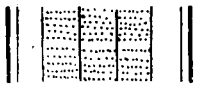
Refer to Couplet No.

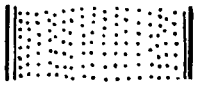
94.(93) Ocelli (eye spots) present .....  95

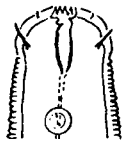
Ocelli absent .....  96

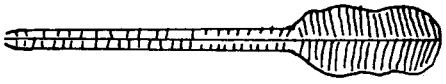
95.(94) Stoma with three equal-sized teeth .....  ..... *Chromadorina*

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

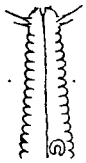
96.(94) Cuticle with lateral longitudinal rows of punctation .....  ..... *Hypodontolaimus*

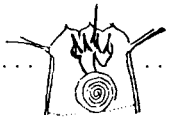
Cuticle without lateral differentiations .....  ..... *Chromadorita*

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

100.(99) Stomal teeth massive .....  ..... *Oncholaimus*

Stomal teeth small .....  ..... *Tobrilus*

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