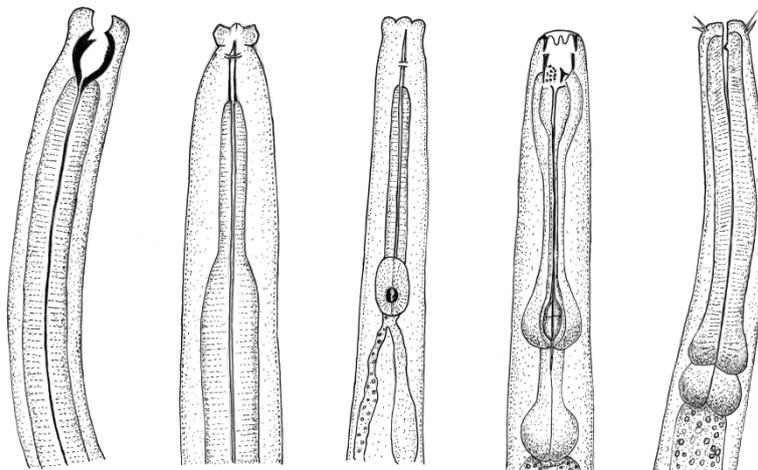


## IDENTIFICATION OF FREE-LIVING NEMATODES FOR ECOLOGICAL STUDIES

An introductory booklet



## Background

In the upper 10 cm of soil there are usually between 200,000 and 5 million nematodes per square metre and 30-60 species may be present. It is relatively easy to extract and count these nematodes but allocating them to 'taxa' is a major challenge for several reasons:

- There are more than 2,000 nematode genera and few nematologists are familiar with all the nematodes that live in soil
- Illustrations and keys normally require the presence of males and females, and they are not always present
- Some keys begin with reference to structures that are either relatively obscure (e.g. phasmids present or absent) or cannot be seen at low magnifications
- The possible level of identification varies because some groups have been studied more than others

Anyone using this booklet should obtain a copy of Yeates et al. 1993: *Journal of Nematology* 25, 315-331. The large Table in this paper lists the feeding habits of about 340 nematode genera and is a fundamental source of information on the food sources of the free-living nematodes that dominate the nematode community in healthy soils. Plant-parasitic nematodes are also included.

As most people would find it impossible to identify all the free-living genera listed by Yeates et al. (1993), this booklet takes a functional approach to nematode identification. Thus, about forty operational taxa are included and they are assumed to include related taxa with similar feeding habits and reproductive features. In general terms, these taxa are 'families'.

On the next page is a scoresheet which lists the operational taxa that would normally be counted in an ecological study. This booklet contains details on how to identify the most common taxa.

Some plant-parasitic nematodes are not listed in the scoresheet because the genera present will depend on the plants growing at the sampling site together with environmental factors such as climate and soil type. Thus, the plant parasites present at a site must be added to the taxon column at the top of the sheet.

The free-living nematodes are listed in the scoresheet by feeding group and coloniser-persister (c-p) value. The c-p scale recognises that closely related taxa have similar anatomical and physiological features and are adapted to specific food sources and environmental conditions (see Ferris et al., 2001: *Applied Soil Ecology* 18, 13-29).

The features of the various c-p groups are listed below.

- **cp1.** Short generation time, small eggs, and high fecundity. Mainly bacterivores that feed continuously on microbes that multiply in enriched conditions. Form dauerlarvae when microbial blooms subside.
- **cp2.** Longer generation times and lower fecundity than the cp1 group. Very tolerant of adverse conditions and may become cryptobiotic if the soil is dry, freezing, or lacking oxygen. Mainly bacterivores and fungivores that continue feeding as resources decline.
- **cp3.** Longer generation times and greater sensitivity to adverse conditions. Fungivores, bacterivores and carnivores.
- **cp4.** Longer generation times, lower fecundity, and greater sensitivity to disturbance. Some fungivores and bacterivores but mainly small omnivores.
- **cp5.** The longest generation times, the largest body sizes, the lowest fecundity, and the greatest sensitivity to disturbance. Predominantly carnivores and omnivores.

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This booklet was prepared for teaching purposes.

Figures on the cover were drawn by Marcelle Stirling.

Top, left to right. Bacterial-feeding rhabditid; fungal-feeding aphelenchid; omnivorous dorylaimid; predatory mononchid.

Bottom, left to right. Five predatory nematodes: Mononchida; Dorylaimida; *Seinura*; Diplogasteridae; Tripylidae.

## SCORESHEET FOR NEMATODE COMMUNITY ANALYSES

cp value	Taxon	Sample details:	Sample details:
Pp	<i>Pratylenchus</i>		
Pp	<i>Meloidogyne</i>		
Pp	<i>Helicotylenchus</i>		
Pp	<i>Paratrichodorus</i>		
Pp	<i>Xiphinema</i>		
Pp			
Pp			
Ba 1	Rhabditidae		
Ba 1	<i>Panagrolaimus</i>		
Ba 1	<i>Diplogastreriana</i>		
Ba 2	Cephalobidae		
Ba 2	<i>Cervidellus</i>		
Ba 2	<i>Acrobeles</i>		
Ba 2	<i>Plectus</i>		
Ba 2	<i>Wilsonema</i>		
Ba 2	<i>Monhystera</i>		
Ba 2	<i>Geomonhystera</i>		
Ba 3	<i>Rhabdolaimus</i>		
Ba 3	<i>Prismatolaimus</i>		
Ba 3	<i>Teratocephalus</i>		
Ba 3	Chromadoridae		
Ba 4	Alaimidae		
Fu 2	<i>Aphelenchus</i>		
Fu 2	<i>Aphelenchoides</i>		
Fu 2	<i>Ditylenchus</i>		
Pa 2	Tylenchidae		
Fu 3	Diphtherophoridae		
Fu 4	Leptonchidae		
Fu 4	<i>Tylencholaimus</i>		
Pa 5	<i>Dorylaimellus</i>		
Pa 5	<i>Axonchium</i>		
Om 4	<i>Eudorylaimus</i>		
Om 5	<i>Mesodorylaimus</i>		
Om 5	<i>Aporcelaimellus</i>		
Om 5	<i>Labronema</i>		
Ca 5	Discolaimidae		
Ca 5	<i>Actinolaimus</i>		
Ca 5	<i>Nygolaimus</i>		
Ca 2	<i>Seinura</i>		
Ca 3	Tripylidae		
Ca 4	Mononchidae		
Ca 4	<i>Ironus</i>		

## PLANT PARASITES

Most plant-parasitic nematodes are in the Order Tylenchida. Details of how they can be identified to genus level are given in a booklet entitled 'Characteristics for differentiating some plant-parasitic nematodes'.

The key characteristics of the Tylenchida are:

- An obvious stylet with two basal knobs
- Oesophagus with a median bulb that is not very obvious at low magnifications

## PLANT ASSOCIATES

These nematodes feed on algae, lichens, and mosses, but as some are associated with roots or feed on root hairs, they are termed plant associates. Although they may feed on plants, these nematodes are very weak plant parasites and do not cause economic damage.

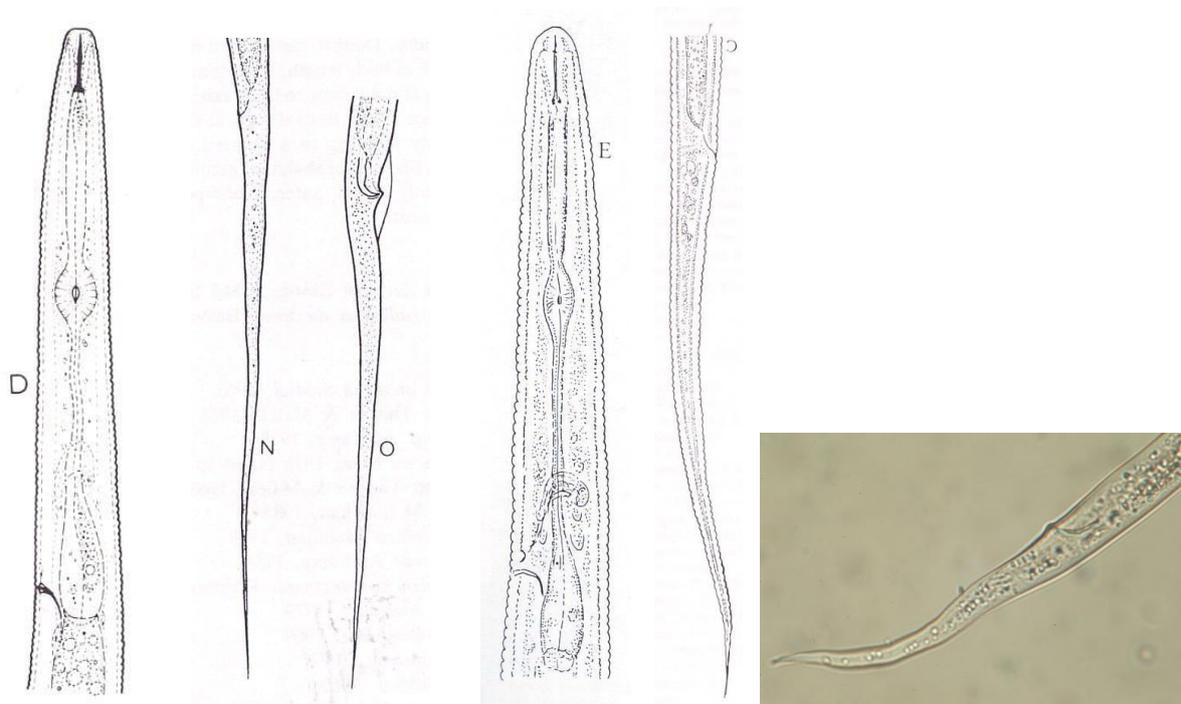
### Fine-tailed Tylenchidae

At a taxonomic level, there are two groups of plant associates but by far the most important are the fine tailed Tylenchidae. Common genera include *Tylenchus*, *Psilenchus* and *Tetylenchus*. As these genera belong to the Order Tylenchida, they are very similar to some of the most important plant parasites.

### Key characteristics

- Length variable but generally 0.4 to 0.7 mm
- Very small stylet (5  $\mu\text{m}$  long) with knobs. The knobs can be seen at 40X and the stylet is just visible at 100 X
- Median bulb that is not very obvious at low magnifications
- Oesophagus abuts intestine
- Filiform tail (although some genera do not have very fine tails)

Some fine-tailed Tylenchidae are often confused with second-stage juveniles of root-knot nematode. These nematodes are similar in size and shape but the tylenchids have a shorter stylet and a finer tail.



*Tylenchus*

*Filenchus*

Male tail

Diagrams from Siddiqi (1985) 'Tylenchida. Parasites of Plants and Insects'. Figs. 16 and 17. Photograph of male tail from the University of California, Davis.

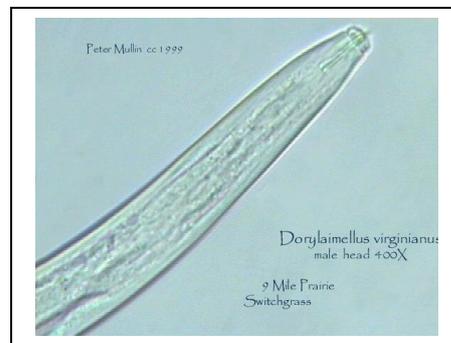
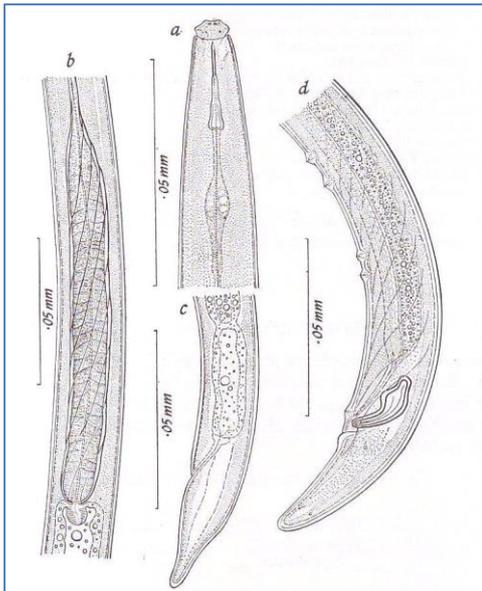
## Belonidiridae

These plant associates are in the Order Dorylaimida, so they have the characteristics of that order. *Dorylaimellus* is relatively common in Australian soils.

### *Dorylaimellus*

#### Key characteristics

- Relatively small nematode
- Short, weak spear with a flanged spear extension similar to *Xiphinema*
- Bottle-shaped oesophagus
- The basal expanded part of the oesophagus is enclosed by a spiral sheath of muscles (often difficult to see at low magnification)
- Round tail



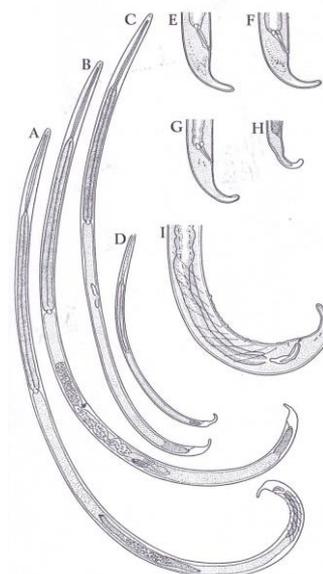
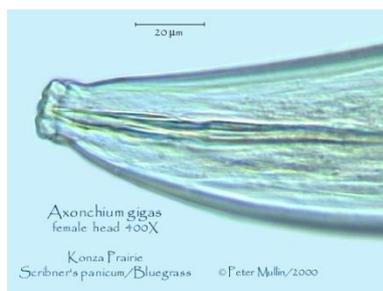
### *Dorylaimellus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 262; Yeates (1979) Nematologica 25, 419-438, Fig. 4. Photos above and below from the University of Nebraska, Lincoln.

### *Axonchium*

#### Key characteristics

- Relatively long nematode (>1.2 mm)
- Spear weak and not very obvious
- Bottle-shaped oesophagus
- Tail may be conoid (as in the figure) or round

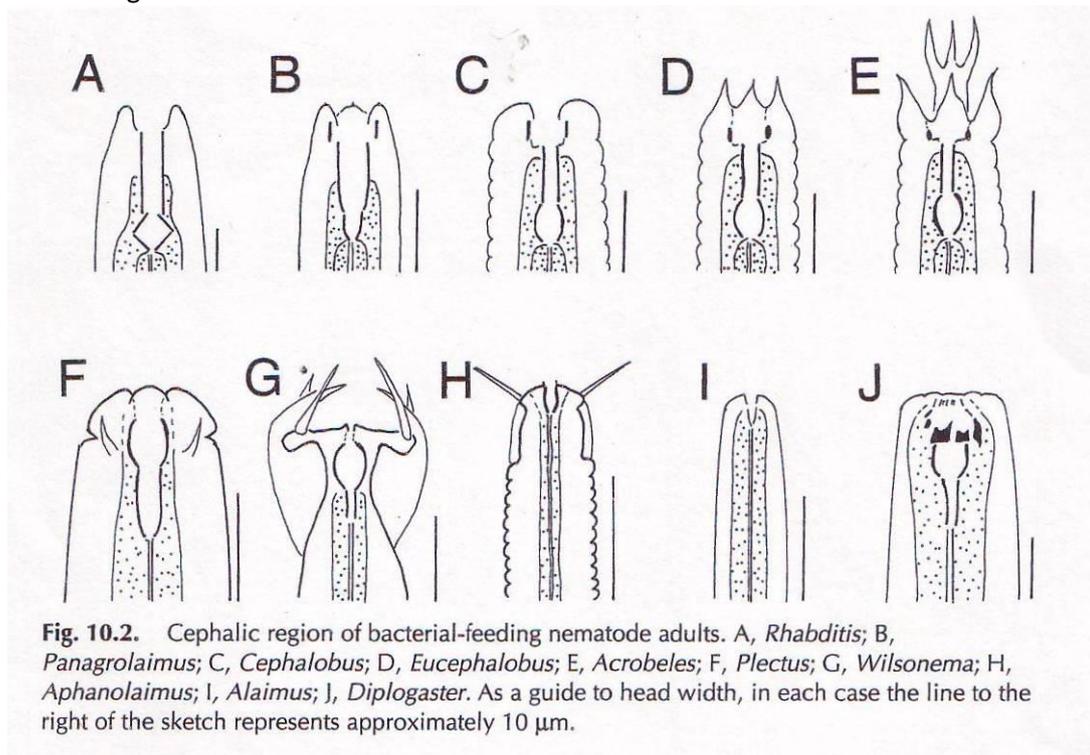


### *Axonchium*

## BACTERIVORES

Bacterivores are the most common trophic group in most soils, particularly agricultural soils.

There are many families and genera and the cephalic region of some of the most common nematodes is shown in the figure below.



**Fig. 10.2.** Cephalic region of bacterial-feeding nematode adults. A, *Rhabditis*; B, *Panagrolaimus*; C, *Cephalobus*; D, *Eucephalobus*; E, *Acrobeles*; F, *Plectus*; G, *Wilsonema*; H, *Aphanolaimus*; I, *Alaimus*; J, *Diplogaster*. As a guide to head width, in each case the line to the right of the sketch represents approximately 10  $\mu\text{m}$ .

From Yeates GW (1998) Feeding in free-living soil nematodes: a functional approach. In: The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes (eds. RN Perry and DJ Wright). CAB International pp. 245 – 269)

### Ecological groups of bacterivores

From an ecological perspective, there are four major groups of bacterivores and each group will be dealt with separately.

#### Enrichment opportunists (Ba1)

**Rhabditidae, Panagrolaimus and Diplogasteridae.**

This group of nematodes have very short life cycles and multiply rapidly when soil is enriched with organic matter. This often happens when soil is tilled, as some of the organic matter on the soil surface is incorporated into the soil by the tillage machinery. Also, organic particles within the profile are broken into smaller particles, increasing the surface area available to decomposing microbes. These nematodes form dauerlarvae when their bacterial food source declines.

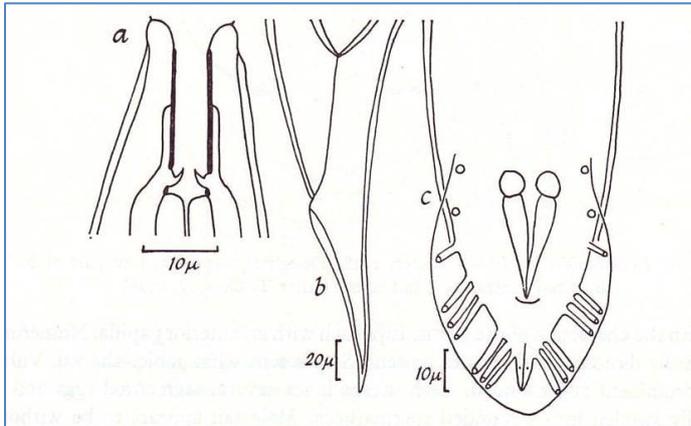
#### Key characteristics of the Rhabditidae and *Panagrolaimus*

- Wide body relative to length and usually move quite quickly
- Basal oesophageal bulb with obvious valves
- Conoid tail
- Non-feeding dauerlarvae often present
  - Straight body with little internal structure and no distinct body contents
  - Rounded head with a narrow stoma that is difficult to see
  - A plug of cuticular material completely covers the buccal cavity (to inhibit feeding)
  - Tail conoid or filiform

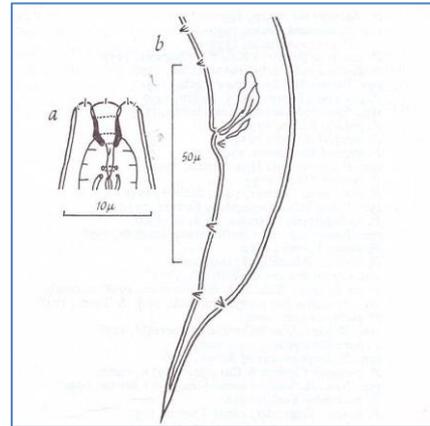
The **Rhabditidae** and ***Panagrolaimus*** are separated by shape of the stoma

- Rhabditidae: Cylindrical (tube-like)
- *Panagrolaimus*: Beaker-shaped

Note: The cylindrical stoma is difficult to see in the dauer larvae of rhabditids. They also have a very pointed tail

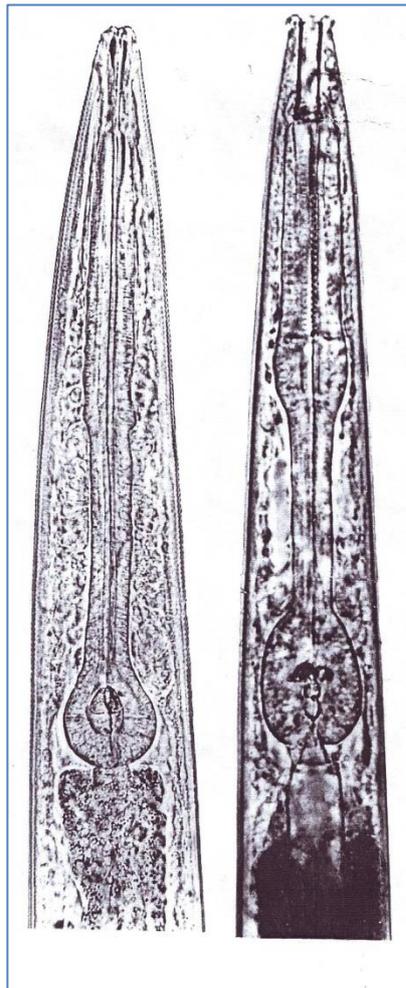


*Caenorhabditis*



*Panagrolaimus*

From: Goodey (1963) Soil and Freshwater Nematodes, Figs. 118 and 135.



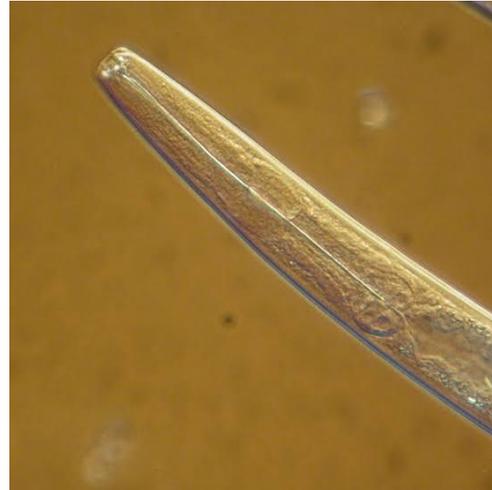
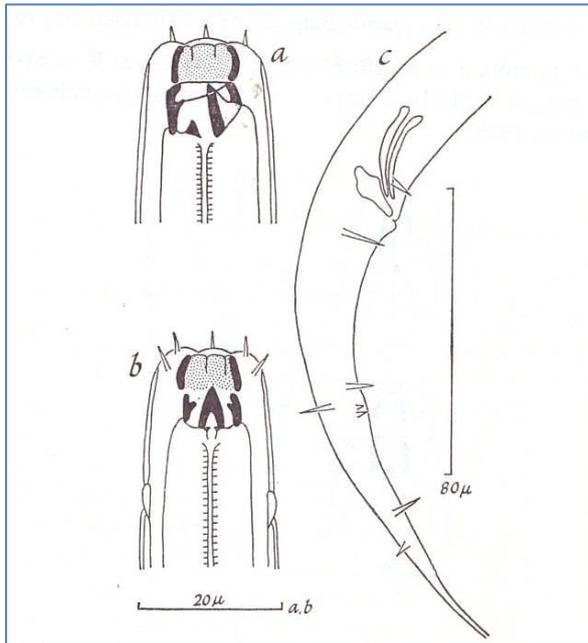
Anterior region of *Panagrolaimus* (left) and *Rhabditis* (right)

From: Mai et al. (1996). Plant-parasitic Nematodes. A Pictorial key to Genera. Plate 2.

## Diplogastridae

These nematodes are not very common in soil. They occur where bacteria are plentiful and are generally associated with decomposing plant material that is in contact with soil. Often found in manure heaps, cow dung, sheep droppings, etc.

The majority of species have small teeth in the stoma. Species with large teeth are predators.



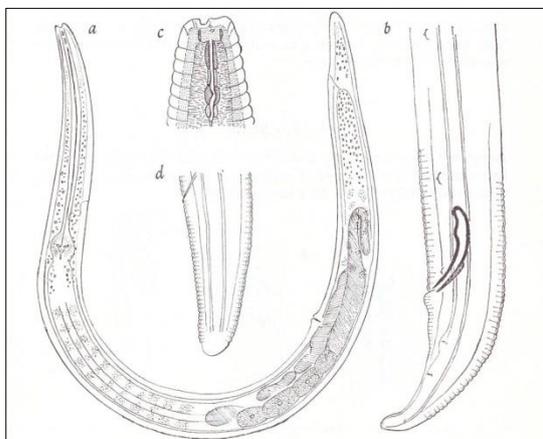
Diplogastridae

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 80. Photo from the University of California, Davis.

## Bacterivores in the c-p group Ba2 Cephalobidae

### Key characteristics

- Very common in soil
- Wide body relative to length
- Distinctly 'broken' stoma
- Basal oesophageal bulb with obvious valves
- Typically round (e.g. *Cephalobus*) or conoid. *Eucephalobus* has pointed tail



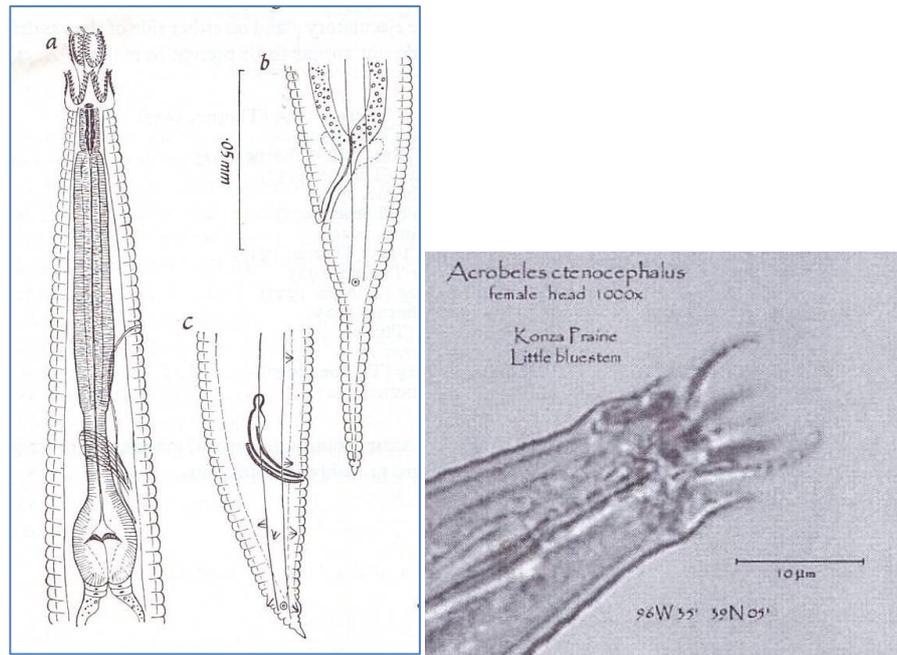
*Cephalobus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 147. Photo from the University of California, Riverside.

## *Acrobeles*

### Key characteristics

- Lips with complex projections



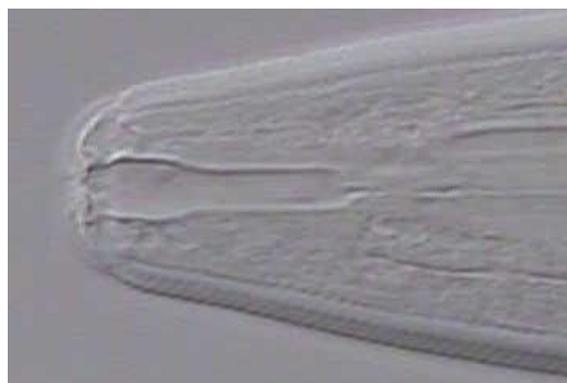
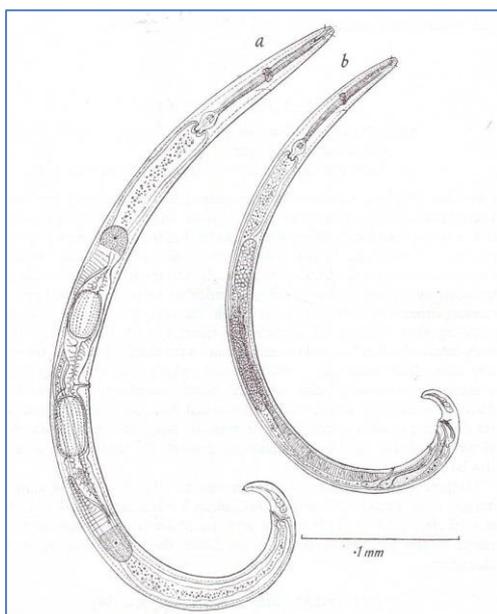
## *Acrobeles*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 155. Photo from the University of Nebraska, Lincoln

## *Plectus*

### Key characteristics

- Setae on head
- Barrel-shaped stoma
- Tail conoid with terminal spinerette
- Tail narrows below the anus



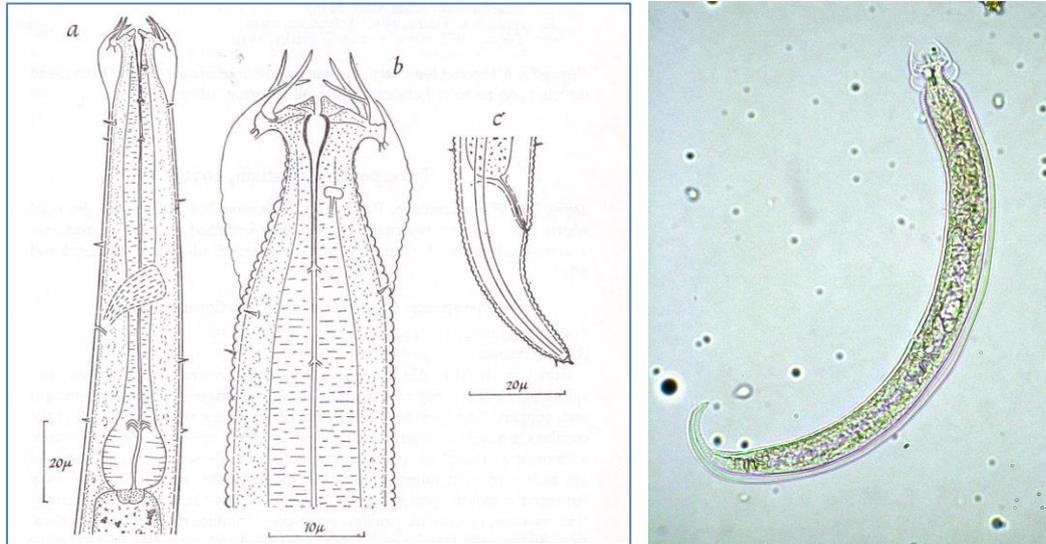
## *Plectus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 158. Photo from the University of California, Riverside.

## *Wilsonema*

### Key characteristics

- A plectid with a body that is similar to *Plectus*. However, the head is unique, as it has obvious flanges on the dorsal and ventral side



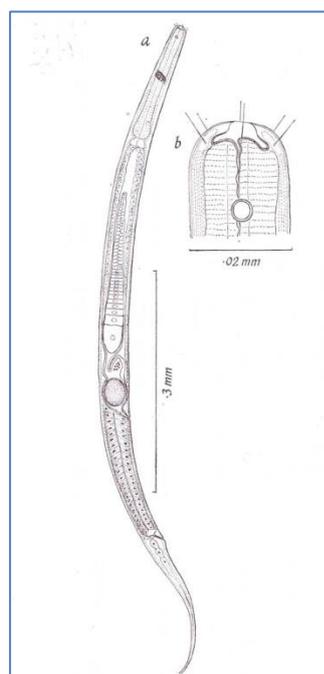
*Wilsonema*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 164. Photo from Jenny Cobon, DAF, Queensland

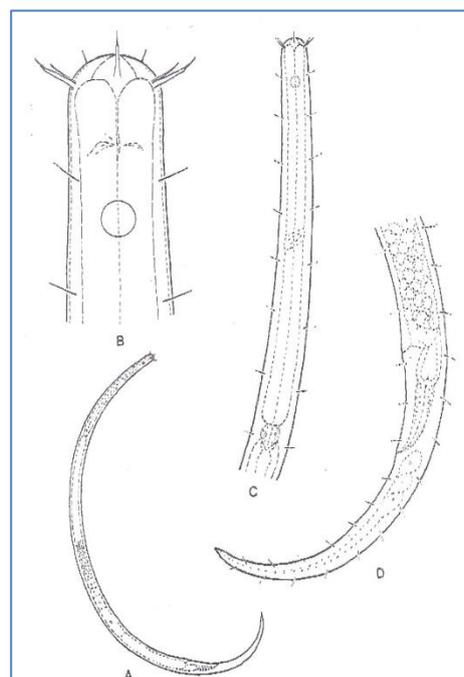
## *Monhystera* and *Geomonhystera*

### Key characteristics

- Small nematodes with shallow stoma and setae on the head
- Prominent circular amphid (can be seen at 400X on some nematodes)
- Cylindrical oesophagus with distinct glands at the junction of oesophagus and intestine
- Uniformly tapering tail
- *Monhystera*: V = 67% of body length.     *Geomonhystera*: V = 80% of body length



*Monhystera*



*Geomonhystera*

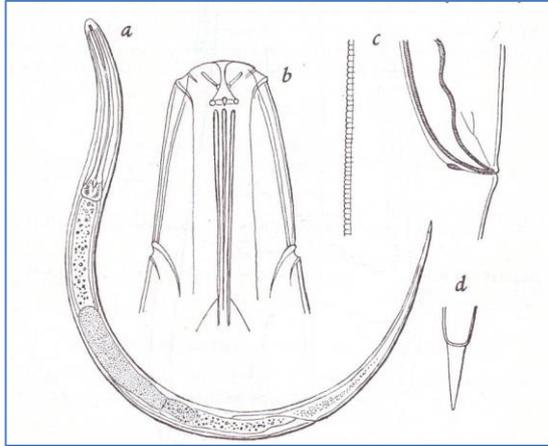
From: Goodey (1963) Soil and Freshwater Nematodes, Fig. 183, and Andrassy (1981) Opusc. Zool. Budapest, 17-18, Fig. 3.

## Bacterivores in the c-p group Ba3

### *Rhabdolaimus*

#### Key characteristics

- Small nematode (<0.5 mm)
- Rounded head with no distinct lips or structure in stoma
- Pear-shaped oesophageal bulb containing valve plates at base of oesophagus
- Tail long and tapering (and is sometimes very long)



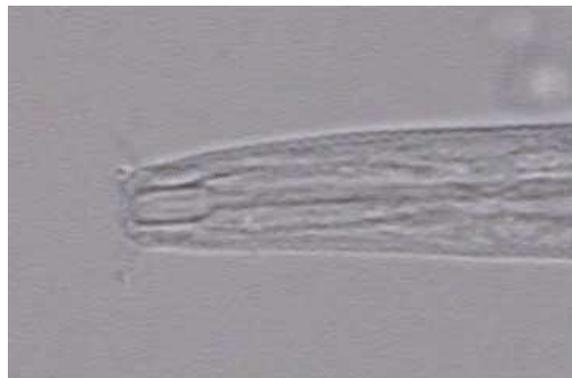
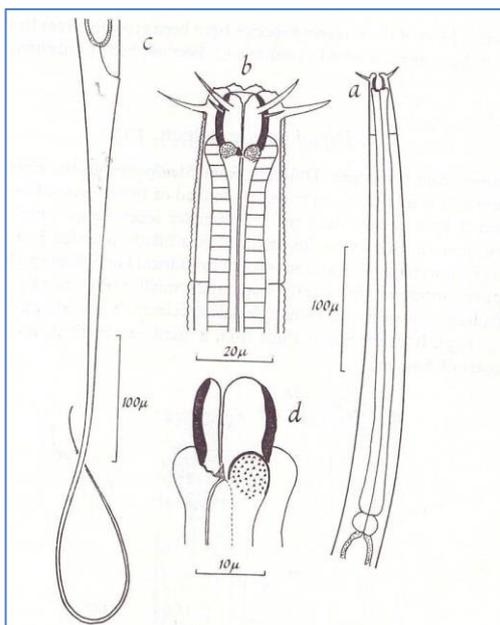
*Rhabdolaimus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 167. Photo from the University of California, Riverside.

### *Prismatolaimus*

#### Key characteristics

- Stoma a distinct, spacious cavity about a head-width wide and long
- Thin setae on head
- Oesophagus cylindrical with obvious cells at oesophageal/intestinal junction
- Very long filiform tail



*Prismatolaimus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 185. Photo from the University of California, Riverside.

## *Teratocephalus*

### Key characteristics

- Head with six well separated lips with strongly cuticularised edges
- Strongly annulated cuticle
- Anterior portion of stoma markedly wider than posterior portion
- Posterior oesophageal bulb and valves weakly developed
- Tail variable at species level (may be slender or conoid)

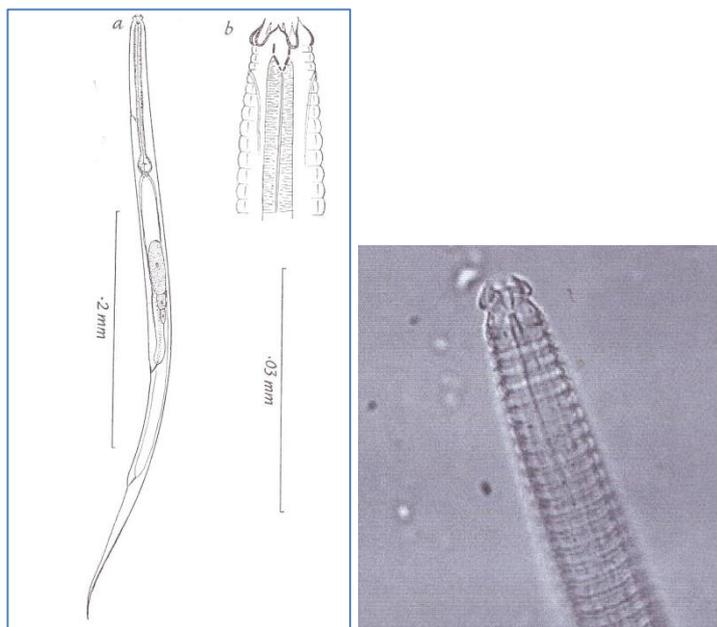


Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 156. Photo from Wageningen University and Research.

## Chromadoridae

### Key characteristics

- Predatory species have cuticularised stoma with teeth
- Setae may be present in head region of predatory species
- Teeth and setae absent in bacterivores
- Spiral amphid
- Spinnerette on tail



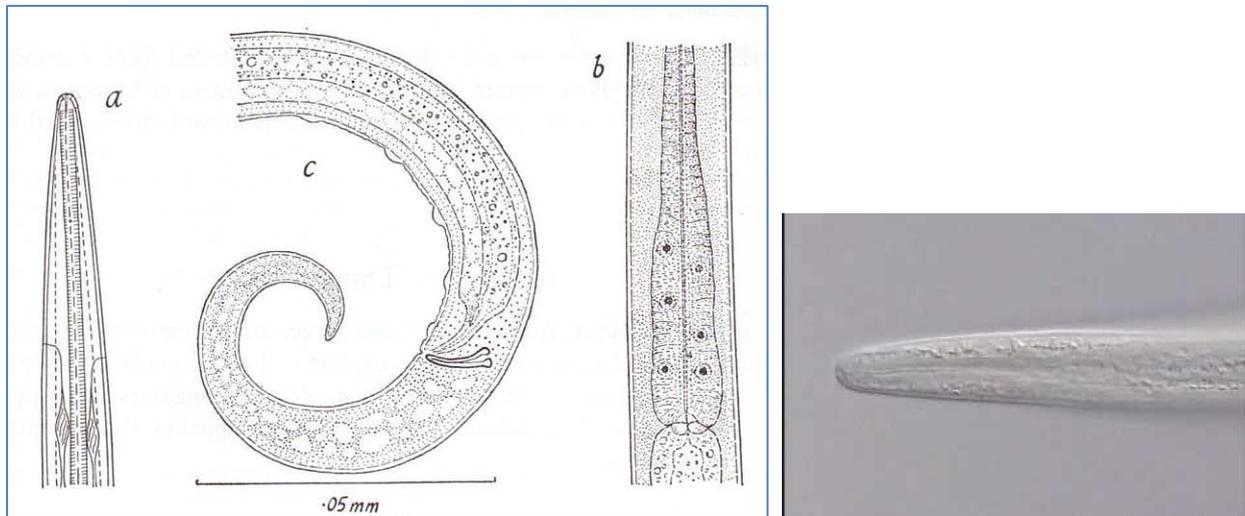
Figure from Yeates (2001) Nematology workshop booklet. Photos from the University of California, Davis

## Bacterivores in the c-p group Ba4

### Alaimidae

#### Key characteristics

- Body long and thin and ventrally curved
- A very small cup-like hole on the head, which leads to the stoma, which is not very obvious
- Oesophagus with weak posterior bulb that is often difficult to see
- Tail curved and pointed



*Alaimus*

Figure from Goodey (1963) Soil and Freshwater Nematodes, Fig. 292. Photo from the University of California, Riverside.

## FUNGIVORES

### *Aphelenchus* and *Apelenchoides*

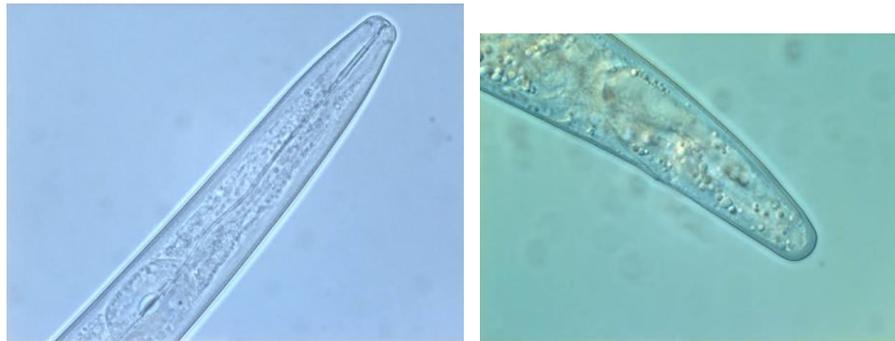
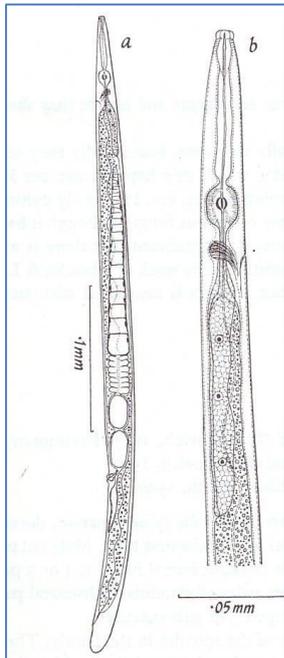
#### Key characteristics

- A strong, squarish median bulb that is obvious at low magnifications
- Relatively weak spear

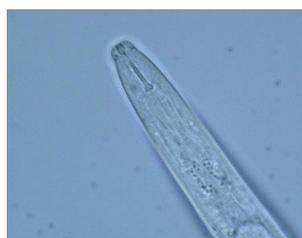
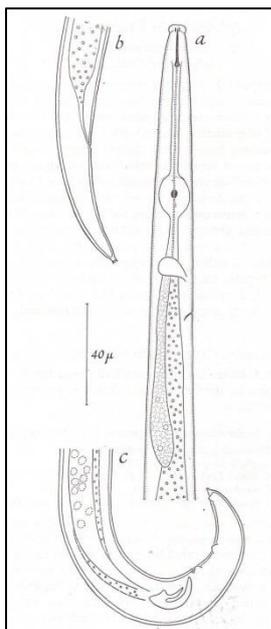
The two genera can be separated by their tail shape.

- *Aphelenchus*: Round tail
- *Aphelenchoides*: Conoid tail

Males are rare in *Aphelenchus avenae*, the most common species in Australia. They are always present in *Aphelenchoides*.



*Aphelenchus avenae*

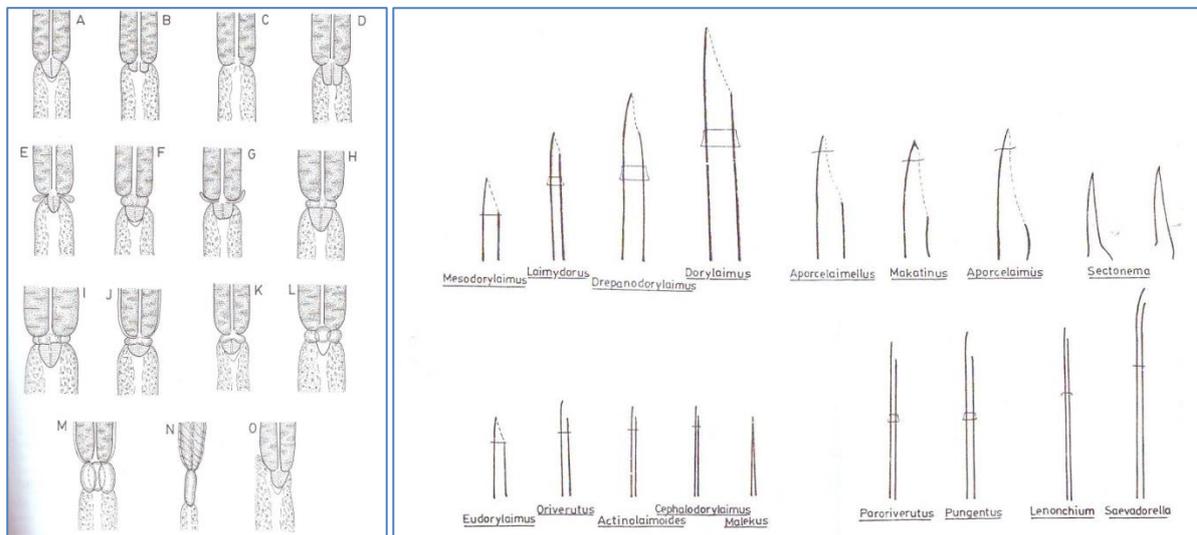
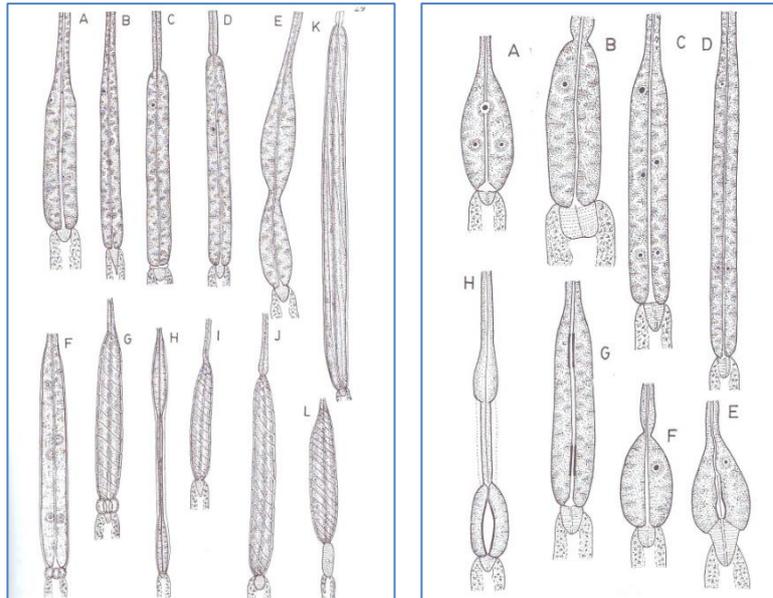


*Aphelenchoides*

## Order DORYLAIMIDA

Most omnivores are dorylaims. However, it is important to remember that two plant-parasitic nematodes (*Xiphinema* and *Paralongidorus*) are also members of the Dorylaimida.

Anyone familiar with *Xiphinema* will be aware of its bottle-shaped oesophageal structure (a narrow cylindrical anterior section with an expanded muscular cylindrical bulb at the base). All dorylaims have a similar type of oesophagus, but it varies markedly in different genera. The oesophago-intestinal junction is also quite variable (see figures below). These nematodes have a feeding spear (odontostyle), but as the figure below shows, it varies in length, width and structure. The odontophore (often referred to as a spear extension) also varies between genera.



From: Jairajpuri and Amad (1992). Dorylaimida. Free-living, Predaceous and Plant-parasitic Nematodes. Figs. 9, 15, 16 and 17.

Because of the huge diversity within the Dorylaimida, considerable taxonomic expertise is required to identify these nematodes to genus level. Thus, in ecological studies, they are sometimes grouped into the four categories, and they are covered on the two pages which follow.

## OMNIVORES

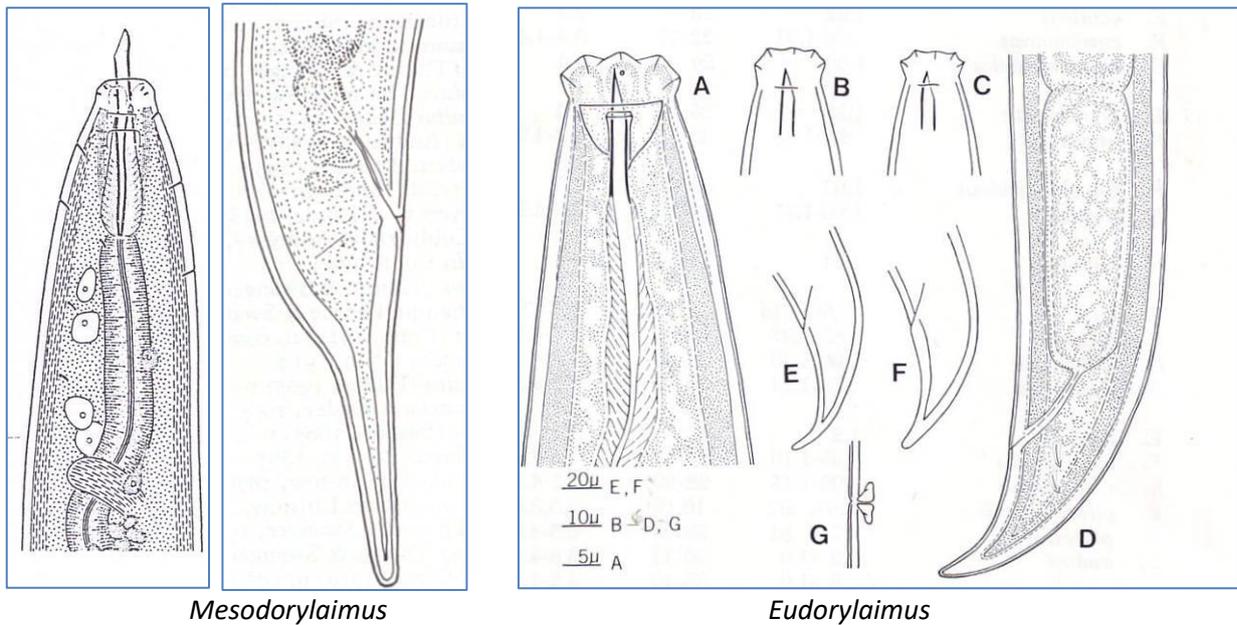
### *Mesodorylaimus* and *Eudorylaimus*

#### Key characteristics

- Bottle-shaped oesophagus (the bulb about half the total length of the oesophagus)

The two genera can be separated by the position of the tail and the tail shape

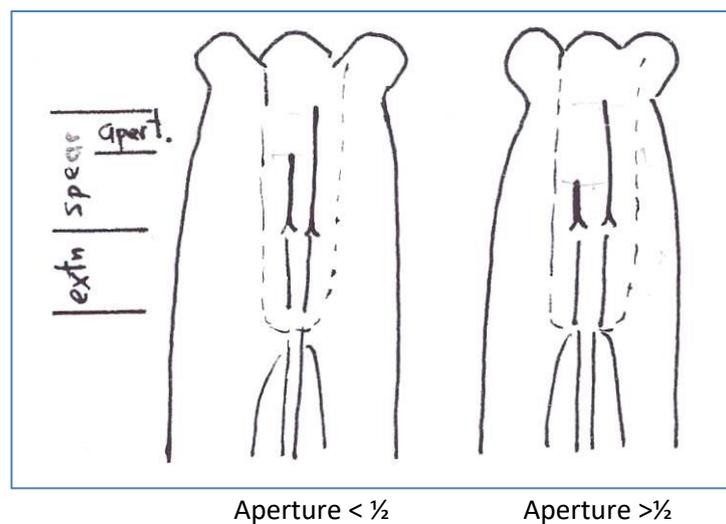
- *Mesodorylaimus*: tail projected along the ventral line
- *Eudorylaimus*: round or conoid tail



*Mesodorylaimus*

*Eudorylaimus*

**Note:** One useful taxonomic character to separate the dorylaims is the length of the aperture of the spear relative to the total length of the spear (see below). In both *Mesodorylaimus* and *Eudorylaimus*, the aperture is less than half the length of the spear



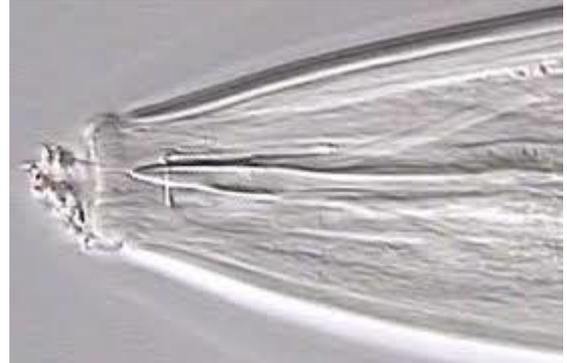
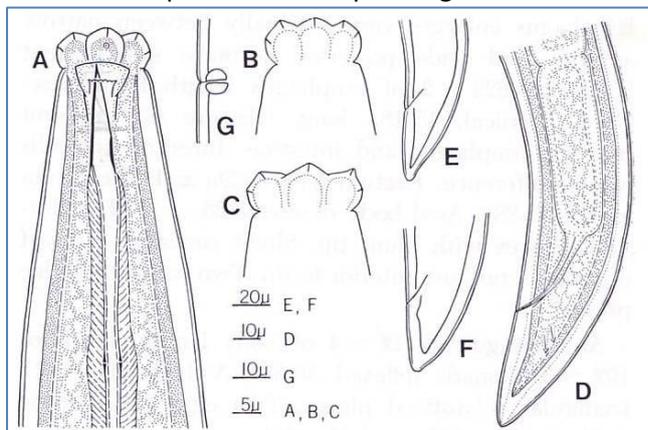
Aperture < ½

Aperture > ½

## *Aporcelaimellus*

### Key characteristics

- Similar to *Mesodorylaimus* and *Eudorylaimus* but a much more robust nematode
- The aperture of the spear is greater than half the length of the spear



*Aporcelaimellus*

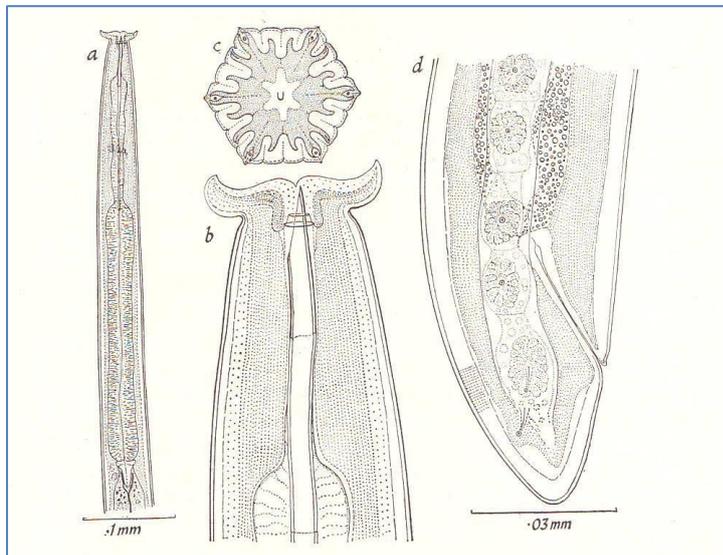
Figure from Tjepkema et al. (1971) Purdue Research Bulletin No. 882.

Photo from the University of California, Riverside

## Discolaimidae

### Key characteristics

- A typical dorylaim, but the lips are extended and much wider than the neck



*Discolaimus*

From: Goodey (1963) Soil and Freshwater Nematodes, Fig. 229. Photo from Wageningen University and Research.

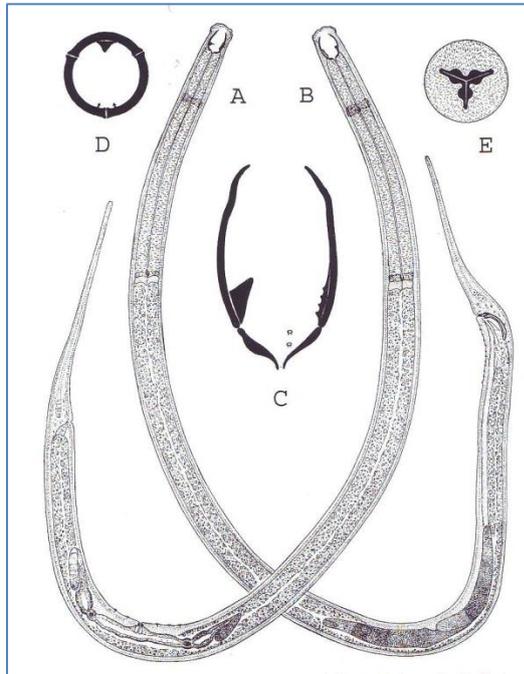
## CARNIVORES

Two groups of predatory nematodes are found in soil. When the nematodes are alive and in water, one common characteristic is that they move very rapidly.

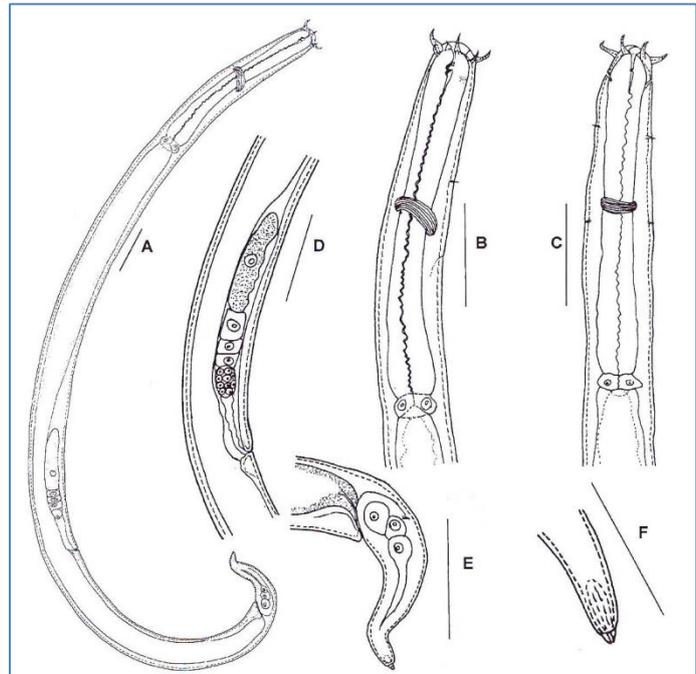
### Mononchidae

#### Key characteristics

- A cuticularised, barrel-shaped open stoma
- Stoma armed with one or two obvious teeth
- Cylindrical oesophagus



Mononchida



Tripylidae

From: Ahmad and Jairajpuri (2010) Mononchida. Predatory Soil Nematodes, Fig. 6.  
*Tripylina* (family Tripylidae) from Zhao (2009) Zootaxa 2238, 1-24, Fig. 3

### Tripylidae

#### Key characteristics

- Simple tube-like stoma with one tooth that is not very obvious
- Cephalic setae present
- Cylindrical oesophagus
- Well-developed oesophago-intestinal junction
- In a dish of water, living nematodes move quickly but the tail is often attached to the bottom of the dish