PLANT AND SOIL NEMATODES

# IDENTIFICATION OF PLANT-PARASITIC NEMATODES TO GENUS LEVEL

**This booklet was prepared for people with limited nematological experience who are extracting nematodes from soil or plant material for diagnostic purposes and need to identify the plant -parasitic nematodes to genus level using a dissecting microscope or low magnifications of a compound microscope**

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Other nematology fact sheets in this series can be accessed at: https://www.appsnet.org/nematodes

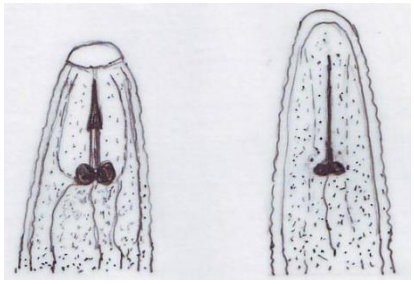
**KEY MORPHOLOGICAL FEATURES OF PLANT-PARASITIC NEMATODES VISIBLE AT LOW MAGNIFICATIONS**

This booklet has been prepared for beginners who wish to identify (to genus level) the plant-parasitic nematodes present in a nematode suspension. It focuses on the limited number of features that are visible at low magnification.

**Stylet**

The stylet is the first feature to look for, as all plant parasites have a stylet.

- Most plant parasites are in the family Tylenchidae and one of the main features of their stylet is that it has knobs
- In most cases, tylenchid stylets are greater than 15 µm long and are easy to see at 40X magnification
    - Lesion and stunt nematode are good examples. Both have stylets about 16 µm long, but the stylet of lesion nematode is stronger and has rounded knobs. The stylet of stunt nematode is not as strong and its knobs are slightly flattened

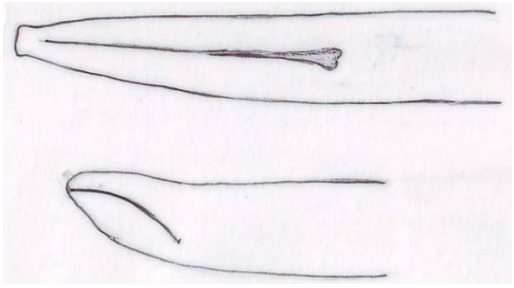
Lesion (*Pratylenchus*)  Stunt (*Tylenchorhynchus*)

- Of the plant parasites, root-knot nematode (*Meloidogyne*) has the smallest stylet (about 10 µm). It is just visible at 40X and can readily be seen at 100X
- If a very small stylet (<8 µm long) is present, the nematode is probably a fine-tailed Tylenchidae. A stylet of this size can only be seen at 100X. Although these tylenchids feed on plants and are in the same family as most plant parasites, they are considered plant associates

One group of plant parasites are in the Order Dorylaimida rather than the family Tylenchidae

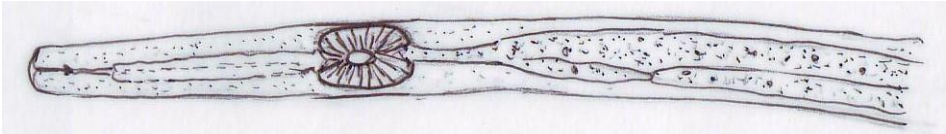
- Stubby root nematode (*Paratrichodorus*) has a curved spear that is often missed by people who are not familiar with nematodes
- Dagger nematode (*Xiphinema*) and needle nematode (*Paralongidorus*) are very long nematodes. They both have a long and obvious spear but dagger nematode has a flanged spear extension (i.e. the structure of the spear differs from the Tylenchidae).

Dagger



Stubby

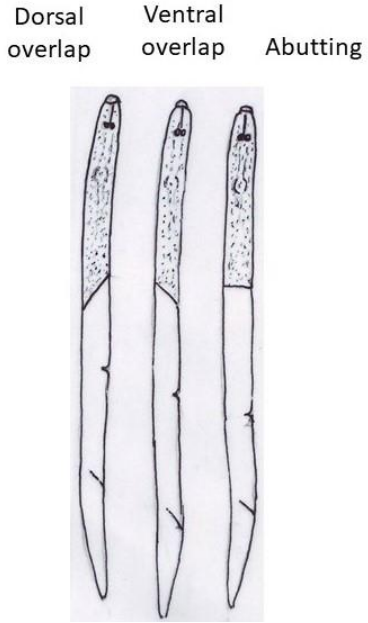
Nematodes which feed on fungi (e.g. *Aphelenchus* and some species of *Aphelenchoides*) also have a stylet. However, they are easy to differentiate from other groups of nematodes because they have an obvious median bulb that is squarish in shape



Once a plant parasite has been found, the following features will help identify it to genus level

**Oesophageal/intestinal junction**

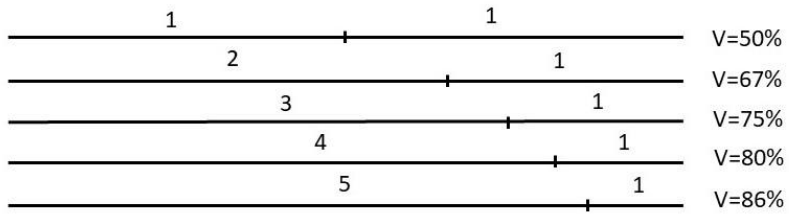
The oesophagus at the anterior end of the nematode is light in colour whereas the intestine is usually dark. It is important to be able to determine whether the oesophagus abuts the intestine or overlaps the intestine ventrally or dorsally. To determine the ventral side, look for the vulva of the female or the copulatory organs (spicules) of the male. If the nematode is a juvenile, it will normally bend inwardly to the ventral side.



**Presence of females and males, and the position and morphology of reproductive organs**

If juveniles are present, but there are no females or males, then the nematode is probably one of the sedentary endoparasites (e.g. root-knot or cyst nematode). In such cases, features of the juvenile (e.g. length, size of the stylet, tail shape) can be used to identify the nematode.

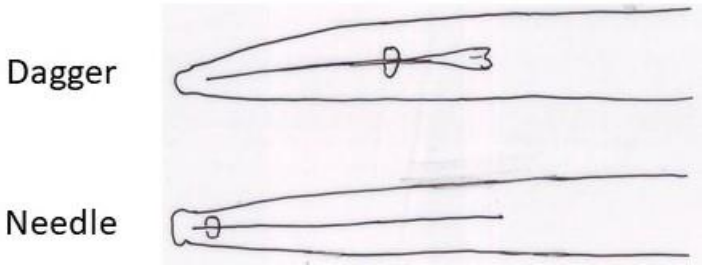
For females, the position of the vulva is important. This can be estimated as a percentage by measuring the length from the lips to the vulva and the length from the vulva to the tail tip, as in the examples below. 1:1 = 50%; 2:1 =67%; 3:1 = 75%; 4:1 = 80%; 5:1 = 86%.




If males are absent, this should be noted, as males are rare in some nematode species. If present, the size and structure of the bursa is sometimes a useful character. The bursa can be seen at the posterior end of the nematode and is used to grasp the body of the female during copulation.

**Guide ring**

Dagger nematode (*Xiphinema*) and Needle nematode (*Paralongidorus*) can be separated by the position of the guide ring (posterior v. anterior)




**Other useful characters**

- The size of the nematode (length and width), tail shape, type of movement, and the shape of a dead nematode are other useful features. Be observant and try to note all these characteristics.

**Removing single nematodes from a suspension in water for morphological or molecular identification**

- Many types of handling needle can be used to remove a nematode from water. One option is to use an eyebrow hair or cut a bristle from a paintbrush or toothbrush and glue it onto a bamboo skewer. Another option is to obtain a dentist's pulp probe
- Place the nematodes in shallow water in a counting slide or Petri dish and view them at low magnification under a stereomicroscope.
- Use the bristle on the handling needle to lift the nematode to the surface of the water. Then hold the bristle horizontally underneath the nematode and move it up quickly to break the surface tension and pull the nematode out of the water
- To remove the nematode, place the bristle in a drop of water on a slide, or if the nematode is required for molecular work, into an Eppendorf tube

**Killing nematodes with fixative so they can be viewed on a slide**

To observe nematodes at magnifications greater than 100X, they must be killed in a way that does not destroy their internal structures

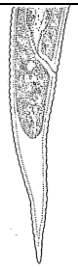
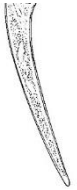
- Several fixatives can be used but one of the most common is formalin (40% formaldehyde). As formalin is a hazardous substance, ensure that appropriate precautions are taken so minimise exposure
- Prepare 4% formalin by adding 10 mL formalin to 90 mL water
- Transfer the nematodes to a drop of water in a glass cavity block and then add a drop of hot 4% formalin

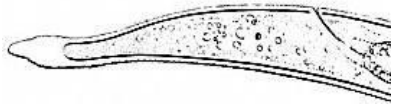
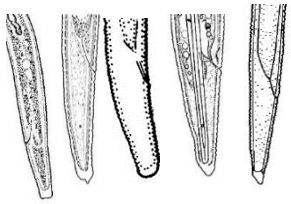
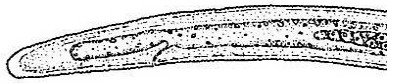
**Preparation of a temporary slide**

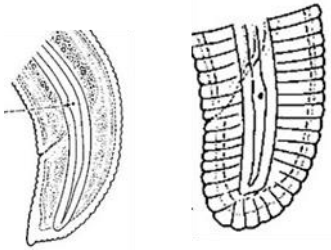
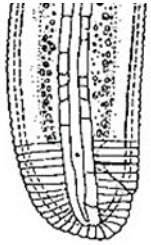
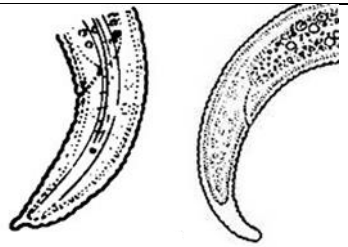
- Heat a metal tube or glass test tube (about 15 mm diameter) and use it to place a ring of paraffin wax on a clean slide
- Add a small drop of fixative to the middle of the ring and then transfer some fixed nematodes into the fixative
- Place a cover slip over the drop and wax ring
- Heat the slide briefly until the wax melts and then place it on a cool surface to allow the wax to set. The nematodes should remain in the clear area of fixative, which will be surrounded by wax
- Ring the coverslip with nail varnish

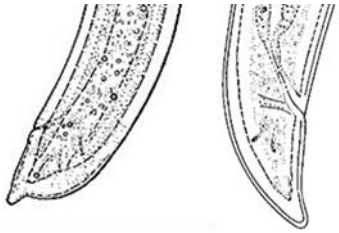
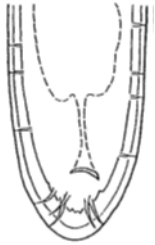
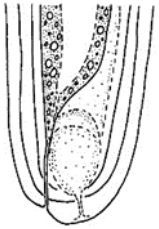
**Preparation of permanent slides in glycerol**

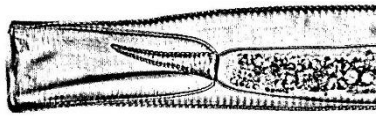
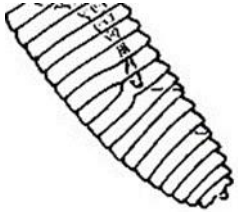
- Kill the nematodes with 4% formalin and allow them to remain in the fixative for a few days
- Transfer the nematodes into a glass cavity block containing 20 mL ethanol, 1 mL glycerol and 79 mL water
- Partly cover the cavity block and place it in a Petri dish. Allow the ethanol and water to evaporate slowly.
- After a few days, decrease the volume, if necessary, and then top up the cavity block with a solution of 5 parts glycerol and 95 parts ethanol. Then place it in a Petri dish as before.
- After the ethanol has evaporated, add pure glycerol
- The nematodes are then ready to be mounted on a slide. This is done in the same way as above, except that a drop of glycerol rather than fixative is placed in the middle of the wax ring

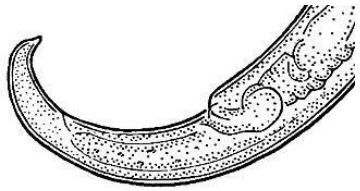
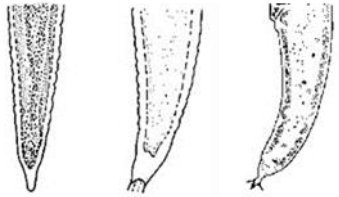
| **COMMON NAME** | **ROOT-KNOT** | **CYST** | **CITRUS** |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Meloidogyne*** | ***Heterodera, Globodera*** | ***Tylenchulus*** |
| **COMMON SPECIES** | Warm climates: *M. javanica, M. incognita, M. arenaria*.<br>Cool climates: *M. hapla, M. fallax* | Found mainly in cool climates. *H. avenae* (cereals), *H. schachtii* (vegetables), *H. trifolii* (clovers).<br>*G. rostochiensis* (potato) is a quarantine pest in Victoria | *T. semipenetrans* |
| **TYPE OF FEMALES** | Sedentary. Saccate female | Sedentary. White females and cysts obvious on root surface. | Saccate, semi-endoparasitic, sedentary females |
| **PRESENCE OF MALES** | Males rare. About 2 mm long (much larger than juveniles) | Males rare or abundant, depending on species | Males common. Have a degenerate stylet. No bursa |
| **SIZE** | J2 about 0.5 mm long | J2 0.5-0.6 mm long | J2 and males 0.4 mm long |
| **STYLET LENGTH** | J2 stylet about 10 µm long and very fine | Obvious stylet (20-28 µm) | J2 stylet about 13 µm long |
| **OESOPHAGUS** | Ventral overlap | Ventral overlap | Abutting |
| **VULVA** | Absent in J2 | Absent in J2 | Absent in J2 |
| **TAIL SHAPE** |  <br>Conoid. Tail tip often held at an angle |  <br>Elongated conical with pointed terminus |   <br>Male tail Female tail |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Move very slowly. J2 often have an empty, hungry look (i.e. dark/clear sections in intestine). Similar to fine-tailed tylenchids but with a longer stylet | J2 broader than *Meloidogyne* and has a longer and stronger stylet | Similar to *Meloidogyne*, but slightly shorter. A stronger stylet and an abutting oesophagus |
| **COMMENTS** | A common and very important pest. Adult females are sedentary and can be found within root galls. Egg masses (about 1 mm in diameter) will be present on galled roots. | A very important pest of cereal and some vegetable crops | J2 and males common in citrus soils, often in high numbers. Also hosted by grapevine.<br>Causes slow decline of citrus |

| **COMMON NAME** | **BURROWING** | **LESION** | **STUNT** |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Radopholus*** | ***Pratylenchus*** | ***Tylenchorhynchus, Merlinius*** |
| **COMMON SPECIES** | *R. similis* | *P. brachyurus, P. coffeae, P. goodeyi, P. neglectus, P. penetrans, P. thornei, P. vulnus, P. zeae* | *T. annulatus, M. brevidens*<br>Lateral fields marked by 4 incisures, but *Merlinius brevidens* has 6 incisures |
| **TYPE OF FEMALES** | Vermiform | Vermiform | Vermiform |
| **PRESENCE OF MALES** | Sexual dimorphism. Males have a degenerate spear and offset head. | Males rare in some species | Males in some species |
| **SIZE** | Adults - 0.8 mm | Adults - 0.7 mm | Adults - 0.7 mm |
| **STYLET LENGTH** | 14 µm | 16 µm | 17 µm (similar to *Pratylenchus*, but not as strong and with knobs more flattened) |
| **OESOPHAGUS** | Dorsal overlap | Ventral overlap | Abutting |
| **VULVA** | 52% | Approx. 67-85% (position varies and is an important characteristic at species level) | 54% |
| **TAIL SHAPE** |  <br>Clavate tail shape | Tail shape varies with species and is an important species characteristic<br>  |  <br>Broadly rounded |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Similar to *Pratylenchus*, but differs in dorsal overlap of oesophagus and V ≈ 50% | Short, strong stylet. Posterior vulva. Squarish head with strong lip region | Similar to *Pratylenchus*, but with roundish head, flattened stylet knobs, abutting oesophagus and median vulva |
| **COMMENTS** | Migratory endoparasite. Wide host range but found mainly on bananas. An important pest. | Migratory endoparasite. Common in soil and root samples on many crops | Ectoparasite. Common, particularly on turf and grasses |

| **COMMON NAME** | **SPIRAL** | **SPIRAL** | **RENIFORM** |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Helicotylenchus*** | ***Rotylenchus*** | ***Rotylenchulus*** |
| **COMMON SPECIES** | Several species, including *H. dihystera* and *H. multicinctus* | *R. brevicaudatus* is common in coastal Qld. and NSW | *R. parvus, R. reniformis* |
| **TYPE OF FEMALES** | Vermiform | Vermiform | Saccate females |
| **PRESENCE OF MALES** | *H. dihystera* - males absent<br>*H. multicinctus* - males present | Males common<br>Obvious bursa envelopes tail | *R. parvus* - males absent/rare<br>*R. reniformis* - males common.<br>Spear & oesophagus degenerate |
| **SIZE** | Adults - 0.8 mm | Adults - 0.8 mm | Small (0.4 mm) |
| **STYLET LENGTH** | 25 µm. Large, strong spear.<br>Lips offset and obvious | 25 µm. Large, strong spear | 12-18 µm |
| **OESOPHAGUS** | Slight ventral overlap | Slight dorsal overlap | Slight ventral overlap |
| **VULVA** | 63% | Obvious vulva 57% | 60-70% in immature females |
| **TAIL SHAPE** |  <br>*H. dihystera* Digitate *H. multicinctus* Rounded |  <br>Broadly rounded |  <br>*R. parvus* *R. reniformis* |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Forms a spiral shape when dead. *H. multicinctus* less likely to form a tight spiral when dead & more likely to be alive when counted | Forms a 'C' or spiral shape when dead | Body often assumes a 'C' shape. Adult female is kidney shaped, but only immature females present in soil. |
| **COMMENTS** | *H. dihystera* is one of the most common nematodes in Australia. Often in high numbers but not a pathogen. *H. multicinctus* can be found on bananas. It is important, but not widespread | Looks similar to *Helicotylenchus* but has dorsal overlap of oesophagus. Males have an obvious bursa | Adult female is sedentary and semi-endoparasitic. Male is non-parasitic. Common in north QLD<br>*R. parvus* - sugarcane<br>*R. reniformis* – cotton, pineapple & other crops |

| **COMMON NAME** | **DAGGER** | **NEEDLE** | **STUBBY-ROOT** |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Xiphinema*** | ***Paralongidorus*** | ***Paratrichodorus*** |
| **COMMON SPECIES** | Many species, including *X. americanum* and *X. elongatum* | *P. eucalypti* | Several species but *P. minor* is widespread |
| **TYPE OF FEMALES** | Vermiform | Vermiform | Vermiform |
| **PRESENCE OF MALES** | Males in some species | Males in some species | Males occur in some species. If males are present, spicule is reduced & bursa is present |
| **SIZE** | Large nematode - adults 2-4 mm long | Very long nematode. Adults 5-10 mm | Adults 0.8 mm, but diameter greater than nematodes of similar length |
| **STYLET LENGTH** | Long stylet (≈100 m) with basal flanges. Guide ring posterior (at base of stylet). Juveniles have developing stylet behind flanges | Long stylet (> 100 µm). Anterior guide ring. | Stylet is 30 µm long, but is bent ('C'-shaped) with no knobs |
| **OESOPHAGUS** | Dorylaimoid (bottle shaped) oesophagus | Dorylaimoid | Dorylaimoid |
| **VULVA** | Variable | Median | 55% |
| **TAIL SHAPE** |  Variable |  Tail is usually round |  Broadley rounded |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Large nematode with long stylet and posterior guide ring | Very long nematode with long stylet and anterior guide ring | 'C' shaped stylet. Thick loose cuticle on some specimens |
| **COMMENTS** | Ectoparasite. Tends to be most common in soil around perennial crops | Ectoparasite. Mainly found in the tropics | Ectoparasite. Common, particularly on turf and grasses |

| COMMON NAME | SHEATH | RING | STING |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Hemicycliophora*** | ***Criconemella, Macroposthonia, Mesocriconema, Ogma*** | ***Ibipora*** |
| **COMMON SPECIES** | | *M. xenoplax* | *I. lolii* |
| **TYPE OF FEMALES** | Vermiform | Vermiform | Vermiform |
| **PRESENCE OF MALES** | Males - rare, without double cuticle and stylet. | Males in some species | Males common |
| **SIZE** | Females - 0.5 mm, enclosed in a double cuticle attached at head, vulva, and tail. Known as a 'sheath' | Stout, fat, heavily annulated nematodes | About 2.5 mm long |
| **STYLET LENGTH** | Long - 90 m | 70-80 m (long obvious spear) | Long, obvious spear (110 µm) |
| **OESOPHAGUS** | Abutting | Abutting | Abutting |
| **VULVA** | 90% | >90% | 52% |
| **TAIL SHAPE** |  Folded skirt of second cuticle partially of fully engulfs tail |  Heavily annulated tail | Round tail |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Double cuticle, folded skirt and long stylet characteristic of juveniles and females | Very sluggish<br>Heavy annulation | Large nematode with a long stylet, pronounced lips and an obvious median bulb |
| **COMMENTS** | Ectoparasite. Most common on turf grasses | Ectoparasite. Common but not extracted efficiently with tray methods due to its inactivity. | A serious pest. Widespread on turfgrass in some areas of NSW and WA. Has been reported from some other states |

| **COMMON NAME** | **PIN** | **LEAF** | |
|---|---|---|---|
| **SCIENTIFIC NAME (genus)** | ***Paratylenchus, Gracilacus*** | ***Aphelenchoides*** | |
| **COMMON SPECIES** | | *A. fragariae, A. ritzemabosi, A besseyi* | |
| **TYPE OF FEMALES** | Vermiform | Vermiform | |
| **PRESENCE OF MALES** | Males in some species | Males common. 'Rose thorn' spicules | |
| **SIZE** | Short (0.5 mm) | Medium sized but slender nematodes - 0.8 mm | |
| **STYLET LENGTH** | *Paratylenchus*: Long, obvious stylet (25 µm)<br>*Gracilacus*: Stylet >30 µm | 10 µm. Knobs difficult to see. | |
| **OESOPHAGUS** | | Aphelenchoid. Squarish median bulb that is very obvious | |
| **VULVA** | Always near posterior end (>80%) | 70% | |
| **TAIL SHAPE** |  |  <br>*A. f* *A. r* *A. b* | |
| **MAIN DIFFERENTIATING CHARACTERS FOR GENUS** | Body narrows distinctly after the vulva (see above) | Fast moving | |
| **COMMENTS** | Ectoparasite. Not very damaging unless present in very high numbers | Ectoparasitic and endoparasitic in above ground tissue.<br>*A. fragariae* - strawberry, ferns<br>*A. ritzemabosi* – chrysanthemum<br>*A. besseyi* - strawberry, grasses | |