Chapter 3

Methodology, morphology and identification

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Methodology

NEMATODE PREPARATION FOR LIGHT MICROSCOPY

Nematode rearing and collection

For taxonomic studies, ten Galleria mellonella larvae are exposed, in a Petri dish (100 × 15 mm) lined with two moistened filter papers, to 2000 infective juveniles (IJ) of a Steinernema species or 200-300 IJ of a Heterorhabditis species. First and second generation adult nematodes are obtained by dissecting infected insects 2-4 days and 5-7 days, respectively, after the host has died. Dissection should be done in Ringer’s solution or 1% saline (NaCl) solution so as to avoid excessive osmotic stress, particularly to the adult stages. Third-stage infective juveniles are obtained when they emerge from the cadavers after 7-10 days.

The recommended infection densities for Steinernema and Heterorhabditis are based on personal experience of the effect of initial inoculation density on morphometrics of the harvested stages (Nguyen, unpubl.). High inoculation densities can result in effects on nematode morphometrics that are significant enough to impact upon accurate species diagnosis.

Observations

All observations and measurements should be performed within a week after collection. For light microscope observation, at least 20 males
and females and infective juveniles are examined live. Additional specimens of different stages may be killed in hot water (60°C) and fixed in TAF (Courtney et al., 1955) or in lactophenol (Franklin & Goodey, 1949). These nematodes can be used further when more observation is needed to confirm the morphology or variation of some structures. Nematodes preserved in different fixatives will assist observation of certain characters. Nematodes fixed in TAF are processed to glycerin by the Seinhorst method (Seinhorst, 1959). Type specimens should always be processed to, and mounted in, glycerin. Cover glass supports are mandatory so as to avoid squashing and flattening the nematodes.

For a microscope with differential interference contrast optics, nematodes processed to glycerin result in the best observation and photographs. Structures to be observed are mentioned in the Morphology section (see p. 68).

Measurements

Either fixed or living nematodes may be measured, although it is essential to state which. A device such as a drawing tube is useful for this step. Experience has shown that it is better to work with fixed adults and living infective juveniles. Living infective juveniles can be mounted in tap water on a glass slide. Cover glass supports must be used to avoid flattening specimens. The slide is sealed with paraffin wax, nail polish or other sealants. The nematodes become immobile after about 20-30 min. If a nematode was actually collected from a host insect (i.e., not by baiting with Galleria), measurements of the nematode reared from the natural host should be collected in order to allow comparison with measurements of the same nematode reared in Galleria.

Nematode Preparation for Scanning Electron Microscopy

Working with fixed nematodes

Adults and IJ fixed by any method can be used for SEM. Transfer nematodes to a working chamber (Fig. 13) in a glass container with 2 ml of 0.1 M sodium cacodylate buffer, or 0.2 M sodium phosphate buffer. Nematodes must be washed five times with this buffer by adding and removing the solution from the glass container. Nematodes should be kept in each change of buffer solution for 10 min. They are post-fixed with 2% osmium tetroxide solution for 12 h at 25°C, washed in five changes of 0.1 M sodium cacodylate buffer for 10 min each change.