

A Simple Technique for Mounting Whole Root-knot Nematode Females

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After extensive investigations of several *Meloidogyne* species, Taylor (4) found that egg-producing females are a reliable nematode life stage to be used for species identification. He noticed that lip regions and excretory pore locations of adult mature females are stable and reliable morphological characters that aid in identification and suggested that the use of these characters in addition to the perineal pattern facilitates more accurate species identification. Most authors of descriptions of new species of *Meloidogyne* also illustrate the anterior portions of adult females, showing lip region profiles, and the location of the excretory pore in addition to the perineal pattern (2,3). This implies that identification based on the morphology of adult mature females should require mounts showing all three characters of the same individual specimen. Taylor also pointed out that about 98% of the *Meloidogyne* populations examined were mixtures of two or more species (4). Therefore, for estimating the incidence of different species in a population, numerous specimens must be mounted for proper identification.

The following procedure for mounting specimens has been used in a survey of *Meloidogyne* species in Florida and has been found practicable and efficient, producing about 70% usable specimens in a minimum of time.

Nematode-infected roots were gently washed free from soil and fixed by heating briefly to the boiling point in a solution of equal parts by volume of glycerine, lactic acid, and distilled water plus acid fuchsin (1). Stain was added by small increments according to the degree of staining desired. Egg-producing females were excised from roots with dissecting needles and transferred to a plastic slide with a few drops of glycerine. The specimens are prepared and mounted as follows:

- 1) A hole is pierced with a fine insect pin (number 0 or smaller mounted on a handle) into the female at midbody to release the body contents (Fig. 1). Very round and voluminous female bodies may need a second hold on the opposite side of the body to prevent body contents from moving to the anterior or posterior part, thus damaging the neck or vulva area (Fig. 1).
- 2) While holding the female down with one dissecting needle, a second needle is used to remove the body contents of the posterior half of the female, especially around the area of the perineal pattern (Fig. 2).
- 3) The female is transferred to a clean area on the slide. A fine scalpel is used to cut away about a quarter of the cuticle close to the perineal pattern without damaging the pattern (Fig. 3).
- 4) The prepared females are transferred to a small drop of glycerine on a glass slide. They are placed in such a way that the cut opening is underneath and in contact with the slide (Fig. 4).
- 5) The coverslip is applied by holding one edge with a needle and releasing it slowly. The slide is sealed with zut or glyceel.

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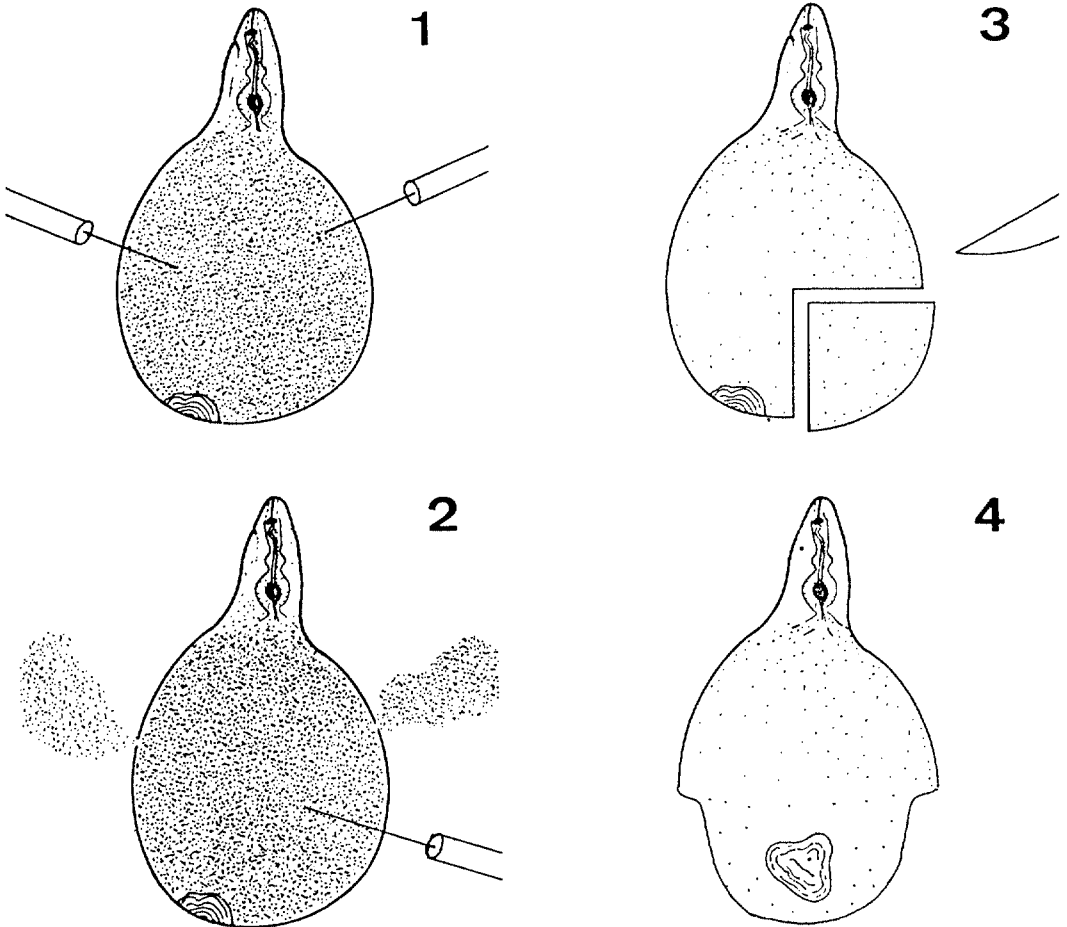


FIG. 1. Piercing female body of root-knot nematode female to remove internal pressure.
 FIG. 2. Removing body content of root-knot nematode female.
 FIG. 3. Cutting root-knot nematode female body to make final mount.
 FIG. 4. Position of root-knot nematode female before the coverslip is placed on it.

With this simple method, other characters in addition to the excretory pore position, lip region, and perineal pattern can be observed from each individual specimen under oil immersion on a compound microscope. Several specimens may be placed on a single slide. Stylet, dorsal gland opening, and details of the perineal pattern can be used for confirmation of species identification.

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