

A Technique for Preparing Perineal Patterns of Root-knot Nematodes for Scanning Electron Microscopy

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The morphology of the perineal pattern in the posterior body region of female root-knot nematodes is one of the most reliable characters for differentiating *Meloidogyne* species. Light microscopy has been used extensively to observe this character. Several recent descriptions of *Meloidogyne* species have shown that the cuticular surface features of patterns are emphasized and better resolved by a scanning electron microscope (SEM).

Few techniques have been described for preparing perineal patterns for SEM observation. Mulvey et al. (4) and Khan and Thames (3) used the whole female to study the perineal pattern, whereas Yik and Birchfield (6) and Eisenback et al. (2) used the excised posterior end. Our objective is to provide a rapid technique for preparing perineal patterns for SEM. Two Portuguese populations (*M. hispanica* Hirschmann and an undescribed *Meloidogyne* sp.) were used in this study.

Perineal patterns were prepared from

live, egg-laying females dissected from fresh root galls. Females were placed in a large drop of 45% lactic acid on a transparent plastic (e.g., perspex) slide, and the perineal patterns were cut and cleaned (5). Up to 10 patterns were then transferred, surface side up, to a drop of 45% lactic acid on a round coverslip (13 mm d), which had been rimmed with a thick ring of glyceel to form a chamber. The chamber was attached to a glass microscope slide by applying a drop of glyceel on the edge. One drop of 2% formalin was then added every 2-3 minutes to the chamber to wash out the lactic acid. After 10 minutes the solution was absorbed with filter paper and the perineal patterns were allowed to dry in a desiccator at room temperature.

The coverslip was detached from the glass slide, mounted onto a SEM stub with double sided adhesive tape, coated with 200 Å of gold, and examined in the SEM.

This technique is an adaptation of the stylet extraction technique described by Eisenback and Hirschmann (1). It is easy to use, it is adequate for perineal patterns (Fig. 1), and it can be used in morphological studies.

Small wrinkles may appear between the lines of the perineal pattern during desiccation from formalin. Because these artifacts could be mistakenly described as new characters, additional light microscopy studies of freshly prepared patterns are recommended, especially for species descriptions.

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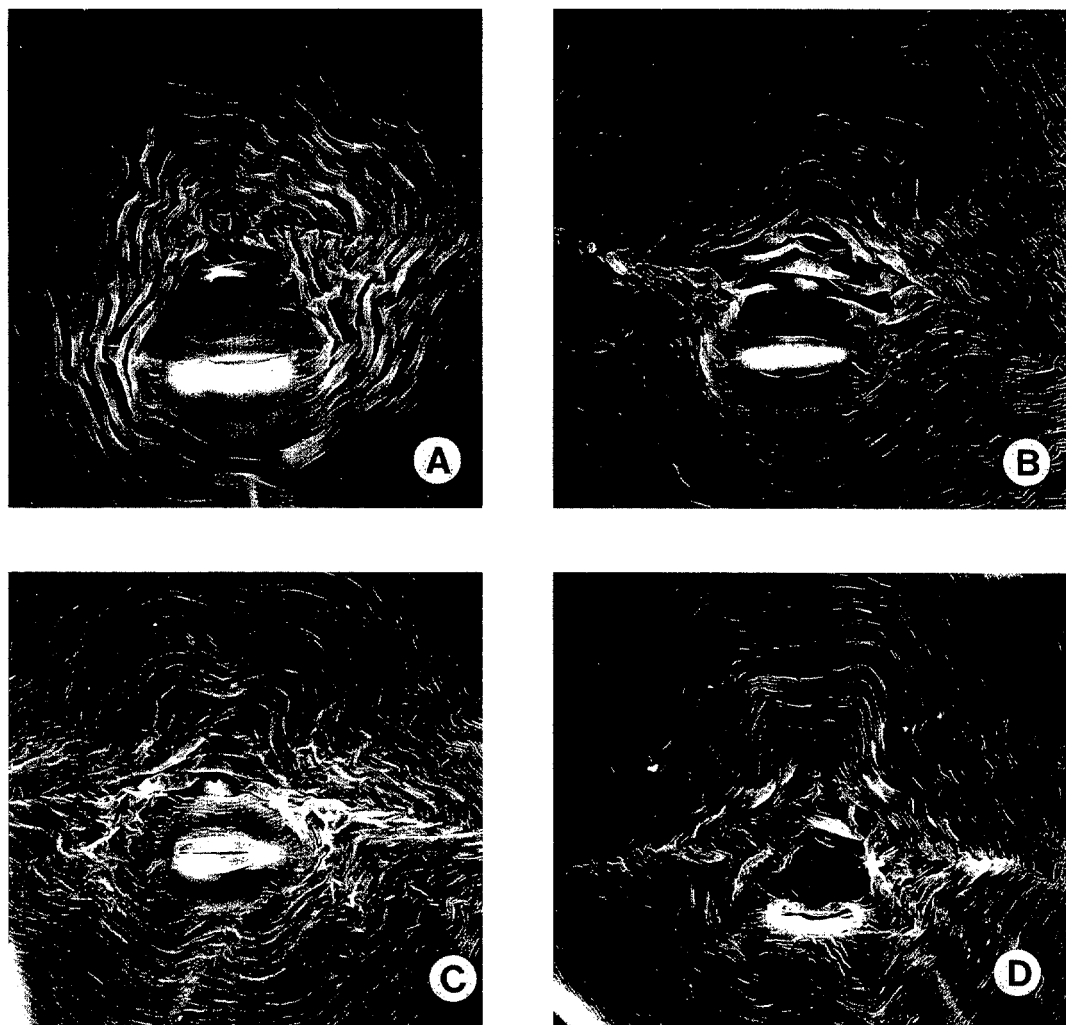


FIG.1. Scanning electron micrographs of female perineal patterns of *Meloidogyne* spp. A) *Meloidogyne hispanica*. B-D) Undescribed *Meloidogyne* species. Scale bars = 20.0 μm .

LITERATURE CITED

1. Eisenback, J. D., and H. Hirschmann. 1982. Morphological comparison of stylets of male root-knot nematodes (*Meloidogyne* spp.). *Scanning Electron Microscopy* 11:837-843.
2. Eisenback, J. D., H. Hirschmann, and A. C. Triantaphyllou. 1980. Morphological comparisons of *Meloidogyne* female head structures, perineal patterns, and stylets. *Journal of Nematology* 12:300-313.
3. Khan, Z. N., and W. H. Thames. 1978. SEM study of perineal patterns of four species of *Meloidogyne*. *Journal of Nematology* 10:292-293 (Abstr.).
4. Mulvey, R. H., P. W. Johnson, J. L. Townshend, and J. W. Potter. 1975. Morphology of the perineal pattern of the root-knot nematodes *Meloidogyne hapla* and *M. incognita*. *Canadian Journal of Zoology* 53: 370-373.
5. Taylor, D. P., and C. Netscher. 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. *Nematologica* 20:268-269.
6. Yik, C. P., and W. Birchfield. 1978. Scanning electron microscopy of perineal patterns of three species of *Meloidogyne*. *Journal of Nematology* 10:118-122.