# Scanning Electron Microscopy of Perineal Patterns of Three species of Meloidogyne

CHOI-PHENG YIK1 and W. BIRCHFIELD2

Abstract: The perineal pattern of Meloidogyne graminicola is oval, characterized by the anastomosing of cuticular striae converging, and elevating at the tail terminus. A deep lateral incisure forming a low arch traverses the rounded perineal pattern of Meloidogyne hapla. Punctations at the tail region of M. hapla are not observed with the scanning electron microscope. Minute crosslines invaginate with the vulvar lips of Meloidogyne incognita. Punctate indentations around the anal opening and bordering the lateral sides of the vulvar slit of M. incognita are revealed. Key Words: Meloidogyne graminicola, Meloidogyne hapla, Meloidogyne incognita, cuticular striae, punctations.

Cuticular markings on the posterior ends of root-knot nematodes are among the most crucial criteria in the identification

Received for publication 31 August 1977.

of *Meloidogyne* spp. Light microscopy has been used extensively to study these perineal patterns (1, 2, 7). Green (3) and Mulvey (4, 5) used the scanning electron microscope (SEM) to study the vulvar cones of cysts and young females of *Heterodera* spp. Mulvey et al. (6) used the SEM to study the perineal patterns of

<sup>&</sup>lt;sup>2</sup> Graduate Research Assistant, Department of Plant Pathology, Louisiana State University, Baton Rouge, Louisiana 70803.

<sup>&</sup>lt;sup>2</sup>Research Plant Pathologist, Agricultural Research Service, United States Department of Agriculture, Baton Rouge, Louisiana 70803.

Meloidogyne hapla and Meloidogyne incognita.

The SEM presents a three-dimensional impression and provides better resolution of cuticular structures of perineal patterns than does light microscopy.

(Kofoid Meloidogyne incognita Chitwood. 1949. White, 1919) and Meloidogyne hapla Chitwood, 1949, are root-knot nematodes commonly parasitizing soybeans, sweet potatoes, and strawberry crops, while Meloidogyne graminicola Golden & Birchfield, 1965, is closely associated with grass weeds in those crops and rice in Louisiana. Supplemental information on the perineal patterns of those three species of root-knot nematodes was obtained from micrographs taken with the SEM in the present study.

## MATERIALS AND METHODS

Root galls caused by M. graminicola on barnyard grass, Echinochloa colonum (L.) Link, galls on strawberry, Fragaria chiloensis Duchesne, caused by M. hapla, and galls on beans, Phaseolus vulgaris L., caused by M. incognita were collected separately from greenhouse cultures. The root galls were

washed, killed, and fixed in cold 1% acid fuchsin lactophenol for 24 to 48 hours.

Mature females of the three species were dissected from their respective galls. Females were transversely bisected, and the posterior halves were trimmed close to the perineal patterns. The posterior ends were placed on 13-mm round cover slips attached off-center to aluminum stubs. The specimens were covered with 200-Å gold coating in a vacuum evaporator operating at 2-4x 10<sup>-5</sup> Torr. The perineal patterns were scanned at 20 KV with an AMR 100 scanning electron microscope and photographed with Polaroid 55 positive/negative film. At least 20 perineal patterns of each species were examined with the SEM.

# RESULTS

Meloidogyne graminicola: The perineal pattern in general is oval or egg-shaped, as shown in Fig. 1-A. There is no lateral line. The cuticular striae are smooth, thick strands in the outer region of the oval pattern. As seen in Fig. 1-B, dorsal to the vulva, the striae anastomose into a tetragonal or pyramidal web that converges at the tail terminus. A semicircular whorl





FIG. 1-(A-B). Scanning electron micrographs of perineal patterns of *Meloidogyne graminicola*. A) Oval shape of perineal pattern. x1000. B) Anastomosing of striae into a tetragonal web (a), converging at the tail terminus (t). Whorls of striae (b) at corners of the vulvar slit, x1800.

consisting of two to three striae is present at each corner of the vulvar slit. All females observed had smooth vulvar lips without invaginations.

Meloidogyne incognita: The general pattern is in the form of a high square arch. Lateral incisures are not consistently observed in the females. However, some females examined had a faint forking of the dorso-ventral cuticular striae, giving the impression of the presence of a lateral line (Fig. 2-A). Cuticular striae are also smooth and thick and appear to be wavy, shorter, and interwoven at the tail terminus. Minute lines perpendicular to the vulvar slit are invaginated into the vulvar lips. In addition, numerous punctate indentations are distributed from the anus to both lateral sides of the vulva (Fig. 2-B).

Meloidogyne hapla: The perineal pattern is round (Fig. 3-A). A deep, arched demarcation (lateral incisure) goes across the perineal pattern, peaking at the tail terminus. Some females examined had the distinct wing on one of the lateral sides (Figs. 3-B, C). Cuticular striae are fine. Fine, minute, undulating, longitudinal folds intersect the striae, giving the perineal

pattern a wrinkled appearance. These folds extend into the lower vulvar lips (Fig. 3-D).

## DISCUSSION

Observations with the SEM showed that the anastomosing of the cuticular striae into a tetragonal web in the central portion of the perineal pattern of M. graminicola is one of its most distinguishing features. Its oval shape further separates it from the round form of M. hapla and the high square arch form of M. incognita.

Fine crosslines invaginating with the vulvar lips of *M. incognita* and the posterior vulvar lips of *M. hapla* are observed. There are punctate indentations bordering the vulva and anus of *M. incognita*. We did not see these crosslines and punctations under the light microscope.

Punctations dorsal to the anal opening that are easily seen with the light microscope are constantly used to identify M. hapla (7). These are not observed with the SEM micrographs on any of the M. hapla specimens we examined. We concur with Mulvey (6) that such structures are subcuticular.





FIG. 2-(A-B). Scanning electron micrographs of perineal patterns of *Meloidogyne incognita*. A) High square arch form of perineal pattern, x1000, B) Indentations (c) bordering lateral sides from anus to vulva and crosslines (d) on vulvar lips. x1400.

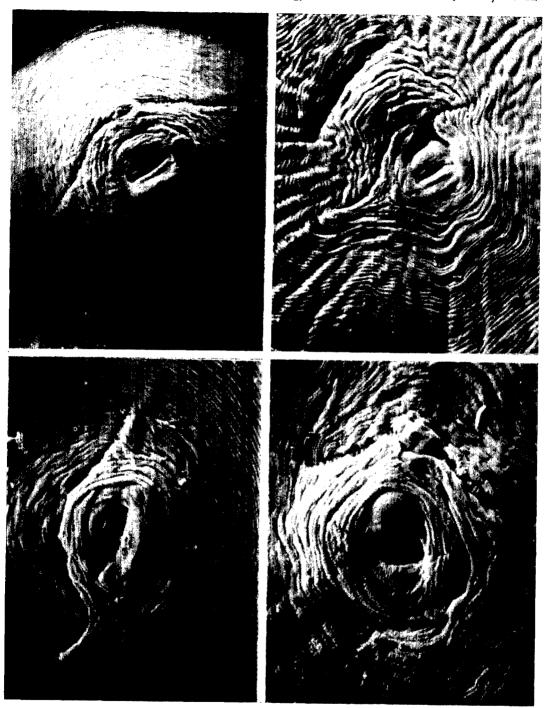


FIG. 3-(A-D). Scanning electron micrographs of perineal patterns of *Meloidogyne hapla*. A) Round form of perineal pattern. x1000. B) & C) Wing appearance  $(\rightarrow)$  on the lateral sides. x600 in B) and x765 in C). D) Lateral incisure (e), crosslines invaginated with the lower vulvar lips (f) and longitudinal folds intersecting the cuticular striae (g). x1800.

The probability that the perineal patterns of root-knot nematodes are the same in any given species is doubtful. However, a typical perineal pattern of the species prevails. Our SEM study of M. graminicola, M. hapla, and M. incognita showed distinctive patterns unique to each species in conformation with patterns observed under the light microscope. Further, the SEM revealed finer morphological features such as punctate indentations, lip striae, and undulating transverse folds not previously reported with light microscopes. Use of the SEM in conjunction with the light microscope will aid the identification of root-knot nematode species, subspecies, and perhaps races, and give a better understanding of the structures already observed under light microscopes.

#### LITERATURE CITED

 ESSER, R. P., V. G. PERRY, and A. L. TAYLOR. 1976. A diagnostic compendium of the genus Meloidogyne (Nematoda: Heteroderidae) Proc. Helminthol. Soc. Wash. 43:138-150.

- GOLDEN, A. M. and W. BIRCHFIELD. 1965.
  Meloidogyne graminicola (Heteroderidae) a
  new species of rootknot nematode from
  grass. Proc. Helminthol. Soc. Wash. 32:228231.
- 3. GREEN, C. D. 1971. The morphology of the terminal areas of round cyst nematode, s. g. Heterodera rostochiensis and allied species. Nematologica 17:34-46.
- MULVEY, R. H. 1973. Morphology of the terminal areas of white females and cysts of the genus Heterodera (s. g. Globodera) J. Nematol. 53:303-311.
- MULVEY, R. H. 1974. Cone top morphology of the white females and cysts of the genus Heterodera (subgenus Heterodera) a cyst forming nematode. Can. J. Zool. 52:77-81.
- MULVEY, R. H., J. P. JOHNSON, J. L. TOWNSHEND, and J. W. POTTER. 1975. Morphology of the perineal pattern of the rootknot nematode, Meloidogyne hapla and Meloidogyne incognita. Can. J. Zool. 53:370-373.
- TAYLOR, A. L., V. H. DROPKIN, and G. C. MARTIN. 1955. Perineal patterns of rootknot nematodes. Phytopathology 54:26-34.
- 8. YIK, C. P. and W. BIRCHFIELD. 1976. The perineal pattern of Meloidogyne graminicola. Paper #8-54. Proc. Am. Phytopathol. Soc. 3:344.