

Description of *Meloidogyne nataliei* n. sp. (Nematoda: Meloidogynidae) from Grape (*Vitis labrusca*) in Michigan, with SEM Observations

A. Morgan Golden, Lindy M. Rose, and George W. Bird¹

Abstract: *Meloidogyne nataliei* n. sp. is described and illustrated from grape (*Vitis labrusca*) in a declining vineyard at Mattawan, Michigan, USA. Infected grape roots exhibit no hyperplastic symptoms. Females protrude from the roots and are surrounded by a massive egg sac containing many eggs. This new species is distinguishable from other species of the genus especially by its large, striking perineal pattern with, usually, two ropelike separated striae on each side extending laterally from the vulval and anal areas. Among other diagnostic characters are the location of the female excretory pore adjacent to or near the base of the head and the heavy larval stylet averaging about 22 μm in length. Examination of males, females, and larvae with the scanning electron microscope confirmed observations made by optical microscopy and revealed diagnostic and other structures in greater detail. Of particular significance was the nature of the male head, with a massive circular labial disc on which is located a rectangular structure surrounding the oral opening, and the six distinct lips appearing as a rosette in en face view. The known distribution of this new species is presently limited to its original location in Michigan. **Key words:** taxonomy, morphology, *Meloidogyne*, root-knot, new species, SEM ultrastructure, grape, *Vitis labrusca*.

In 1977, soil and root samples of grape (*Vitis labrusca* cv. Concord) obtained from a badly declining vineyard at Mattawan, Michigan, were submitted to the Michigan State University Cooperative Extension Diagnostic Laboratory. The roots were heavily parasitized by a nematode protruding from the root surface. There were no visible hyperplastic symptoms, and females generally were surrounded by a massive egg sac containing many eggs. Males were also present in the egg sac. Nematode morphology indicated that this population represented a new species in the genus *Meloidogyne*. The nematode is described herein as such, but it is obviously far different from most other root-knot nematode species. A preliminary report on this nematode was recently given (5).

MATERIALS AND METHODS

Specimens were obtained from collections of grape roots from the original in-

festated vineyard. Larvae and males for morphological examination were usually recovered from fresh infected roots and egg sacs kept in petri dishes with a small amount of water. Some were recovered from soil by sieving and Baermann funnel extraction. Females were later dissected from these roots after fixation overnight in 3% formaldehyde solution.

The procedures used in measuring, drawing, and preparing specimens were essentially those used by Golden and Birchfield (3) except that some fixed females were cut and mounted in clear lactophenol solution. Photomicrographs of perineal patterns were made with an automatic 35-mm camera attached to a compound microscope having an interference contrast system, and those of infected roots and whole females were made with a 8.3 \times 10.8-cm sheet-film camera attached to a dissecting microscope. For scanning electron microscopy, living specimens of males, females, and larvae were fixed in 3% glutaraldehyde solution with 0.05 M phosphate buffer (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO₂, sputter-coated with 20–30 nm layer of gold-palladium, and examined with a scanning electron microscope (SEM).

Meloidogyne nataliei n. sp.

Females (30): Length 731–1,247 μm (mean 933 μm , standard deviation [SD] 101 μm); width 383–834 μm (568 μm , SD 103);

Received for publication 12 December 1980.

¹Respectively, Nematologist, Nematology Laboratory, Plant Protection Institute, USDA SEA AR, Beltsville, MD 20705; Senior Laboratory Technician, Nematology Diagnostic Laboratory, Department of Entomology, Michigan State University, East Lansing, MI 48824; and Professor, Department of Entomology, Michigan State University, East Lansing, MI 48824.

Appreciation is extended for the technical assistance of Donna M. S. Ellington, Support Scientist, and Paula Crowley, Laboratory Technician (in laboratory of senior author), and to Bruce Ingber, Laboratory Technician, Electron Microscopy Laboratory, USDA SEA AR, Beltsville, MD 20705, for SEM technical assistance. Appreciation is also extended to John Davenport for collecting type material, and to Natalie Knobloch for preliminary taxonomic observations.

a = 1.3–2.5 (1.7, SD 0.3); b = 5.0–8.3 (6.7, SD 0.9); stylet 21.1–22.4 μm (21.8 μm , SD 0.4); width of stylet knobs 5.2–6.9 μm (6.1 μm , SD 0.5); dorsal esophageal gland orifice (DGO) 3–6 μm (4.1 μm , SD 0.9) from base of stylet; center of median bulb 43–68 μm (56 μm , SD 6) from anterior end; vulva slit length 31–45 μm (38 μm , SD 4); distance from vulva slit to anus 49–77 μm (60 μm , SD 7).

Holotype (female): Length 835 μm ; width 465 μm ; a = 1.8; b = 6.0; stylet 21.9 μm ; DGO 4.7 μm from base of stylet; width of stylet knobs 6.0 μm ; vulva slit length 37 μm ; distance from vulva slit to anus 49 μm ; distance between phasmids 83 μm ; excretory pore 9.6 μm from anterior center tip of head.

Description of females: Body pearly white, globular to pear-shaped, and usually with exceptionally long neck (Figs. 15, 16, 41). Vulva terminal and generally on a small protuberance located in a median plane with the neck. Esophageal and anterior region commonly appearing as illustrated (Figs. 6, 17, 18, 19). Head offset from neck, bearing a labial cap and a prominent cephalic annule which sometimes is subdivided by a single striae. Cephalic framework distinct; stylet exceptionally strong with knobs sloping posteriorly. Excretory pore prominent (Figs. 6, 19) and located near base of head, 6–11 μm (9 μm , SD 2) from anterior center tip of head. Massive egg sac extruded posteriorly, often three or more times the size of the female (Figs. 39, 40, 42), and containing 50–400 eggs. Cuticle thick (Fig. 15), measuring 10–21 μm (16 μm , SD 4). Perineal pattern (Figs. 7–11, 20–26) large, highly distinctive, with heavy striae forming a rounded arch, and usually with two separated ropelike striae extending laterally from vulval and anal areas and forming a lateral field which decreases in size and prominence as its length increases. Phasmids (Figs. 9, 12) prominent, commonly located at a level opposite or just above the distinct anus and separated by a distance of 62–107 μm (78 μm , SD 14).

Males (30): Length (1,191–1,757 μm (1,489 μm , SD 127); a = 33–45 (39, SD 3.3); b = 4.2–5.9 (5.0, SD 0.5); c = 86–125 (101, SD 13.6); stylet 28.4–29.2 μm (28.9 μm , SD

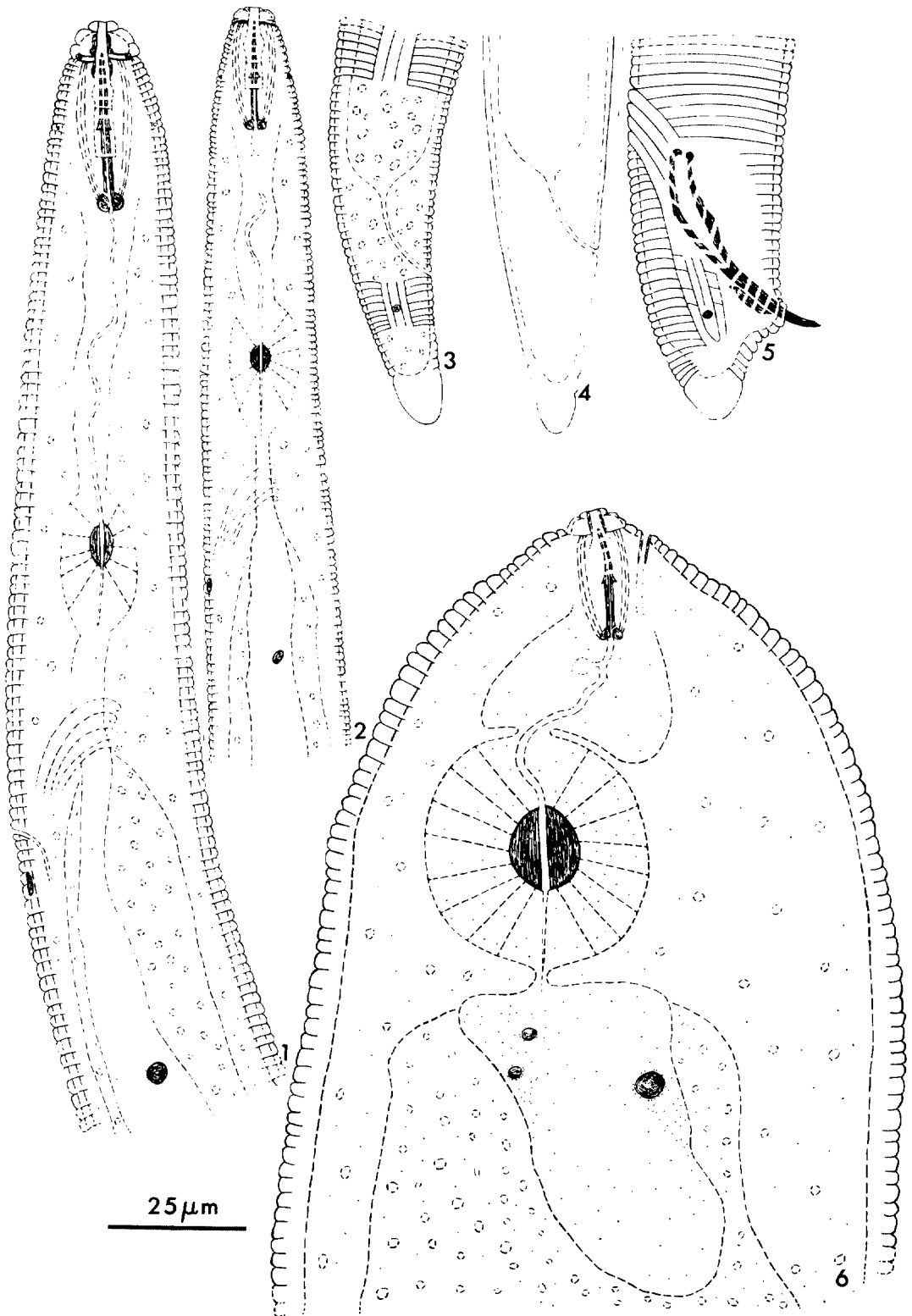
0.21); DGO 4.0–6.5 μm (5.0 μm , SD 0.8) from base of stylet; center of median bulb 71–95 μm (85 μm , SD 5.6) from anterior end; spicules 41.3–44.3 μm (43 μm , SD 0.8); gubernaculum 6–11 μm (9.7 μm , SD 1.6); tail 12–18 μm (15 μm , SD 2.2).

Allotype (male): Length 1,474 μm ; a = 35.7; b = 3.7; c = 107; stylet 28.4 μm ; DGO 4.3 μm from base of stylet; spicules 43 μm ; gubernaculum 9 μm ; tail 14 μm .

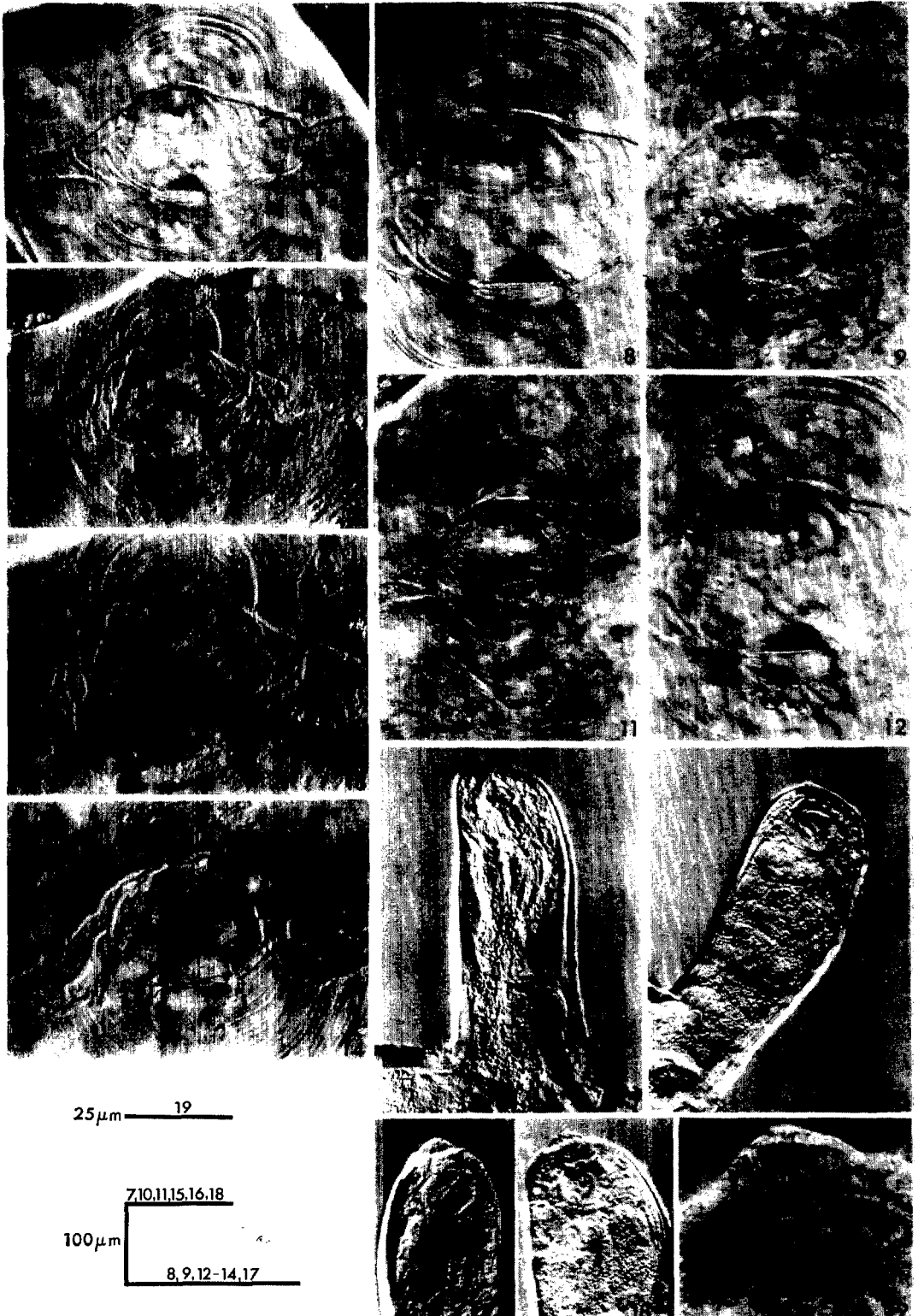
Description of males: Body long, vermiform, tapering slightly at both extremities. Midbody width 33–43 μm (38 μm , SD 3.2); body annules distinct, measuring about 2 μm . Head markedly offset, with massive labial disc, prominent lips, and post labial annule often with a discontinuous stria (Figs. 1, 34–37). Cephalic sclerotization heavy. Lateral field (Fig. 5) approximately 1/4 body width at midbody, with five lines, not areolated. Stylet, stylet knobs, stylet guide, cephalids, hemizonid, and anterior portion commonly appear as illustrated (Fig. 1). Excretory pore anterior to hemizonid, ranging about 6–20 μm anteriorly. Testis one. Spicules (Fig. 5) arcuate, tips sharply pointed. Gubernaculum boat-shaped, distally with small projection extending dorsally. Distinct phasmids generally at level of cloaca opening; tail short, reduced immediately past cloaca opening, with narrowly rounded terminus, appearing about as illustrated (Figs. 5, 38).

Second-stage larvae (32): Length 539–641 μm (599 μm , SD 32); a = 20.4–28.7 (23.9, SD 2.1); b = 3.2–4.1 (3.6, SD 0.3); c = 19–26 (22, SD 1.6); stylet 21.9–22.8 μm (22.4 μm , SD 0.2); DGO 3.0–4.3 μm (3.8 μm , SD 0.4) from base of stylet; median bulb center 60–83 μm (68 μm , SD 4.4) from anterior end; head width (hw) 7.8–9 μm (8.5 μm , SD 0.4); head height (hh) 3.0–3.4 μm (3.3 μm , SD 0.2); hw/hh ratio 2.4–3.0 (2.6, SD 0.2); tail length 22.9–30 μm (27.0, SD 1.6); hyaline tail terminal 7.3–10.8 μm (8.4 μm , SD 0.9); caudal ratio A 1.0–1.3 (1.1, SD 0.1); caudal ratio B 1.1–1.7 (1.4, SD 0.2).

Description of larvae: Body vermiform, tapering at both ends but more so posteriorly. Head offset, bluntly rounded, more than twice as wide as high, with heavy cephalic sclerotization, and one postlabial annule without striae (Figs. 2, 30–33). Body annules fine but distinct. Lateral field (Fig.



Figs. 1-6. Drawings of *Meloidogyne nataliei* n. sp. 1) Anterior region of male. 2-4) Larvae: consecutively, anterior region, tail region showing phasmid and lateral field, and tail outline. 5) Male posterior, showing phasmid, lateral field, spicules, and gubernaculum. 6) Anterior region of female.



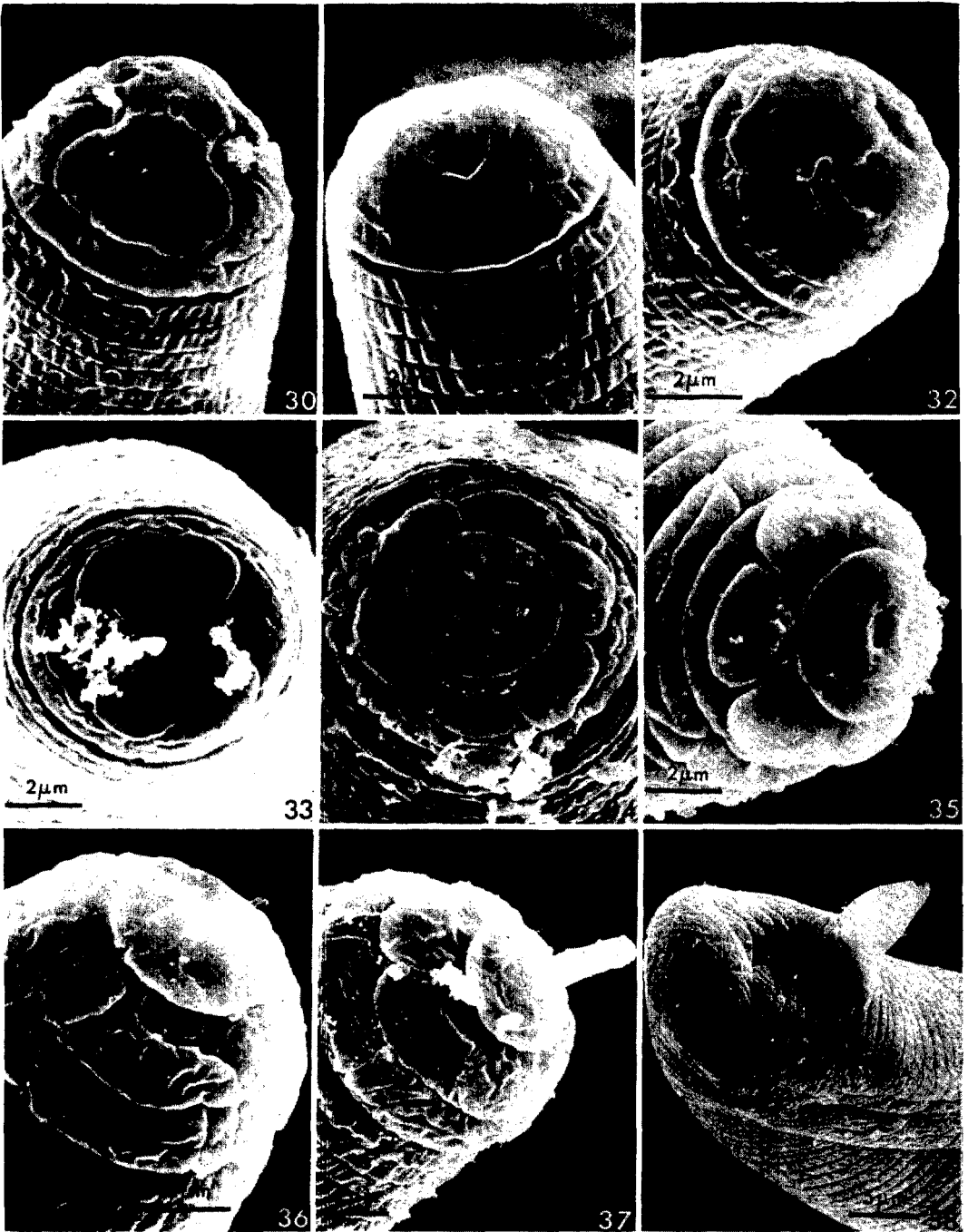
Figs. 7-19. Photomicrographs of females of *Meloidogyne nataliei* n. sp. 7-14) Perineal patterns (9 and 12 slightly deeper focus showing phasmids also). 15-16) Whole neck region. 17-19) Anterior neck region.



Figs. 20-29. Scanning electron micrographs of females of *Meloidogyne nataliei* n. sp. 20-26) Perineal patterns. 27,28) Head region. 29) En face view.

3) with four lines, without areolation, and about 1/4 body width, the latter being 22-29 μm (26 μm , SD 1.7) at its widest part. Anterior portion with associated structures commonly appearing as illustrated (Fig. 2). Stylet strong, with stylet guide as shown. Excretory pore rather faint, located in front

of hemizonid at a level from the center to the anterior portion of median bulb, and 60-65 μm (62 μm , SD 2.5) from anterior end. Hemizonid distinct, situated 22-36 μm (32 μm , SD 6) posterior to center of median bulb. Phasmids (Fig. 3) large, located in anterior 1/4 of tail. Rectum not dilated.



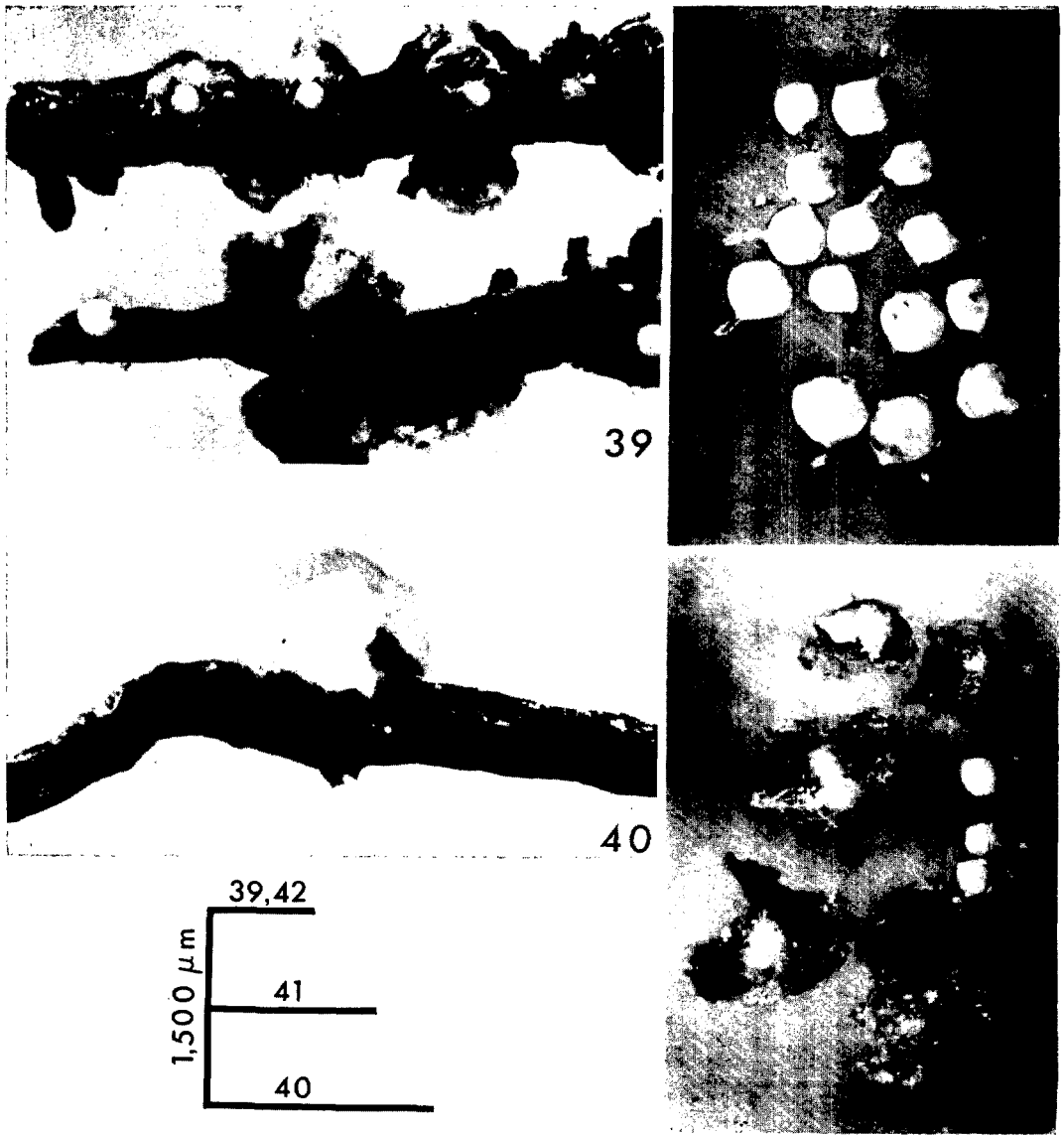
Figs. 30-38. Scanning electron micrographs of larvae and males of *Meloidogyne nataliei* n. sp. 30-33) Larvae, head regions and heads. 34-37) Males, head regions and heads. 38) Male, posterior portion.

Tail short, with exceptionally short hyaline tail terminal and a narrowly rounded terminus often appearing as illustrated (Figs. 3, 4).

Eggs (25): Length 147-186 μm (168 μm, SD 12); width 50-62 μm (56 μm, SD

3); L/W ratio 2.6-3.6 (3, SD 0.3). Egg shell hyaline, without visible markings, as seen with optical microscopy.

Holotype (female): Collected by G. W. Bird and L. M. Rose, 21 December 1979 in a vineyard at Mattawan, Michigan, USA.



Figs. 39-42. Photomicrographs of specimens of *Meloidogyne nataliei* n. sp. on or isolated from roots of grape. 39,40) Infected roots with females protruding, some with massive egg sacs attached. 41) Whole females. 42) Whole females and egg sacs with eggs.

Slide T-335t, USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

Allotype (male): Slide T-336t. Same data as holotype. USDANC, Beltsville, Maryland, USA.

Paratypes (males, females, larvae, and eggs): USDANC, Beltsville, Maryland, USA; Nematology Collection, Department of Entomology, Michigan State University, East Lansing, Michigan, USA; University of California Nematode Survey Collection (UCNSC) Davis, California, USA; Nema-

tology Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, England; Canadian National Collection of Nematodes, Ottawa, Canada; Laboratoire des Vers, Muséum National d'Histoire Naturelle, Paris, France; Institute voor Dierkunde, Laboratorium voor Morfologie en Systematiek der Dieren, Ledeganckst, 35, B-9000, Gent, Belgium; Laboratory voor Nematologie, Binnehave 15, Wageningen, Netherlands; and National Nematode Collection, Division of Nematology, Indian

Agricultural Research Institute, New Delhi, India.

Type host and locality: Roots of grape (*Vitis labrusca* cv. Concord) from a vineyard at Mattawan, Michigan, USA.

Diagnosis: The perineal patterns, as described and illustrated herein, especially with the ropelike striae extending laterally from the vulval and anal areas, readily differentiate *Meloidogyne nataliei* n. sp. from all other species in the genus. In addition, the location of the female excretory pore near the base of the head and the exceptionally large larval stylet are among the other characters which distinguish this species.

SEM examination of specimens confirmed observations made with optical microscopy and revealed much more detail. The very large size of perineal pattern, the extent of the laterally directed striate, and the nature of the other striae are evident. Figures 27–29 show some details of female heads. (Figs. 20–26). Anterior views of larvae (Figs. 30–33) and males (Figs. 34–37) revealed the nature of the amphidial openings, the lips, and presence of a massive labial disc in the males. The male labial disc is circular, appears raised above and separated from the lips, and on its uppermost surface a rectangular structure (Figs. 34, 35) surrounds the oral aperture and contains the inner labial sensilla. The six lips are about equal in size, the submedian lips are distinct and fully or partially separate, and in a head-on view, the head appears as a rosette. Thus, the male head of *M. nataliei* n. sp. is quite unlike males of seven other *Meloidogyne* species on which SEM examinations have been recently reported (1, 2, 4).

On its only known host at this time, *M. nataliei* n. sp. does not form galls or knots; the females protrude from the root surface similar to a cyst nematode and become surrounded by a massive egg sac containing many eggs (Figs. 39, 40, 42). In these respects, this new species resembles *M. camelliae* Golden 1979 (2), a parasite of *Camellia japonica* L., but morphologically these two species are very different. Investigations are being conducted in Michigan on the biology, host range, and other aspects of this remarkable nematode on grape. Its known distribution is presently limited to the type area at Mattawan, Michigan.

The species name is given in honor of Mrs. Natalie Knobloch, a recently retired nematologist of the Department of Entomology, Michigan State University, East Lansing, Michigan.

LITERATURE CITED

1. Eisenback, J. D., and Hedwig Hirschmann. 1980. Morphological comparison of *Meloidogyne* males by scanning electron microscopy. *J. Nematol.* 12:23-32.
2. Golden, A. Morgan. 1979. Descriptions of *Meloidogyne camelliae* n. sp. and *M. querciana* n. sp. (Nematoda: Meloidogynidae), with SEM and host-range observations. *J. Nematol.* 11:175-189.
3. Golden, A. M., and W. Birchfield. 1972. *Heterodera graminophila* n. sp. (Nematoda: Heteroderidae) from grass with a key to closely related species. *J. Nematol.* 4:147-154.
4. Golden, A. M., J. H. O'Bannon, G. S. Santo, and A. M. Finley. 1980. Description and SEM observations of *Meloidogyne chitwoodi* n. sp. (Meloidogynidae), a root-knot nematode on potato in the Pacific Northwest. *J. Nematol.* 12:319-327.
5. Rose, Lindy. 1980. Comparative morphology of an undescribed species of the Meloidogyninae from *Vitis* L., in Michigan. *J. Nematol.* 12:236 (Abstr.).