Journal of Nematology 21(4):453–461. 1989.

Further Details and SEM Observations on *Meloidogyne marylandi* (Nematoda: Meloidogynidae)

A. Morgan Golden[1]

Abstract: Specimens of *Meloidogyne marylandi* from Bermuda grass and a population from Zoysia grass were examined and compared morphologically by light and electron microscopy. The populations probably are conspecific and the differences noted in the Zoysia population, mainly those of second-stage juveniles (J2) with shorter tails, are considered normal variations rather than representing another form. Scanning electron microscope observations provided additional details of the perineal pattern and head of females and head and lateral fields of second-stage juveniles. Relationship of *M. marylandi* to closely related species is given. This species is currently known to occur only in Maryland, and populations previously reported from this state as *M. graminis* are now considered to be *M. marylandi.* Other reports of *M. graminis* in the United States now need to be reconfirmed by examination of voucher or recollected specimens.

Key words: host, *Meloidogyne marylandi,* morphology, population, root-knot nematode, taxonomy, turf grass.

The root-knot nematode species *Meloidogyne marylandi* Jepson & Golden, 1987 (3) was recently described from what proved to be a species complex with *M. graminis* (Sledge & Golden, 1964) Whitehead, 1968. *Meloidogyne marylandi* was found on Bermuda grass (*Cynodon dactylon* (L.) Pers.) in College Park, Maryland; *M. graminis* was originally described as a *Hypsoperine* species (5) from St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze) in Winter Haven, Florida. It was later transferred from *Hypsoperine* to *Meloidogyne* (6). In 1964 Bell and Krusberg (1) found "major infestations" of a root-knot nematode causing injury on Bermuda grass at College Park and on Zoysia grass (*Zoysia japonica* Steudel) at Reisterstown, Maryland. This nematode was identified by A. M. Golden as *M. graminis,* although at that time differences were noted in the second-stage juveniles (1). Recently a limited comparative examination of populations from Bermuda and Zoysia grasses indicated that second-stage juveniles from Zoysia grass had shorter tails than those from Bermuda grass. These differences caused concern about other possible differences and the conspecificity of these two populations.

This paper gives results of a detailed morphological study of *M. marylandi* populations on Zoysia grass and Bermuda grass in Maryland.

Materials and Methods

Most specimens used in this study were collected originally from Bermuda grass (*Cynodon dactylon* (L.) Pers.) in College Park and Zoysia grass (*Stenotaphrum secundatum* (Walter) Kuntze cv. Emerald) in Calverton, Maryland, and increased on these hosts in a growth chamber at Beltsville at 25 C for 14 hours of light and at 20 C for a 10-hour dark period. Some additional specimens were collected and examined directly from Zoysia field plots at Calverton.

Second-stage juveniles for morphological examination were recovered from live infected roots or from egg sacs kept in petri dishes with a small amount of tap water. Females were later dissected from the roots after fixation overnight in 3% formaldehyde solution. The procedures used for measuring and preparing specimens were essentially the same as those used by Golden and Birchfield (2), except that some fixed females were cut and mounted in a lactophenol solution. All photomicrographs of females, second-stage juveniles, and eggs were made with an automatic 35 mm camera attached to a compound microscope

Received for publication 15 November 1988.

[1] Nematologist, Nematology Laboratory, Plant Sciences Institute, USDA ARS, Beltsville, MD 20705.

Appreciation for technical assistance is extended to Donna M. S. Ellington, Support Scientist, and Paula Crowley, Laboratory Technician, Nematology Laboratory, USDA ARS, Beltsville, MD.

TABLE 1. Measurements (μm) of females of *Meloidogyne marylandi* from two hosts and two locations in Maryland.

	Bermuda culture College Park† (n = 25)		Zoysia culture Calverton (n = 30)	
	Range	Mean ± SD	Range	Mean ± SD
Body length	525.1–923.2	747.4 ± 110.6	427.0–692.4	566.5 ± 64.3
Body width	207.7–421.2	297.0 ± 46.8	248.1–427.0	314.4 ± 42.1
a	1.8–3.3	2.5 ± 0.4	1.5–2.3	1.8 ± 0.2
Stylet length	14.2–14.8	14.4 ± 0.3	12.8–14.0	13.5 ± 0.4
Stylet base to DGO	3.5–4.7	3.9 ± 0.4	1.8–2.9	2.5 ± 0.4
Stylet knob width			3.5–4.7	4.2 ± 0.4
Head tip to excretory pore	8.8–19.5	13.5 ± 3.5	8.3–20.6	12.3 ± 3.3
Head tip to median bulb valve	115.1–165.2	139.8 ± 10.9	84.4–118.0	97.6 ± 9.9
Vulva slit length	20.7–29.5	25.1 ± 2.8	20.6–26.5	24.3 ± 2.3
Vulva slit to anus	11.8–15.3	12.8 ± 1.1	11.8–16.5	13.7 ± 1.7
Cuticle thickness, on neck			7.1–11.8	9.4 ± 1.5
on midbody			8.7–20.1	13.3 ± 2.7

† From Jepson and Golden (3).

with differential interference contrast. For the scanning electron microscopy (SEM), living specimens were fixed in 3% glutaraldehyde solution buffered with 0.05 M phosphate (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO_2, and sputter-coated with a 20–30-nm layer of gold-palladium.

All measurements are in micrometers (μm) unless otherwise stated.

SYSTEMATICS

Meloidogyne marylandi
Jepson & Golden, 1987
(Figs. 1–38)

Females: Measurements of 55 females in Table 1.

Body color usually pearly white, old specimens sometimes light brown. Shape globular to elongate; neck distinct, often to one side of a median plane through vulva. Posterior vulva located on small protuberance. Stylet short. Stylet knobs distinct, rounded, usually sloping posteriorly (Fig. 5). Head not offset, variable in shape, framework distinct, generally labial cap prominent, and one head annule (Figs. 6, 23, 24). Excretory pore usually near base of unprotruded stylet. Body cuticle thick, thinner near anterior end of neck (Figs. 1, 5). Median bulb prominent, usually near base of neck or sometimes posterior (Figs. 1, 2). Perineal pattern ovoid to round. Striae wavy, coarse well spaced, wavy striae usually forming a low, rounded dorsal arch; some patterns with a higher, squarish dorsal arch (Figs. 14–22, 26–31). Perivulval region without striae, although occasionally some striae may extend to each end of the vulva.

Males: Not found.

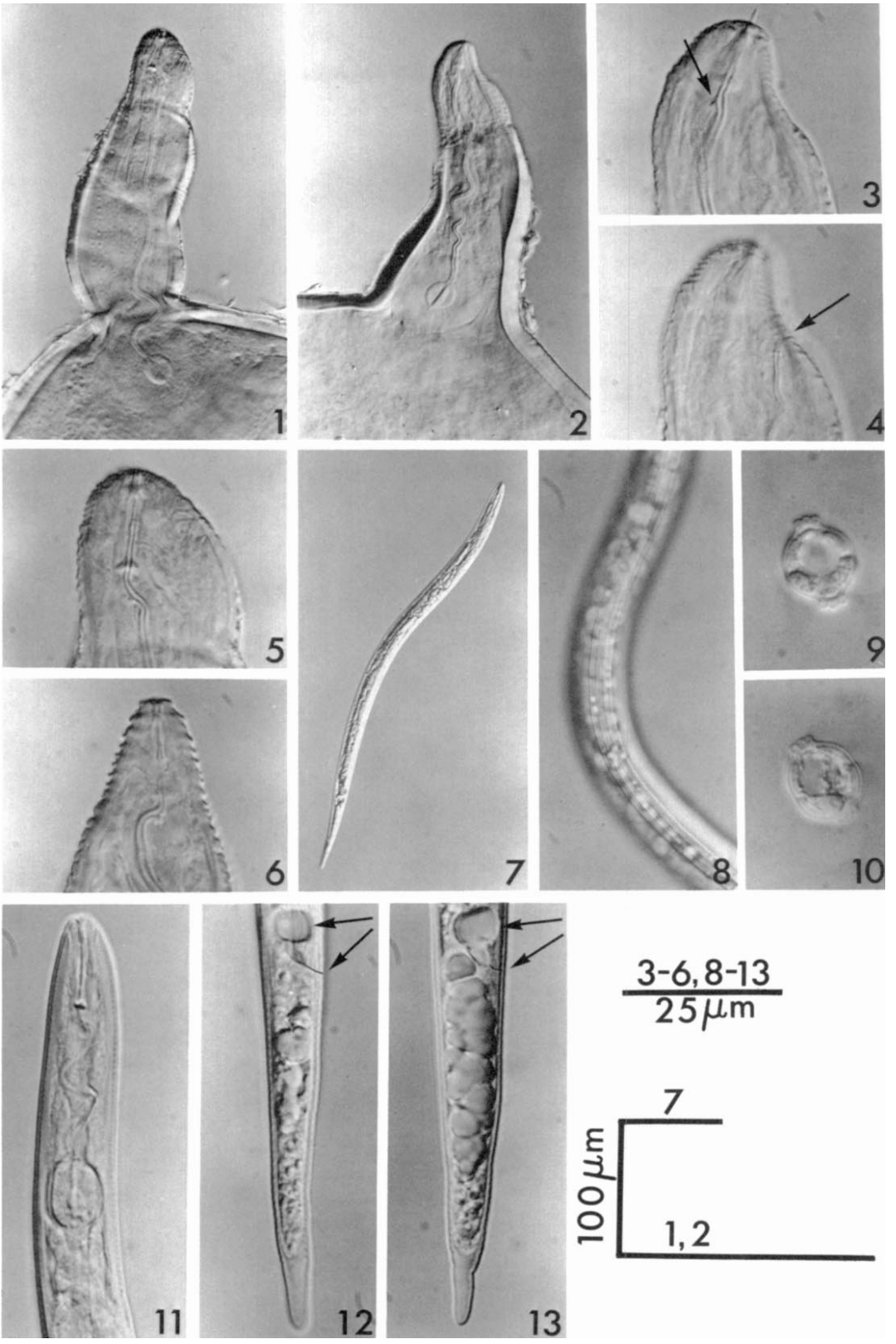
Second-stage juveniles: Measurements of 97 juveniles in Table 2.

Body vermiform, tapering at both extremities, but tapers more posteriorly (Fig. 7). Head not offset, with labial disc; lip region without annulation (Figs. 32–34). Stylet delicate, with small rounded knobs (Fig. 11). Cuticular annulation fine, distinct. Lateral field prominent, with four incisures; some areolation, especially in anterior portion (Figs. 8–10, 35–37). Excretory pore 3–4 annules anterior to hemizonid. Rectum inflated (Figs. 12, 13). Phasmids small, indistinct, and at about 70% tail length from terminus. Tail tapering gradually, terminus bluntly rounded (Fig. 12).

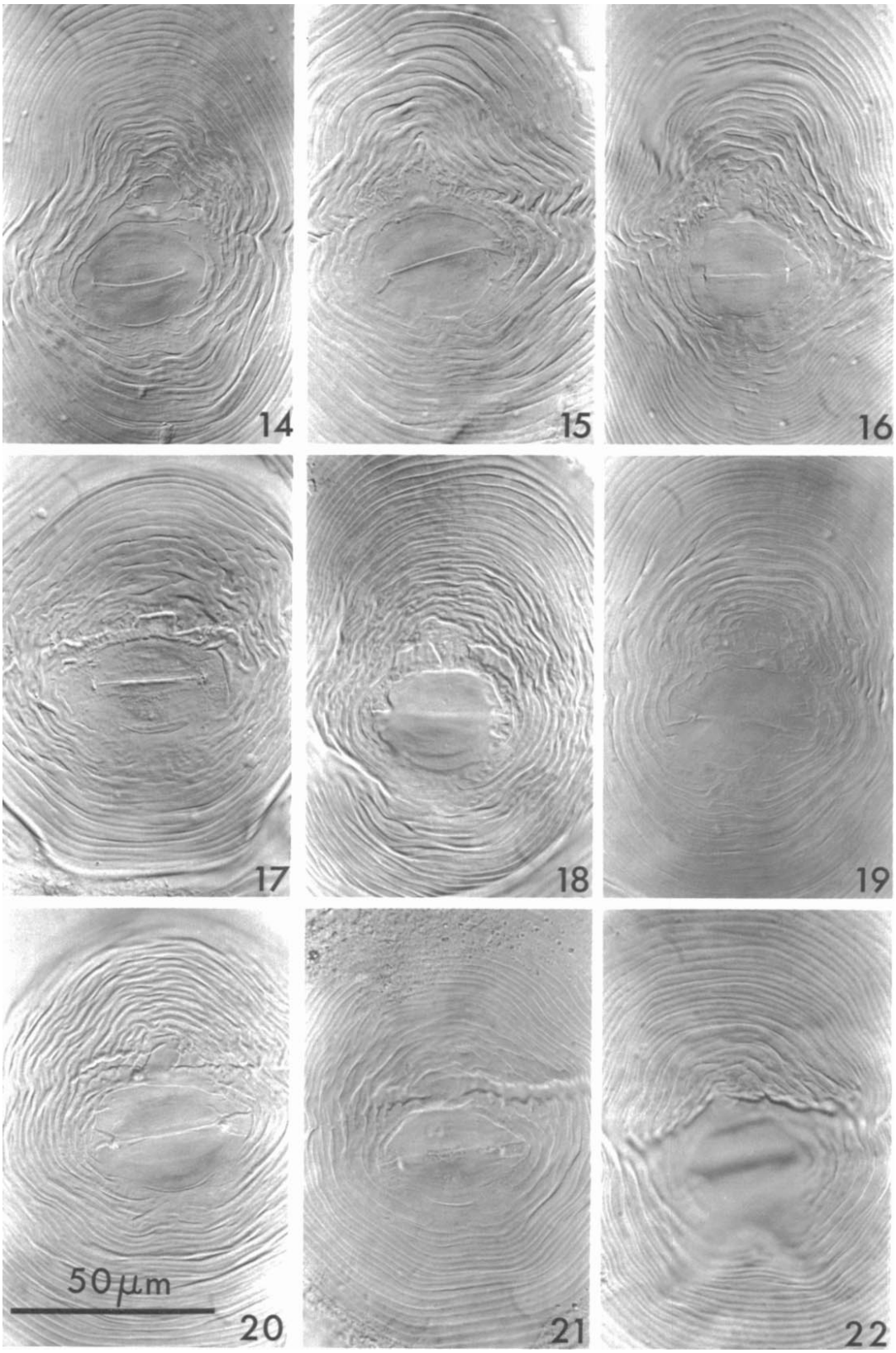
Eggs: Measurements of 55 eggs in Table 3.

Egg shell hyaline, without visible markings (Fig. 38).

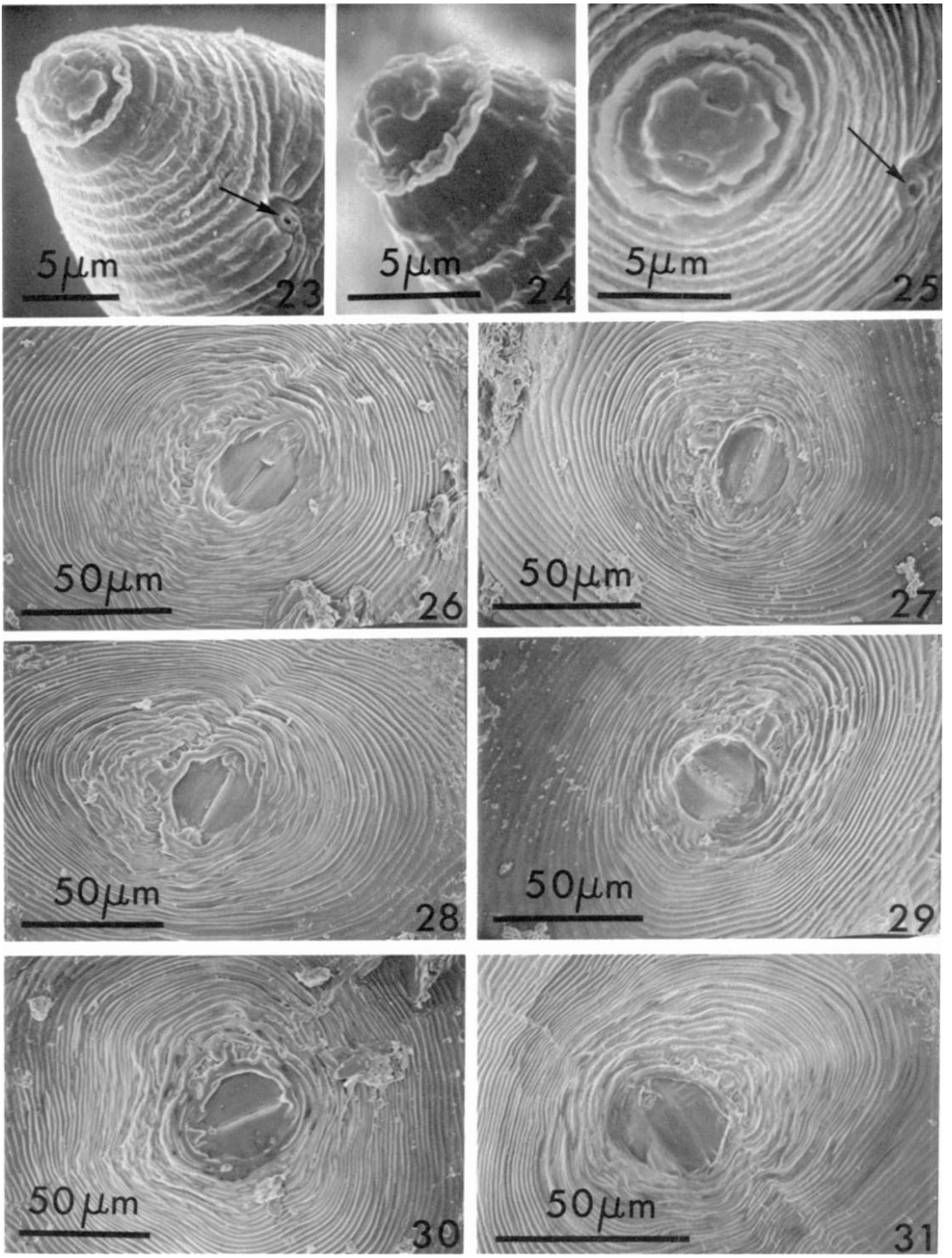
The type host and locality of *M. marylandi* are roots of Bermuda grass (*Cynodon dactylon* (L.) Pers.), from the University of Maryland golf course at College Park, Maryland, USA.



FIGS. 1–13. Photomicrographs of *Meloidogyne marylandi*. 1–6) Females. 1, 2) Anterior end. 3–6) Anterior region of neck showing stylet, dorsal gland outlet (arrow), and excretory pore (arrow). 7–13) Second-stage juveniles. 7) Whole specimen. 8) Lateral field, on surface. 9, 10) Midbody sections showing lateral field. 11) Anterior region. 12, 13) Tail and inflated rectum (slightly accentuated).



FIGS. 14–22. Photomicrographs of nine different perineal patterns of *Meloidogyne marylandi.*

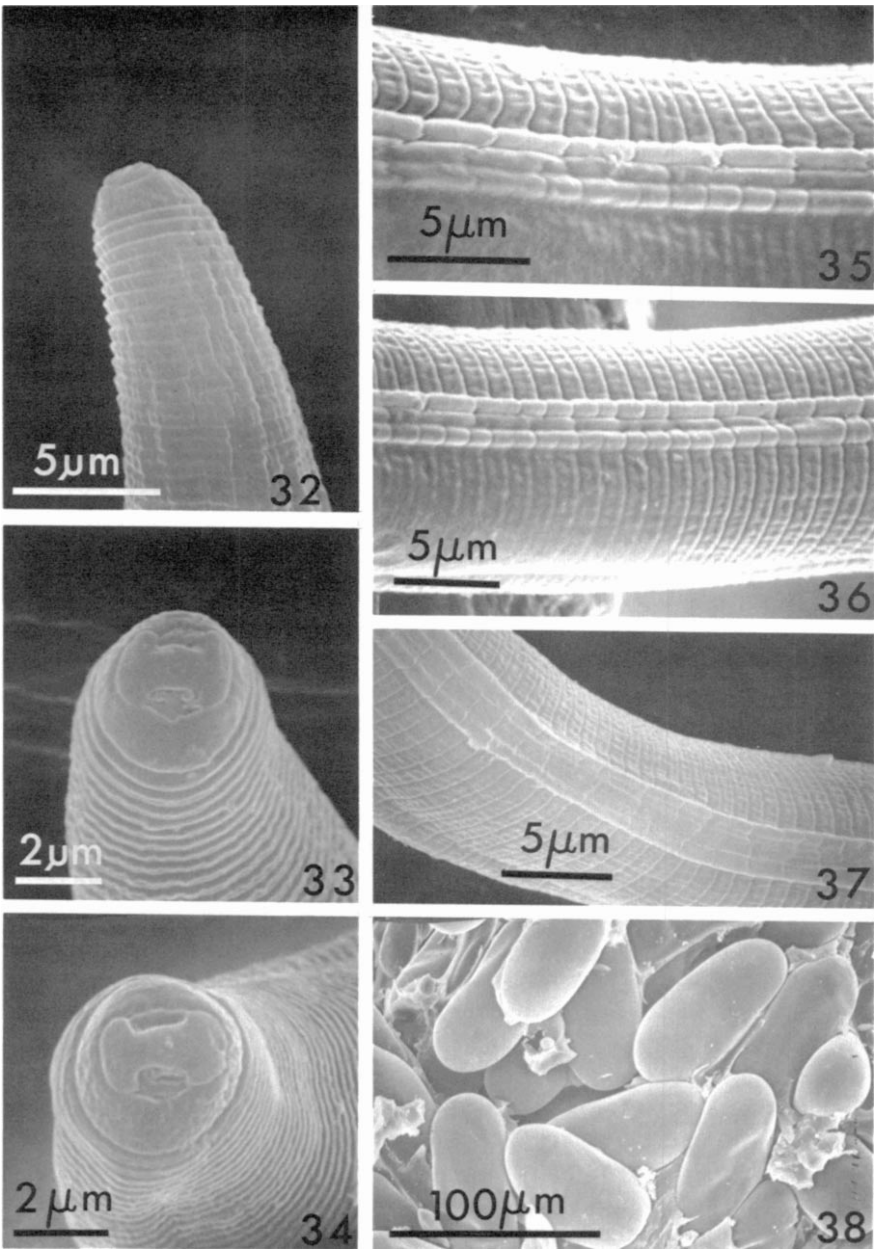


FIGS. 23–31. Scanning electron micrographs of females of *Meloidogyne marylandi.* 23–25) Head region. 23) Head and excretory pore at surface (arrow). 24) Head. 25) En face and excretory pore (arrow). 26–31) Perineal patterns of six different specimens.

TABLE 2. Measurements (μm) of second-stage juveniles of *Meloidogyne marylandi* from two hosts and two locations in Maryland.

	Bermuda culture College Park† (n = 40)		Zoysia culture Calverton (n = 37)		Zoysia field plots Calverton (n = 20)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Body length	384.8–488.5	424.6 ± 20.5	323.8–397.6	370.1 ± 13.7	367.8–411.8	395.1 ± 12.5
Body width at midbody	13.6–17.1	14.8 ± 0.6	13.0–18.3	15.2 ± 1.7	14.8–17.7	16.2 ± 0.8
Head width	4.7–5.4	5.3 ± 0.1	4.1–5.3	5.0 ± 0.3	4.7–5.3	5.3 ± 0.1
Head height	1.8–2.4	2.3 ± 0.1	1.8–2.4	2.3 ± 0.2	2.3–2.9	2.6 ± 0.3
Head width/height ratio	2.0–3.0	2.3 ± 0.2	2.0–3.0	2.2 ± 0.2	1.8–2.2	2.1 ± 0.2
a	25.2–33.1	28.8 ± 1.5	20.1–28.4	24.5 ± 2.4	22.9–26.4	24.5 ± 1.0
b	1.9–2.7	2.2 ± 0.2	1.8–2.6	2.0 ± 0.2	1.8–2.2	1.9 ± 0.1
c	6.6–7.7	7.0 ± 0.3	5.9–7.4	6.7 ± 0.3	6.4–7.3	6.8 ± 0.2
Stylet length	10.8–11.8	11.4 ± 0.3	10.0–11.8	11.2 ± 0.4	10.6–11.2	10.8 ± 0.3
Base of stylet to DGO	2.4–3.0	2.4 ± 0.2	1.8–2.9	2.4 ± 0.2	1.8–2.9	2.4 ± 0.1
Head tip to median bulb valve	50.2–60.8	57.6 ± 2.3	44.2–51.9	48.9 ± 1.8	49.0–54.3	51.3 ± 1.4
Head tip to base of esophageal gland lobe	172.9–221.3	194.9 ± 11.6	154.6–207.7	187.5 ± 16.5	182.9–212.4	200.2 ± 7.7
Tail length	52.5–68.4	60.6 ± 3.4	46.0–61.9	54.9 ± 3.6	55.5–60.2	57.9 ± 1.6
Hyaline tail terminal	9.4–13.8	11.8 ± 1.1	9.4–12.9	11.4 ± 0.9	10.0–12.4	11.5 ± 0.6
Caudal ratio A	2.0–3.3	2.6 ± 0.3	2.0–3.2	2.5 ± 0.2	2.0–2.6	2.4 ± 0.2
Caudal ratio B	3.0–5.8	4.2 ± 0.8	2.7–5.2	3.9 ± 0.5	3.0–4.2	3.9 ± 0.3
Width of tail at hyaline portion	3.5–4.8	4.5 ± 0.3	3.5–5.3	4.6 ± 0.3	4.7–5.3	4.7 ± 0.1
Width of tail 5 μm from tail tip	2.4–3.5	2.9 ± 0.3	2.4–3.5	2.9 ± 0.2	2.9–3.5	3.0 ± 0.1

† From Jepson and Golden (3).



FIGS. 32–38. Scanning electron micrographs of second-stage juveniles and eggs of *Meloidogyne marylandi*. 32–37) Juveniles. 32) Lateral view of head region. 33, 34) En face. 35–37) Lateral field. 36) Anterior region, showing areolation and reduction in lines near beginning. 38) Eggs.

The diagnosis and relationships of *M. marylandi* to the four species below were given by Jepson and Golden (3). Some of those details and additional ones by the author are included here.

Meloidogyne marylandi differs from the following: 1) *M. graminis*, with second-stage juvenile average tail length 78 μm, hyaline tail terminus 18.5 μm; female stylet length average 12.5 μm, perineal pattern usually with prominent lateral lines. 2) *M. maritima* Jepson, 1987, with second-stage juvenile tail conically tapering, with constriction near finely rounded terminus, hyaline region length average 13.6 μm; inner portion of female perineal pattern with very coarse,

TABLE 3. Measurements (μm) of eggs of *Meloidogyne marylandi* from two hosts and two locations in Maryland.

	Bermuda culture College Park† (n = 30)		Zoysia culture Calverton (n = 25)	
	Range	Mean ± SD	Range	Mean ± SD
Length	79.7–100.3	91.6 ± 5.2	83.8–100.8	93.7 ± 4.1
Width	39.5–52.5	46.6 ± 4.2	35.5–49.7	41.8 ± 3.4
L/W ratio	1.6–2.4	2.0 ± 0.2	1.8–2.6	2.2 ± 0.2

† From Jepson and Golden (3).

widely spaced striae and lateral lines marked at folded and twisted junction of the dorsal and ventral striae. 3) *M. aquatilis* Ebsary & Eveleigh, 1983, with second-stage juvenile hyaline tail terminus slightly clavate, usually with internal disc-like structure; excretory pore 7–9 annules anterior to hemizonid; female stylet length average 11.5 μm, perineal pattern with high, truncate dorsal arch, discontinuous striae, and without distinct lateral lines. 4) *M. naasi* Franklin, 1965, with second-stage juvenile tail 70 μm average in length, narrows posteriorly to a finely pointed tip which may be irregular and occasionally forked; rectum undilated; excretory pore posterior and adjacent to hemizonid; female perineal pattern with large, prominent phasmids.

Unknown in *M. marylandi*, males have been described for these four species, and they also show some distinctive features.

Examination of specimens by SEM confirmed the LM observations and showed greater detail of the structures observed with LM. The nature of the female lips, head annules, and perineal patterns was more clearly revealed. Anterior views of the second-stage juveniles showed greater details of the amphidial openings and lips, the absence of striations on the large head annule, and better definition of the lateral field.

Voucher specimens from this study are deposited in the USDA Nematode Collection, Beltsville, Maryland.

DISCUSSION

Details given in this report on the Maryland nematode populations from Bermuda grass in College Park and from Zoysia grass in Calverton indicate they are conspecific. Initially there was some doubt about this because of the shorter tail in the second-stage juveniles from the Calverton population (Table 2); however, subsequent collections from Zoysia grass field plots at Calverton showed the tail to be closer to the tail length of the College Park Bermuda grass populations recently described as *M. marylandi* (3). This slight difference in tail length is attributed to normal variation within the species, particularly in the absence of other significant differences.

Reexamination of specimens in the USDA Nematode Collection, which tentatively had been identified in 1964 as *M. graminis* on Bermuda and Zoysia grasses in Maryland (1), showed them to be *M. marylandi*. The latter species is currently known only from Maryland; on the other hand, *M. graminis* has been reported on turf grasses by various workers from nine states in the south, midwest, and California (7). Also, host range tests of selected Gramineae and evaluation of several cultivars and lines of Bermuda and Zoysia grasses for resistance to *M. graminis* have been reported (4,7). In view of our better understanding of the *M. graminis* species complex on turf grasses, it would seem advisable and appropriate to reexamine voucher specimens or collect new specimens from these populations, including the host and resistance evaluation tests, and reconfirm the identity of the nematode species involved.

LITERATURE CITED

1. Bell, A. A., and L. R. Krusberg. 1964. Occurrence and control of a nematode of the genus *Hypsoperine* on Zoysia and Bermuda grasses in Maryland. Plant Disease Reporter 48:721–722.

2. Golden, A. M., and W. Birchfield. 1972. *Heterodera graminophila* n. sp. (Nematoda: Heteroderi-

dae) from grass with a key to closely related species. Journal of Nematology 4:147–154.

3. Jepson, S. B., and A. M. Golden. 1987. *Meloidogyne marylandi* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing grasses. Pp. 263–265 *in* S. B. Jepson. Identification of root-knot nematodes (*Meloidogyne* species). C.A.B. International, Wallingford, United Kingdom.

4. Murray, J. J., T. E. Poole, and S. A. Ostazeski. 1986. Techniques for determining reproduction of *Meloidogyne graminis* on Zoysiagrass and Bermudagrass. Plant Disease 70:559–560.

5. Sledge, E. B., and A. M. Golden. 1964. *Hypsoperine graminis* (Nematoda: Heteroderidae), a new genus and species of plant-parasitic nematode. Proceedings of the Helminthological Society of Washington 31:83–88.

6. Whitehead, A. G. 1968. Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. Transactions of the Zoological Society of London 31:263–401.

7. Williams, A. S., and C. W. Laughlin. 1968. Occurrence of *Hypsoperine graminis* in Virginia and additions to the host range. Plant Disease Reporter 52: 162–163.