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Morphological and Molecular Characterization of *Meloidogyne mayaguensis* Isolates from Florida[1]

J. Brito,[2] T. O. Powers,[3] P. G. Mullin,[4] R. N. Inserra,[2] and D. W. Dickson[5]

Abstract: The discovery of *Meloidogyne mayaguensis* is confirmed in Florida; this is the first report for the continental United States. *Meloidogyne mayaguensis* is a virulent species that can reproduce on host cultivars bred for nematode resistance. The perineal patterns of *M. mayaguensis* isolates from Florida show morphological variability and often are similar to *M. incognita.* Useful morphological characters for the separation of *M. mayaguensis* from *M. incognita* from Florida are the male stylet length values (smaller for *M. mayaguensis* than *M. incognita*) and J2 tail length values (greater for *M. mayaguensis* than *M. incognita*). *Meloidogyne mayaguensis* values for these characters overlap with those of *M. arenaria* and *M. javanica* from Florida. Enzyme analyses of Florida *M. mayaguensis* isolates show two major bands (VS1-S1 phenotype) of esterase activity, and one strong malate dehydrogenase band (Rm 1.4) plus two additional weak bands that migrated close together. Their detection requires larger amounts of homogenates from several females. Amplification of two separate regions of mitochondrial DNA resulted in products of a unique size. PCR primers embedded in the COII and 16S genes produced a product size of 705 bp, and amplification of the 63-bp repeat region resulted in a single product of 322 bp. Nucleotide sequence comparison of these mitochondrial products together with sequence from 18S rDNA and ITS1 from the nuclear genome were nearly identical with the corresponding regions from a *M. mayaguensis* isolate from Mayaguez, Puerto Rico, the type locality of the species. *Meloidogyne mayaguensis* reproduced on cotton, pepper, tobacco, and watermelon but not on peanut. Preliminary results indicate the *M. mayaguensis* isolates from Florida can reproduce on tomato containing the *Mi* gene. Molecular techniques for the identification of *M. mayaguensis* will be particularly useful in cases of *M. mayaguensis* populations mixed with *M. arenaria, M. incognita,* and *M. javanica,* which are the most economically important root-knot nematode species in Florida, and especially when low (<25) numbers of specimens of these species are recovered from the soil.

Key words: isozyme, *Meloidogyne arenaria, M. incognita, M. javanica, M. mayaguensis,* mitochondrial DNA, molecular diagnosis, morphology, nematode, root-knot nematode, taxonomy.

Meloidogyne mayaguensis Rammah and Hirschmann, 1988 is a highly virulent root-knot nematode originally described from a population collected in Puerto Rico from eggplant (*Solanum melongena*). This nematode was initially considered to be *M. arenaria* because of a similar chromosome number (44–45 vs. 40–48) and round-to-ovoid perineal patterns. According to the original description, the nematode differs from *M. arenaria* and other *Meloidogyne* species based on its unique enzyme phenotype and several morphological characters. The female perineal patterns are round to dorsal-ventral ovoid with lateral lines seldom distinguishable, lacking wings, and with discontinuous, forked ventral striae. The male head region lacks annulation, which is sometimes present in *M. arenaria* males. The original description made no mention of similarity of some important morphological (perineal patterns) and biological (host range) characteristics with *M. incognita* (Rammah and Hirschmann, 1988).

The economic importance and geographical distribution of *M. mayaguensis* remained unknown for some years after its description. In the late 1980s, severe damage and occurrence of root-knot nematode infected roots were observed in West Africa on tomato 'Rossol' with the *Mi* gene (Prot, 1984) and on root-knot nematode resistant soybean 'Forrest' and sweet potato 'CDH' (Fargette, 1987), growing in fields infested with putative *M. arenaria* or *M. incognita,* respectively. Esterase phenotype and RFLP analyses of these root-knot nematodes provided profiles fitting those of *M. mayaguensis* (Fargette and Braaksma, 1990; Fargette et al., 1996). Subsequently, *M. mayaguensis* was reported from Brazil (Carneiro et al., 2001), the Caribbean basin (Carneiro et al., 2000; Decker and Rodriguez Fuentes, 1989; Trudgill et al., 2000), South Africa (Willers, 1997), and in glasshouses in France (Blok et al., 2002). These observations indicated that the host range of this species is similar to that of *M. incognita* and includes a large number of vegetable and field crops.

Until this time there have been no reports of the presence of this nematode in North America. Late in 2001, routine regulatory samples collected from ornamental nurseries in south Florida were found infected with a root-knot nematode with perineal patterns morphologically similar to *M. incognita* but with an esterase and malate dehydrogenase phenotype similar to *M. mayaguensis.* The perineal patterns from some females, however, had a high trapezoidal dorsal arch similar to that of *M. incognita.* Specimens of *M. mayaguensis* with a high trapezoidal dorsal arch have been reported from populations collected in the Ivory Coast in West Africa (Fargette and Braaksma, 1990) and Brazil (Carneiro et al., 2001). Due to the known economic significance of this species, it was necessary to determine if *M. mayaguensis* was indeed established in Florida.

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[1] Journal Series No. 14094, Agricultural Research Division, University of Nebraska.

[2] Nematologists, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, P.O. Box 147100, Gainesville, FL 32614-7100.

[3] Associate Professor, Plant Pathology, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

[4] Postdoctoral Associate, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

[5] Professor, Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0620.

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E-mail: tpowers@unl.edu

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The objectives of this study with five isolates of *M. mayaguensis* from Florida were to: (i) compare the perineal patterns and morphometrics of selected stages of these isolates with those reported in the literature for *M. mayaguensis* and also with Florida populations of *M. incognita, M. arenaria,* and *M. javanica,* which are the most economically important root-knot nematodes in the state; (ii) determine their enzyme phenotypes; (iii) compare and expand the database of DNA markers useful for species identification; and (iv) elucidate any host preferences.

Materials and Methods

Nematode origin: Representatives of four *M. mayaguensis* populations were collected from unidentified ornamental roots and one from roots of the ornamental shrub, *Tibouchina elegans.* Their origins and designations were Broward County (DPI: N01-00283), Palm Beach County (N01-00304 and N02-00818), and Dade County (N01-00514). Single egg mass isolates of populations 283, 514, and 818 were made and designated as isolates 1, 4, and 5, respectively. Two isolates, 2 and 3, were obtained from population 304 to determine intraspecific variation due to a large variability in the J2 body length of this population. *Meloidogyne arenaria* race 1 was originally collected from peanut (*Arachis hypogaea*), and *M. incognita* race 4 and *M. javanica* from tobacco (*Nicotiana tabacum*) plants growing at the Green Acres Research Farm, University of Florida, Alachua County, Florida. *Meloidogyne incognita* race 2 was obtained from tobacco plants in Alachua County. Second-stage juveniles (J2) extracted from a single egg mass isolate produced by a female of each field population that showed esterase and malate dehydrogenase profiles fitting those of *M. arenaria, M. incognita, M. javanica,* or *M. mayaguensis* were reared on tomato (*Lycopersicon esculentum* 'Rutgers') and maintained in separate greenhouses. Type locality juveniles of *M. mayaguensis* were obtained from original cultures maintained at North Carolina State University under the designation Puerto Rico—E180 (Esbenshade and Triantaphyllou, 1985). In 1993, specimens of this nematode sent to the University of Nebraska were frozen as individual juveniles in 15 µl of distilled water in PCR reaction tubes.

Morphometric and morphological analyses: Morphometric characters for J2 (Esser et al., 1976; Jepson, 1987) were examined for isolates 1 to 4 using live specimens relaxed with low heat and mounted in water agar (Esser, 1986). The morphometrics of J2 *M. incognita* races 2 and 4 from Florida were examined and compared with those of *M. mayaguensis* J2 from Florida, and those reported in the original description and by Fargette and Braaksma (1990) in West Africa. Lengths of J2 tails, male stylets, and perineal patterns were obtained from *M. arenaria* and *M. javanica* Florida populations and compared with *M. mayaguensis* isolates. Perineal patterns were mounted in glycerin, examined, and photographed (Hartman and Sasser, 1985). Measurements also were taken from adult specimens of isolates 1, 2, and 4 (Esser, 1986). Stylet length of mature females was measured using live specimens that were seen occasionally moving their stylets. These torpid specimens always have intact stylets, whereas dead or fixed specimens often have damaged stylets lacking tips, which are not recognizable when using a compound microscope.

Enzyme analysis: A single female from the three isolates (1, 2, and 4) was crushed in 10 µl of sample buffer (0.5 M Tris HCl, pH 6.8, 10% glycerol, 0.05% bromophenol blue [w/v]) (BioRad, Hercules, CA). The extracts were analyzed by electrophoresis using polyacrylamide gel made up of 4% stacking gel, pH 6.8, and 7% separating gel, pH 8.8 with Tris-glycine buffer (BioRad) (Davis, 1964; Ornstein, 1964). At least 52 females from each isolate were subjected to electrophoresis. An isolate of *M. javanica* from Florida was used as a standard to ensure the system was working properly. Electrophoresis was carried out at in a Mini-protean III (BioRad) at 4 °C and 100 V for 15 minutes and then the voltage was set at 200 V for 27 minutes. The activity of esterase (Est) and malate dehydrogenase (Mdh) was detected following procedures described by Harris and Hopkinson (1976). Enzyme phenotypes were categorized as described by Esbenshade and Triantaphyllou (1985; 1990).

DNA analysis: Four different PCR primer sets, two derived from mitochondrial DNA and two from nuclear ribosomal genes, were used in these analyses. Primers 1108 5′-TACCTTTGACCAATCACGCT-3′ and C2F3 5′-GGTCAATGTTCAGAAATTTGTGG-3′ (Powers and Harris, 1993) amplify a region of variable size in *Meloidogyne* between the mitochondrial cytochrome oxidase subunit II gene and the large (16S) ribosomal gene. Primers 63 VNL 5′-GAAATTGCTTTATTGTTAC-TAAG-3′ and 63 VTH 5′-TAGCCACAGCAAAATAGT-TTTC-3′ (Stanton et al., 1997) amplify a region of 63-bp repeats located in the mitochondrial genome of many *Meloidogyne* spp. (Lunt et al., 1998). The ITS1 primer set, which amplifies the first transcribed spacer of the nuclear ribosomal gene repeats, has been extensively investigated together with ITS2 as a diagnostic marker for nematodes (Powers et al., 1997; Vrain et al., 1992). The 18S primer set used in this study combines primer 18S 1.2 5′-GGCGATCAGATACCGCCCTAGTT-3′ and the reverse complement of rDNA2 from Vrain et al. (1992), 5′-TACAAAGGGCAGGGACGTAAT-3′. This primer amplifies a 638-bp portion of the 3′ region of the 18S nuclear ribosomal gene. Amplifications were conducted as described in Powers and Harris (1993), and amplified DNA was sequenced at Davis Sequencing LLC (Davis, CA). Nucleotide sequences of *M. mayaguensis* have been submitted and assigned GenBank Accession numbers AY446961–AY446982.

Host differentials: Five plants each of differential host plants (Taylor and Sasser, 1978) were inoculated with 5,000 eggs for each isolate (1, 2, and 4). Forty-four days after inoculation plants were harvested. Root systems were washed and stained with phloxine B (Holbrook et al., 1983). Galls and egg mass indicies were determined according to Taylor and Sasser (1978).

Results and Discussion

Morphometric and morphological analyses: Morphometrics of the J2, males, and females of the *M. mayaguensis* Florida isolates and the original *M. mayaguensis* description are listed in Tables 1, 3, and 4. Selected morphometrics of J2, males, and females of *M. incognita* races 2 and 4 are listed in Tables 2 and 5.

Second-stage juveniles: Morphometrics of *M. mayaguensis* J2 from Florida isolates did not differ from those reported in the original description. However, some of the morphological characters of diagnostic significance (Esser et al., 1976; Jepson, 1987; Taylor, 1987), such as tail length, showed smaller low range values in Florida isolate 2 than reported in the original description (47 µm vs. 49.2 µm) (Table 1). These differences indicate variability in the morphological characters of J2 among *M. mayaguensis* populations from Florida. This variability was confirmed by examining isolate 3 originating from a single egg mass collected from the same field population as that of isolate 2. Isolate 3 had the smallest low range value of J2 body and tail length, which were 377 µm and 43 µm, respectively, compared with 390.4 µm and 49.2 µm from the original description (Table 1). The ranges of mean values for body length across the four isolates from Florida (419.2–473 µm) were greater than those reported for eight West African isolates of *M. mayaguensis* (ranging from 416.5–441.3 µm) (Fargette and Braaksma, 1990). Ranges of Florida isolates of *M. mayaguensis* overlapped with those of *M. incognita* races 2 and 4 (Tables 1, 2). However, across isolates *M. mayaguensis* had greater values than those of *M. incognita* (races 2 and 4) for the following measurements, respectively: range of mean body length (419–473 µm vs. 388.5–406.6 µm); high range of body length

Table 1. Morphometrics (mean, standard deviation, and range) of second-stage juveniles of four isolates of *Meloidogyne mayaguensis* from Florida and the original description.[a]

Character	Isolate 1	Isolate 2[b]	Isolate 3[c]	Isolate 4	Original description[d]
Body length	461.1 ± 15.9 (433.0–481.0)	449.2 ± 26.4 (421.0–483.0)	419.0 ± 20.5 (377–450)	473.0 ± 15.0 (439.0–491.5)	453.6 ± 28.4 (390.4–528.0)
Body width	15 ± 0.4 (14.5–16.1)	15.6 ± 0.6 (14.7–16.1)	15.3 ± 0.5 (14.7–16.0)	15.7 ± 0.5 (14.7–16.1)	14.7 ± 0.5 (13.8–15.8)
Body width at anus	10.6 ± 0.3 (10.0–11.2)	10.6 ± 0.5 (9.8–11.7)	10.8 ± 0.5 (10.2–11.2)	11.0 ± 0.4 (10.2–11.6)	10.9 ± 0.5 (10.2–12.2)
Stylet length	10.9 ± 0.3 (10.4–11.5)	10.8 ± 0.3 (10.2–11.4)	10.9 ± 0.2 (10.5–11.2)	10.9 ± 0.3 (10.2–11.4)	11.6 ± 0.3 (11.1–12.2)
Stylet base to head end	15.0 ± 0.5 (14.5–15.6)	15.3 ± 0.5 (14.2–16.1)	14.9 ± 0.5 (13.7–15.6)	15.3 ± 0.3 (14.7–15.7)	15.2 ± 0.3 (14.8–15.8)
DGO	3.8 ± 0.3 (2.9–4.1)	3.4 ± 0.3 (2.9–3.9)	2.8 ± 0.3 (2.0–3.4)	3.8 ± 0.3 (3.2–4.7)	3.9 ± 0.2 (3.3–4.3)
Head end to metacorpus valve	59.2 ± 1.7 (56.5–61.7)	58.2 ± 3.0 (52.9–63.7)	56.5 ± 2.8 (50.0–60.7)	61 ± 2.2 (56.5–64.6)	58.2 ± 1.8 (55.2–62.9)
Excretory pore to head end	92.4 ± 4.0 (88.2–98.0)	92.0 ± 19.0 (85.2–97.0)	89.4 ± 3.2 (83.3–96.0)	96.2 ± 3.3 (93.0–99.9)	87.6 ± 3.3 (79.9–97.9)
Tail length	56.4 ± 2.9 (50.5–61.2)	53.1 ± 3.1 (47.0–59.7)	49.5 ± 3.7 (43.1–56.8)	56.7 ± 2.3 (51.9–61.2)	54.4 ± 3.6 (49.2–62.9)
Pharynx	78.0 ± 3.5 (72.5–89.0)	75.1 ± 3.2 (68.1–80.3)	75.1 ± 2.0 (70.5–76.4)	78.1 ± 2.4 (73.5–81.3)	—
Pharyngeal overlap	99.4 ± 11.5 (82.3–128.0)	113.7 ± 18.2 (107.5–192.5)	97.9 ± 19.8 (63.7–127.0)	121.3 ± 11.7 (96.0–141.0)	—
Hyaline tail terminus length	11.1 ± 2.6 (5.0–14.7)	11.4 ± 1.9 (8.5–14.7)	10.3 ± 1.8 (7.8–14.7)	11.3 ± 1.4 (9.3–13.7)	—
a	30.8 ± 1.2 (28.3–32.5)	28.8 ± 1.5 (27.0–31.2)	27.5 ± 1.4 (25.0–31.1)	30.2 ± 1.3 (28.1–33.1)	30.9 ± 1.9 (26.4–34.7)
Body length/head end to metacorpus valve	7.8 ± 0.3 (7.1–8.3)	7.7 ± 0.3 (7.1–8.3)	7.4 ± 0.4 (6.7–8.3)	7.8 ± 0.3 (7.2–8.3)	7.8 ± 0.4 (6.8–8.8)
b	5.9 ± 0.3 (5.2–6.3)	6.0 ± 0.2 (5.6–6.4)	5.6 ± 0.2 (5.1–5.9)	6.1 ± 0.2 (5.7–6.4)	—
c	8.2 ± 0.4 (7.6–8.6)	8.5 ± 0.4 (7.8–9.1)	8.5 ± 0.6 (7.4–9.9)	8.4 ± 0.2 (8.0–8.7)	8.3 ± 0.4 (7.0–9.2)
c′	5.3 ± 0.3 (4.8–5.9)	5.0 ± 0.3 (4.4–5.6)	4.6 ± 0.4 (3.9–5.4)	5.1 ± 0.3 (4.5–5.3)	5.0 ± 0.4 (4.3–5.7)

[a] Measurements (µm) were taken using 20 specimens from each isolate.
[b] Single egg mass isolate 2 obtained from population N01-00304.
[c] Single egg mass isolate 3 obtained from population N01-00304.
[d] Rammah and Hirschmann, 1988.

TABLE 2. Morphometrics (mean, standard deviation, and range) of second-stage juveniles of two races of *Meloidogyne incognita* from Florida.[a]

Character	Race 2	Race 4
Body length	388.5 ± 18.8 (348.5–424.0)	406.5 ± 12.9 (372.0–426.0)
Body width	14.7 ± 0.4 (14.2–16.0)	15.1 ± 0.5 (14.2–16.0)
Body width at anus	10.7 ± 0.5 (9.7–11.4)	11.1 ± 0.3 (10.5–11.7)
Stylet length	10.7 ± 0.3 (10.2–11.2)	10.6 ± 0.2 (10.2–10.9)
Stylet base to head end	14.9 ± 0.3 (14.5–15.6)	14.9 ± 0.2 (14.5–15.6)
DGO	3.1 ± 0.3 (3.8–2.8)	3.3 ± 0.3 (2.8–3.8)
Head end to metacorpus valve	57.1 ± 1.4 (54.8–59.7)	55.9 ± 2.1 (53.5–59.7)
Excretory pore to head end	71.4 ± 1.7 (68.6–74.4)	83.6 ± 1.9 (80.0–86.2)
Tail length	49.6 ± 3.3 (44.1–52.9)	48.7 ± 1.3 (46.0–50.0)
Pharynx	71.4 ± 1.7 (68.6–74.4)	70.9 ± 2.2 (67.5–73.5)
Pharngeal overlap	107 ± 6.8 (100–127)	114.4 ± 7.4 (102.5–127.0)
Hyaline tail terminus length	11.5 ± 1.7 (9.7–13.7)	10.3 ± 1.2 (8.3–12.2)
a	26.4 ± 1.4 (23.4–28.1)	27.0 ± 1.3 (24.0–29.0)
Body length/head end to metacorpus valve	6.8 ± 0.3 (6.1–7.3)	7.3 ± 0.3 (6.9–7.8)
b	5.4 ± 0.2 (4.8–5.8)	5.7 ± 0.2 (5.2–6.1)
c	7.8 ± 0.3 (7.1–8.5)	8.4 ± 0.2 (8.0–8.8)
c′	4.7 ± 0.3 (4.1–5.1)	4.4 ± 0.1 (4.1–4.7)

[a] Measurements (µm) were taken using 20 specimens from each population.

(450–491.5 µm vs. 424–426 µm); range of mean tail length (49.5–56.7 µm vs. 48.7–49.6 µm); and high range of tail length (56.8–61.2 µm vs. 50–52.9 µm) (Tables 1, 2). Overlapping values were more frequent between the two races of *M. incognita* and isolate 2 of *M. mayaguensis* because of their small body size (Table 1). The body and tail lengths of *M. mayaguensis* J2 from Florida were between those of *M. incognita* and *M. arenaria* (Cliff and Hirschmann, 1985). The great variability observed in the morphometric parameters of *M. mayaguensis* populations complicate the morphological separation of this species from *M. incognita* if only J2 are available. Our observations of the J2 of the two races of *M. incognita,* which were identified with morphological, enzymatic, and host preference procedures, indicate that body and tail length values of J2 >425 and 53 µm respectively, are out of range for this species and fit those of *M. mayaguensis* and other root-knot nematodes species, with values of these parameters greater than those of *M. incognita.* This observation is supported by Esser et al. (1976), who reported high range values of body length for *M. incognita,* which are smaller (393 µm) than those observed for race 2 (424 µm) and race 4 (426 µm) in this study. Also, the mean values of tail length (48.7 µm) and tail hyaline portion (8.9 µm) reported by Jepson (1987) for *M. incognita* are smaller than or similar to those found in this study for race 2 (49.6 µm and 11.5 µm) and race 4 (48.7 µm and 10.3 µm) (Table 2). This observation further corroborates our assessment. However, greater high range values (than those found in our study) for body (463 µm) and tail (62 µm) length of *M. incognita* J2 are reported by Eisenback et al. (1981). We do not know how common these large values are in *M. incognita* populations. These values indicate that morphometric separation of *M. incognita* J2 from those of *M. mayaguensis* may be unreliable for certain isolates.

The mean, standard deviation, and range values of J2 tail lengths of *M. arenaria* and *M. javanica* from Florida were 61.4 ± 4.4 (52.0 to 68.5) and 51.2 ± 2.2 (47.5 to 55.5), respectively. These values are similar to *M. mayaguensis* J2 across four isolates 53.9 (43.1 to 61.2) (Table 1) and are not useful for the separation of these three species.

Males: The morphometrics of *M. mayaguensis* males from Florida isolates were similar to those reported in the original description except for the stylet length values, which were smaller across the three populations (17.5 to 21.7 µm vs. 20.7 to 24.6 µm) (Table 3). Smaller values of stylet length (19 to 21 µm) than those of the original description also were reported by Fargette and Braaksma (1990) for *M. mayaguensis* populations from West Africa (Table 6). The stylet length values for *M. incognita* races 2 and 4 males were 23.9 (21.7 to 25.4) and 23.5 (21.5 to 26 µm), respectively (Table 5). The low ranges of this parameter (21.5 µm) for *M. incognita* males overlap the high ranges (21.7 µm) of *M. mayaguensis* stylet length of males from Florida making a clear separation of the two species difficult. The low ranges of this parameter for *M. incognita* races 2 and 4 from Florida were smaller than those reported by Eisenback et al. (1981) and Jepson (1987) for *M. incognita,* which were 24 (23 to 25 µm) and 24 to 26 µm, respectively (Table 6). However, in the isolates studied from Florida stylet length values >22 µm were out of range for *M. mayaguensis.* Both means (23.9 to 23.5 µm) and high range values (25.4 to 26 µm) of this parameter for *M. incognita* races 2 and 4 are greater than those observed in *M. mayaguensis* isolates from Florida 19 (17 to 21.7 µm) (Table 3) and West Africa 19 to 21 µm (range of mean values of eight populations) (Fargette and Braaksma, 1990) (Table 6). The results of these observations in Florida and West Africa indicate that stylet length of male has important diagnostic value for the separation of Florida and West Africa populations of *M. mayaguensis* from *M. incognita.* High variability was observed in the male body length (Table 3).

The mean, standard deviation, and range values of stylet lengths for *M. arenaria* and *M. javanica* males from Florida were 21.6 ± 1.3 (19.6 to 68.5) and 20.6 ±

TABLE 3. Morphometrics (mean, standard deviation, and range) of males of three isolates of *Meloidogyne mayaguensis* from Florida and the original description.[a]

Character	Isolate 1	Isolate 2	Isolate 4	Original description[b]
Body length	995.5 ± 97.0 (856.5–1140.5)	1049 ± 183.6 (782–1397)	990.0 ± 193.3 (695.5–1501.0)	1,503.0 ± 141.9 (1,175.0–1,742.0)
Body width	27.0 ± 1.8 (24.1–31.5)	31.1 ± 2.8 (26.4–34.3)	29.1 ± 3.7 (24.5–38.2)	37.8 ± 3.1 (32.2–44.4)
Body width at stylet base	15.6 ± 0.7 (14.2–16.7)	16.6 ± 1.2 (14.7–19.1)	16.1 ± 1.5 (14.2–19.5)	18.9 ± 0.8 (17.4–20.5)
Body width at excretory pore	23.4 ± 1.7 (20.0–26.5)	27.4 ± 2.2 (23–30.5)	24.8 ± 3.2 (21.0–34.3)	29.0 ± 1.6 (25.9–31.8)
Stylet length	19.7 ± 0.8 (17.5–20.8)	19.4 ± 1.0 (17.5–21.0)	19.3 ± 1.0 (17.5–21.7)	22.0 ± 0.8 (20.7–24.6)
Stylet knob height	2.3 ± 0.2 (1.9–2.8)	2.5 ± 0.2 (2.0–3.0)	2.3 ± 0.2 (2.0–2.9)	3.0 ± 0.3 (2.4–3.7)
Stylet knob width	4.6 ± 0.3 (3.9–5.0)	4.7 ± 0.5 (3.0–5.0)	4.3 ± 0.3 (3.8–5.0)	5.0 ± 0.3 (4.3–5.6)
DGO	4.6 ± 0.4 (3.9–5.0)	3.8 ± 0.5 (3.0–4.8)	4.6 ± 0.3 (4.1–5.0)	4.1 ± 0.4 (3.3–5.0)
Head end to metacorpus valve	86.0 ± 3.8 (78.0–93.1)	80.2 ± 4.7 (71.5–87.7)	82.2 ± 6.2 (72.5–92.0)	92.1 ± 4.3 (84.4–102.0)
Excretory pore to head end	138.3 ± 14.8 (117.5–183.0)	135 ± 15.8 (119.0–174.0)	166.4 ± 8.8 (111.5–178.3)	(147.2–180.8)
Tail length	11.2 ± 1.0 (9.8–13.5)	10.2 ± 1.4 (7.8–11.7)	11.4 ± 1.4 (8.8–15.1)	14.3 ± 1.1 (11.3–16.3)
Spicule length	26.0 ± 1.6 (23.5–29.4)	27.3 ± 1.2 (25.4–29.4)	25.5 ± 1.4 (23.8–28.4)	28.3 ± 1.5 (24.4–31.3)
Gubernaculum length	6.9 ± 0.4 (6.1–7.7)	6.9 ± 0.7 (8.3–5.8)	6.8 ± 0.4 (6.0–7.8)	7.1 ± 0.6 (6.1–9.3)
a	36.8 ± 2.6 (31.5–40.9)	35.2 ± 5.2 (21.9–42.9)	33.8 ± 3.1 (28.4–39.3)	39.9 ± 3.9 (31.1–49.6)
Body length/head end to metacorpus valve	11.6 ± 0.9 (10.3–13.1)	13.6 ± 2.1 (9.7–17.5)	12.0 ± 2.0 (9.3–15.3)	16.3 ± 1.5 (14.1–19.1)
Stylet knob width/height	2.0 ± 0.2 (1.7–2.4)	1.9 ± 0.2 (1.5–2.2)	1.9 ± 0.1 (1.7–2.2)	1.7 ± 0.2 (1.3–2.0)
c	89.1 ± 10.0 (73.2–102.7)	108.3 ± 19.6 (76.4–128.9)	87.4 ± 14.6 (128.3–68.2)	105.7 ± 10.0 (85.8–124.3)

[a] Measurements (µm) were taken using 20 specimens from each population.
[b] Rammah and Hirschmann, 1988.

0.7 (19.5 to 22), respectively. These values are similar to *M. mayaguensis* males across three Florida isolates 19.4 (17.5 to 21.7) (Table 3) and are not useful for the separation of the three species.

Females: The selected morphometrics for *M. mayaguensis* females did not differ from those reported in the original description and the isolates from West Africa (Tables 4, 6). Our measurements indicate that stylet length of *M. mayaguensis* females from Florida ranges from 13.5 µm to 15 µm across the three isolates (Tables 4, 6). Ranges of stylet length values for *M. incognita* from Florida were 14.2 to 16.8 µm across the two races (2 and 4) (Table 5). The low ranges of stylet length values (13.5 to 13.9 µm) for *M. mayaguensis* were

TABLE 4. Selected morphometrics (mean, standard deviation, and range) of live females of three isolates of *Meloidogyne mayaguensis* from Florida and the original description.[a]

Character	Isolate 1[b]	Isolate 2[c]	Isolate 4[d]	Original description[e]
Stylet length	14.31 ± 0.36 (13.90–15.00)	14.50 ± 0.34 (13.70–15.00)	13.97 ± 0.30 (13.50–14.70)	15.8 ± 0.8 (13.8–16.8)
DGO	4.34 ± 0.33 (3.9–4.9)	4.43 ± 0.50 (3.40–5.30)	4.88 ± 0.46 (4.0–6.0)	4.8 ± 0.8 (3.5–6.7)
Vulval slit length	26.5 ± 1.6[f] (23.5–29.4)	26.0 ± 2.0[g] (22.0–30.0)	26.3 ± 1.2[g] (24.9–29.4)	26.1 ± 1.9 (20.9–30.4)

[a] Morphometrics in µm.
[b] $n = 14$.
[c] $n = 15$.
[d] $n = 17$.
[e] Rammah and Hirschmann, 1988.
[f] $n = 28$.
[g] $n = 22$.

TABLE 5. Selected morphometrics (mean, standard deviation, and range) of live females and males of two races of *Meloidogyne incognita* from Florida.

Character[a]	♀ Race 2[b]	♀ Race 4[c]	♂ Race 2[d]	♂ Race 4[d]
Stylet length	14.69 ± 0.43 (14.20–15.60)	15.87 ± 0.48 (14.80–16.80)	23.9 ± 1.1 (21.7–25.4)	23.48 ± 1.3 (21.50–26.00)
DGO	4.03 ± 0.41 (3.50–4.90)	4.00 ± 0.30 (3.40–4.40)		
Vulval slit length[d]	21.33 ± 3.22 (14.70–26.90)	21.90 ± 2.48 (17.60–26.90)		

[a] Morphometrics in µm
[b] $n = 15$.
[c] $n = 19$.
[d] $n = 20$.

smaller than those of *M. incognita* female races 2 and 4 (14.2 and 14.8 µm, respectively) (Tables 4–6). Based on these results *M. mayaguensis* females having a stylet length ≤14 µm can be separated from those of *M. incognita* and, conversely, *M. incognita* females with a stylet length ≥15 µm can be discriminated from *M. mayaguensis*. Using this character, females having stylet lengths between 14 and 15 µm cannot be reliably identified. The separation of the two based on stylet length is supported by smaller means for stylet lengths (11.7 to 13.3 µm) of females reported for isolates of *M. mayaguensis* studied in West Africa (Fargette and Braaksma, 1990) (Table 6). However, greater high ranges (16.8 µm) and mean (15.8 µm) for this character are listed in the original description, complicating the separation of the two species using this morphological character. The range value of the stylet length for *M. incognita* (14.2 to 15.6 and 14.8 to 16.8 µm for races 2 and 4, respectively) (Table 5) was smaller than those (16 to 18 µm) reported by Jepson (1987) and similar to those (15 to 17 µm) listed by Eisenback et al. (1981) (Table 6). The vulva slit length values of *M. mayaguensis* females from Florida did not differ from those reported in the original description. The range of the means across the three isolates (26 to 26.5 µm) and low ranges (22 to 24.9 µm) of this character for *M. mayaguensis* females from Florida (Table 4) were greater than those of *M. incognita* races 2 and 4 (20 to 21 µm and 14.7 to 17.6 µm, respectively) (Tables 4, 5). Variability of this character is reported in the original description (20 to 30 µm) and in *M. mayaguensis* isolates from West Africa (22.1 to 26.3 µm), which weakens its diagnostic value.

The perineal patterns of females of the three isolates of *M. mayaguensis* from Florida were similar, in part, to those reported in the original description. Approximately 70% of *M. mayaguensis* females from Florida showed round or dorso-ventrally ovoid perineal patterns (Fig. 1A). The remaining 30% (50% in isolates 2 and 3) showed a high trapezoidal dorsal arch (Fig. 1B) similar to that of *M. incognita*. Specimens with these perineal patterns were indistinguishable from those of *M. incognita*. Other shapes of the dorsal arch observed in the three isolates include high arch trapezoidal on one side and oval on the other (Fig. 2A) and low trapezoidal arch without or with distinct lateral lines (Fig. 2B,C). Specimens with high trapezoidal dorsal arch are

TABLE 6. Range of morphological characters useful for the separation of *Meloidogyne mayaguensis* (*Mm*) from *Meloidogyne incognita* (*Mi*) from Florida.

Differential characters	*Mm* from Florida[a]	*Mm* from West Africa[b]	*Mm* from Puerto Rico[c]	*Mi* races 2 and 4 from Florida[d]	*Mi*[e]	*Mi*[f]
			Second-stage juveniles			
	($n = 80$)	($n = 151$)	($n = 35$)	($n = 40$)	n = undefined	n = undefined
Body length	377–491	416.5–441.3	390–520	348–424	346–463	—
Tail length	43.1–61.2	Not reported	49.2–62.9	44.1–52.9	42–62	48.7
			Males			
	($n = 60$)	($n = 239$)	($n = 30$)	($n = 40$)	n = undefined	n = undefined
Stylet length	17–21.7	19–21	20.7–24.6	21.5–26	23–25	24–26
			Females			
	($n = 45$)	($n = 91$)	($n = 35$)	($n = 30$)	n = undefined	n = undefined
Stylet length	13.5–15	11.7–13.7	13.8–16.8	14–16.8	15–17	16–18
Vulva slit length	22–30	22.1–26.3	12.7–21.1	14.7–26.9	Not reported	Not reported

[a] Cumulated values across four isolates.
[b] Cumulated values across eight isolates (Fargette and Braaksma, 1990).
[c] Rammah and Hirschmann, 1988.
[d] Cumulated values for the two races (two isolates).
[e] Eisenback et al., 1981.
[f] Jepson, 1987.

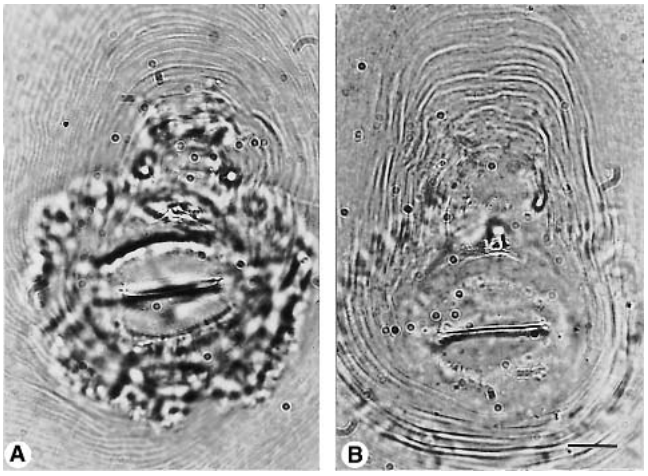

FIG. 1. Photomicrographs of perineal patterns of isolate 2 of *Meloidogyne mayaguensis* female from Florida. A) A perineal pattern fitting that reported in the original description of this species. B) A perineal pattern showing similarity to that of *Meloidogyne incognita.* Scale bar for both figures = 12 µm.

reported in *M. mayaguensis* from West Africa and also Brazil (Carneiro et al., 2001; Fargette and Braaksma, 1990).

Because of the similarity of J2 morphometrics and female perineal patterns of *M. mayaguensis* with those of *M. incognita,* we cannot exclude the possibility that *M. mayaguensis* has been undetected for many years in Florida and identified as *M. incognita* in analyses of Florida regulatory and survey samples. The separation of *M. incognita* from *M. mayaguensis* may be possible by employing a compound microscope to examine the morphometric characteristics of J2, males, and females using large number of specimens (>25 specimens). The major morphometric characters of diagnostic values for the separation of these two species are summarized in Table 6.

Perineal patterns of *M. arenaria* and *M. javanica* are useful for the separation of these two species from *M. mayaguensis. Meloidogyne mayaguensis* perineal patterns usually have a high dorsal arch unlike *M. arenaria* and *M. javanica.* They usually lack lateral lines that are present in *M. javanica.* However, distinct lateral lines similar to *M. javanica* were observed occasionally in *M. mayaguensis* specimens (Fig. 2C).

The identification of only a few specimens of each of these species or of mixed populations of *M. mayaguensis* with *M. arenaria, M. incognita,* or *M. javanica* can be carried out only tentatively, and species identification is not reliable. These tentative diagnoses need to be supported by molecular analysis.

Enzyme analysis: Isolates 1, 2, and 4 showed an esterase phenotype VS1-S1 (Fig. 3A). This phenotype is reported for other *M. mayaguensis* isolates (Carneiro et al., 2001; Fargette et al., 1996; Rammah and Hirschmann, 1988). This phenotype also was observed in an isolate of *M. enterolobii* from China (Yang and Eisenback, 1983). One very strong band (Rm: 1.4) (Fig. 3B) and two other weak bands of Mdh activity (data not shown) were observed in all three isolates. However, these two minor bands require a large amount of homogenates from several females for their detection. The same strong band (Rm: 1.4) of Mdh activity was observed also in two populations of this nematode from Brazil (Carneiro et al., 2001) and one from Martinique (Carneiro et al., 2000). It also was reported in four populations of *M. chitwoodi,* and one population each of *M. enterolobii, M. graminicola, M. naasi, M. oryzae,* and *M.*

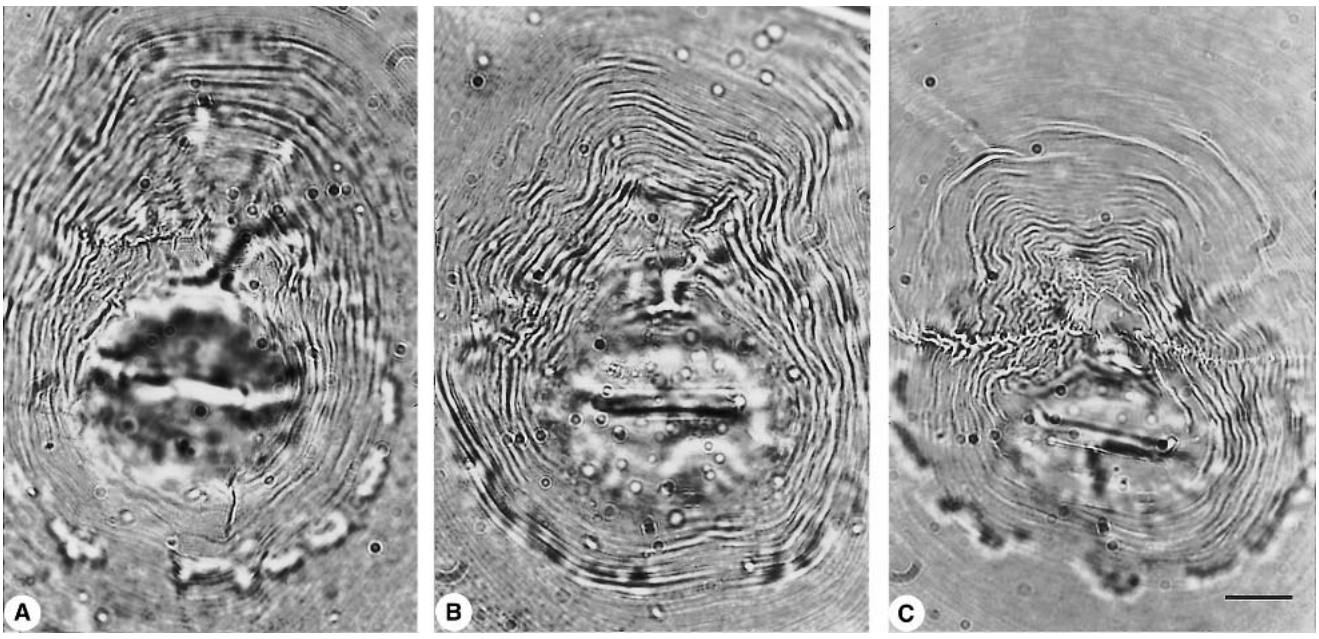

FIG. 2. Micrograph of perineal patterns of *Meloidogyne mayaguensis* Florida populations. A–C) Frequent variability observed among perineal patterns. Scale bar for all figures = 12 µm.

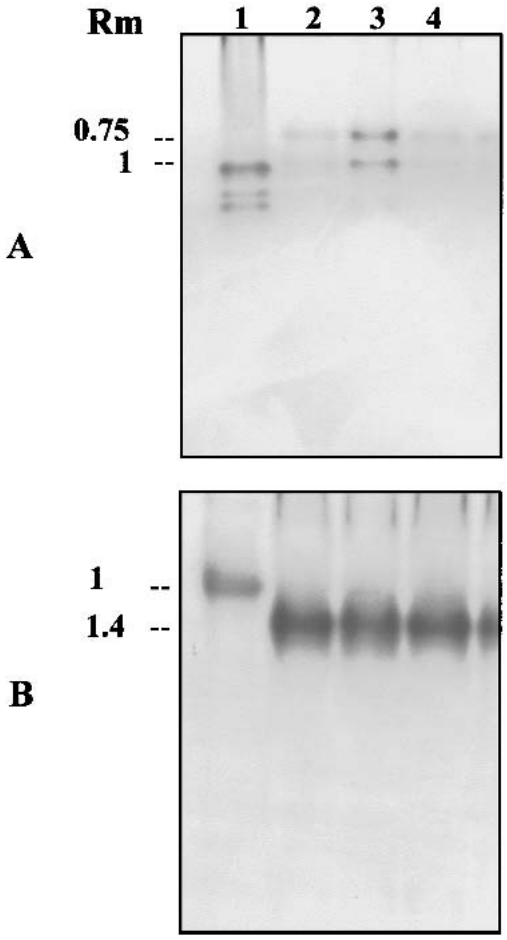

FIG. 3. Enzyme phenotypes found in three isolates of *Meloidogyne mayaguensis* from Florida. A) Esterase phenotypes, VS1-S1. Lane 1 = extract of a single female of *Meloidogyne javanica;* Lanes 2, 3, and 4: extract of a single female of *M. mayaguensis* isolates 1, 2, and 4, respectively. B) Malate dehydrogenase phenotypes. Lane 1: extract of a single female of *M. javanica;* Lanes 2, 3, and 4: extract of a single female of *M. mayaguensis* isolates 1, 2, and 4, respectively.

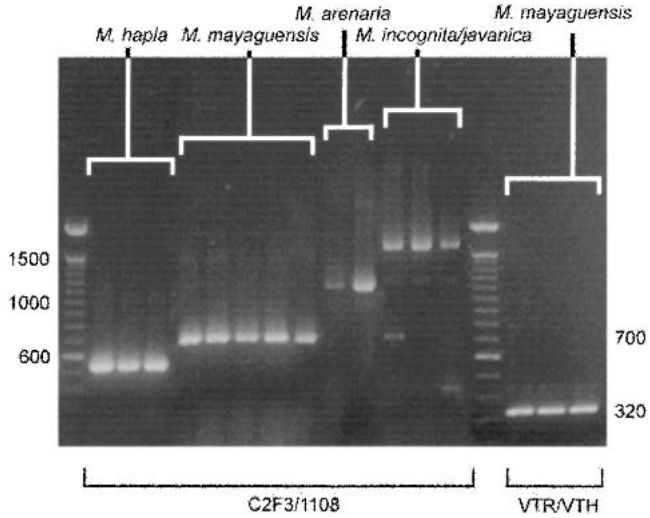

FIG. 4. Amplification products generated from individual juveniles with mitochondrial primer sets C2F3/1108 and 63VNL/63VTH. Primer set C2F3/1108 amplifies a unique 705-bp product in *Meloidogyne mayaguensis.* Primer set 63VNL/63VTH generates a 322-bp product. Other *Meloidogyne* species generate multiple products or no product with this primer set.

platani (Esbenshade and Triantaphyllou, 1985). These findings indicate at Mdh enzyme has low specificity and is of limited diagnostic value for the separation of *M. mayaguensis* from those species.

DNA analysis: Blok et al. (2002) describe two features of the mitochondrial genome that distinguish *M. mayaguensis* from other major species of *Meloidogyne.* For the region of variable size bounded by the primers in the COII and 16S RNA genes, *M. mayaguensis* produces a unique size of 705 bp (Fig. 4). We confirmed this observation by amplifying the 705-bp product from 160 individual juvenile specimens representing the five Florida isolates and the type material from Puerto Rico. Fifteen of these amplification products were sequenced for comparison of Florida isolates with Puerto Rico and the published sequence of a French glasshouse isolate (Blok et al., 2002). Slight nucleotide variation ranging from 0.1% to 1.8% was observed among the 15 sequences. There was little evidence to suggest that separate and distinct Florida and French populations exist in nature, yet the finding of 15 mitochondrial haplotypes (mitochondrial sequences with at least one confirmed nucleotide substitution) indicates that extensive, but low-level variation exists in the species. The two haplotypes from Puerto Rican specimens displayed the largest genetic distances in comparisons among haplotypes, possibly indicating a lack of gene flow between populations from Florida and Puerto Rico. Additional samples will be necessary to determine if geographical population structure exists for the species.

The second distinguishing feature observed by Blok et al. (2002) pertained to the amplification of a single 322-bp fragment using the primer set that targets the 63-bp tandem repeat region of the *Meloidogyne* mitochondrial genome. Typically, the major *Meloidogyne* species produce multiple fragments or no fragments in amplifications with this primer set (Lunt et al., 1998). We tested 14 *M. mayaguensis* juveniles from two isolates. All amplifications produced the predicted 322-bp fragment (Fig. 4).

Two primer sets designed to amplify conserved and rapidly evolving regions of the nuclear ribosomal repeats, 18S and ITS1 respectively, were tested for their potential to discriminate *M. mayaguensis.* Only three nucleotides differed between the 638-bp segment of 18S in *M. mayaguensis* and *M. incognita; M. incognita* and *M. arenaria* are identical for this region. No intraspecific variation was observed among six *M. mayaguensis* 18S sequences. The close 18S similarity between these species contrasts with the greater distance when *M. mayaguensis* is compared with *M. graminis* (9 nucleotide substitutions), *M. chitwoodi* (11 substitutions), and *M. hapla* (14 substitutions). ITS1 nucleotide sequence also dem-

onstrates the similarity between *M. mayaguensis* isolates from Puerto Rico and Florida; however, difficulties with alignments due to differences in amplicon size make interspecific calculations of similarity tentative. We are continuing to acquire ITS1 sequence to better assess the diagnostic potential of this marker.

Host differentials: Isolates 1, 2, and 4 from Florida showed similar host preference to that of *M. incognita* race 4 (Taylor and Sasser, 1978). Fargette (1987) reported similar results for *M. mayaguensis* from West Africa. However, an isolate of *M. mayaguensis* from Puerto Rico showed the same host range as *M. incognita* race 2, which does not reproduce on cotton (Rammah and Hirschmann, 1988). In this study all the isolates reproduced on cotton, tobacco, pepper, and watermelon but not on peanut. The reproduction of all isolates was much higher on tobacco than on cotton. These results show that there is variability in the physiological host response among isolates of *M. mayaguensis,* which indicates the possibility of host races. Isolate 2 was able to reproduce on BHN (+*Mi*) tomato cultivars in growth chamber experiments. Experiments to determine reproductive capabilities of each of the isolates at multiple temperatures are now under way.

The experiments described above clearly indicate that *M. mayaguensis* is established in southern Florida. They also illustrate the need for molecular methods to monitor the distribution and dispersal of this potentially severely damaging pathogen.

Literature Cited

Blok, V. C., J. Wishart, M. Fargette, K. Berthier, and M. S. Phillips. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. Nematology 4:773–781.

Carneiro, R. M. D. G., M. R. A. Almeida, and P. Quénéhervé. 2000. Enzyme phenotypes of *Meloidogyne* spp. isolates. Nematology 2:645–654.

Carneiro, R. M. D. G., W. A. Moreira, M. R. A. Almeida, and A. C. M. M. Gomes. 2001. Primeiro registro de *Meloidogyne mayaguensis* em Goiabeira no Brasil. Nematologia Brasileira 25:223–228.

Cliff, G. M., and H. Hirschmann. 1985. Evaluation of morphological variability in *Meloidogyne arenaria.* Journal of Nematology 17:445–459.

Davis, B. J. 1964. Disk electrophoresis. II. Method and application to human serum proteins. Annals of the New York Academy of Science 121:404–427.

Decker, H., and M. E. Rodriguez Fuentes. 1989. The occurrence of root gall nematodes *Meloidogyne mayaguensis* on *Coffee arabica* in Cuba. Wissenschaftliche Zeitschrift der Wilhelm-Pieck, Universität, Rostock, Naturwissenschaftliche Reihe 38:32–34.

Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A guide to the four most common species of root-knot nematodes (*Meloidogyne* species). Cooperative Publication, Department of Plant Pathology, North Caroline State University, and U.S. Agency for International Development, Raleigh, N.C.

Esbenshade, P. R., and A. C. Triantaphyllou. 1985. Use of enzyme phenotype for identification of *Meloidogyne* species. Journal of Nematology 17:6–20.

Esbenshade, P. R., and A. C. Triantaphyllou. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. Journal of Nematology 22:10–15.

Esser, R. P. 1986. A water agar *en face* technique. Proceedings of the Helminthological Society of Washington 53:254–255.

Esser, R. P., V. G. Perry, and A. L. Taylor. 1976. A diagnostic compendium of the genus *Meloidogyne* (Nematoda: Heteroderidae). Proceedings of the Helminthological Society of Washington 43:138–150.

Fargette, M. 1987. Use of esterase phenotype in the taxonomy of the genus *Meloidogyne.* 2. Esterase phenotypes observed in West African populations and their characterization. Revue de Nématologie 10:45:56.

Fargette, M., and R. Braaksma. 1990. Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne.* 3. A study of some "B" race lines and their taxonomic position. Revue de Nématologie 13:375–386.

Fargette, M., M. S. Phillips, V. C. Blok, R. Waugh, and D. L. Trudgill. 1996. An RFLP study of relationships between species, isolates, and resistance-breaking lines of tropical species of *Meloidogyne.* Fundamental and Applied Nematology 19:193–200.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. New York: North–Holland Publishing.

Hartman, K. M., and J. N. Sasser. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. Pp. 69–77 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne,* vol. II. Biology and control. Raleigh, NC: North Carolina State University Graphics.

Holbrook, C. C., D. A. Knauft, and D. W. Dickson. 1983. A technique for screening peanut for resistance to *Meloidogyne arenaria.* Plant Disease 57:957–958.

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). Wallingford: C.A.B. International.

Lunt, D. H., L. E. Whipple, and B. C. Hyman. 1998. Mitochondrial DNA variable number tandem repeats (VNTRs): Utility and problems in molecular ecology. Molecular Ecology 7:1441–1455.

Ornstein, L. 1964. Disk electrophoresis. I. Background and theory. Annals of the New York Academy of Science 121:321–349.

Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. Journal of Nematology 25:1–6.

Powers, T. O., T. C. Todd, A. M. Burnell, P. C. B. Murray, C. C. Fleming, A. L. Szalanski, B. J. Adams, and T. S. Harris. 1997. The internal transcribed spacer region as a taxonomic marker for nematodes. Journal of Nematology 29:441–450.

Prot, J. C. 1984. A naturally occurring resistance-breaking biotype of *Meloidogyne arenaria* on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol. Revue de Nématologie 7:23–28.

Rammah, A., and H. Hirschmann. 1988. *Meloidogyne mayaguensis* n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. Journal of Nematology 20:58–69.

Stanton, J., A. Hugall, and C. Moritz. 1997. Nucleotide polymorphism and an improved PCR-based mtDNA diagnostic for parthenogenetic root-knot nematodes (*Meloidogyne* spp.). Fundamental and Applied Nematology 20:261–268.

Taylor, A. L. 1987. Identification and estimation of root-knot nematodes species in mixed populations. Bulletin 12, Florida Department of Agriculture, Division of Plant Industry, Gainesville.

Taylor, A. L., and J. N. Sasser. 1978. Biology, identification, and control of root-knot nematodes (*Meloidogyne* spp.). Cooperative Publication. Department of Plant Pathology, North Carolina State University, and U.S. Agency for International Development, Raleigh, N.C.

Trudgill, D. L., G. Bolla, V. C. Blok, A. Daudi, K. G. Davies, S. R. Gowen, M. Fargette, J. D. Madulu, T. Mateille, W. Mwagenui, C. Nestscher, M. S. Phillips, A. Sawadogo, C. G. Trivino, and E. Voyoukallou. 2000. The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. Nematologica 2:823–845.

Vrain, T. C., D. A. Wakarchuk, A. C. Levesque, and R. I. Hamilton. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15:563–573.

Willers, P. 1997. First record of *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988: Heteroderidae on commercial crops in the Mpumalanga province, South Africa. Inligtingsbulletin-Instituut vir Tropiese en Subtropiese Gewasse 294:19–20.

Yang, B., and J. D. Eisenback. 1983. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. Journal of Nematology 15:381–391.