# MORPHOLOGICAL, BIOCHEMICAL, AND MOLECULAR DIAGNOSTICS OF *MELOIDOGYNE* SPP. ASSOCIATED WITH *MUSA* SPP. IN COLOMBIA

D. H. Riascos[1*], A. T. Mosquera-Espinosa[2], F. Varón de Agudelo[3], J. M. O. Rosa [4], C. M. G. Oliveira[4], and J. E. Muñoz[5]

[1]Estudiante de Doctorado Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira, Colombia; [2]Departamento de Ciencias Naturales y Matemáticas, Pontificia Universidad Javeriana, Cali, Colombia; [3]Profesor Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira-Colombia; [4]Laboratorio de Nematologia, Instituto Biológico, Campinas, São Paulo, Brasil; [5]Profesor titular Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, Palmira, Colombia; *Corresponding author: dhriascoso@unal.edu.co

## ABSTRACT

Riascos, D. H., A. T. Mosquera-Espinosa, F. Varón de Agudelo, J. M. O. Rosa, C. M. G. Oliveira, and J. E. Muñoz. 2019. Morphological, biochemical, and molecular diagnostics of *Meloidogyne* spp. associated with *Musa* spp. in Colombia. Nematropica 49:229-245.

Root-knot nematodes (*Meloidogyne* spp.) are one of the most destructive plant-parasitic nematodes of *Musa* spp. around the world. The objective of this study was to identify species of *Meloidogyne* associated with plantain (*Musa* AAB Simmonds) and banana (*Musa acuminata* AAA) crops from Colombia in different zones of Valle del Cauca, Quindío, Risaralda, and Caldas using morphological, biochemical, and molecular diagnostics. Each population of *Meloidogyne* was cultured on tomato (*Solanum lycopersicum* cv. Santa Clara). Nematodes were then extracted from cultures and species identification was conducted using perineal patterns and esterase phenotypes. Molecular identification was performed by amplifying and sequencing the *D2-D3* expansion segments of the 28S nuclear ribosomal RNA gene as well as partial region of cytochrome oxidase subunit I (*COI*), and NADH dehydrogenase subunit 5 (*Nad5*) genes of mitochondrial DNA. Phylogenetic analysis were performed using maximum likelihood estimation and Bayesian inference for the three target regions. Results confirmed *M. incognita, M. acrita, M. arenaria,* and *M. hispanica* are associated with *Musa* spp. This is the first report of *M. acrita* and *M. hispanica* attacking *Musa* spp. of Colombia.

*Key words:* banana, *Meloidogyne acrita, Meloidogyne arenaria, Meloidogyne hispanica, Meloidogyne incognita,* plantain

## RESUMEN

Riascos, D. H., A. T. Mosquera-Espinosa, F. Varón de Agudelo, J. M. O. Rosa, C. M. G. Oliveira, and J. E. Muñoz. 2019. Diagnóstico morfológico, bioquímico y molecular de *Meloidogyne* spp. asociado con *Musa* spp. en Colombia. Nematropica 49:229-245.

*Meloidogyne* está entre los nematodos parásitos de plantas que más limita la producción de *Musa* spp. en el mundo. Para un control eficiente de poblaciones de *Meloidogyne*, el presente estudio tuvo como propósito identificar especies de este nematodo en cultivos de plátano (*Musa* AAB Simmonds) y de banano (*Musa acuminata* AAA) establecidos en diferentes zonas de producción de los departamentos de Valle del Cauca, Quindío, Risaralda y Caldas, Colombia, através de diagnóstico morfológico, bioquímico y

molecular. Para tal fin, diferentes poblaciones de *Meloidogyne* fueron multiplicadas en plántulas de tomate (*Solanum lycopersicum* cv. Santa Clara) para su identificación basado en la configuración de los patrones perineales y fenotipos de esterasa. La identificación molecular se realizó por amplificación y secuenciación del segmento de expansión *D2-D3* del 28S del ARN ribosomal, la región parcial de los genes citocromo oxidasa subunidad I (*COI*) y NADH dehidrogenasa subunidad 5 (*Nad5*) del ADN mitocondrial. Se realizaron análisis filogenéticos con los métodos de máxima verosimilitud e inferencia bayesiana para los tres loci. Los resultados mostraron que *M. incognita*, *M. acrita M. arenaria* y *M. hispanica* están asociados a Musaceae en el área de estudio. Este es el primer reporte de *M. acrita* y *M. hispanica* en plátano para Colombia.

*Palabras claves*: banano, *Meloidogyne acrita, Meloidogyne arenaria, Meloidogyne hispanica, Meloidogyne incognita,* plátano

## INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) have a broad host range (Fassuliotis, 1970; Carpenter and Lewis, 1991; Noe, 1991; Knight *et al*., 1997; López-Pérez *et al*., 2011; García and Sanchez-Puerta, 2012; Kaur and Attri, 2013; Khanal *et al*., 2016) and are distributed from tropical to temperate regions of the world. Every year, approximately 100 billion USD of agricultural crops are lost to *Meloidogyne* spp. worldwide (Ibrahim *et al*., 2011). Moens *et al*. (2006) stated that root-knot nematodes are one of the major yield-limiting factors of plantain and banana (*Musa* spp.) production. Similarly, De Waele and Davide (1998) mentioned that *M. incognita*, *M. javanica*, and *M. arenaria* occur with the highest frequency in *Musa* spp. around the world. Other species of *Meloidogyne* less frequently found in *Musa* spp. are *M. cruciani*, *M. hispanica,* and *M. graminicola* (Cofcewicz *et al*., 2005; Zhou *et al*., 2015). In Colombia, *M. incognita, M. javanica,* and *M. arenaria* have been found in different production zones of *Musa* spp. (Jaraba *et al*., 2008; Múnera, 2008; Navarro *et al*., 2011).

The symptoms induced by *Meloidodyne* spp. in susceptible cultivars of banana and plantain include severe galling (13 to 20 mm in diam.), stunting, narrow leaves with pale yellow color, thin pseudostems, reduced numbers of secondary and tertiary roots, reduced root hairs, and small fruit bunches (Loos, 1959; Davide, 1996; De Waele and Davide, 1998). Significant yield losses of 45 and 57% can occur when population levels of root-knot nematode are 10,000 and 20,000 individuals per plant, respectively (Davide, 1996).

Because host plant resistance is the preferred method for plant-parasitic nematode management (Khanal *et al*., 2018), understanding nematode variability at the molecular level is important in breeding programs (Khanal *et al*., 2019). Furthermore, the genus *Meloidogyne* has 98 species (Jones *et al*., 2013) and successful management of root-knot nematodes is dependent on accurate species identification (Eisenback, 1982). Identification of a species using classical morphological methods is a difficult task, not only due to the occurrence of mixtures of two or more species in the same field, but also because morphological and morphometric characteristics can overlap among species (Janssen *et al*., 2016; Khanal *et al*., 2016). To solve this problem in the taxonomic studies of *Meloidogyne*, biochemical diagnosis using esterase and/or malate dehydrogenase isozyme profiles and molecular diagnosis using ribosomal RNA and mitochondrial DNA are recommended in addition to morphological information (Carneiro *et al*., 2000). In Colombia, three studies have identified a few species of *Meloidogyne* in *Musa* spp. to date; two are based on morphological and morphometric analyses (Jaraba *et al*., 2008; Navarro *et al*., 2011), and one is through integrative taxonomy (Múnera, 2008). Further studies employing morphological, biochemical, and molecular diagnostics are necessary to complement the taxonomic information currently available and to identify additional species of *Meloidogyne* associated with plantain and banana in production zones that have not previously been surveyed. The objectives of this study were: (i) to identify the species of *Meloidogyne* associated with *Musa* spp. in Valle del Cauca, Quindío, Risaralda and Caldas of Colombia using morphological, biochemical, and molecular diagnostics, and (ii) to elucidate the

phylogenetic relationship of *Meloidogyne* spp. associated with *Musa* spp. from the above-mentioned regions.

## MATERIALS AND METHODS

*Sampling and extraction of nematodes*

Roots and soil samples were collected from plantain and banana crops in different production zones of Colombia including Valle del Cauca (municipalities of Palmira, Caicedonia, Argelia and Buenaventura), Quindío (Calarcá and Córdoba), Risaralda (La Celia), and Caldas (Neira and Manizales). Composite samples were collected from 15 crops, and each sample represented 15–20 plants/ha (Table 1). *Meloidogyne* was extracted from the root samples by using a blending and sieving method, and from soil by using a decanting method (Ravichandra, 2014).

*Morphological identification*

One-month-old tomato cv. Santa Clara seedlings were individually inoculated with second-stage juveniles (J2) of *Meloidogyne* from three crops; one from banana in Calarcá, Quindío (Code sample S6) and two from plantain in Calarcá and Córdoba, Quindío (Code samples S4 and S5, respectively), and maintained in a greenhouse for 2-3 months until gall formation. Adult females of *Meloidogyne* were extracted from galled root tissue and perineal patterns were prepared as described by Hartman and Sasser (1985). Microphotographs were taken using a light microscope equipped with Differential Interference Contrast-DIC (DM2500, Leica, Germany), and the species were identified.

*Biochemical identification*

The three populations of *Meloidogyne* raised on tomato (S4, S5, and S6) were identified using esterase phenotype as described by Oliveira *et al*. (2012). For each population, young females were transferred to an Eppendorf tube (one or five females/tube/population) with 24 µl of extraction buffer (20% Sucrose, 1% Triton X-100, 3 µl 1% Bromophenol). The females were disrupted with the aid of a sterilized glass rod, and the resulting macerate was subjected to a 6% polyacrylamide gel electrophoresis at 8ºC and 180 volts for 4 hr. *Meloidogyne javanica* was used as reference positive control. After electrophoresis, the gel was transferred to 0.5 L of phosphate buffer pH 6.2 (solution A: 0.1 M $NaH_2PO_4$ + solution B: 0.1 M $Na_2HPO_4$) for 10 min, stained for esterase activity (100 mL phosphate buffer, 0.1 g Fast blue RR, 0.06 g β – Naftil) for 2 hr, and fixed with discoloring solution (30% absolute ethanol and 5% acetic acid) for 4 hr. The analysis was performed two times, and results were reproducible.

*Molecular identification*

DNA extraction of second-stage juveniles (J2) of *Meloidogyne* from 14 crops of *Musa* spp. was performed using a proteinase K method. An individual nematode mounted on a sterilized slide was crushed with a sterile scalpel and transferred to a tube with 15 µl of worm lysis buffer (50 mM KCl, 10 mM Tris pH 8.0, 15 mM $MgCl_2$, 0.5% Triton x-100, 4.5% Tween-20, 0.09% Proteinase K). The tubes were incubated at -80°C for 15 min, followed by 65°C for 1 hr and 95°C for 15 min. The tubes were then centrifuged at 16,000 rpm for 1 min, and stored at -20°C until further use.

Three loci were amplified by Polymerase Chain Reaction (PCR): *D2–D3* expansion segment of large subunit-LSU of 28S ribosomal DNA using forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and the reverse primer D3B (5′-TCCTCGGAAGGAACCAGCTACTA-3′) (De Ley *et al.*, 1999); the mitochondrial cytochrome oxidase subunit I (*COI*) partial region using the forward primer JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and the reverse primer JB4.5 (5′-TAAAGAAAGAACATAATGAAAATG-3′) (Bowles *et al*., 1992); and the NADH dehydrogenase subunit 5 (*Nad5*) partial gen of the mitochondrial DNA using the forward primer NAD5F2 (5′-TATTTTTTGTTTGAGATATATTAG-3′) and the reverse primer NAD5R1 (5′-CGTGAATCTTGATTTTCCATTTTT-3′) (Janssen *et al*., 2016). The PCR conditions were initial denaturation for 2 min at 94ºC followed by 40 cycles of 45 sec at 94°C, 45 sec at 55°C, and 1 min at 72°C and final extension for10 min at 72ºC for *D2–D3* expansion segment; initial denaturation for 2 min at 94ºC followed by 40 cycles of 45 sec at 94°C, 45 sec at 54°C, and 1 min at 72°C and final extension for 10 min at 72ºC for *COI*; and initial

Table 1. Detail on the samples collected in *Musa* spp. crops of Valle del Cauca, Quindío, Risaralda, and Caldas from 2016 to 2018 and used for identification of root-knot nematode species.

| State | Locality | Latitude | Longitude | Altitude (m) | Species and variety of *Musa* | Year | Sample code |
|---|---|---|---|---|---|---|---|
| Risaralda | La Celia | 5º00´06.21”N | 76º00´32.41”O | 1380 | (*Musa* AAB Simmonds) Dominico Hartón | 2016 | S1 |
| Risaralda | La Celia | 4º59´51.77”N | 76º00´40.39”O | 1380 | (*Musa* AAB Simmonds) Dominico Hartón | 2016 | S2 |
| Risaralda | La Celia | 5º00´22.58”N | 75º59´46.53”O | 1380 | (*Musa* AAB Simmonds) Dominico Hartón | 2016 | S3 |
| Quindío | Córdoba | 4º24´17.25”N | 75º42´55.43”O | 1700 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S4 |
| Quindío | Calarcá | 4º31´46.20”N | 75º37´17.96”O | 1573 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S5 |
| Quindío | Calarcá | 4º31´46.20”N | 75º37´17.96”O | 1573 | (*Musa* acuminata AAA) Gros Michel | 2017 | S6 |
| Caldas | Neira | 5º10´04.88”N | 75º31´14.55”O | 1969 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S7 |
| Caldas | Manizales | 5º05´56.19”N | 75º25´27.87”O | 2153 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S8 |
| Valle del Cauca | Bolo-Palmira | 3º27´07.12”N | 76º18´15.61’’O | 1000 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S9 |
| Valle del Cauca | Rozo-Palmira | 3º35´19.51”N | 76º18´06.22”O | 1000 | (*Musa* AAB Simmonds) Dominico Hartón | 2017 | S10 |
| Valle del Cauca | Rozo-Palmira | 3º35´19.51”N | 76º18´06.22”O | 1000 | (*Musa acuminata* AAA) Gros Michel | 2017 | S11 |
| Valle del Cauca | Zabaletas-Buenaventura | 3º45´15.71”N | 76º58´21.61”O | 658 | (*Musa* AAB Simmonds) Dominico Hartón | 2018 | S12 |
| Valle del Cauca | Bajo Calima-Buenaventura | 3º57´55.19”N | 77º01´17.80”O | 295 | (*Musa* AAB Simmonds) Dominico Hartón | 2018 | S13 |
| Valle del Cauca | Caicedonia | 4º21´25.67”N | 75º49´31.77”O | 1100 | (*Musa* AAB Simmonds) Dominico Hartón | 2018 | S14 |
| Valle del Cauca | Argelia | 4º44´06.21”N | 76º07´39.08”O | 1752 | (*Musa* AAB Simmonds) Dominico Hartón | 2018 | S15 |

denaturation for 2 min at 94ºC followed by 40 cycles of 60 sec at 94°C, 60 sec at 45°C, and 90 sec at 72°C and final extension for 10 min at 72ºC for Nad5. The PCR products were subjected to a 2% agarose gel electrophoresis at 130 volts for 30 min, visualized using a transilluminator, and purified using polyethylene glycol 6000 precipitation method (Schmitz and Riesner, 2006). Sequencing in both directions was done by BIONNER (Korea).

*Phylogenetic analysis*

Consensus sequences were aligned and edited using the software Geneious (Kearse *et al*., 2012). Basic Local Alignment Search Tool for nucleotide (BLASTn) at the National Center for Biotechnology Information (NCBI) was used to confirm the species identity. Other sequences were downloaded for the three loci from GenBank, including sequences of *Pratylenchus scribneri*, *Pratylenchus brachyurus,* and *Aphelenchoides besseyi* as outgroups to estimate molecular phylogenies for *D2-D3*, *COI,* and *Nad5*, respectively. MAFFT v7 (Katoh *et al*., 2002) was used to align the sequences with the protocol Q-INS-i for D2-D3 and *Nad5* and the protocol E-INS-i for *COI* and jModelTest v2.1.7 (Posada, 2008) was used to find the best nucleotide substitution model for each locus based on the Akaike Information Criterion corrected for small sample sizes. Afterwards, Maximum Likelihood (ML) was used to estimate a phylogeny for each locus, using 250 bootstraps and the general time reversible model with allowance for gamma distribution of rate variation (GTR + Γ) in RAxML v8 (Stamatakis, 2014). The phylogeny of *Meloidogyne* was also inferred using MrBayes v3.2.6 (Ronquist *et al.*, 2012), the general time reversible model with allowance for gamma distribution of rate variation and a proportion of invariant sites (GTR + Γ + I) for *D2-D3*, the GTR + Γ model for *COI*, and the GTR + I model for *Nad5*. For each locus, two independent Metropolis-coupled Markov chain Monte Carlo (MCMCMC) searches were performed for 2 million generations sampling every 2,000 steps. Convergence was assessed using Tracer v1.5 (burn-in = 20% of the samples), and by examining the average standard deviation of split frequencies among parallel chains. Consensus tree for each locus from the posterior distribution of 1,600 phylogenies was also calculated.

## RESULTS

*Morphological identification*

Two types of perineal patterns, corresponding with *M. incognita* and *M. acrita*, were observed in the populations of *Meloidogyne* cultured on tomato. The perineal patterns of *M. incognita* in samples from Calarcá and Córdoba (Quindío) were characterized by the presence of a high squarish dorsal arch with wavy striae and the absence of lateral lines (Fig. 1A and B). On the other hand, perineal patterns of *M. acrita* in a sample from Calarcá (Quindío) were distinguished by the presence of a high squarish dorsal arch, and a lateral field with sub-lateral incisures weakly demarcated by broken and forked striae (Smooth Breaks Few – SBF) (Fig. 1C and Table 2). Reduced morphological variation was observed among the perineal patterns of each population.

*Biochemical identification*

Two esterase phenotypes were observed in populations of *Meloidogyne* cultured on tomato (Fig. 1D). Esterase phenotype I1 corresponding to *M. incognita* was identified in plantain and banana populations from Calarcá and Córdoba (Quindío) while the esterase phenotype F1 corresponding to *M. acrita* was only identified in a population associated with a plantain crop from Calarcá (Quindío). In the same plantain crop where the esterase profile of *M. acrita* was recorded, the esterase profile of *M. incognita* also occurred (Fig. 1D and Table 2).

*Molecular identification*

Thirty-three consensus sequences of the *D2-D3* expansion segment, 41 consensus sequences of the *COI* gene, and 11 of the *Nad5* gene were obtained from the populations of *Meloidogyne* collected from plantain and banana in Colombia. The *D2-D3* and *COI* sequences had 99% similarity to the reference sequences of *M. incognita, M. javanica, M. arenaria, M. konaensis, M. polycephannulata, M. morociensis, M. luci, M.*

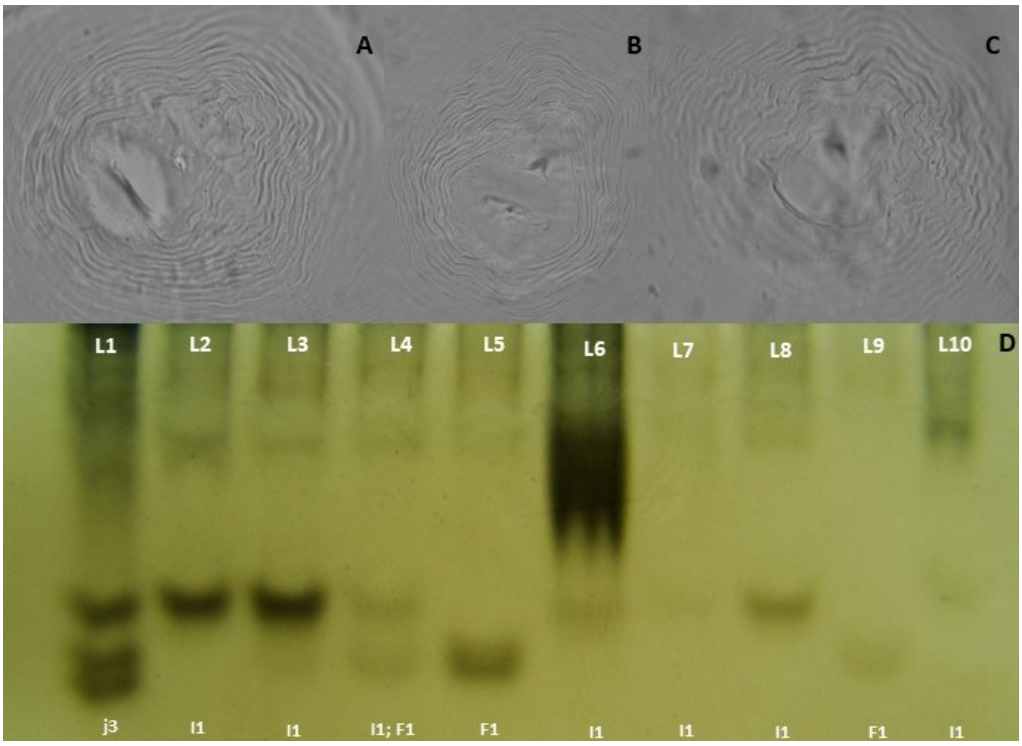


Figure 1. Morphological and biochemical identification of *Meloidogyne* spp. associated with plantain and banana. A and B) Perineal pattern of *M. incognita*. C) Perineal pattern of *M. acrita*. D) Esterase phenotypes of populations of *Meloidogyne* associated with *Musa* spp. in Colombia. Line 1 to 5: Material from five different females of each population was loaded in each well. Line 6 to 10: material from a single female in each well. Line 1. *M. javanica* (J3), Line 2. *M. incognita* (I1) associated with rhizosphere of banana in Calarcá (Quindío), Line 3. *M. incognita* (I1) associated with roots of banana in Calarcá (Quindío), Line 4. *M. incognita* (I1) and *M. acrita* (F1) associated with rhizosphere of plantain in Calarcá (Quindío), Line 5. *M. acrita* (F1) associated with roots of plantain in Calarcá (Quindío), Line 6. *M. incognita* (I1) associated with rhizosphere of plantain in Córdoba, Line 7. *M. incognita* (I1) associated with rhizosphere of banana in Calarcá (Quindío), Line 8. *M. incognita* (I1) associated with roots of banana in Calarcá (Quindío), Line 9. *M. acrita* (F1) associated with rhizosphere of plantain in Calarcá (Quindío) and Line 10. *M. incognita* (I1) associated with roots of plantain in Calarcá (Quindío).

*paranaensis, M. phaseoli, M. lopezi, M. arabicida,* and *M. izalcoensis* "tropical root-knot nematodes" or *M. incognita* group. Only one sequence of the *D2-D3* expansion segment from Manizales (Caldas) had 100% similarity with *M. hispanica* (EU443606, KF501128). Four sequences of the *Nad5* gene were obtained from Rozo-Palmira (Valle del Cauca) with 99% to 100% similarity to *M. incognita* collected from *Dioscorea* spp. (KY522769, KY522768, KY522758, KY522757, KY522756, and KU372361) and *Syngonium* (KU372361). Five sequences of the *Nad5* gene were obtained from Calarcá (Quindío) and two from Zabaletas-Buenaventura (Valle del Cauca) with 100% similarity to *M. arenaria* collected from *Calathea* and *Echiocactus grusonii* (KU372349 and KU372350, respectively). All sequences obtained in the present study were deposited in NCBI (Table 2).

*Phylogenetic analysis*

The trees estimated using maximum likelihood and Bayesian inference for the *D2-D3* expansion segment suggested that all sequences from the Valle del Cauca, Quindío, Risaralda, and Caldas regions clustered in the same clade with reference sequences of *M. incognita, M. javanica,* and *M. arenaria* (bootstrap support or BS = 88%,

Table 2. Root-knot nematode species identified from *Musa* spp. crops in Valle del Cauca, Quindío, Risaralda, and Caldas using morphological, biochemical and molecular diagnostics.

| Sample code | Esterase profile | Perineal patterns number done | RKN species | rRNA-based technique Sequences *D2-D3* (Accession numbers) | mtDNA-based technique Sequences *COI* (Accession numbers) | mtDNA-based technique Sequences *N*ad5 (Accession numbers) |
|---|---|---|---|---|---|---|
| S1 | - | - | *Meloidogyne* spp. | MN072399, MN072400, MN072401, MN072402 | MN095064, MN095065, MN095066, MN095075, MN095076, MN095077 | - |
| S2 | - | - | *Meloidogyne* spp. | - | MN095078, MN095079, MN095080, MN095081, MN095082, MN095083, MN095084, MN095085, MN095086, MN095088, MN095089 | - |
| S3 | - | - | *Meloidogyne* spp. | MN072405 | MN095067, MN095087, MN095090, MN095091, MN095092 | - |
| S4 | I1 | 10 | *M. incognita* | MN072414, MN072415, MN072416 | - | - |
| S5 | I1; F1 | 20 | *M. incognita*; *M. acrita*; *M. arenaria* | MN072417 | - | MN106028, MN106029, MN106030, MN106031, MN106032 |
| S6 | I1 | 10 | *M. incognita* | - | - | - |
| S7 | - | - | *Meloidogyne* spp. | MN072409, MN072410, MN072411, MN072412, MN072413 | - | - |
| S8 | - | - | *M. hispanica* | MN072418 | - | - |
| S9 | - | - | *Meloidogyne* spp. | MN072403, MN072404, MN072406, MN072407, MN072408 | MN095093, MN095094, MN095095, MN095096, MN095097 | - |
| S10 | - | - | *M. arenaria* | MN072419, MN072420, MN072421, MN072422, MN072423 | - | MN106033, MN106034, MN106035, MN106036 |
| S11 | - | - | *Meloidogyne* spp. | MN072424, MN072425 | - | - |
| S12 | - | - | *M. arenaria* | MN072426, MN072427 | MN095068, MN095069, MN095098, MN095099, MN095100, MN095101 | MN106026, MN106027 |
| S13 | - | - | *Meloidogyne* spp. | MN072428, MN072429 | MN095070, MN095071, MN095102, MN095103 | - |
| S14 | - | - | *Meloidogyne* spp. | MN072430, MN072431 | MN095072, MN095073, MN095104 | - |
| S15 | - | - | *Meloidogyne* spp. | MN072432 | MN095074 | - |

posterior probability or PP = 1). The consensus sequence of *M. hispanica* of Caldas grouped in another clade with the reference sequences of the same species (BS = 99%, PP = 0.99) (Figs. 2 and 3). As with the *D2-D3* segment phylogenies, in the phylogenies that were estimated using the *COI* gene, all the sequences obtained from Valle del Cauca, Quindío, Risaralda and Caldas regions grouped in the same clade with reference sequences of *M. incognita, M. javanica*, and *M. arenaria* (BS = 100%, PP = 1) (Figs. 4 and 5).

In the trees we estimated using the *Nad*5 gene, all the sequences collected from Rozo-Palmira (Valle del Cauca) grouped with reference sequences of *M. incognita*, although the support was low (BS = 48, PP = 0.83). The sequences generated from populations collected in Calarcá (Quindío) and Zabaletas-Buenaventura (Valle del Cauca) clustered in the same clade with reference sequences of *M. arenaria* (BS = 95%, PP = 0.99) (Figs. 6 and 7).


Figure 2. Maximum likelihood phylogeny of *Meloidogyne* spp. based on the *D2–D3* expansion segment of 28S rRNA and 250 bootstraps. The outgroup (*Pratylenchus scribneri*) is shown in gray font; sequences obtained in this study appear in bold typeface. Values at the nodes represent the bootstrap support. The scale represents the number of substitutions per site.

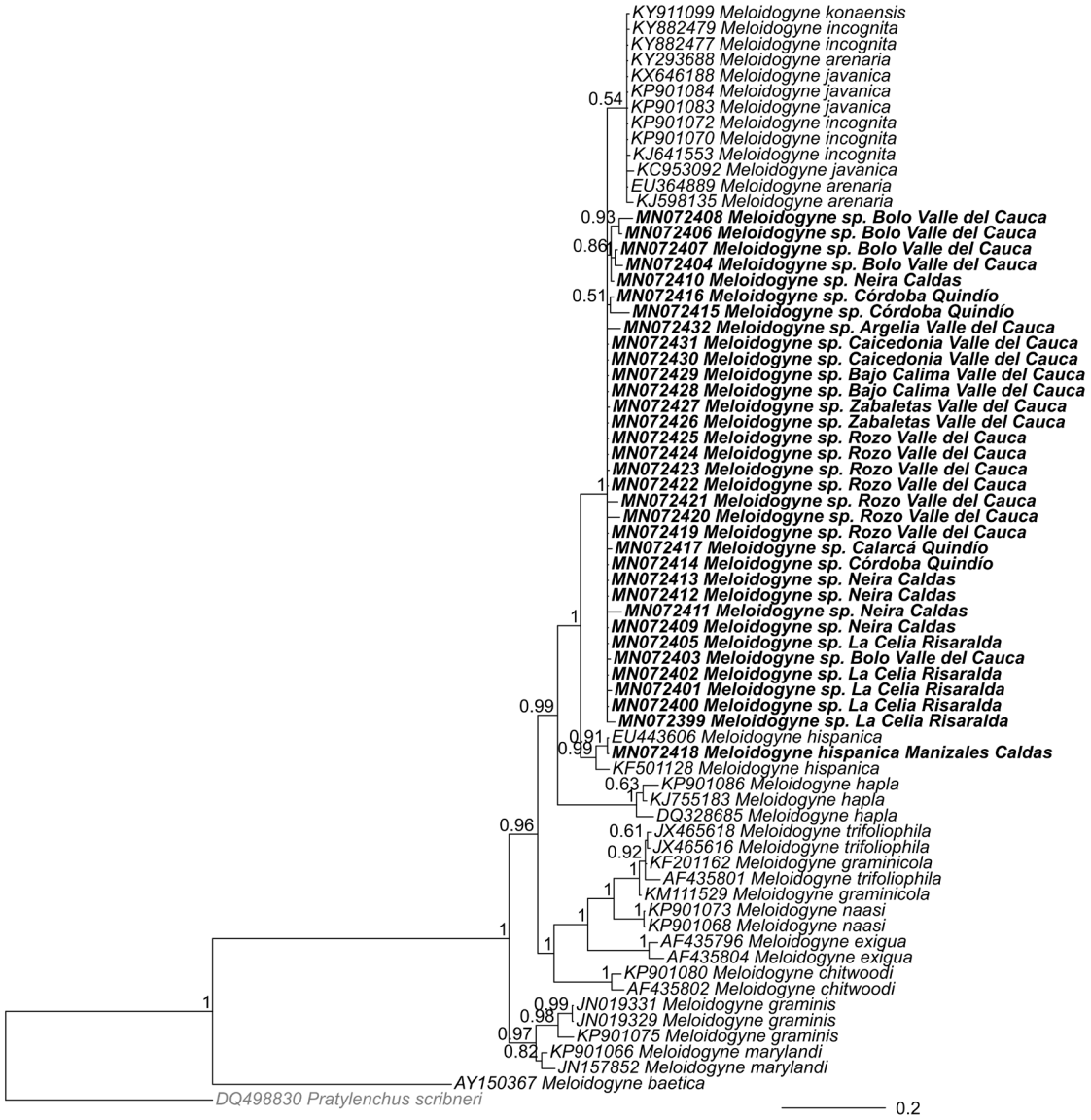


Figure 3. Bayesian phylogeny of *Meloidogyne* based on *D2–D3* expansion segment of 28S rRNA. The phylogeny is a consensus tree from a posterior distribution of 1,600 trees that were inferred in MrBayes. The outgroup (*Pratylenchus scribneri*) is shown in gray font. The sequences obtained in this study appear in bold typeface. Values at the nodes represent the posterior probability. The scale represents the number of substitutions per site.

## DISCUSSION

Independent of the agro-ecological differences, results from this study support that *Meloidogyne* was present in all the production zones of *Musa* spp. sampled in the Valle del Cauca, Quindío, Risaralda, and Caldas regions of Columbia. These findings confirm the wide geographical distribution of *Meloiodogyne* spp. on *Musa* spp. in Colombia (Villegas, 1989; Guzmán and Castaño, 2004; Torrado and Castaño, 2009). The *D2-D3* expansion segment and *COI* did not discriminate between *M. incognita, M. javanica, M. arenaria, M. konaensis, M. polycephannulata, M. morociensis, M. luci, M. paranaensis, M. phaseoli, M. lopezi, M. arabicida,* and *M. izalcoensis*. However, morphological characteristics of the perineal pattern, esterase phenotype, and the *Nad5* gene, confirmed that *M. incognita, M. acrita,* and *M. arenaria* were

associated with *Musa* in Colombia. All populations that multiplied on tomato had perineal patterns and esterase phenotype I1 corresponding to *M. incognita* (Chitwood, 1949; Carneiro *et al*., 2000; Oliveira *et al*., 2011), with the exception of a population that had a perineal pattern and the esterase phenotype F1 similar to those reported for *M. acrita* (Bergé and Dalmasso, 1975; Esbenshade and Triantaphyllou, 1985; Chitwood, 1949; Esser *et al.* 1976; Eisenback and Triantaphyllou, 1991; Kaur and Attri, 2013). In each sample, the perineal patterns corresponded to the esterase phenotypes, confirming the utility of these tools for the identification of species of *Meloidogyne*, especially in cases where morphometric and molecular data are insufficient to discriminate between species (Sasser *et al*., 1983; Carneiro *et al*., 1996; García and Sanchez-Puerta, 2012).

Due to small differences in the configuration of perineal patterns, *M. acrita* has been considered

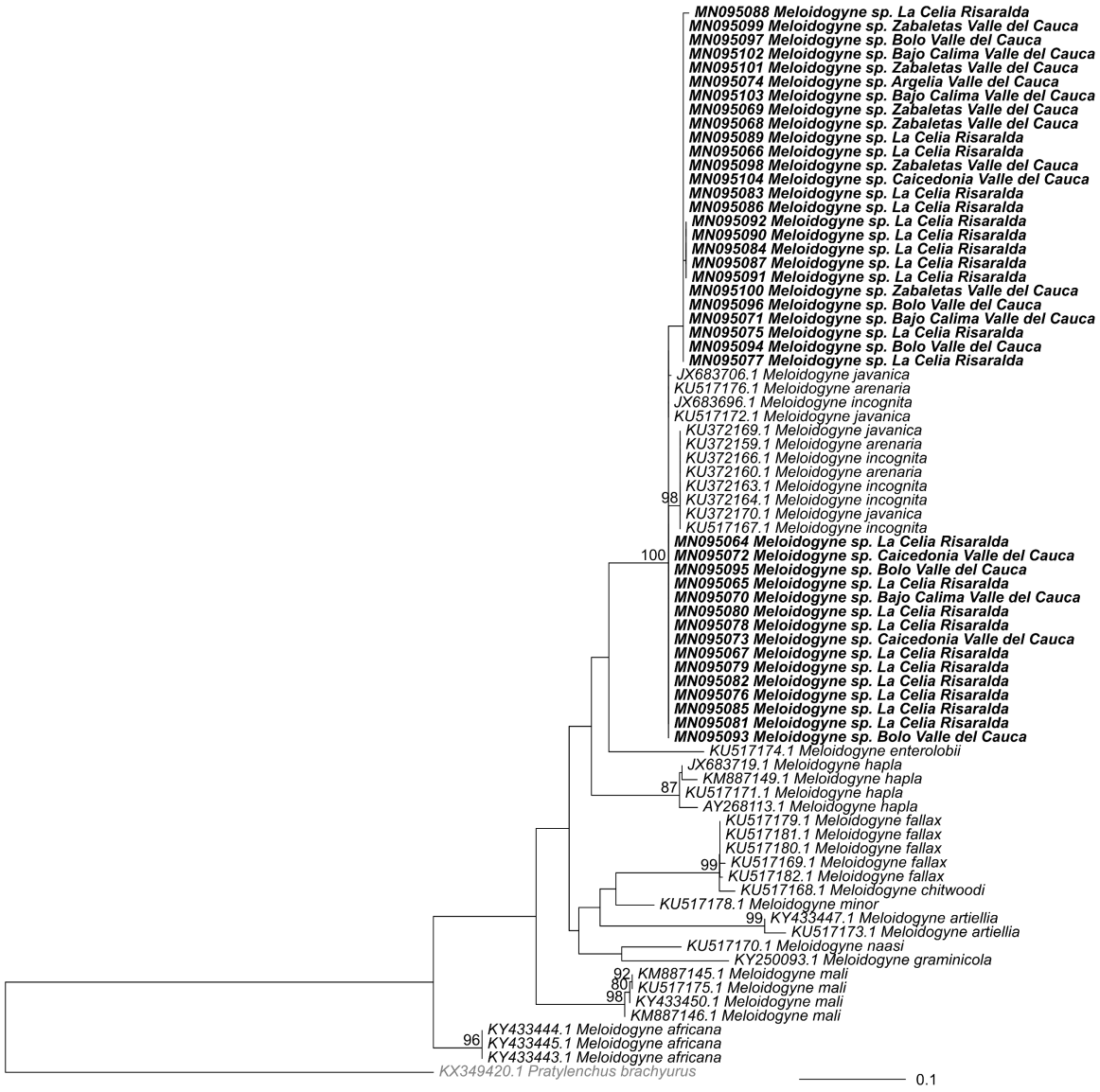


Figure 4. Maximum likelihood phylogeny of *Meloidogyne* based on cytochrome oxidase subunit I (*COI*) and 250 bootstraps. The outgroup (*Pratylenchus brachyurus*) is shown in gray font. The sequences obtained in this study appear in bold typeface. Values at the nodes represent the bootstrap support. The scale represents the number of substitutions per site.

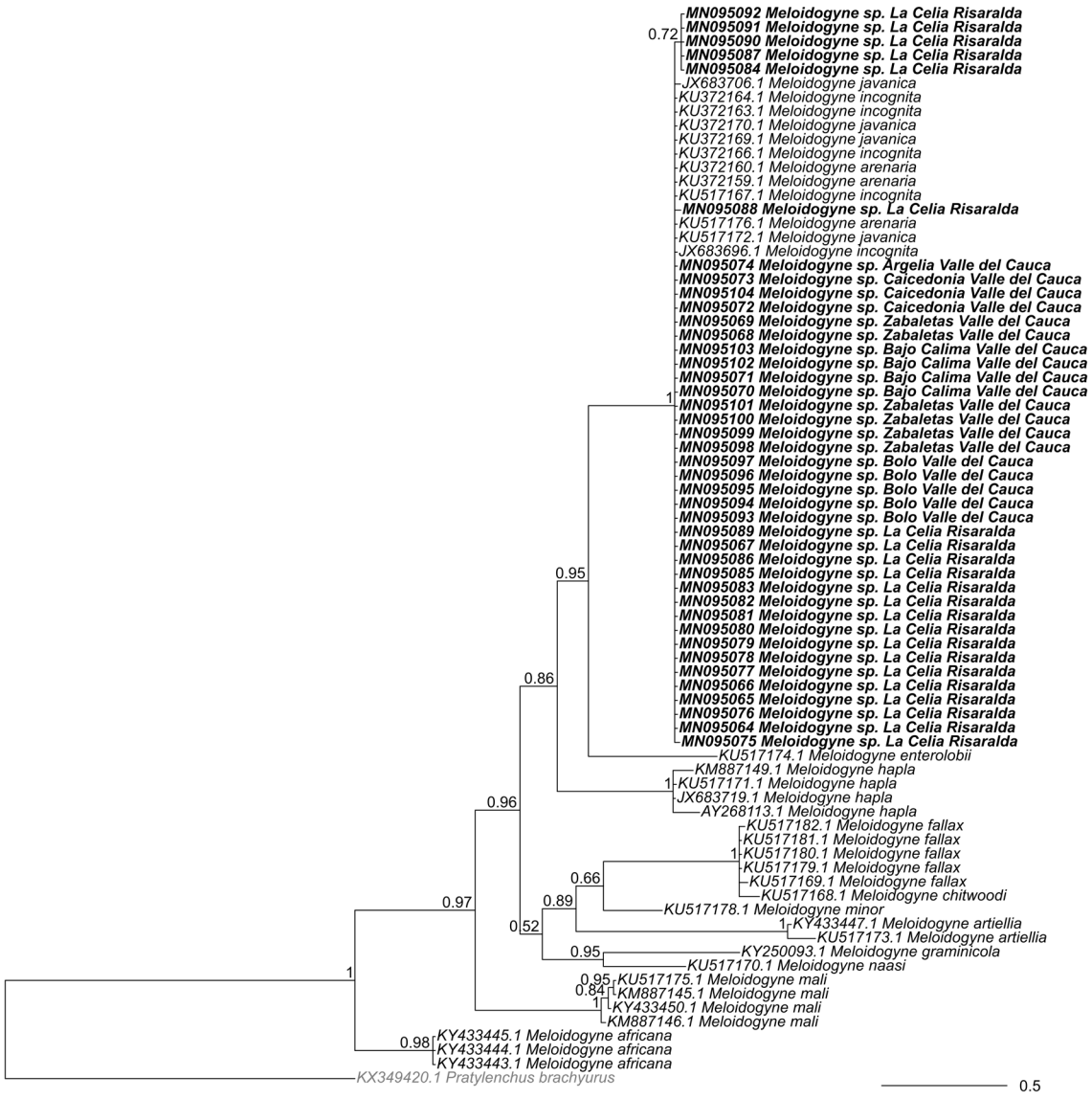


Figure 5. Bayesian phylogeny of *Meloidogyne* based on cytochrome oxidase subunit I (*COI*). The phylogeny is a consensus tree from a posterior distribution of 1,600 trees that were inferred in MrBayes. The outgroup (*Pratylenchus brachyurus*) is shown in gray font. The sequences obtained in this study appear in bold typeface. Values at the nodes represent the posterior probability. The scale represents the number of substitutions per site.

as a variety, subspecies, or synonym of *M. incognita* (Golden and Birchfield, 1978; Triantaphyllou and Sasser, 1960). However, Esser *et al.* (1976) reported that *M. incognita var acrita* should be recognized as *M. acrita* based on hololectotype and paralectotypes deposited in the USDA nematode collection in Beltsville, MD. We consider *M. acrita* as a valid species due to the differences in perineal pattern and electrophoretic profile of esterase to that of *M. incognita*. Results from this study suggest that *M. incognita, M. acrita,* and *M. arenaria* can occur simultaneously in the same crop, which is in agreement with Janssen *et al*. (2016).

This research confirmed that the *D2-D3* expansion segment of ribosomal RNA and mitochondrial *COI* do not have sufficient resolution to discriminate between closely related species namely "tropical root-knot nematodes" or *M. incognita* group. However, *Nad5* is a good molecular marker to differentiate *M. incognita, M. javanica,* and *M. arenaria*. In this study the

sequence data for *Nad5* gene had homology of 99-100% with reference populations of *M. incognita* and *M. arenaria* from *Dioscorea* spp., *Syngonium,* and *Calathea,* biochemically supported (esterase and malate dehydrogenase isozyme profiles) by Janssen *et al*. (2016) and Kolombia *et al*. (2017).

The occurrence of *M. incognita* and *M. arenaria* in samples collected from Columbia are in agreement with previous reports of these plant-parasitic nematodes in *Musa* crops of the world, including Colombia (Crozzoli *et al*., 1995; Carneiro *et al*., 1996, 2000; Jaraba *et al*., 2008; López-Pérez *et al*., 2011; Navarro *et al*., 2011; Múnera, 2008; Daneel *et al*., 2015; Lara and Nuñez, 2016). Esterase phenotypes I1 and I2 corresponding to *M. incognita* have been found in *Musa* spp. crops from Martinique and French Guiana. However, in this research only I1 was found (Carneiro *et al*., 1996, 2000; Cofcewicz *et al*., 2005).

Esterase phenotype I1 has also been observed in other crops of economic importance including

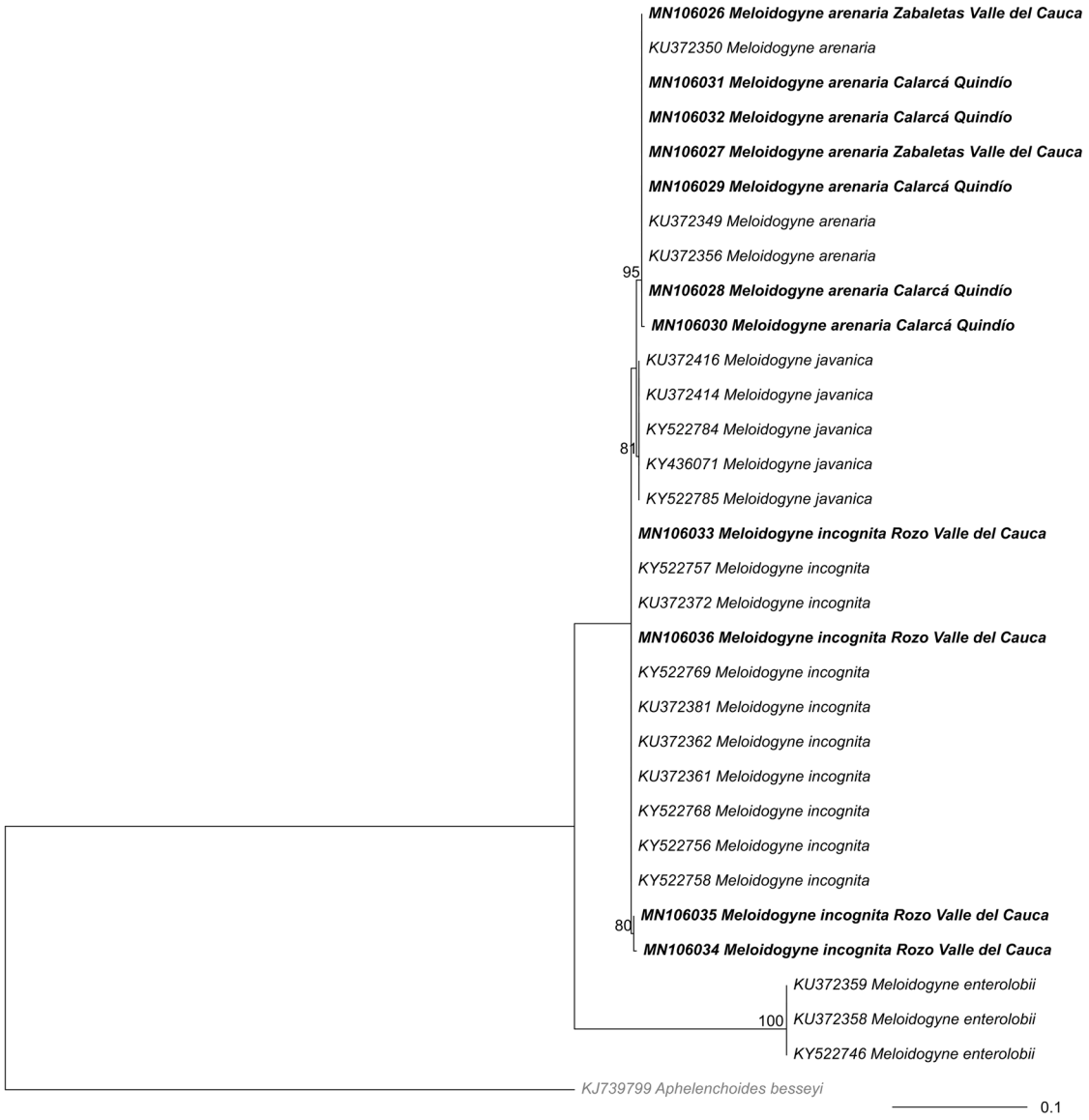


Figure 6. Maximum likelihood phylogeny of *Meloidogyne* based on *Nad5* and 250 bootstraps. The outgroup (*Aphelenchoides besseyi*) is shown in gray font. The sequences obtained in this study appear in bold typeface. Values at the nodes represent the bootstrap support. The scale represents the number of substitutions per site.

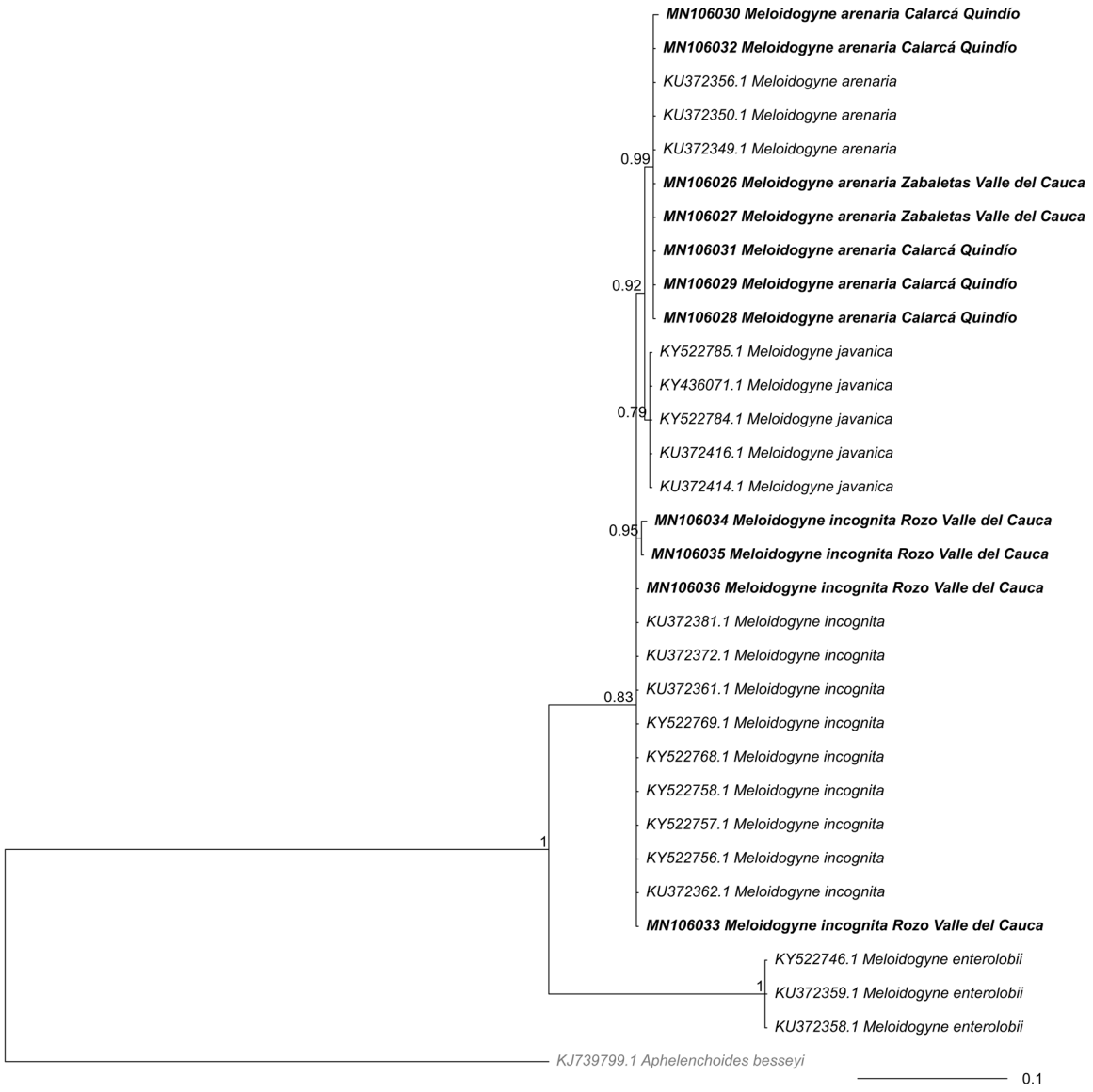


Figure 7. Bayesian phylogeny of *Meloidogyne* based on *Nad5*. The phylogeny is a consensus tree from a posterior distribution of 1,600 trees that were inferred in MrBayes. The outgroup (*Aphelenchoides besseyi*) is shown in gray font. The sequences that were obtained in this study appear in bold typeface. Values at the nodes represent the posterior probability. The scale represents the number of substitutions per site.

cherry (*Malpighia punicifolia*), pineapple (*Ananas comosus*), soybean (*Glycine max*), fig (*Ficus carica*), chayote (*Sechium edule*), and cucumber (*Cucumis sativus*) (Carneiro *et al*., 2000). Previously, *M. acrita* was reported parasitizing banana crops in the Bocas District of the Republic of Panama. Experimentally, it was proven that *M. acrita* contributes to the expression of "vascular wilt diseases of bananas" caused by *Fusarium oxysporum* f. *cubense* (Loos, 1959). However, this study is the first report of this plant-parasitic nematode in *Musa* spp. in Colombia.

Based on the molecular analysis of *D2-D3* expansion segment, this study is the first to report the presence of *M. hispanica* in a plantain crop in Colombia. This result is consistent with the report of this species in a banana crop in Martinique (Cofcewicz *et al*., 2005). *D2-D3* expansion segment was useful in identifying species of *Meloidogyne*, including *M. hapla, M. chitwoodi, M. exigua, M. marylandi, M. graminis,* and *M. naasi* (Ye *et al*., 2015). *Meloidogyne hispanica*, is

a polyphagous species pathogenic in onion, lettuce (*Lactuca sativa*), carnation (*Dianthus caryophyllus*), beet (*Beta vulgaris*), spinach (*Spinacia oleraceae*), melón (*Cucumis melo*), cucumber, bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), corn (*Zea mays*), tobacco (*Nicotiana tabacum*), tomato, and potato (*Solanum tuberosum*) (Nobre *et al*., 2012).

Although *M. incognita, M. acrita,* and *M. arenaria* were recorded in Quindío, *M. incognita* and *M. arenaria* in Valle del Cauca, and *M. hispanica* in Caldas, it is possible that these species are widespread in Colombia due to human dispersion of plantain and banana among localities based on previous reports for *M. incognita* and *M. arenaria* in the Arauca, Magdalena, Antioquia, Córdoba, and Quindío departments (Jaraba *et al.*, 2008; Múnera, 2008; Navarro *et al.*, 2011). Vegetative propagation of infected material is a very effective means of dispersing nematodes and other pests.

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