PLANT-PARASITIC NEMATODES ASSOCIATED WITH YAMS (*DIOSCOREA* SPP.) AND IDENTIFICATION OF *MELOIDOGYNE* AND *PRATYLENCHUS* SPECIES IN THREE YAM-GROWING REGIONS OF COSTA RICA

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ABSTRACT

Humphreys-Pereira, D. A., L. Flores-Chaves, L. Salazar, and L. Gómez-Alpízar. 2017. Plant-parasitic nematodes associated with yams (*Dioscorea* spp.) and identification of *Meloidogyne* and *Pratylenchus* species in three yam-growing regions of Costa Rica. Nematropica 47:120-134.

Yam (*Dioscorea* spp.) and yampee (*D. trifida*) tubers showing symptoms of dry rot and galls have been observed frequently in storage facilities and yam fields from the North, Atlantic, and South (Brunca) regions of Costa Rica. A survey of plant-parasitic nematodes associated with yams and yampee was performed in these three regions. The analyses showed that the most frequent nematodes in tubers were *Scutellonema* and *Aphelenchoides* (50% of the samples), followed by *Meloidogyne* (47%), *Tylenchus* (43%), *Pratylenchus* (33%), and *Helicotylenchus* (23%). Female perineal patterns and molecular techniques such as PCR-RFLP and PCR with species-specific primers were used to identify *Meloidogyne* species. *Meloidogyne javanica* and *M. incognita* were found concomitantly in white yam (*D. rotundata*) and *M. incognita* in three yampee samples. Species-specific primers for *Pratylenchus* species allowed the identification of *P. coffeae* and *P. brachyurus* in both yellow yam (*D. cayenensis*) and yampee. *Pratylenchus coffee* was found in the three regions of Costa Rica. Furthermore, *P. coffeae* and *P. brachyurus* were also found concomitantly in two samples. A Bayesian analysis based on the D2-D3 region of the *28S* gene placed one population extracted from greater yam (*D. alata*) and one from yampee in clades with *P. zeae* and *P. gutierrezi*, respectively. The present study is the first report of *Meloidogyne* and *Pratylenchus* species in yams and yampee in the major yam production areas of Costa Rica using fast and accurate molecular methods.

Key words: *28S* gene, dry rot, galls, mtDNA, PCR-RFLP, perineal patterns

RESUMEN

Humphreys-Pereira, D. A., L. Flores-Chaves, L. Salazar, y L. Gómez-Alpízar. 2017. Nematodos parásitos de plantas asociados a ñames (*Dioscorea* spp.) e identificación de especies de *Meloidogyne* y *Pratylenchus* en tres regiones productoras de ñame en Costa Rica. Nematropica 47:120-134.

Tubérculos de ñames (*Dioscorea* spp.) y yampí (*D. trifida*) con síntomas de pudrición seca y agallas se han observado frecuentemente en bodegas de almacenamiento y empacadoras en las regiones Norte, Atlántica y Sur (Brunca) de Costa Rica. Se realizó un estudio de nematodos fitoparásitos asociados a ñames y yampí en estas regiones. Los análisis mostraron que los nematodos más frecuentes en tubérculos fueron *Scutellonema* y *Aphelenchoides* (50% de las muestras), seguido de *Meloidogyne* (47%), *Tylenchus* (43%), *Pratylenchus* (33%) y *Helicotylenchus* (23%). Las especies de *Meloidogyne* presentes se identificaron mediante diseños perineales y técnicas moleculares como PCR-RFLP y PCR-cebadores específicos. *Meloidogyne javanica* y *M. incognita* se encontraron asociadas a un mismo hospedante, ñame blanco (*D. rotundata*), mientras que *M. incognita* en cuatro muestras de yampí. El uso de cebadores específicos para especies de *Pratylenchus* permitió la detección de *P. coffeae* y *P. brachyurus* en ambos hospedantes, ñame amarillo (*D. cayenensis*) y yampí. *Pratylenchus coffee* se detectó en las tres regiones productoras de ñame en Costa Rica. Adicionalmente, dos muestras mostraron la presencia concomitante de *P. coffeae* y *P. brachyurus*. El análisis de inferencia bayesiana basado en la región D2-D3 del gen *28S* agrupó una población extraída de ñame grande (*D. alata*) y otra de yampí en clados con *P. zeae*

y *P. gutierrezi*, respectivamente. En adición corroboró la identificación de *P. coffeae* y *P. brachyurus*. El presente estudio es el primer reporte de especies de *Meloidogyne* y *Pratylenchus* asociados a ñames y yampí en las áreas de mayor producción de estos cultivos en Costa Rica con el uso de técnicas moleculares más rápidas y precisas.

Palabras clave: 28S gen, pudrición seca, agallas, mtDNA, PCR-RFLP, diseños perineales

INTRODUCTION

Commercial yam (*Dioscorea* spp.) production in Costa Rica started at the beginning of the 1980s with *D. alata* cv. Antillano in the Atlantic region (Región Huetar Atlántica) (Palmer 1994). Currently, three cultivars are commercially produced, *D. alata* cv. Diamantes 22 (greater yam), *D. cayenensis* cv. Diamantes 2004 (yellow yam), and *D. rotundata* cv. Diamantes 2006 (white yam/habanero yam). In addition, *D. trifida* (yampee, yampí or papa china) is also produced in Costa Rica, but no commercial cultivar is available. Farmers select and cultivate yampee by color and tuber shape. Yams are mostly grown in the Atlantic and North (Región Huetar Norte) regions and to a lesser extent in the South region (Región Brunca) of Costa Rica. Greater yam is the most widely grown yam species in the country, followed by yampee, and small quantities of yellow and white yams. Yam production plays an important role in the Costa Rican economy, e.g., in 2015, 11,640 T of yams and 1,126 T of yampee were exported and brought a value of $11.9 million and $1.2 million, respectively (Alvarado-Valverde, 2016).

Plant-parasitic nematodes negatively affect the marketability of the yam tubers in fields or during storage (Acosta and Ayala, 1976; Baimey *et al*., 2009; Mudiope *et al*., 2012; Humphreys-Pereira *et al*., 2014). The yam nematode, *Scutellonema bradys* (Steiner and LeHew) Andrássy and the root-lesion nematode, *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, are the two main causal agents of the dry rot disease of yams (Ayala and Acosta, 1971; Acosta and Ayala, 1975, 1976; Castagnone-Sereno and Kermarrec, 1988; Bridge *et al*., 2005). General symptoms of the dry rot disease include brown-dark areas in the infested tubers, cracks and flaking off the tuber skins (Bridge *et al*., 2005). Severe necrosis and deep cracks in *D. rotundata* tubers can be observed when an initial population of 600 *S. bradys* or *P. coffee* individuals is used in inoculation tests and complete deterioration of the tubers is obtained with 1000 nematodes (Acosta and Ayala, 1975). Root-knot nematodes (*Meloidogyne* spp.) also adversely affect the tuber marketability due to the induction of galls on the surface and the presence of brown and necrotic lesions below the tuber skins (Fawole, 1988).

In spite of the negative impact of plant-parasitic nematodes on yams, at present there are few studies related to these pathogens in Costa Rica. López and Salazar (1988a) identified *M. javanica* in the tubers of greater yam using morphological techniques based on perineal patterns. Recently, Humphreys-Pereira *et al*. (2014) described the presence of the yam nematode, *S. bradys*, in four yam species from the North and Atlantic regions of Costa Rica. The accurate identification of the nematode species associated with yams is essential for the development of breeding programs, for screening tolerance or resistance, and for development of effective management methods that are compatible with the environment. The objectives of the present study were to: (a) determine the distribution and quantify the plant-parasitic nematodes associated with yams and yampee in the main yam production areas of Costa Rica, (b) identify with molecular tools the *Meloidogyne* and *Pratylenchus* species associated with four species of *Dioscorea*.

MATERIALS AND METHODS

Sampling, nematode populations, and characterization of perineal patterns

Soil and root samples from 27 yam fields and tuber samples from 30 fields were collected in different geographical localities from the Atlantic, North, and South regions of Costa Rica. Tuber samples were collected mostly after harvest or from the planting materials (tubers) for the next growing season. Five to ten tubers were randomly picked from the harvest bags in each sampling location. Soil and roots were sampled from fields (size no more than 1 ha) where the tuber crop was 2- to 5-mo-old. Each field was divided roughly in two parts; five root systems and the soil surrounding the roots were collected in a zig-zag pattern on each part, forming two composite samples (5 plants each; total of 10 plants per field). Each plant was randomly selected, and the root systems were pulled up from the first 20 cm of the soil using a shovel. The tubers were carefully washed, and symptoms related to nematode damage were described. Root and tuber samples were processed as in Humphreys-Pereira *et al*. (2014). Nematodes were extracted from 100 cm^3

of soil per composite sample (representing the two parts of the field) using the floatation-centrifugation method (Caveness *et al*., 1955; Alvarado and López, 1985). All plant-parasitic nematodes found in the samples were identified to genus based on morphology. Relative frequency of each genus was calculated as in Barker (1985). The *Meloidogyne* populations from roots or tubers representing each field were established as greenhouse cultures on two tomato (*Solanum lycopersicum* cv. Hayslip) pots, by inoculating all juveniles from the counting dishes. Twenty *Meloidogyne* females from each field were prepared for perineal pattern description. Perineal patterns were prepared following the method of Franklin (1962), modified by Taylor and Netscher (1974) and mounted in lactophenol. The perineal patterns were observed in an inverted microscope (Olympus IX51®) equipped with differential interference contrast (DIC).

Molecular identification

DNA was extracted from a pool of 30-40 *Meloidogyne* females of each field (total of four populations) and from 40 *Pratylenchus* individuals (juveniles and adults) extracted from roots (total of 13 populations) as in Humphreys *et al*. (2012). Positive controls of *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* (Humphreys *et al*., 2012) and *P. brachyurus*, *P. gutierrezi*, and *P. penetrans* extracted from roots of pineapple, coffee and lily, respectively, were included in this study (Table 1). For *Meloidogyne*, the mitochondrial DNA region between the 3' end of the cytochrome oxidase subunit II (*cox2*) and the 16S *rRNA* genes were amplified using primers 1108/C2F3 (Powers and

Table 1. Populations of *Meloidogyne* and *Pratylenchus* associated with yams from different areas of Costa Rica used for molecular identification.

Code	Host	Collection area
MY1	Tubers of white yampee (*Dioscorea trifida*)	Ticabán, La Rita, Pococí, Limón, AR[z]
MY2	Tubers of white yampee (*D. trifida*)	San Isidro del General, Pérez Zeledón, SR
MY3	Roots of white yampee (*D. trifida*)	Las Brisas, San Vito, Coto Brus, SR
MW	Tubers of white yam (*D. rotundata* cv. Diamantes 2006)	Hojancha, Cariari, Pococí, Limón, AR
Mar (*M. arenaria*)	Roots of pothos (*Epipremmum aureum*)	La Guácima, Alajuela
Mi (*M. incognita*)	Roots of tomato (*Solanum lycopersicum*)	La Lima, Cartago
Mj (*M. javanica*)	Roots of liriope (*Liriope* sp.)	Santa Bárbara, Heredia
Mh (*M. hapla*)	Roots of strawberry (*Fragaria vesca*)	Llano Grande, Cartago
PÑC1	Tubers of yellow yam (*Dioscorea cayenensis native*)	Cahuita, Talamanca, AR
PÑC4	Tubers of yellow yam (*D. cayenensis* cv. Diamantes 2004)	Carolina, Cariari, Pococí, AR
PÑC7	Tubers of yellow yam (*D. cayenensis* cv. Diamantes 2004)	Palmitas, La Rita, Pococí, AR
PÑC10	Tubers of yellow yam (*D. cayenensis* cv. Diamantes 2004)	Hojancha, Cariari, Pococí, AR
PÑC13	Tubers of yellow yam (*D. cayenensis* native)	Cahuita, Talamanca, AR
PÑC16	Roots of yellow yam (*D. cayenensis* cv. Diamantes 2004)	Montealegre, El Amparo, Los Chiles, NR
PÑH1	Tubers of white yam (*D. rotundata* cv. Diamantes 2006)	Hojancha, Cariari, Pococí, AR
PÑH3	Tubers of white yam (*D. rotundata* cv. Diamantes 2006)	Colimas, Cariari, Pococí, AR
PY1	Roots of white yampee (*D. trifida*)	Valle Hermoso, Sabalito, Coto Brus, SR
PY2	Roots of white yampee (*D. trifida*)	Valle Hermoso, Sabalito, Coto Brus, SR
PY3	Roots of white yampee (*D. trifida*)	Montealegre, El Amparo, Los Chiles, NR
PY9	Roots of white yampee (*D. trifida*)	Montealegre, El Amparo, Los Chiles, NR
PÑA1	Roots of greater yam (*D. alata* cv. Diamantes 22)	Hojancha, Cariari, Pococí, AR
PPI	Roots of pineapple (*Ananas comosus* cv. MD-2)	Upala, Alajuela
PCAF1	Roots of coffee (*Coffea arabica*)	Santa Rosa, Poás, Alajuela
PL1	Roots of lily (*Lilium* sp.)	San José de la Montaña, Heredia

[z]AR= Atlantic region; SR= South region; NR= North region.

Harris, 1993). The amplification reaction included 0.4 µM of each primer, 1× PCR reaction buffer (High Fidelity, Thermo Fisher Scientific, Watham, CA), 1 µl of 20 mg/ml BSA (Thermo Fisher Scientific), 3 mM $MgCl_2$ (Thermo Fisher Scientific), 0.08 mM dNTP mix (Thermo Fisher Scientific), 1 unit of High Fidelity PCR Enzyme Mix (Thermo Fisher Scientific) and 3 µl of the DNA preparation in a final volume of 25 µl. The PCR amplification was performed in a Perkin-Elmer GeneAmp® 9700 thermal cycler (Perkin Elmer Applied Biosystems, Foster City, CA) following conditions described by Jeyaprakash *et al*. (2006). PCR-RFLP was performed using the restriction enzyme *Hinf*I (Thermo Fisher Scientific) following manufacturer's instructions. PCR using SCAR species-specific primers for *M. incognita* (Finc/Rinc), *M. javanica* (Fjav/Rjav) and *M. arenaria* (Far/Rar) were performed as in Zijlstra *et al*. (2000).

The species-specific primer sets PC1/PC2 (Uehara *et al*., 1998) and 18S/ACM7R (Vrain *et al*., 1992; Machado *et al*., 2007) were used for the identification of *P. coffeae* and *P. brachyurus*, respectively. The amplification reaction included 0.8 µM of each primer, 1× PCR reaction buffer (Thermo Fisher Scientific), 2 mM $MgCl_2$ (Thermo Fisher Scientific), 0.16 mM dNTP mix (Thermo Fisher Scientific), 1 unit of Taq polymerase (Thermo Fisher Scientific) and 3 µl of the DNA preparation in a final volume of 25 µl. We followed the amplification conditions described by Uehara *et al*. (1998) and Machado *et al*. (2007). Variability in the D3 region of the large subunit (LSU) *28S rDNA* in *Pratylenchus* was studied using the primer set D3A/D3B (Al-Banna *et al*., 1997). The amplification reaction was performed as described before for the species-specific primers but only with DNA samples from populations PÑC1, PÑC4, PÑC7, PÑC10, PÑC13, PÑH1, PÑH3, PY1, PY2 and PÑA1 (Table 1). The amplification conditions consisted of an initial denaturation at 94ºC for 5 min, followed by 35 cycles of 94ºC for 30 s, 61ºC for 60 s, and 72ºC for 1 min. The final extension was at 72ºC for 7 min using a Perkin-Elmer GeneAmp® 9700 thermal cycler (Perkin Elmer Applied Biosystems). PCR products were purified using the QIAquick PCR Purification kit (Qiagen) and sequenced bidirectionally at Macrogen (Seoul, Korea) with primers D3A/D3B. The new D3 sequences were uploaded to the GenBank with the accession numbers KX792096-KX792101. *Pratylenchus* sequences generated in this study were aligned with sequences retrieved from the GenBank by using ClustalW (Thompson *et al*. 1994), with the parameters presented in Bioedit 7.0.9 (Hall, 1999). The phylogenetic relationship within the genus *Pratylenchus* was inferred using a Bayesian analysis with the program Mrbayes 3.2 (Ronquist *et al*., 2012) and with the substitution model GTR + I + G. The Bayesian analysis was conducted with four MCMC chains (three heated, one cold) using 10^6 generations and sampled every 1,000 generations. The first 2,500,000 generations (2,500 trees) were discarded as burn-in. Posterior probabilities (PP) are shown in the nodes.

RESULTS

Description of tuber symptoms

Tubers of white yam infected with *Meloidogyne* showed large galls on the entire tuber surface (Fig. 1A). Brown-reddish or dark necrotic small spots were observed below the tuber skin (Fig. 1B). Individual females with egg masses were found in the spots. Conversely, yampee tubers infected with *Meloidogyne* had small galls only in the anterior end (Fig. 1C). Within the galls, females with egg masses were surrounded by brown-reddish lesions. Tubers of yellow and white yams had symptoms of dry-rot. Symptoms include dark brown to black lesions below the tuber skin, necrotic areas, flaking off (Fig. 1D), and deep cracks (Figs. 1E, G). Lesions in yellow yam tubers were extended approximately 12 mm (Fig. 1F). Nematode extractions from the tubers showed that *Pratylenchus* was associated with these symptoms (see section below).

Frequency and population density of nematode genera

Seventeen genera of nematodes were identified in soil surrounding the yam roots, whereas 13 and 12 genera were found in roots and tubers, respectively (Tables 2-5). In soil samples (27 fields), the most frequent plant-parasitic nematodes associated with yams were *Helicotylenchus* (present in 93% of the samples) followed by *Tylenchus* (85%), *Pratylenchus* (63%), *Ditylenchus* (52%), and *Aphelenchoides* (48%) (Table 2). The predominant nematode genera in root samples were *Tylenchus* (70%), *Ditylenchus* (70%), *Pratylenchus* (67%), *Aphelenchoides* (52%), *Helicotylenchus* (44%), and *Meloidogyne* (30%) (Table 2). In yam tubers, *Scutellonema* and *Aphelenchoides* occurred in 50% of the samples collected from storage facilities or from the planting materials for the next crop cycle (total = 30 samples). Other frequent genera in tubers were *Meloidogyne* (47%), *Tylenchus* (43%), *Pratylenchus* (33%), and *Helicotylenchus* (23%).

The most abundant nematode in soil (average number of nematodes per 100 cm^3 soil) was *Helicotylenchus* with a population density of 54

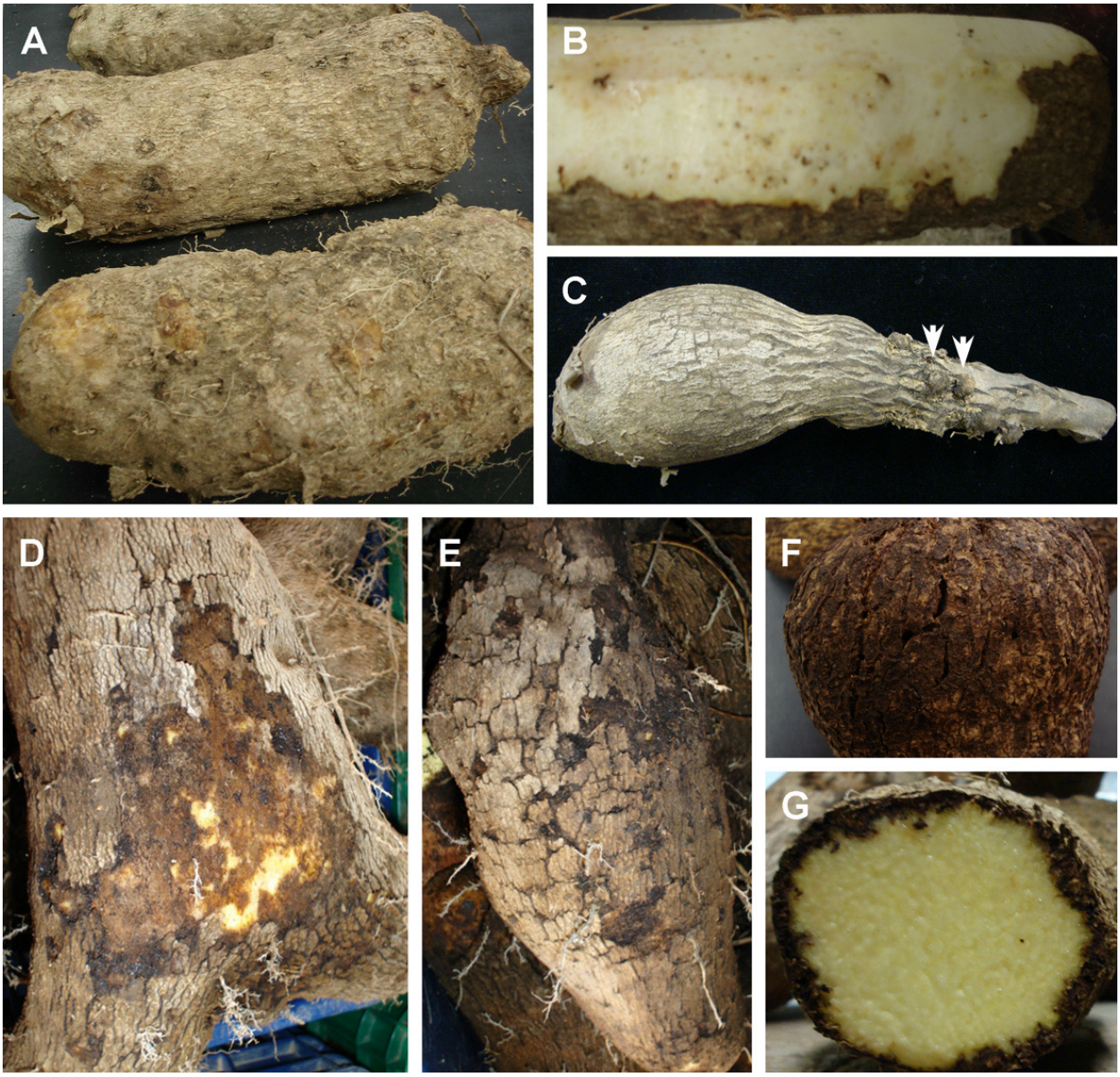

Fig. 1. Symptoms produced by *Meloidogyne* (A-C) and *Pratylenchus* (D-G) on yams and yampee. A-B: Tubers of white yam with galls and necrotic spots below the tuber skin; C: Yampee tuber with small galls in the anterior part; D: Flaking off of the epidermis in yellow yam tuber; E-F: Cracking in the surface area of yellow and white yam tubers, respectively; G: Dry rot below the outer skin of yellow yam tuber.

Table 2. Relative frequency and population densities of plant-parasitic nematodes in soil (100 cm^3), roots and tubers (100 g) of yams (*Dioscorea* spp.) in Costa Rica.

	Soil		Roots		Tuber	
Genera	Relative frequency[y]	Population density[z]	Relative frequency	Population density	Relative frequency	Population density
Aphelenchoides	48	2 (1-8)	52	110 (5-970)	50	88 (4-840)
Aphelenchus	41	8 (1-37)	30	162 (5-515)	13	55 (1-204)
Boleodorus	4	2	-	-	-	-
Criconematidae	44	11 (1-26)	11	7 (5-10)	3	2
Ditylenchus	52	2 (1-6)	70	95 (5-375)	17	3 (1-7)
Helicotylenchus	93	54 (1-338)	44	54 (5-395)	23	2 (1-5)
Hemicycliophora	7	15 (1-30)	15	18 (10-30)	3	1
Heteroderids	-	-	11	7 (5-10)	10	4 (1-6)
Meloidogyne	26	29 (1-178)	30	761 (10-5025)	47	291 (1-2683)
Paratylenchus	4	1	4	20	3	384
Pratylenchus	63	23 (1-134)	67	1208 (5-7890)	33	58204 (7-223048)
Rotylenchulus	15	5 (1-8)	11	5	-	1(1-2)
Scutellonema	11	4 (2-6)	22	104 (5-320)	50	26185 (577-108096)
Trichodoridae	-	-	4	5	-	-
Tylenchorhynchus	4	17	-	-	-	-
Tylenchulus	4	1	-	-	-	-
Tylenchus	85	12 (1-105)	70	29 (5-105)	43	9 (1-63)
Ultratenella	4	3	-	-	-	-
Xiphinema	4	6	-	-	-	-
Number of samples	27		27		30	

[y]Percentage of samples in which the genus was found.
[z]Mean (min-max).

Table 3. Population densities of plant-parasitic nematodes in soil (100 cm^3) of several yam species from three regions of Costa Rica.

	Atlantic region		North region			South region
Genera	*D. alata* (6)[y]	*D. cayenensis* (4)	*D. alata* (8)	*D. cayenensis* (1)	*D. trifida* (2)	*D. trifida* (6)
Aphelenchoides	1 (1-3)[z]	4 (1-8)	1	-	-	1
Aphelenchus	22 (6-37)	3 (1-5)	-	-	-	6 (2-8)
Boleodorus	-	-	2	-	-	-
Criconematidae	11 (1-26)	16 (1-26)	7 (1-20)	-	-	-
Ditylenchus	3 (1-6)	1	2 (1-6)	-	-	2 (1-3)
Helicotylenchus	126 (7-338)	79 (2-224)	31 (7-60)	4	3	5 (1-18)
Hemicycliophora	-	-	30	-	1	-
Meloidogyne	-	89 (1-178)	2 (1-2)	1	1	19
Paratylenchus	-	1	-	-	-	-
Pratylenchus	20 (2-54)	63 (25-134)	6 (1-11)	-	2 (1-2)	1
Rotylenchulus	6 (5-7)	1	8	-	-	-
Scutellonema	-	-	4 (2-6)	-	-	-
Tylenchorhynchus	-	-	17	-	-	-
Tylenchulus	-	-	1	-	-	-
Tylenchus	10 (7-16)	16 (9-27)	23 (4-105)	5	1	4 (1-11)
Ultratenella	-	3	-	-	-	-
Xiphinema	-	6	-	-	-	-

[y]Number of yam fields.
[z]Mean (min-max).

Table 4. Population densities of plant-parasitic nematodes in roots (100 g) of several yam species from three regions of Costa Rica.

	Atlantic region		North region			South region
Genera	*D. alata* (6)[y]	*D. cayenensis* (4)	*D. alata* (8)	*D. cayenensis* (1)	*D. trifida* (2)	*D. trifida* (6)
Aphelenchoides	210 (5-970)[z]	90 (15-240)	47 (15-80)	-	-	25 (10-35)
Aphelenchus	505 (495-515)	40	20	-	-	56 (5-145)
Criconematidae	-	-	5	-	-	8 (5-10)
Ditylenchus	89 (25-195)	170 (70-270)	161 (15-376)	5	-	23 (10-35)
Helicotylenchus	26 (5-80)	8 (5-10)	105 (10-395)	-	-	5
Hemicycliophora	-	-	30	10	15	-
Heteroderids	5	-	5	10	-	-
Meloidogyne	-	150	10	10	25	1,962 (20-5,025)
Paratylenchus	-	20	-	-	-	-
Pratylenchus	125 (30-285)	2,410 (25-5,140)	17 (5-25)	315	4,243 (595-7,890)	912 (5-2,040)
Rotylenchulus	5	-	-	-	-	10 (5-15)
Scutellonema	23 (5-40)	-	147 (20-320)	140	-	-
Trichodoridae	-	-	5	-	-	-
Tylenchus	28 (5-75)	12 (7-14)	42 (15-105)	-	30	27 (10-45)
Tylenchus	10 (7-16)	16 (9-27)	23 (4-105)	5	1	4 (1-11)

[y]Number of yam fields.
[z]Mean (min-max).

Table 5. Population densities of plant-parasitic nematodes in tubers (100 g) of several yam species from three regions of Costa Rica.

	Atlantic region			North region			South region
Genera	*D. alata* (10)[y]	*D.cayenensis* (5)	*D. rotundata* (2)	*D. trifida* (1)	*D. alata* (3)	*D. trifida* (2)	*D. trifida* (7)
Aphelenchoides	244 (10-840)[z]	11	-	-	-	-	25 (10-35)
Aphelenchus	-	-	-	-	-	-	56 (5-145)
Criconematidae	-	-	-	-	-	-	8 (5-10)
Ditylenchus	3	-	-	-	5	-	23 (10-35)
Helicotylenchus	3	-	2	-	-	-	5
Hemicycliophora	-	-	-	-	10	15	-
Heteroderids	-	-	1	-	10	-	-
Meloidogyne	2 (1-3)	-	2683	8	10	25	1,962 (20-5,025)
Paratylenchus	-	-	384	-	-	-	-
Pratylenchus	-	71,474 (19,884-134,241)	112,279 (1,509-223,048)	-	315	4,243 (595-7,890)	912 (5-2,040)
Rotylenchus	-	-	-	-	-	-	10 (5-15)
Scutellonema	31,899 (3,029-108,096)	-	577	31,792	140	-	-
Tylenchus	2 (1-3)	1	9	-	-	-	-

[y]Number of yam fields.
[z]Mean (min-max).

nematodes (ranged from 1 to 338). *Meloidogyne* and *Pratylenchus* showed population densities of 29 (ranged from 1 to 178) and 23 (ranged from 1 to 134) nematodes, respectively. In roots, *Pratylenchus* and *Meloidogyne* had the highest average population densities with 1,208 nematodes per 100 g of roots (ranged from 5 to 7,890) and 761 (from 10 to 5,025), respectively, followed by *Aphelenchus* with 162 nematodes (ranged from 5 to 515). *Scutellonema* presented the highest average population density in tubers with 26,185 nematodes (ranged from 577 to 108,096) per 100 g of tuber. *Pratylenchus* had a population density of 58,204 individuals per 100 g of tuber (ranged from 7 to 223,048), and *Meloidogyne* had 291 individuals per 100 g (ranged from 1 to 2,683).

Scutellonema was not detected in the South region of Costa Rica, but *Meloidogyne* and *Pratylenchus* were found on population densities ranging from 20 to 5,025 individuals and from 5 to 2,040 individuals per 100 g of roots of *D. trifida*, respectively (Table 4). In the same region and host, *Meloidogyne* had the highest population density on tubers, ranging from 2 to 1,117 individuals per 100 g of tuber (Table 5). In the North region, the *Pratylenchus* population density in *D. trifida* ranged from 595 to 7,890 individuals per 100 g of roots, whereas in *D. alata*, population ranged from 5 to 25. The population density of *Meloidogyne* was low in the three types of samples (soil, roots, and tubers) from the three hosts in the North region (Tables 3-5). In this region, *Scutellonema* was found in high population densities on *D. alata* tubers (from 4,244 to 32,155 individuals per 100 g of tuber) and low in one root sample of *D. cayenensis* (140 individuals per 100 g of roots). In the Atlantic region, roots samples of *D. cayenensis* showed population densities of *Pratylenchus* ranged from 25 to 5,140 individuals per 100 g of roots, whereas in tubers of the same yam species, population densities ranged from 19,884 to 134,241 individuals per 100 g of tuber. Population densities were also high in tubers of *D. rotundata* ranging from 1,509 to 223,048 individuals per 100 g of tuber and in one tuber sample of *D. trifida* collected from the same region (31,792 individuals per 100 g of tuber). *Scutellonema* was found in high population densities on tubers of *D. alata* collected from the Atlantic region (ranged from 3,029 to 108,096 individuals per 100 g of tuber). In one tuber sample of *D. trifida* collected from the Atlantic region, 31,792 *Scutellonema* individuals were found together with 8 *Meloidogyne* individuals. In some samples, mixed populations were found in *D. rotundata*, e.g., *Meloidogyne* and *Pratylenchus* with 2,683 and 1,509 individuals per 100 g of tuber, respectively, and *Pratylenchus* and *Scutellonema* with 223,048 and 577 individuals per 100 g of tuber, respectively.

Morphological identification of Meloidogyne *spp.*

Perineal patterns of females extracted from white yam had a general shape dorso-ventrally ovoid. Forty percent with dorsal arches moderately high to high without lateral lines (Fig. 2A) and 60% with low to high dorsal arches and with lateral lines in both sides (Fig. 2B). The striae varied from fine to coarse, smooth or wavy and continuous or broken. Females extracted from yampee had dorso-ventrally ovoid perineal patterns and moderately high to high dorsal arches (Fig. 2C-D). The striae ranged from fine to coarse or smooth to wavy. Lateral lines were not observed.

Molecular identification of Meloidogyne *and* Pratylenchus

PCR amplification of the mitochondrial DNA region between the *cox2* and 16S *rRNA* genes from three *Meloidogyne* populations extracted from yampee (MY1, MY2, and MY3), one from white yam (MW), and the controls *M. incognita* (MI) and *M. javanica* (MJ) yielded a single band of ~1500 bp. Samples of *M. hapla* (MH) and *M. arenaria* (MA) resulted in PCR products of ~500 bp and ~1100 bp, respectively (Fig. 3A). PCR-RFLP using the restriction enzyme *Hinf*I showed three restriction patterns. The first pattern consisted in two DNA fragments of ~300 bp and ~1200 bp in samples MY1, MY2, MY3, and the control *M. incognita*. No digestion was observed in PCR products of *M. javanica*. Population MW showed a digestion pattern with three DNA fragments. Two fragments matched with *M. incognita* (band sizes of ~350 and ~1250 bp) and the third band was not digested by *Hinf*I, as in PCR products of *M. javanica* (Fig. 3B). PCR using species-specific primers for *M. incognita* (Finc/Finc) yielded a single band of ~1200 bp in samples MY1, MY2, MY3, MW, and the positive control (Fig. 4A). *Meloidogyne javanica*-specific primers Fjav/Rjav resulted in a band of ~700 bp in population MW and the control (Fig. 4B). A PCR product of ~400 bp was obtained only in the positive control using *M. arenaria*-specific primers Far/Rar. The presence of two types of perineal patterns in *Meloidogyne* extracted from white yam (MW), the PCR-RFLP, and the species-specific primer results clearly showed the presence of concomitant species, *M. incognita* and *M. javanica*, in white yam samples from the Atlantic region. The same techniques

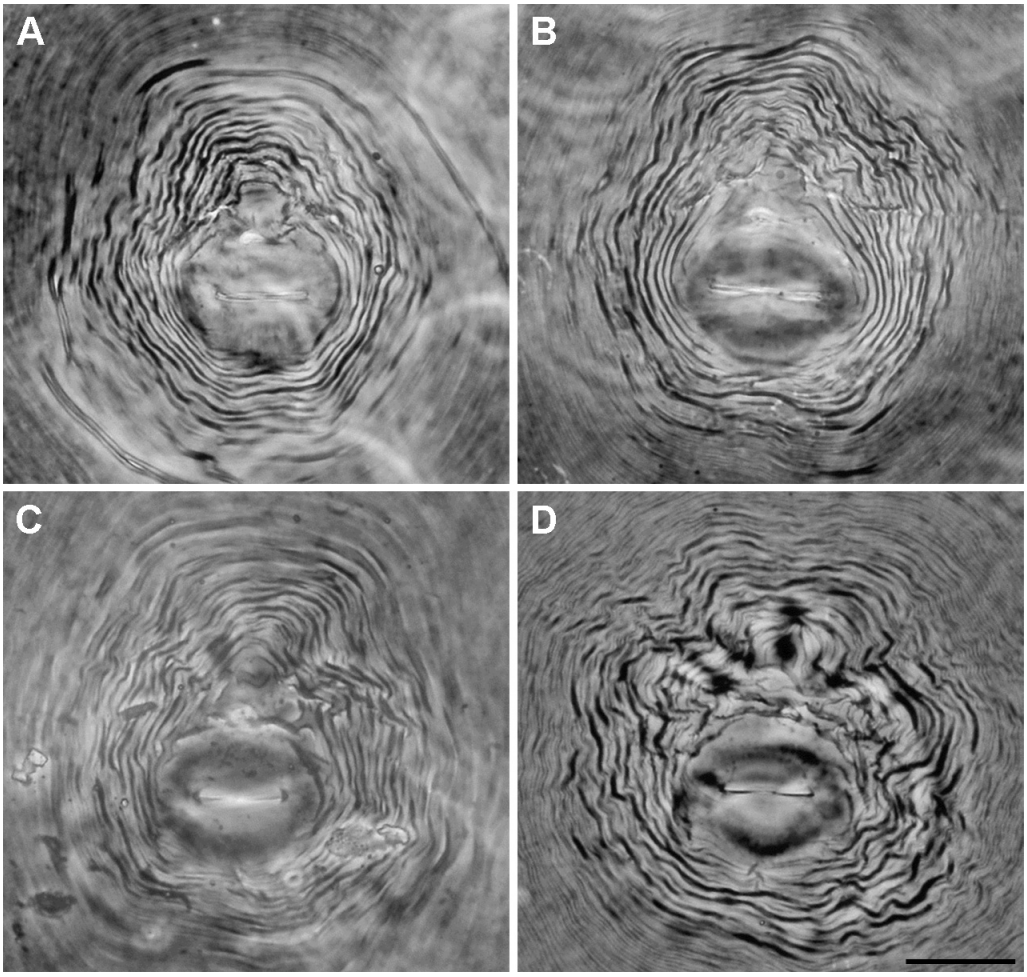

Fig. 2. Photomicrographs of *Meloidogyne* spp. perineal patterns extracted from white yam (A-B) and yampee (C-D) from Costa Rica. (Scale bar = 25 µm).

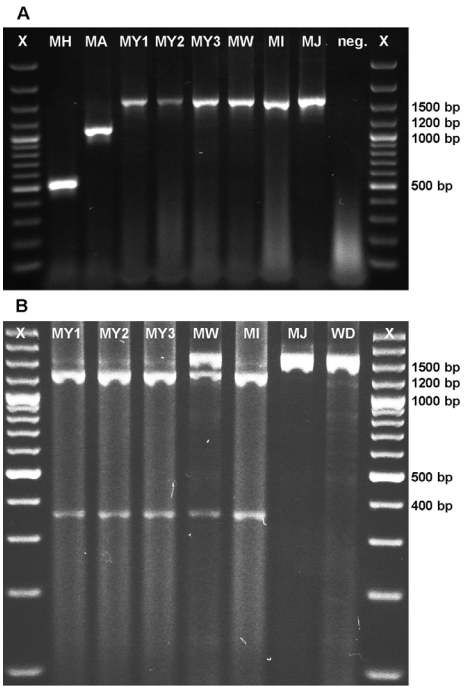

Fig. 3. Amplification products of DNA from *Meloidogyne* species using COII and 16S *rRNA* primers (A) and restriction pattern produced by *Hinf*I (B). *M. hapla* (MH), *M. arenaria* (MA), populations of *Meloidogyne* spp. extracted from yam and yampee (MY1, MY2, MY3, MW), *M. incognita* (MI), *M. javanica* (MJ) and sample MW without digestion (WD). X = 100 bp DNA ladder (Fermentas), neg. = negative control.

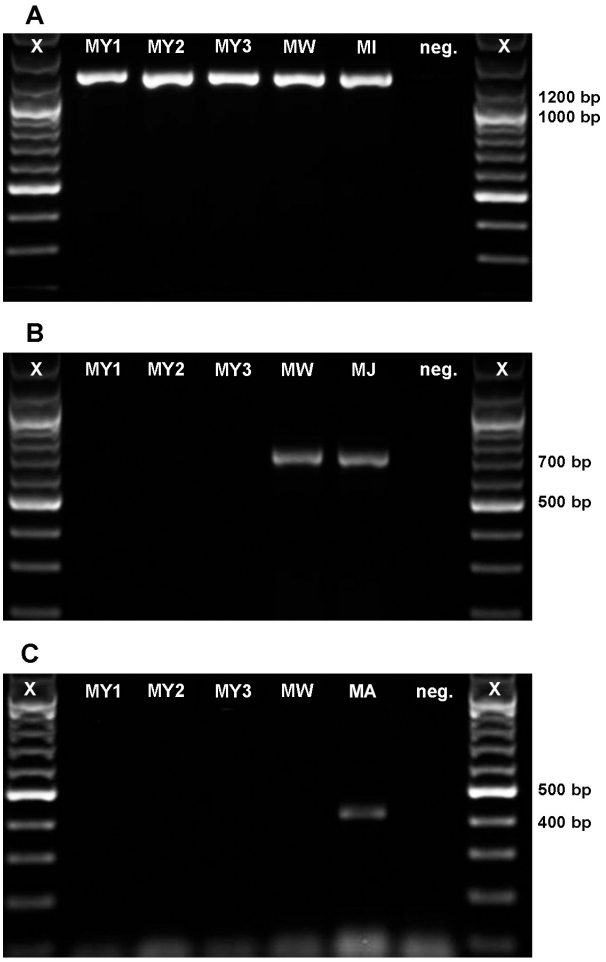

Fig. 4. Amplification products of DNA from *Meloidogyne* species using primers Finc/Rinc (A), Fjav/Rjav (B), and Far/Rar (C). Populations of *Meloidogyne* spp. extracted from yam and yampee (MY1, MY2, MY3, MW), *M. incognita* (MI), *M. javanica* (MJ), *M. arenaria* (MA). X = 100 bp DNA ladder (Fermentas), neg. = negative control.

allowed the identification of *M. incognita* from yampee in the South and Atlantic regions.

PCR using species-specific primers was performed to identify the *Pratylenchus* species in the 13 populations extracted from tuber and root samples of *Dioscorea* spp. *P. coffeae*-specific primers PC1/PC2 yielded a single product of ~700 bp in six *Pratylenchus* populations extracted from yellow yam (PÑC1, PÑC4, PÑC7, PÑC10, PÑC13, and PÑC16), two from white yam (PÑH1 and PÑH3) and one from yampee (PY2) (Fig. 5A). The amplification product using ACM7R/18S primers for *P. brachyurus* showed a single band of ~300 bp in one population extracted from yellow yam (PÑC16), three from yampee (PY2, PY3, and PY9) and the *P. brachyurus* control obtained from pineapple (Fig. 5B). These results revealed the presence of concomitant species in two hosts, yellow yam (sample PÑC16) and yampee (sample PY2). No amplification using primers PC1/PC2 and ACM7R/18S was observed on populations PY1 and PÑA1. To elucidate their identity, sequencing of the D3 region of the large subunit (LSU) 28S rDNA was performed with primers D3A/D3B. Additionally, DNA from eight more populations was included. D3 sequences from PY2, PÑC1, PÑC4, PÑC7, PÑC10, PÑC13, and PÑH1 were identical to each other (GenBank accession number KX792101) and differed by a single nucleotide (A rather than G at position 318) from sequence PÑH3 (KX792100). Query in the GenBank produced the highest similarity to sequences of *P. coffeae* (99-100% identity). For the sequence PY1 (KX792097) and the control *P. gutierrezi* (PCAF1, KX792096) similarity was highest (100% identity) to a sequence of *P. gutierrezi* (AF170442). The sequence PÑA1 (KX792099) matched with two sequences of *P. zeae* (EU130894 and JN020932) based on the highest similarity values (99% identity).

The phylogenetic analysis based on the D3 region among the *Pratylenchus* species extracted from yams and yampee with other root-lesion nematode sequences confirmed the identity of these species (Fig. 6). The two *P. coffeae* haplotypes identified in this study formed a monophyletic group with two *P. coffeae* sequences from other countries (PP = 84). The sequence PÑA1 extracted from greater yam was placed with *P. zeae*, highly supported with a PP of 100%. The PYI and PCAF1 sequences extracted from yampee and coffee, respectively, grouped (PP = 98) with a sequence of *P. gutierrezi* (AF170442). Importantly, two sequences of *P. gutierrezi* retrieved from the GenBank (AF170440 and AF170441) and an unidentified *Pratylenchus* (EU130899) formed a different clade (PP = 99). Sequence PL1 (KX792098) was placed in the monophyletic group composed of *P. penetrans* sequences (PP = 100).

DISCUSSION

This study showed the frequency of plant-parasitic nematodes and distribution of *Meloidogyne* and *Pratylenchus* species in the main yam-growing regions of Costa Rica. Linked to the present work, we detected and studied the intraspecific variability of the yam nematode, *Scutellonema bradys*, in 15 localities from Costa Rica (Humphreys-Pereira *et al*., 2014). This nematode was found in *D. alata*, *D. rotundata*, and *D. trifida* in the Atlantic region, and in *D. alata* and *D. cayenensis* in the North region, but it was not observed in the South region of Costa Rica (Humphreys-Pereira *et al*., 2014). Importantly,

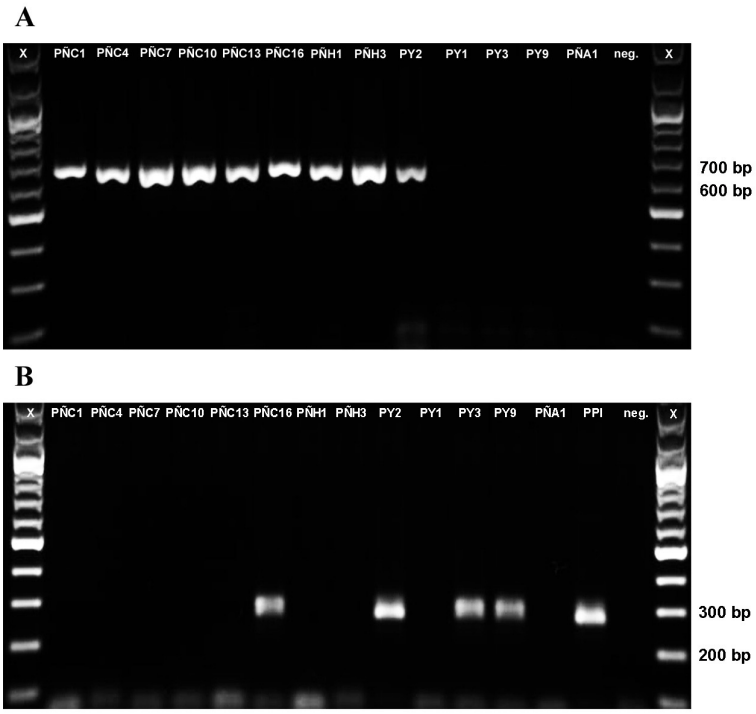

Fig. 5. Amplification products of DNA from *Pratylenchus* species using primers PC1/PC2 (A) and ACM7R/18S (B). Populations of *Pratylenchus* spp. extracted from yam and yampee (PÑC1, PÑC4, PÑC7, PÑC10, PÑC13, PÑC16, PÑH1, PÑH3, PY2, PY1, PY3, PY9, PÑA1) and *Pratylenchus* sp. extracted from pineapple. X = 100 bp DNA ladder (Fermentas), neg. = negative control.

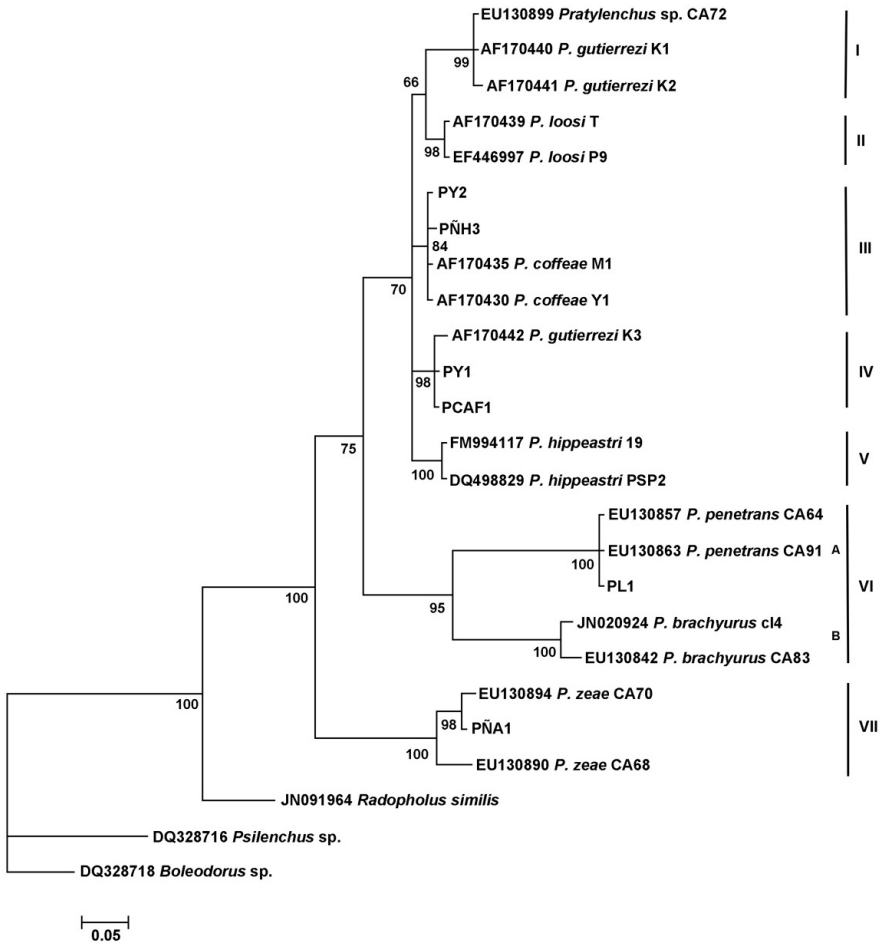

Fig. 6. Consensus tree showing the relationship of *Pratylenchus* spp. sequences based on the D3 region of 2*8S* rRNA gene. The consensus tree was generated from Bayesian Inference analysis using the GTR+I+G model. Posterior probability values are given in the clades.

S. bradys was found on 50% of the tuber samples, mostly associated with symptoms of the dry rot in greater yam (*D. alata*). The presence of this nematode in the four yam species cultivated by Costa Rican farmers complicates its management and the implementation of cultivar rotation. Recently, Claudius-Cole and Fawole, (2016) studied the ability of *S. bradys* to reproduce on several tropical cover crops, and demonstrated that *Cajanus cajan* could function as a trap crop, an alternative to reduce the damage from *S. bradys* populations. Commonly, yam farmers in Costa Rica leave the field fallow for about 2 to 3 mo between crop cycles (specifically in *D. alata*), which could be used to grow non-host or poor hosts to reduce populations.

Aphelenchoides was also found in half of the tuber samples, but in low population densities (4-840 nematodes/100 g). Kermarrec and Anais, (1973) found the foliar nematode, *Aphelenchoides besseyi*, associated with necrotic lesions in the foliage and tubers of *D. trifida* in Guadeloupe. The genus *Aphelenchoides* is widely diverse, consisting of several trophic groups as fungivores, predators, and plant-parasites depending on the species present (Sánchez-Monge *et al*., 2015). Therefore, species identification and pathogenicity assays are essential to determine their impact on yams. Similarly, the genus *Ditylenchus* includes mycophagous nematodes and few are plant parasites (Duncan and Moens, 2013), and members of the genus *Aphelenchus* feed on fungal hyphae (Yeates *et al*., 2009). Both *Ditylenchus* and *Aphelenchus* were found associated to yams.

The genus *Pratylenchus* was associated with yams in high population densities (up to 223,048 nematodes/100 g) in tubers of yellow yam. Four *Pratylenchus* species were detected on roots and tubers using molecular techniques. *Pratylenchus coffeae* was the predominant species (9 samples) and mostly associated with yellow yam, followed by *P. brachyurus* (4 samples) commonly found in yampee (*D. trifida*). One population each of *P. zeae* and *P. gutierrezi* were found in *D. alata* and *D. trifida*, respectively. *Pratylenchus coffeae* is considered one of the main causal agents of the dry rot in yams worldwide (Acosta and Ayala, 1975; 1976; Castagnone-Sereno and Kermarrec, 1988; Bridge *et al*., 2005), whereas *P. brachyurus* has been found in roots and tubers in Côte d'Ivoire, Fiji and Tonga, Guatemala, Mexico, and Nigeria (Miege, 1957; Bridge, 1988; Jenkins and Bird, 1962; Unny and Jerath, 1965; Roman, 1977; Bridge *et al*., 2005). In Costa Rica, *P. brachyurus* was initially identified in pineapple (Lopez and Salazar, 1990) and *P. zeae* in rice and sugarcane (Lopez and Salazar 1988b, 1990). There is no information regarding the damage caused by *P. zeae* on yams worldwide. Yams are commonly rotated with corn and cassava in Costa Rica, suitable hosts for *P. zeae* and *P. brachyurus*, respectively (McSorley *et al*. 1983; Olowe and Corbett, 1976). The two identical sequences of *P. gutierrezi* were collected from Poás, Alajuela (the coffee sample) and from the South region (the yampee sample) of Costa Rica. This nematode had not been identified in these two areas before. In the original description, *P. gutierrezi* was collected from San Antonio, Alajuela (Golden *et al*., 1992). Recently, Zamora-Araya *et al*. (2016) identified *P. gutierrezi* in Cinco Esquinas, Alajuela, and separated *P. gutierrezi* from *P. panamaensis* by using molecular markers and phylogenetic analyses. Importantly, the yampee sample was collected within a coffee plantation, a known host for this nematode. Therefore, caution must be taken with the use of intercropping as a management tool.

The genus *Meloidogyne* was found in 47% of all yam tuber samples associated mostly with small galls on the anterior part of yampee tubers or with large galls on white yam tubers (galls covered most of the tuber). The damage on white yam tubers was associated with higher *Meloidogyne* population densities (average 2,683 nematodes/100 g) than in yampee tubers (193 nematodes/100 g) which could explain the observed damage. Atu *et al*. (1983) determined that an inoculum level of 1,250 eggs of *Meloidogyne* reduced the market value by 40%. Coyne *et al*. (2006) in a survey on market stalls in West Africa observed less tuber galling on yellow yam that on white yam and water/greater yam. Mudiope *et al*. (2012) in a pathogenicity assay determined that white yam was the most susceptible to *Meloidogyne* followed by yellow yam, and water yam. In our study, galls on tubers or roots were not observed on *D. alata* (cv. Diamantes 22) and *D. cayenensis* (cv. Diamantes 2004). The absence of galls on Costa Rica´s cultivars might indicate certain tolerance to root-knot nematodes. López and Salazar (1988a) identified *M. javanica* in greater yam, but in cv. Antillano (*D. alata*), the only cultivar produced in Costa Rica (collected in the Atlantic region) in the 1980s. Later, cv. Diamantes 22 (*D. alata*) was substituted for the cv. Antillano because its tolerance to Anthracnose and possibly to *Meloidogyne*. Tuber galls were not observed in the north region, but a more extensive sampling must be performed on *D. trifida*.

The tolerance of the yam cultivars and yampee materials available for the growers in Costa Rica has not been evaluated against nematodes. This study provides valuable information for future projects on developing resistant cultivars and showed the impact of the genera *Pratylenchus*, *Scutellonema*, and

Meloidogyne on tuber quality. The yam nematode, *S. bradys*, was not found in the south region. A larger survey should be done to confirm its absence in this region, which can be recommended as a seed production area. Therefore, caution must be taken when farmers move vegetative material from other regions to the south region. Future efforts should be focused on determining the critical population densities of plant-parasitic nematodes in Costa Rican yam cultivars and their effect on the reduction of the tuber quality. Furthermore, it is necessary to quantify the damage caused by nematodes on yam tubers in the field and as well as in postharvest.

LITERATURE CITED

Acosta, N., and A. Ayala. 1975. Pathogenicity of *Pratylenchus coffeae*, *Scutellonema bradys, Meloidogyne incognita*, and *Rotylenchulus reniformis* on *Dioscorea rotundata*. Journal of Nematology 7:1-6.

Acosta, N., and A. Ayala. 1976. Effects of *Pratylenchus coffeae* and *Scutellonema bradys* alone and in combination on Guinea Yam (*Dioscorea rotundata*). Journal of Nematology 8:315-317.

Al-Banna, L., V. Williamson, and S. Gardner. 1997. Phylogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26S rDNA. Molecular Phylogenetics and Evolution 7:94-102.

Alvarado-Valverde, A. L. 2016. Exportaciones: Raíces y tubérculos, periodo 1999-2016. Consejo Nacional de Producción (CNP). Online https://simacr.go.cr/index.php/exportaciones-ryt

Alvarado, M., and R. López. 1985. Extracción de algunos nematodos fitoparásitos mediante modificaciones de las técnicas de centrifugación-flotación y embudo de Baerman modificado. Agronomía Costarricense 9:175-180.

Atu, U., S. Odurukwe, and R. Ogbuji. 1983. Root-knot nematode damage to *Dioscorea rotundata*. Plant Disease 67:814-815.

Ayala, A., and N. Acosta. 1971. Observations on yam (*Dioscorea alata*) nematodes. Nematropica 1:39-40.

Baimey, H., D. Coyne, and N. Labuschagne. 2009. Pathogenicity of *Scutellonema bradys* populations from different geographical areas in Benin on yam (*Dioscorea* spp.). Crop Protection 28:715-721.

Barker, K. R. 1985. Sampling nematode communities. Pp. 3-17 *in* K. R. Barker, C. C. Carter, and J. N. Sasser (eds.). An Advanced Treatise on *Meloidogyne*. Volume II: Methodology. Raleigh, NC: North Carolina State University Graphics.

Bridge, J. 1988. Plant-parasitic nematode problems in the Pacific islands. Journal of Nematology 20:173-183.

Bridge, J., D. Coyne, and C. Kwoseh. 2005. Nematode parasites of tropical root and tuber crops (excluding potatoes). Pp. 221-258 *in* M. Luc, R. Sikora, and J. Bridge (eds.). Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK: CABI Publishing.

Castagnone-Sereno, P., and A. Kermarrec. 1988. Association between *Pratylenchus coffeae* and *Scutellonema bradys* in yam tubers under agronomic conditions in the French West Indies. Nematropica 18:155-157.

Caveness, F. E., and H. Jensen. 1955. Modification of the centrifugal flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proceedings of the Helminthological Society of Washington 22:87-89.

Claudius-Cole, A. O., and B. Fawole. 2016. Reproduction and biology of *Scutellonema bradys* in roots of tropical cover crops. Nematropica 46:22-30.

Coyne, D. L., A. Tchabi, H. Baimey, N. Labuschagne, and I. Rotifa. 2006. Distribution and prevalence of nematodes (*Scutellonema bradys* and *Meloidogyne* spp.) on marketed yam (*Dioscorea* spp.) in West Africa. Field Crops Research 96:142-150.

Duncan, L. W., and M. Moens. 2013. Migratory endoparasitic nematodes. Pp. 144-178 *in* R. N. Perry, and M. Moens (eds.). 2nd Ed. Plant Nematology. Wallingford, UK: CABI Publishing.

Fawole, B. 1988. Histopathology of root-knot nematode (*Meloidogyne incognita*) infection on white yam (*Dioscorea rotundata*) tubers. Journal of Nematology 20:23-28.

Franklin, M. T. 1962. Preparation of posterior cuticular patterns of *Meloidogyne* spp. for identification. Nematologica 7:336-337.

Golden, A. M., R. López, and H. Vílchez. 1992. Description of *Pratylenchus gutierrezi* n. sp. (Nematoda: Pratylenchidae) from Coffee in Costa Rica. Journal of Nematology 24:298-304.

Hall, T. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Humphreys-Pereira, D. A., V. M. Williamson, S. Lee, D. L. Coyne, L. Salazar, and L. Gómez-Alpízar. 2014. Molecular and morphological characterisation of *Scutellonema bradys* from yam in Costa Rica and development of specific primers for its detection. Nematology 16:137-147.

Humphreys, D. A., V. M. Williamson, L. Salazar, L. Flores-Chaves, and L. Gómez-Alpízar. 2012. Presence of *Meloidogyne enterolobii* Yang & Eisenback (= *M. mayaguensis*) in guava and acerola from Costa Rica. Nematology 14:199-207.

Jenkins, W. R., and G. W. Bird. 1962. Nematodes associated with wild yam, *Dioscorea* sp., with special reference to the pathogenicity of *Meloidogyne incognita*. Plant Disease Reporter 46:858-860.

Jeyaprakash, A., M. S. Tigano, J. Brito, R. M. D. G. Carneiro, and D. W. Dickson. 2006. Differentiation of *Meloidogyne floridensis* from *M. arenaria* using high-fidelity PCR amplified mitochondrial AT-rich sequences. Nematropica 36:1-12.

Kermarrec, A., and A. Anais. 1973. Presencia en Guadalupe de *Aphelenchoides besseyi* Christie 1942 (Nematoda, Aphelenchoididae) en foliage del mapuey: *Dioscorea trifida* L. Turrialba 23:389-393.

López, R., and L. Salazar. 1988a. Nuevos huéspedes de *Meloidogyne javanica* (Nemata: Meloidogynidae) en Costa Rica. Agronomía Costarricense 12:241-244.

López, R., and L. Salazar. 1988b. Nematodos asociados al arroz (*Oryza sativa* L.) en Costa Rica. VII. *Pratylenchus zeae*. Agronomía Costarricense 12:183-190.

López, R., and L. Salazar. 1990. Morfología de algunos *Pratylenchus* spp. (Nemata:Pratylenchidae) encontrados en Costa Rica, vista con el microscopio electrónico de rastreo. Agronomía Costarricense 14:189-196.

Machado, A., L. Ferraz, and C. Oliveira. 2007. Development of a species-specific reverse primer for the molecular diagnostic of *Pratylenchus brachyurus*. Nematropica 37:249-257.

McSorley, R., S. K. O'Hair, and J. L. Parrado. 1983. Nematodes of cassava, *Manihot esculenta* Crantz. Nematropica 13:261-287.

Miège, J. 1957. Influences de quelques caractères des tubercules semences sur la levée et le rendement des ignames cultivées. Journal d'Agriculture Tropicale et de Botanique Appliquée 4:315-342.

Mudiope, J., D. L. Coyne, E. Adipala, and H. A. L. Talwana. 2012. Damage to yam (*Dioscorea* spp.) by root-knot nematode (*Meloidogyne* spp.) under field and storage conditions in Uganda. Nematropica 42:137-145.

Olowe, T., and D. C. M. Corbett. 1976. Aspects of the biology of *Pratylenchus brachyurus* and *P. zeae*. Nematologica 22:202-211.

Palmer, P. 1994. "Wa' apin man": La historia de la costa talamanqueña de Costa Rica, según sus protagonistas. San José, CR: Editorial de la Universidad de Costa Rica, 402 p.

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.

Román, J., and C. Sosa-Moss. 1977. Observaciones sobre la asociación de *Pratylenchus brachyurus* con la pudrición seca del barbasco *Dioscorea floribunda* en la región de Mexico. Nematropica 7:25-26.

Ronquist, F., M. Teslenko, P. van der Mark, D. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539-542.

Sánchez-Monge, A., L. Flores, L. Salazar, S. Hockland, and W. Bert. 2015. An updated list of the plants associated with plant-parasitic *Aphelenchoides* (Nematoda: Aphelenchoididae) and its implications for plant-parasitism within this genus. Zootaxa 4013:207-224.

Taylor, D. P., and C. Netscher. 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica 20:268-269.

Thompson, J., D. Higgins, and T. Gidson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Uehara, T., T. Mizukubot, A. Kushida, and Y. Momota. 1998. Identification of *Pratylenchus coffeae* and *P. loosi* using specific primers for PCR amplification of ribosomal DNA. Nematologica 44:357-368.

Unny, K. L., and M. L. Jerath. 1965. Parasitic nematodes on *Dioscorea* spp. in Eastern Nigeria. Plant Disease Reporter 49:875-876.

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

Yeates, G. W., H. Ferris, T. Moens, and W. H. Van der Putten. The role of nematodes in

ecosystems. Pp. 1-44 *in* M. J. Wilson, and T. Kakouli-Duarte (eds.) 1st Ed. Nematodes as environmental indicators. Wallingford, UK: CABI Publishing.

Zamora-Araya, T., W. Peraza-Padilla, A. Archidona-Yuste, C. Cantalapiedra-Navarrete, G. Liébanas, J. E. Palomares-Rius, and P. Castillo. 2016. Root-lesion nematodes of the genus *Pratylenchus* (Nematoda: Pratylenchidae) from Costa Rica with molecular identification of *P. gutierrezi* and *P. panamaensis* topotypes. European Journal of Plant Pathology 145:973-998.

Zijlstra, C., D. T. H. M. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology 2:847-

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