DIVERSITY OF ROOT-KNOT NEMATODES PARASITIZING COFFEE IN CENTRAL AMERICA

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ABSTRACT

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The diversity of root knot nematodes parasitizing coffee orchards in Central America was newly assessed through a broad regional survey. Populations of *Meloidogyne* spp. were identified by their esterase phenotype. Eleven esterase phenotypes were observed and nine species identified. *Meloidogyne exigua* was the most widely distributed while *M. arabicida, M. arenaria, M. hapla, M. izalcoensis* and *M. paranaensis* appeared to be much more localized. Concerning *M. paranaensis*, only observed in Guatemala, the two-band esterase phenotypes prevailed. About *M. arenaria*, the one-band esterase phenotype (A1) was observed for the first time on coffee in one population in Guatemala. A three-band esterase phenotype (A3) was observed in two populations in El Salvador and could belong to *M. morocciensis*. The presence of *M. enterolobii* on coffee in Central America was confirmed with one population from Guatemala and another one from Costa Rica as a new report. Based on esterase diagnosis identifications of *M. incognita* were made for the first time on coffee in the region: in Costa Rica, El Salvador and Guatemala. Taken together, the results of the survey revealed a high number of root knot nematode species present on coffee throughout Central America.

Key words: Central America, *Coffea arabica, Coffea canephora*, electrophoresis, esterase, root-knot nematode

RESUMEN

Villain, L., J. L. Sarah, A. Hernández, B. Bertrand, F. Anthony, P. Lashermes, P. Charmetant, F. Anzueto, P. Figueroa and R. M. D. G. Carneiro. 2013. Diversidad de nematodos agalladores asociados al cultivo de café en Centro América. Nematropica 43:194-206.

La diversidad de los nematodos agalladores parasitando plantaciones de café en Centroamérica fue nuevamente evaluada a través de un amplio monitoreo. Poblaciones de *Meloidogyne* spp. fueron identificadas por sus esterasas. Once fenotipos de esterasas fueron observados y nueve especies fueron identificadas. *M. exigua* apareció como la más distribuida mientas que *M. arabicida, M. arenaria, M. hapla, M. izalcoensis* y *M. paranaensis* resultaron mucho más localizadas. Para *M. paranaensis* únicamente observada en Guatemala, prevaleció el fenotipo de esterasas de dos bandas. Para *M. arenaria*, se observó por primera vez en café el fenotipo de esterasa de una banda (A1) para una población de Guatemala. Se observó también un fenotipo de esterasas de tres bandas (A3) en dos poblaciones de El Salvador, el cual podría corresponder a *M. morocciensis*. Se confirmó la presencia de *M. enterolobii* sobre café en Centroamérica, con una población de Guatemala y otra de Costa Rica como nueva notificación. Se realizaron las primeras identificaciones basadas en fenotipos de esterasas de *M. incognita* sobre café en esta región: en Costa Rica, El Salvador y Guatemala. Todos estos datos revelan un gran número de especies de nematodos agalladores presentes en café a través de Centroamérica.

Palabras clave: Centroamérica, *Coffea arabica, Coffea canephora*, electroforesis, esterasas, nematodos agalladores.

INTRODUCTION

Coffee is grown on nearly one million hectares in Central America with *Coffea arabica* as the main cultivated species for which this region produces 18% of world exports (FAO, 2012). This crop plays an important social role in the region since it employs almost a quarter of the active rural population and because the majority of coffee producers are small family farmers (Anonymous, 2002). Coffee originating in Africa began its economic expansion in Central America at the end of the 19th century and serious damage caused by nematodes was reported in this region at the beginning of the 20th century, e.g., in Guatemala by Alvarado (1935). Currently, *Pratylenchus* spp. and *Meloidogyne* spp. are considered as a major threat to coffee in Central America (Campos and Villain, 2005; Villain *et al*., 2008). Some old surveys of nematodes on coffee were in Guatemala (Schieber and Sosa, 1960; Schieber, 1966), Honduras (Pinochet and Ventura, 1980) and Costa Rica (Figueroa, 1988) with species diagnostics based on morphological criteria only and especially on perineal patterns for root-knot nematodes (RKN). However, species identification based on this feature is difficult and even uncertain for some species. For example, similar perineal patterns are encountered in *M. paranaensis* Carneiro, Carneiro, Abrantes Santos and Almeida, *M. izalcoensis* Carneiro, Almeida, Gomes and Hernandez, *M. enterolobii*, Yang and Eisenback (syn. *M. mayaguensis*, Rammah and Hirschmann) and *M. incognita*, (Kofoid and White) Chitwood, which all parasitize coffee trees (Carneiro and Cofcewicz, 2008). The use of isoenzymes, and particularly esterases, has proved to be very useful in identifying RKN (Esbenshade and Triantaphyllou, 1985, 1990; Carneiro *et al*., 2000; Cofcewicz *et al*., 2004; Carneiro and Cofcewicz, 2008). Some characterizations of RKN parasitizing coffee trees in Central America have been made using this feature (Carneiro *et al.,* 2004; Hernández *et al*., 2004a) but they were limited to only few populations. Our work constitutes the first regional scale survey of RKN and aims to update the knowledge about diversity of *Meloidogyne* species parasitizing coffee trees in different coffee producing regions in Central America using esterase phenotypes for identification.

MATERIALS AND METHODS

The whole surveyed area is bounded by latitude 15°N in Guatemala and by latitude 9°N in Costa Rica (Fig. 1). Number of collected samples was: in Guatemala, 49 on 9 farms; in El Salvador, 26 on 26 farms; in Honduras, 7 on 7 farms and in Costa Rica, 17 on 16 farms. The survey was mainly focused on major coffee growing areas where *Meloidogyne* spp. infestations have been regularly reported with grower concerns though often without species-specific identification: the Pacific slope of the volcanic chain Sierra Madre in the South West of Guatemala; the Izalco volcano massif in the South West of El Salvador; the eastern province of El Paraíso bordering Nicaragua of Honduras; the Central Valley (provinces of Alajuela and Heredia) and the Turrialba Valley of Costa Rica. Additionally, samples were collected in regions previously poorly surveyed for coffee parasitic nematodes and/or where RKN reports are absent or rare in the northern (Verapaces), south-eastern (Santa Rosa), north-eastern (Chiquimula, Zacapa) and north-western (Huehuetenango) regions of Guatemala; as well as in the province of Guanacaste and the Perez Zeledon Valley in Costa Rica. Sampling in the field was done between 2003 and 2005. Each sample was composed of roots collected on 5-6 coffee-trees. For each coffee-tree, roots, mainly non-woody roots, were sampled from 20 to 60 cm from the root crown on each side of the tree trunk within the row and perpendicularly to the row, in the first 30 cm of soil depth. In addition, roots of cultivated *Musa* AAB and AAA, and of *Impatiens* sp., a very common weed in coffee plantations throughout Central America, were sampled in some of the surveyed coffee fields. Data on geographical location, altitude, and RKN host plants of the sampling sites are summarized in Table 1. During sampling, symptoms were observed both on white unlignified and woody roots, including the tap root by excavating along it or, in some cases, by uprooting the coffee trees.

Meloidogyne spp. populations were characterized by isoenzyme esterase analysis according to the

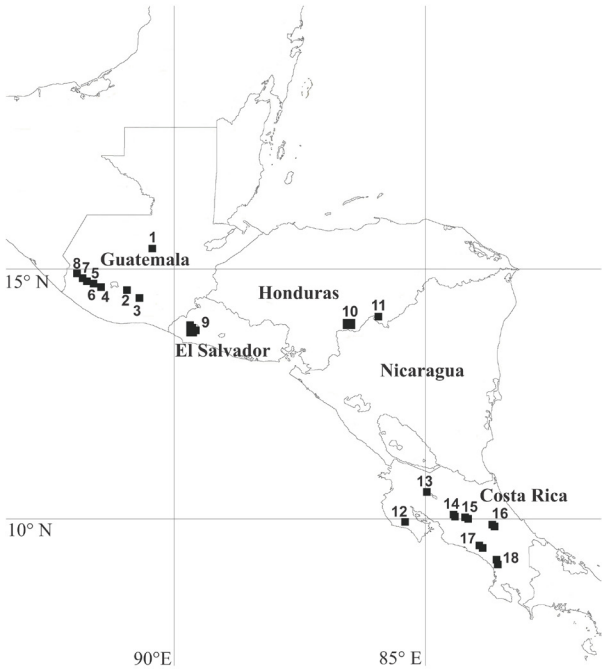

Fig. 1. Map of Central America with farm locations where root samples were collected in coffee orchards, in Costa Rica, El Salvador, Guatemala and Honduras. Sampling location numbers are reported for each farm in Table 1.

following methodology. Twenty egg masses were removed from roots collected in the field and inoculated in susceptible cultivars of tomato (*Solanum lycopersicum* cv. Nainemor and cv. Santa Cruz) and coffee (*Coffea arabica* cv Mundo novo and cv. Caturra) and reared in the greenhouse. All populations were increased on tomato. For each population about thirty young females (milky white in colour) were collected from this host and their esterases were extracted and characterized using polyacrylamid gel electrophoresis according to the technique described either by Carneiro and Almeida (2001) on a hand-made unit or by Hernandez *et al*. (2004a) on a Cl-18 Permatron and Mini-protean II electrophoresis system (Bio-Rad). For more reliable identification of esterase phenotypes, esterase extract from a reference population of *M. javanica* (Treub, 1885, Chitwood, 1949) was included in each electrophoresis gel for direct comparison. As proposed by Esbenshade and Triantaphyllou (1985, 1990) and Carneiro *et al.* (2000), esterase phenotypes (Est) were designated by letters suggesting the related species (when known), or the rate of migration of the major bands (S = slow and F = fast) and a numeral indicating the number of bands. Additionally, perineal patterns of twenty females were observed for each one of the populations from El Salvador.

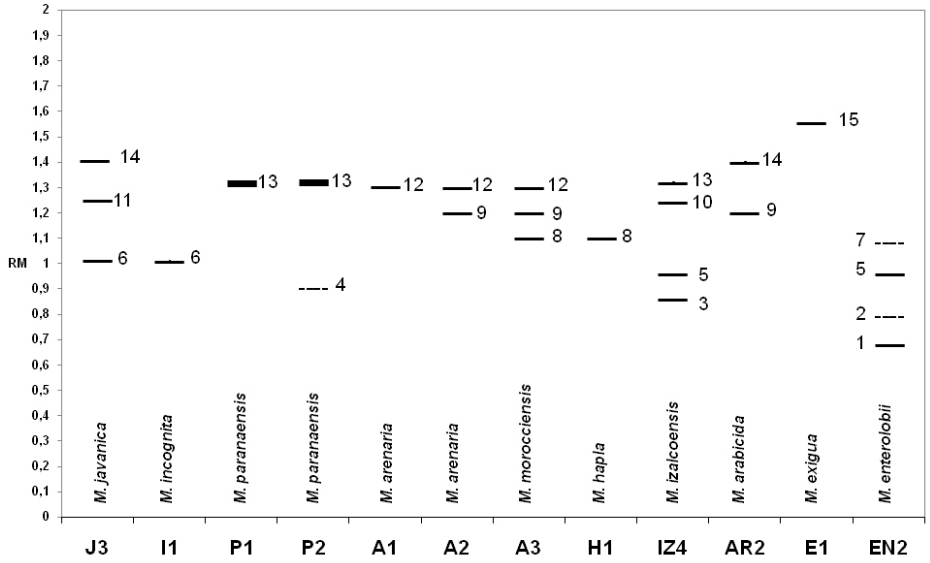

Fig. 2. Diagram of the esterase phenotypes observed among the *Meloidogyne* spp. populations analyzed. The observed esterase bands with their respective rate of migration (RM) were 1: RM = 0.68, 2: RM = 0.79, 3: RM = 0.86, 4: RM = 0.90, 5: RM = 0.96, 6: RM = 1.01, 7: RM = 1.08, 8: RM = 1.10, 9: RM = 1.20, 10: RM = 1.24, 11: RM = 1.25, 12: RM 1.30, 13: RM = 1.32, 14: RM = 1.40, 15: RM = 1.55.

RESULTS AND DISCUSSION

Eleven esterase phenotypes were observed among the analyzed samples compared to Est J3 (Rm: 1.00, 1.24, 1.36) of *M. javanica* (Fig. 2) and were attributed to nine RKN species according to the previous characterizations made by Esbenshade and Triantaphyllou (1985, 1990), Carneiro *et al*. (2000) as well as Carneiro and Cofcewicz (2008). These phenotypes were Est E1 (Rm: 1.50): *M. exigua*; Est P1 (Rm: 1.32) and P2 (Rm: 0.90, 1.32): *M. paranaensis*; Est AR2 (Rm: 1.20, 1.40): *M. arabicida*; Est IZ4 (Rm: 0.86, 0.96, 1.24, 1.30): *M. izalcoensis*; Est I1 (Rm: 1.00): *M. incognita*; Est A1 (Rm: 1.30) and Est A2 (Rm: 1.20, 1.30): *M. arenaria*; Est H1 (Rm: 1.10): *M. hapla* and Est EN2 (Rm: 0.68, 0.96) with two minor bands (Rm: 0.79, 1.08): *M. enterolobii* (= *M. mayaguensis* is now considered as a junior synonym of *M. enterolobii* Xu *et al.,* 2004; Hunt and Handoo, 2009; Castagnone-Sereno, 2012). An Est A3 (Rm: 1.10, 1.20, 1.30) was also observed which is commonly attributed to *M. arenaria* but should be assigned to *M. morocciensis*, as discussed below (Carneiro *et al*., 2008). Table 1 summarizes the species diagnosis of the populations of RKN collected along with their geographical location, altitude and host.

Meloidogyne exigua (Est E1)

In Honduras, *M. exigua* was detected in all sampled farms distributed throughout the surveyed province of El Paraíso, indicating that this RKN species may be widespread in this region. The region of El Paraíso bordering Nicaragua is, until now, the only region where RKN have been reported on coffee. A more complete analysis has already been performed of samples from all the important agricultural regions of Honduras using a diagnosis based on the perineal pattern, which may be an appropriate feature in the particular case of *M. exigua* diagnosis (Pinochet and Ventura, 1980). Among the samples collected from 51 crop species, these authors found *M. exigua* only on two coffee root samples both of which were collected in the region of El Paraíso but none in the western region of Honduras,

which is currently the most important coffee growing area. *Meloidogyne* populations from three coffee root samples from the same region of El Paraíso were also identified by their esterase phenotypes as *M. exigua* by Hernández *et al*., 2004a. It should be mentioned that the El Paraíso coffee growing region borders a coffee growing area in Nicaragua where *M. exigua* has also been observed (Campos and Villain, 2005).

One population from Guatemala presented the Est E1 phenotype of *M. exigua*. This result agrees with field observations of symptoms commonly connected with *M. exigua* parasitism: well-formed spherical galls without large swellings or cracked roots which appear serially like rosaries. The detection of a single population of *M. exigua* from Guatemala, is the first diagnosis of this species based on the esterase phenotype for this country. Schieber and Sosa (1960) previously reported *M. exigua* in Guatemala based on the perineal pattern and even described the species as being the main RKN on coffee in Guatemala. However, in a later paper, Schieber (1966) published photographs of swollen and corky coffee roots which the author attributed respectively to *M. exigua* and *Pratylenchus coffeae* attacks. In fact, we now know that in Guatemala these symptoms are caused by *M. paranaensis* (Campos and Villain, 2005, Villain *et al*., 2008), probably in association with *Fusarium* sp. as demonstrated in the case of corky roots caused by the association of *M. arabicida* and *F. oxysporum* in Costa Rica (Marbán-Mendoza *et al*., 1989; Bertrand *et al*., 2000). Another survey in Guatemala failed to detect any populations of *M. exigua* (Hernández *et al*., 2004a). At the present time, with only one population detected, the presence of *M. exigua* on coffee in Guatemala can be considered as rare, but this new infestation nevertheless merits careful attention. In El Salvador, no population of *M. exigua* was detected in our survey, although this species was reported as parasitizing coffee by the same authors cited above for Guatemala (Schieber and Sosa, 1960). However, the same observations made about their sighting of *M. exigua* in Guatemala also apply to the sighting in El Salvador. The fact that *M. exigua* was not found on any of the many samples taken in the south-western region indicates that this species is probably not present on coffee there. Another study also failed to detect *M. exigua* in different regions of El Salvador (Hernández *et al*., 2004a).

In Costa Rica, *M. exigua* (Est E1) was found to be widely distributed through the Central Valley, the major coffee growing region (Alajuela, Heredia and Cartago provinces). Indeed, *M. exigua* was previously widely reported in the Central Valley (López, 1985; Flores and López, 1989; López and Salazar, 1989a, Avelino *et al*., 2009). *Meloidogyne exigua* was also detected in the Pacific South area of San Jose province, in Perez Zeledón county. This detection is in agreement with previous observations of *M. exigua* in the same area, on coffee (López, 1985) but also on a native tree (*Miconia* sp.) in primary forests (López and Vilchez, 1991). *Meloidogyne exigua* has also been detected on coffee in other counties: in south-western Costa Rica, in Buenos Aires county (López, 1985) and Coto Brus county, bordering Panama (Avelino *et al*., 2009). The spreading of this species in the Turrialba valley is in agreement with the results of other surveys (López, 1985; Avelino *et al*., 2009). On the other hand, the absence of *M. exigua* in all the samples collected in the north-western coffee growing area of Costa Rica, (Guanacaste province), is in agreement with the absence of reports in the literature concerning the presence of this species on coffee in this province.

Meloidogyne exigua appears to be widespread on coffee in many Latin America coffee growing countries including major coffee producers like Colombia and Brazil (Campos and Villain, 2005; Gaitán *et al*., 2008; Ferraz, 2008). This species is likely native to the continent since it was detected in a primary forest in Costa Rica (López and Vilchez, 1991), in Brazil in the Atlantic mountain forest in Rio de Janeiro state, and in the Amazon forest in Mato Grosso state (Lima *et al*., 2005; Silva *et al*., 2008), as well as in Martinique island, French West Indies (Quénéhervé *et al*., 2011). According to the results of the present survey and to previous monitoring, the eastern region of El Paraíso in Honduras bordering Nicaragua could currently be the northern continental limit of the main distribution of *M. exigua*, as the species appears to be absent in El Salvador and exceptional in Guatemala (only one population detected). To our knowledge, *M. exigua* has not been reported in Mexico. For the record, at these latitudes of the Caribbean, *M. exigua* has been reported: i) on coffee in the Dominican Republic (Schieber and Grullon, 1969); ii) in the French West Indies: on coffee and natural forest in Martinique (Goodey *et al*., 1965; Kermarec and Scotto la Massèse, 1972; Quénéhervé, *et al*., 2011) and on Citrus in Guadeloupe (Scotto la Massèse, 1969).

Meloidogyne paranaensis (Est P1, Rm: 1.32 and Est P2, Rm: 0.9, 1.32)

This species is considered as a major threat for coffee crop in Brazil since its description in the Parana state (Carneiro *et al*., 1996; Campos and Villain, 2005). Corky root symptoms similar to those observed in cases of coffee root parasitism by *M. paranaensis* were already observed at the beginning of the 20th century in Guatemala (Alvarado, 1935). Symptoms caused by *M. paranaensis* on coffee are very impressive: more swelling than galling with the whole root system being affected. In time, these swellings turn corky on woody roots including on the tap root, where the cork layer can extend right up to the collar.

The present survey appears to confirm *M. paranaensis* as the predominant RKN parasitizing coffee trees in Guatemala, at least in the south west, the major coffee growing region. In accordance with Carneiro *et al.* (2004), our results confirm the presence in Guatemala

Table 1. Geographical data on root knot nematode infested coffee orchards from where samples were collected with their respective esterase phenotype (EST) and corresponding species diagnosis.

Country[w]	Province, County (District)	Farm (SLN[x])	Altitude (m.s.l.)	Host[y]	EST	Diagnosis
GTM	Alta Verapaz, Santa Cruz Verapaz	Don Bosco (1)	1200	*C. arabica* (2)	EN2	*M. enterolobii*
					H1	*M. hapla*
	Chimaltenango, Acatenango	Los Cerritos (2)	1650	*C. arabica*	E1	*M. exigua*
	Escuintla, Palín	La Providencia (3)	1170	*C. arabica* (2)	P1	*M. paranaensis*
					P1	*M. paranaensis*
		Raguay (3)	1260	*C. arabica* (3)	P1	*M. paranaensis*
				C. canephora[z] (4)	P1	*M. paranaensis*
				C. canephora[z] (2)	A2	*M. arenaria*
				Musa AAB	I1	*M. incognita*
	Suchitepéquez, San Francisco Zapotitlán	Elviras (4)	1050	*C. arabica*	P2	*M. paranaensis*
	Quetzaltenango, Colomba	Los Manaques (5)	820	*C. arabica* (3)	P2	*M. paranaensis*
				C. arabica	I1	*M. incognita*
				C. canephora[z]	P2	*M. paranaensis*
	Quetzaltenango, El Palmar	El Faro (6)	1200	*C. canephora*[z] (4)	A1	*M. arenaria*
	San Marcos, La Reforma	Neuva América (7)	1000	*C. canephora*[z] (6)	P2	*M. paranaensis*
				Impatiens sp.	A1	*M. arenaria*
	San Marcos, San Rafel Pie de la Cuesta	Panoramá (8)	1080	*C. canephora*[z] (5)	P2	*M. paranaensis*
				C. canephora (3)	P2	*M. paranaensis*
				C. arabica (2)	P2	*M. paranaensis*
				Musa AAB (2)	P2	*M. paranaensis*
SLV	Sonsonate, Cruz Grande	Las Palmeras (9)	550	*C. arabica*	IZ4	*M. izalcoensis*
		La Gloria (9)	550		IZ4	*M. izalcoensis*
		La estrella (9)	600		IZ4	*M. izalcoensis*
		Samaria (9)	600		IZ4	*M. izalcoensis*
		Santa Rita (9)	620		IZ4	*M. izalcoensis*
		San Roberto (9)	640		IZ4	*M. izalcoensis*
		El Carmen (9)	750		IZ4	*M. izalcoensis*
		San Luis (9)	770		IZ4	*M. izalcoensis*
					A2	*M. arenaria*
		Santa Rosa (9)	800		IZ4	*M. izalcoensis*
		Nuevos Horizontes (9)	1100		IZ4	*M. izalcoensis*
	Sonsonate, Huiscoyolate	El Angel (9)	550	*C. arabica*	IZ4	*M. izalcoensis*
		Las Mercedes (9)	430		IZ4	*M. izalcoensis*
		Las Merceditas (9)	430		IZ4	*M. izalcoensis*
	Sonsonate, Las Lajas	Las Lajas (9)	1000	*C. arabica*	IZ4	*M. izalcoensis*
	Sonsonate, Los Naranjos	Santa Marta (9)	1520	*C. arabica*	H1	*M. hapla*
	Sonsonate, San Isidro	San Nicolás (9)	1030	*C. arabica*	H1	*M. hapla*
	Sonsonate, Talcomunca	Grano de Oro (9)	850	*C. arabica*	H1	*M. hapla*
					A3	*M. morocciensis*
		El Carmen (9)	840		IZ4	*M. izalcoensis*
		Santa Elisa (9)	540		IZ4	*M. izalcoensis*
	Sonsonate, Teshcal	San Miguelito (9)	450		IZ4	*M. izalcoensis*
		San José (9)	640		IZ4	*M. izalcoensis*
		La Esperanza (9)	700		IZ4	*M. izalcoensis*
	Sonsonate, Tunalmiles	Las Mercedes (9)	520	*C. arabica*	IZ4	*M. izalcoensis*
		Laurelar (9)	820		IZ4	*M. izalcoensis*
		El Carrizal (9)	940		IZ4	*M. izalcoensis*
		El Gran Chaparral (9)	1000		IZ4	*M. izalcoensis*
					A3	*M. morocciensis*
HND	El Paraíso, El Paraíso	Unknown (10)	600	*C. arabica*	E1	*M. exigua*
		J. Sandoval (10)	650		E1	*M. exigua*
		Encarnación (10)	1170		E1	*M. exigua*
	El Paraíso, Trojes	La Unión (10)	800	*C. arabica*	E1	*M. exigua*
		Carlos Falk (10)	800		E1	*M. exigua*
		Asoproat (11)	830		E1	*M. exigua*
		Oscar Alemán (11)	1140		E1	*M. exigua*

Table 1. Geographical data on root knot nematode infested coffee orchards from where samples were collected with their respective esterase phenotype (EST) and corresponding species diagnosis (continued).

Country[w]	Province, County (District)	Farm (SLN[x])	Altitude (m.s.l.)	Host[y]	EST	Diagnosis
CRC	Guanacaste, Hojancha	O. Loría (12)	750	*C. arabica*	EN2	*M. enterolobii*
	Guancaste, Tilarán (Tierras morenas)	Miramac (13)	650	*C. arabica*	I1	*M. incognita*
				Musa AAB	I1	*M. incognita*
	Alajuela, Palmares	V. Julio Araya (14)	1050	*C. arabica*	I1	*M. incognita*
	Alajuela, San Ramón (San Isidro)	La Canaria (14)	1000	*C. arabica*	E1	*M. exigua*
	Heredia, Barva (San Pedro)	Cicafé (15)	1100	*C. arabica* (3)	E1	*M. exigua*
	Heredia, Heredia (San Isidro)	San Isidro (15)	1100	*C. arabica*	E1	*M. exigua*
	Cartago, Jiménez (Juan Viñas)	Juan Viñas (16)	1200	*C. arabica*	AR2	*M. arabicida*
	Cartago, Turrialba (Atirro)	Atirro (16)	700	*C. arabica*	E1	*M. exigua*
	Cartago, Turrialba (Unknown)	La Isabel (16)	700	*C. arabica*	E1	*M. exigua*
	Cartago, Turrialba (Unknown)	La Calera (16)	700	*C. arabica*	E1	*M. exigua*
	San José, Dota (Copey)	El Salao (17)	Unknown	*C. arabica*	E1	*M. exigua*
	San José, Río Nuevo (Los Angeles)	M. Corrales (17)	700	*C. arabica*	E1	*M. exigua*
	San José, Perez Zeledón (Cajón)	P. C. barrientos (18)	600	*C. arabica*	I1	*M. incognita*
		Monte Box (18)	600	*C. arabica*	E1	*M. exigua*
					EN2	*M. enterolobii*
	San Jose, Perez Zeledón (Pejiballe)	E. Badilla (18)	700	*C. arabica*	I1	*M. incognita*
		Los Durán (18)	750	*C. arabica*	I1	*M. incognita*

[w]ISO country codes (GTM = Guatemala; SLV = El Salvador; HND = Honduras; CRC = Costa Rica)
[x]Sampling location number referred in Fig. 1.
[y]Sample number is indicated in brackets when several samples were collected on a given host and on a given location.
[z]*C. canephora* sampled roots from rootstocks with *C. arabica* grafted.

of the two known esterase phenotypes, Est P1, and Est P2, for *M. paranaensis* among which Est P2 appears to be the most widespread among the populations we studied. Indeed, the Est P1 phenotype was observed on all coffee root samples of both own-rooted *C. arabica* and *C. canephora* rootstocks from just one farm with no mixture with the Est P2 phenotype. This farm is located only about 3 km from the other farm where an Est P1 population was detected earlier (Carneiro e*t al*., 2004; Hernández *et al*., 2004a). So the distribution range of this Est P1 phenotype appears to be limited to a very small area in Guatemala. Conversely, in Brazil, up to now, Est P1 is the only phenotype observed among all the *M. paranaensis* populations sampled in different states and regions (Carneiro *et al*., 2004; Carneiro *et al*., 2005b; Carneiro and Cofcewicz, 2008). A comparative study on the pathogenesis of these two different esterase phenotype biotypes of *M. paranaensis* on a range of coffee genotypes is thus required, despite the fact that molecular diversity appears to be low among populations of *M. paranaensis* and even between populations of the two Est phenotypes (Carneiro *et al*., 2004). Another observation of this study leads to consider pathogenic differences between these two *M. paranaensis* esterasic phenotypes. Among the coffee orchards sampled in Guatemala and presenting a widespread presence of *M. paranaensis* on coffee roots, two of them counted with association of *Musa* AAB. One of them was the orchard infested with the P1 where no *M. parananensis* was detected on *Musa* AAB. Whereas in the other field where the Est P2 phenotype was present, *M. paranaensis* was present on *Musa* AAB roots. Even though its pathogenicity was not checked on coffee, the presence of an Est P2 population of *M. paranaensis* on *Musa* AAB trees leads to recommendations that coffee growers pay particular care when planting *Musa* spp. in coffee fields since excising lateral shoots or excavating corms of plantain trees (the most common method of propagation used by farmers) is an efficient way of disseminating nematodes (Gowen *et al*., 2005).

Additionally, on this same farm where *M. paranaensis* (Est P2) appears to be widely distributed on both *C. arabica* and *Musa* AAB roots, this nematode was also detected in a commercial *C. canephora* plantation at an impressive root infestation rate with general plant exhaustion and a high mortality rate among stumped trees. This observation is in accordance with previous studies revealing the susceptibility of many *C. canephora* lines to *M. parananensis* and underlines the need to use selected resistant germplasm such as the cv. Nemaya if using *C. canephora* rootstocks to control this RKN in *C. arabica* crop (Bertrand and Anthony, 2008). *Meloidogyne paranaensis* was recently detected in Espirito Santo State in Brazil and represents an emerging serious damaging pest on *C. canephora* cv Conilon crop in Brazil (Barros *et al*., 2011).

In the present survey as well as previous studies (Hernández *et al*., 2004a; Carneiro *et al*., 2004), *M. paranaenis* was not detected in any other country in Central America besides Guatemala. In addition to the presence of *M. paranaensis* in Guatemala and Brazil, there are other interesting reports concerning this nematode: one putative *M. konaensis* population collected on coffee in Hawaii was finally identified as *M. paranaensis* because of its Est P1 phenotype and its molecular and morphological profiles matching those of *M. paranaensis* (Carneiro *et al*., 2004; Carneiro and Cofcewicz, 2008). One population in Peru and one in Surinam, as well as four populations in Brazil, all

collected on coffee, presented the same esterase (Est P1), malate dehydrogenase, superoxide dismutase and glutamate oxaloacetate transaminase phenotypes as *M. paranaensis*, which, at that time, had not yet been described, so these populations were thought to belong to an unidentified species (Esbenshade and Triantaphyllou, 1985). The erratic distribution of *M. paranaensis* in South and Central America, and in the Caribbean and Hawaii is probably linked to the transport of coffee trees but also to the transport of other crop vegetative germplasm like plantains, on which we observed *M. paranaensis* in Guatemala. The coffee crop was first introduced in the Americas via Martinique (French West Indies) in the 18th century, from where it spread to the rest of the Caribbean and then to Central America (Mauro, 1991). Indeed *M. paranaensis* was recently detected in a primary forest in Martinique (Quénéhervé and Van den Berg, 2009; Quénéhervé, 2009; Quénéhervé *et al.*, 2011). Coffee was introduced into Guatemala in the middle of the 18th century from the Antilles, but it is not known whether it was in the form of seeds or of young coffee trees (Wagner, 2001). As far as Hawaii is concerned, coffee is reported to have been introduced on several occasions. The first successful introduction of coffee was made in 1825 with 30 live coffee plants from Rio de Janeiro state, Brazil (Goto, 1982), but no *M. paranaensis* has been detected in this state up to now (Campos and Villain, 2005; Ferraz, 2008). Coffee germplasm was imported from Guatemala in 1892 but it seems to have been seeds (Goto, 1982). It would be interesting to monitor the presence (or absence) of *M. paranaensis* in the primary forests close to coffee growing areas in countries where this RKN species has already been detected. The fact that a plant nematode is native or introduced has major implications for recommendation of control.

Meloidogyne arabicida (Est AR2)

The presence of *M. arabicida* which causes similar corky root symptoms to those caused by *M. paranaensis* was confirmed in Turrialba valley in Costa Rica where it was originally described (López and Salazar, 1989b). Additionally, a generalized infestation of a 15-year-old coffee plantation with typical corky root symptoms similar to those caused by *M. arabicida* was observed during this survey on a farm located in the San Isidro valley. The plants in this particular parcel came from a nursery located in Turrialba. Because of the risk due to transporting samples to other coffee growing regions and the concern the spreading of this species represents for Costa Rican coffee grower corporation, no sample was taken from this farm. Due to the high degree of pathogenicity of this RKN on *C. arabica* linked with its association with Fusarium oxysporum (Marbán-Mendoza *et al*., 1989; Bertrand *et al*., 2000) and the apparent dissemination of the nematode via nursery seedlings, local authorities should pay careful attention to the coffee planting material being transported from this region. Currently, with the exception of its detection on a few farms outside Turrialba valley that are apparently due to the introduction of nursery seedlings from Turrialba, this species appears to be limited to this region. And even within this region, *M. arabicida* has never been reported on other crops.

Meloidogyne incognita (Est I1)

This species was detected in different regions of Costa Rica. This species was observed once in the Central Valley and once at one of the two survey sites in the Guanacaste cordillera, on both coffee and *Musa* AAB trees present in two neighbouring plots. In the San Isidro valley also called Valle de El General (Perez Zeledón county), different populations sampled on coffee presented the *M. incognita* Est I1 phenotype. In contrast, *M. incognita* was not detected on coffee in the Turrialba valley. Although in Costa Rica *M. incognita* has already been identified in coffee nurseries based on the perineal pattern (Figueroa, 1988), our result is the first report of *M. incognita* on coffee in Costa Rica confirmed by esterase phenotypes. It should be noted that this species has been detected on many different crops in Costa Rica and is the main plant parasitic nematode on tobacco (Lopez, 1978) in the province of Alajuela, on tomato (Candanedo *et al*., 1988) in the province of Guanacaste, and on celery (Incer and Lopez; 1979) in the province of Alajuela. This is in agreement with our detection of *M. incognita* on coffee in the same three areas. In particular, the presence of *M. incognita* at four of the seven sites sampled in the region of El Valle de El General indicates that this species of RKN could be widespread in this important coffee growing region of Costa Rica, a region which, according to the literature, has not been yet sufficiently surveyed for nematode parasitism on coffee. It is interesting to note that on one farm in the province of Guanacaste, *M. incognita* was detected in two neighbouring plots, one planted with coffee and the other with *Musa* AAA. Cross pathogenicity tests should be carried out to assess virulence of *M. incognita* from these different hosts on coffee genotypes and vice-versa.

In Guatemala, *M. incognita* was also observed in roots of *Musa* AAB plants in a coffee field where only an Est P1 *M. paranaensis* population was detected on the coffee root samples. *M. incognita* was also detected in one sample of *C. arabica* roots in Guatemala on a farm where the other samples were all identified as *M. paranaensis*. *M. incognita* was not detected in El Salvador. Unlike our results on populations in the region of El Paraíso, Honduras (all diagnosed as *M. exigua*), in a previous survey, *M. incognita* was detected on one sample of coffee roots in the same region mixed with *M. exigua* (Pinochet and Ventura, 1980). However this particular identification should be interpreted with caution since it was based on perineal patterns. The identifications of *M. incognita* in Costa Rica and Guatemala are the first based on esterase diagnosis

on coffee in Central America. In South America, *M. incognita* is a common RKN species on coffee, especially in Brazil where it causes serious damage in several states (Campos and Villain, 2005; Ferraz, 2008), and in Colombia, where its presence appears to be more sporadic (Gaitán *et al*., 2008). In Mexico, a corky root syndrome on coffee trees, very similar to that observed in Guatemala and Brazil with *M. paranaensis* or in Costa Rica with *M. arabicida*, has been observed for many years in the state of Veracruz and has been attributed to *Meloidogyne incognita* in association with *Fusarium* and *Trichoderma* (Teliz-Ortiz *et al*., 1993; García *et al*., 1997; Marbán-Mendoza, 2009). However these identifications were based on perineal patterns so esterase and molecular analyses of this RKN population are required for a reliable identification of the species involved in this corky-root syndrome.

M. izalcoensis (Est Iz4)

This species appears to be widely distributed on coffee in the south-western Izalco volcano massif (Table 1) where it was recently described on coffee (Carneiro *et al*., 2005a). However, this species has not yet been detected in any other part of El Salvador (Villain *et al*., 2008) so its presence appears to be limited to a small area. The symptoms observed on plants infested by *M. izalcoensis* in the field look like swellings on woody roots including the tap root or on non-lignified white secondary roots, the woody one getting some times lightly corky with necrosis. All perineal patterns of these population were similar to the one described for *M. incognita.*

M. enterolobii (= M. mayaguensis) (Est EN2)

This species was detected once in Guatemala on a sample collected from a few coffee trees in the northern Verapaces region and twice in Costa Rica in one population from the San Isidro Valley (Perez Zeledón county) in association with *M. exigua* and on one population from the Guanacaste province. This latter population was discovered on just one coffee tree in an abandoned coffee plantation. Despite extensive sampling in the vicinity of the sampled infested tree, none of the surrounding coffee trees showed symptoms like those observed on this infested coffee tree: large galls and swellings without cracking of the cortex. In addition to esterase phenotypes, the identification of both populations from Guatemala and Costa Rica as *M. enterolobii* was confirmed by molecular markers: AFLP, ISSR, RAPD and SCAR (Tigano *et al*., 2010).

Hernández *et al*. (2004a) already detected the presence of *M. enterolobii*, diagnosed on isozyme (Esterases, MDH, SOD, GOT) analysis and perineal patterns, on coffee from the same farm in the northern region of Guatemala. Additionally, this species was only reported as *M. mayaguensis* on coffee in Cuba where it appears to be the most damaging RKN on coffee (Sampedro *et al*., 1989; Rodriguez *et al*., 1995). On the contrary, the population of *M. enterolobii* from Guatemala seems to be less aggressive on coffee in the field, causing very little galling on only a few coffee trees and with no symptoms on growth and fruit load. These field observations are in agreement with those of Hernández *et al.* (2004b) who did not succeed in developing a population of *M. enterolobii* from Guatemala on coffee after rearing it on tomato. The coffee orchard in which this population was collected was planted for five years after uprooting a secondary forest. Consequently, this population of *M. enterolobii* may have transferred to the coffee trees, which appears to have been a poor host for this nematode. In Costa Rica, field observations of the impact of *M. enterolobii* on coffee were not possible since only one infested tree was observed in an abandoned orchard. This population from Costa Rica was conserved and reproduced well on some *C. arabica* cultivars, unlike another population of *M. enterolobii* on guava which appeared to be a weak parasite of coffee (Muniz *et al*., 2009). Some intraspecific diversity could explain the variability of pathogenicity observed on different populations of this species. Consequently and because of the damage caused by this nematode on coffee in Cuba (Sampedro *et al*., 1989; Rodriguez *et al*., 1995), careful attention should be paid to *M. enterolobii,* which has been detected in primary forests in Martinique, French West Indies (Quénéhervé, 2009) and in Brazil (Lima *et al*., 2005). Moreover, this species appears to be a serious emerging threat for different crops like guava in Brazil (Randig *et al*., 2009; Gomes *et al*., 2011) with the ability to develop on different crop genotypes carrying resistance genes to major *Meloidogyne* species (Castagnone-Sereno, 2012).

M. arenaria (Est A1 and Est A2)

The Est A1 phenotype was observed in one population on coffee in Guatemala. Perennial patterns were not observed. The symptoms caused by this population are very severe even on *C. canephora* rootstocks: large root swellings (without corky formation) on the whole root system including the tap root up to the collar and with dramatic induction of adventitious roots. The Est A1 phenotype of *M. arenaria* was also observed on a sample of *Impatiens* sp., a very common weed in Central American coffee plantations. The sample of Impatiens came from another farm located in the same area. However, this Est A1 phenotype was not detected on the coffee root samples collected in the same field, where only *M. paranaensis* (Est P2) was found. The other esterase phenotype of *M. arenaria*, Est A2, was observed once in Guatemala on one sample of *C. canephora* rootstock roots where the other samples from coffee roots were all identified as *M. paranaensis* (Est P1). The Est A1 phenotype, which is considered by Carneiro *et al.* (2008) to be an enzymatic variant of *M. arenaria*, appears to be much less common than

the Est A2 phenotype (Esbenshade and Triantaphyllou, 1985; Cofcewicz *et al*., 2005, Carneiro *et al*., 2008). To our knowledge, this observation in a population in Guatemala is the first report of the Est A1 phenotype on coffee. Careful attention should be paid to this population because of the impressive root symptoms observed in the field. Since perineal patterns were not observed, this population is currently undergoing genetic and morphologic studies to confirm its taxonomic status.

The Est A2 phenotype was observed in Guatemala on two samples of *C. canephora* rootstock roots from one coffee plot from where four other root samples carried Est P1 *M. paranaensis* populations. This same Est A2 phenotype of *M. arenaria* was also detected in one sample from the region of Izalco volcano massif in El Salvador. For this population, perineal patterns were in accordance to those described for *M. arenaria*. The Est A2 phenotype was previously observed on coffee in El Salvador but in the south-eastern province of Usulután (Carneiro *et al*., 2004; Hernández *et al*., 2004a). So although infrequent, this phenotype was nevertheless observed in three distant coffee growing regions on the pacific slope of the Sierra Madre in Guatemala and El Salvador.

More generally, *M. arenaria* appears to be rare on coffee in Central America and probably elsewhere in the world, as it has only been reported in Cuba (Rodriguez *et al*., 1995) and Jamaica (Whitehead, 1968). However, attention should be paid to this species due to the high reproductive fitness observed on *C. arabica* cv. Catuai and cv. Sarchimor of an Est A2 phenotype population collected on coffee in the eastern region of El Salvador (Hernández *et al*., 2004b).

Meloidogyne morocciensis (Rm: 1.10, 1.20, 1.30)

The three band esterase phenotype commonly named as Est A3 since it was previously considered to be specific to *M. arenaria* (Esbenshade and Triantaphyllou, 1985), was observed on two samples of coffee roots from El Salvador. However, this Est A3 phenotype was observed in a new species, *M. morocciensis*, described on peach rootstock (*Prunus persica* cv. Missouri) in Morocco (Rammah and Hirschmann, 1990). More recently, a study of the diversity of *M. arenaria* including morphological, morphometrical features, four enzymatic phenotypes, chromosome number and molecular clustering (RAPD and ISSR) pointed to the need to review the taxon previously recognized as *M. arenaria*, and more specifically suggested that the Est A3 phenotype combined with a malate deshydrogenase N1 phenotype should instead be attributed to *M. morocciensis* (Carneiro *et al*., 2008). These two populations from El Salvador were then identified as such. Like in our study, the populations with an Est A3 phenotype presented perineal patterns characteristic of *M. arenaria* but these authors underlined the highly variability of this feature among putative *M. arenaria* populations and considered it not to be useful for the identification of this species. Additional studies on these populations with an Est A3 phenotype collected on coffee in El Salvador should be conducted including other isozyme phenotypes particularly malate deshydrogenase, chromosome number and molecular marker analysis to confirm the taxonomical status of these populations. These data contribute in indicating that *M. morocciensis* could be a cosmopolitan species and that all putative *M. arenaria* with an Est A3 phenotype collected over the world should be reviewed.

Meloidogyne hapla (Est H1)

This RKN more adapted to temperate climates, was observed in one sample from Guatemala, from the same cool highland region from where *M. enterolobii* was detected. *Meloidogyne hapla* was also observed in El Salvador on three farms located at high altitudes [850, 1030 and 1520 meters above sea level (m.s.l.)]. Perineal patterns of these populations were in accordance to the type pattern of *M. hapla*. Such as in Guatemala, the symptoms observed in the field were the same as those reported by Lordello (1982) in Brazil: large spherical galls with occasional induction of adventitious roots. This species was previously detected once on coffee in the same region in Guatemala (Hernández *et al.*, 2004a). *Meloidogyne hapla* appears to be a very occasional parasite of coffee in Central America and only at higher altitudes, as is the case throughout the world with reports of its presence in Brazil and Tanzania, but with very little associated damage (Lordello, 1982; Bridge, 1984), and more recently in Hawaii where on the contrary significant damage was reported (Handoo *et al.*, 2005). In Costa Rica, although *M. hapla* has not been yet detected on coffee, this RKN is reported to be widespread on vegetables in the highlands of the central volcanic range (López and Salazar, 1978).

General considerations on RKN geographical distribution, origin and identification in Central America

Although the relation between RKN species and altitude was not an aim of this work, some trends are suggested for the main species observed at higher frequencies (Table 1). *M. exigua*, *M. paranaensis, M. incognita* and *M. izalcoensis* appear to be present at a wide range of altitudes (600 to 1650; 820 to 1200; 600 to 1260 and 430 to 1100 m.s.l., respectively) covering most of the range of altitudes at which *C. arabica* grows in Central America (600 to 1400 m.s.l.). Logically, *M. hapla*, a species linked to temperate climates, was observed more frequently at higher altitudes (1030, 1200 and 1400 m.s.l.) with one population found at a lower altitude (850 m.s.l.). *M. enterolobii* was present only in three samples but the fact that this species was detected at 600, 750 and 1200 m.s.l., i.e. at a wide range of altitudes, is in agreement with the apparently

cosmopolitan distribution of this species -including reports of the junior synonym species *M. mayaguensis*- in both tropical and temperate regions. For the other species, not enough data were available to allow us to draw any conclusions concerning their altitude preferences. In Guatemala, no populations of RKN were detected in the exploratory samples collected from the south-eastern (Santa Rosa), eastern (Chiquimula and Zacapa) and the western (Huehuetenango) regions. The absence of RKN in the13 samples from these three regions is in agreement with the very few reports of *Meloidogyne* spp. on coffee from these regions by the diagnostic laboratory of the National Association of Coffee, Anacafé. Moreover, when RKN was observed, it was always with very low population levels (unpublished data). This could be related to the nature of the clay soils in this region in contrast to the Andosols in the south-western region along the Sierra Madre Cordillera, where the majority of RKN parasitism on coffee was observed (Anonymous, 1984).

Wide interspecific diversity of RKN was observed on coffee in Central America but it is difficult to determine at this point if it is indigenous and/or if it results from introductions. As part of Mesoamerica, Central America is one of the major biodiversity hotspots (Myers *et al.* 2000) and is recognized to be an important biological corridor where many flora and fauna species from both North and South America converged (Raven and Axelrod, 1974; Sarkar *et al.*, 2009).

Meloidogyne exigua appears to be endemic to the American continent, the most widely distributed species on coffee throughout the continent (Campos and Villain, 2005) and very dependent on coffee outside the primary forests where it has been found, despite the fact *C. arabica* was introduced into America since it originated in the Ethiopian highlands (Anthony *et al.*, 2002). In parallel, it's worth taking into account that this meiotic parthenogenetic species is, according to phylogeny studies, the more closed to ancestral amphimictic RKN (Castagnone-Sereno, 2006). In contrast, other species like *M. paranaensis*, appear to have a very fragmented range of distribution probably related to human activities and more particularly to the spread of the coffee crop and eventually other crops with successive germplasm exchanges starting in the Caribbean and continuing in Central America. Other species like *M. izalcoensis* or *M. arabicida* appear to have a remarkably limited distribution area even though they are highly aggressive on most *C. arabica* cultivars. This may be due to a process of evolution and specialisation facilitated by the extremely mountainous relief of Central America (as has been argued for Globodera speciation in the Andes mountains) by opportunistic parasitizing of new host groups caused by orogeny (Grenier *et al*., 2010). Indeed, the diversity of RKN parasitizing coffee, which is a recent crop in Central America and was mainly established in natural forest areas in the highlands, could reflect the original diversity of RKN throughout this mountainous tropical region.

To determine the indigenous versus introduced status of the RKN species parasitizing coffee trees, more representative surveys are required of all coffee producing regions in Central America including preserved natural areas, particularly those bordering coffee growing areas representing different types of phytocenoses from tropical dry forest to rainy forest. This would improve our understanding of the actual distribution of the different RKN on coffee in Latin America with implications in terms of quarantine and preventive measures, such as the use of forest soils for coffee nurseries, since they are frequently believed by farmers to be free of economically important plant parasitic nematodes. More globally for tropical crops, this information could help forecast hazards linked to dissemination of known emerging RKN species such as *M. paranaensis* or *M. enterolobii* (Quénéhervé, 2009). Species-specific SCAR markers have been developed for the three major RKN that parasitize coffee in Latin America, *M. incognita, M. exigua* and *M. paranaensis* (Randig *et al*., 2002) as well as for *M. hapla* (Zijlstra, 2000) and more recently for M. enterolobii (Tigano *et al*., 2010). On the other hand, the SCAR markers developed for *M. arenaria* (Zijlstra *et al*., 2000) appear to be unreliable for routine diagnosis and taxonomic entities currently recognised as "*M. arenaria*" should thus be reviewed (Carneiro *et al*., 2008). However in many cases, SCAR markers would be valuable tools for more exhaustive surveys and their use in Multiplex PCR amplifications would also allow much easier detection of the presence of species mixtures even in populations present at very low densities because of the high sensitivity of this analysis based on PCR (Randig *et al*., 2002; Carneiro *et al*., 2005b). This technique also makes it possible to work directly on populations present in the roots of coffee trees rather than on bred-on-tomato populations originating from a small number of collected egg masses, which can cause biases, as mentioned above. However isoenzyme analysis and particularly esterase phenotype analysis continue to be valuable ways of detecting unknown taxa as was the case of the recent description of *M. izalcoensis* in El Salvador (Carneiro *et al*., 2005a). Additionally, the pathogenicity on coffee and the geographic range still need to be determined for some RKN only sporadically reported on coffee in this region, such as *M. enterolobii* (= *M. mayaguensis*), *M. incognita* or *M. arenaria* and probably *M. morocciensis*. The interspecific diversity of RKN species parasitizing coffee in Central America requires broad-range screening of species to identify sources of resistance with a broad spectrum of resistance in wild *Coffea* spp. germplasm. The time-consuming and labour-intensive pathogenic characterization and screening for resistance in coffee germplasm can be facilitated by high through-put methods (Villain *et al*. 2010).

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