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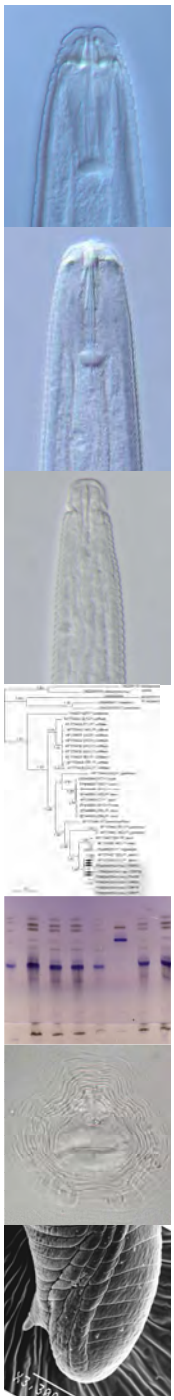

Biodiversity of phytoparasitic nematodes associated with Musaceae and fruit crops in Colombia

Biodiversidad de nematodos fitoparásitos asociados con Musaceae y cultivos frutales en Colombia

Gladis Emilia Múnera Uribe

Promoter: Prof. Dr. Wilfrida Decraemer
Copromoter: Dr. Wim Bert

Academic year 2007-2008

Thesis submitted in fulfillment of the requirements for the degree of Doctor in Science (Biology)

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Dedicatory

Elisa, only you knew and shared all stone of this slope.

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General Introduction

General Introduction

Colombia is located on the Northwestern corner of South America between the Pacific and Atlantic Oceans. It is the 26th largest nation in the world, comprises 115 million hectares (1,138,910 sq km), 5 percent of which are under cultivation (pastures not included). Colombia is predominantly an agrarian country and apart from few exceptions it is self-sufficient. Approximately 80% of people live in urban areas, most of them situated at Andes Region.

Colombian topography is characterized by the Andean Cordillera range (30% of country area), situated in the West- central part of the country, and stretches from South to North almost along the whole of the country. Between mountains, there are high plateaus and fertile valleys which are crossed by the major river system. The country is 100% tropical, situated between 4° to 12° at South and North of Equator line, respectively. Altitude ranges between 0 to 5.700 meters above sea level which combined with other climatic factors (i.e. all year long the rainy season alternates with the dry season) allows/provides all climate conditions for the production of a wide range of both tropical and temperate-zone crops and several harvests a year are obtained. Variety of crops for both export and domestic consumption are produced.

Coffee, cut flowers and banana productions range between second to third largest on the world. Other important crops yielded are plantain, sugarcane, tobacco, cotton, rice, sorghum, soybean, maize, palm oil, cocoa, bean, cassava, potatoes, grapes, vegetables, citrus fruits and native fruits. Dietary requirements also include numerous types of indigenous fruits. Domestic markets consume the bulk of the production of all crops mentioned above (exceptions the three first) but some of them are also exported.

Agriculture production is affected by many factors. Although plant–parasitic nematodes are often not as important as some other biotic and non-biotic constrains on crop production in the tropics, they can nevertheless cause extensive damage and substantial yield losses (Sasser & Freckman, 1987). Prevention or alleviation of these losses would contribute to increase food production and cash income (De Waele & Elsen, 2007).

Data about nematode studies in Colombia mentioned in this section and in chapter 3 only include taxa and crops related to this thesis. However, a lot of papers on other nematode taxa (plant-parasitic as e.g. *Xiphinema* –Siddiqi & Lenne, 1990- and entomopathogenic nematode descriptions) have been published; most of them in Spanish.

Colombian nematological research has been focused mainly on root-knot nematodes or the genus *Meloidogyne*. About 50 percent of the Colombian nematode literature has been produced by means of thesis research at level of bachelor. Topics most frequently dealt with are: identification to genus level of nematodes associated with different crops (mainly coffee, fruit crops, banana, cut flowers, potatoes, tobacco, bean, sorghum, soybean and vegetables), genetic resistance, chemical and biological control, yield losses, interaction with other organisms (Muñoz *et al.*, 2003).

Taxonomical studies are scanty, most of them deal with the genus *Meloidogyne*. Hereby, the perineal pattern and host differential tests are the methods more widely used for nematode identification (Corrales, 1996; Gaviria & Navarro, 2003; Lozada *et al.*, 2002; Múnera, 2002c, 2003a; Múnera & Navarro, 2000a, 2002; Múnera & Saldarriaga, 2003; Vargas *et al.*, 2002). Morphometric data, with a few exceptions are not recorded. Scanning electron microscope, protein analysis, specific primers and phylogenetic methods were not yet used. Molecular studies on *Meloidogyne* were rarely conducted (Vanegas, 2006).

However, plant-parasitic nematodes from Colombia have been included in two pioneer contributions, i.e. an extensive study on *Meloidogyne* (using enzyme phenotypes for identification of species) and one on the genus *Radopholus* (revision of the genus *Radopholus*) (resp. Esbenshade & Triantaphyllou, 1985c; Sher, 1968b). Additionally, one *Pratylenchus* species, *P. neobrachyurus,* was described by Siddiqi from the grass *Andropogon guyanus* at Carimagua- Casanare, east of Colombia (Siddiqi, 1994).

Four major fruit crops are grown in temperate climate in Colombia, *Solanum quitoense* Lam., *Cyphomandra betacea* (Cav.) Sendt. (syn. *Solanum betaceum* Cav.), *Passiflora ligularis* Juss., *Rubus glaucus* Benth. and plantain (*Musa* AAB) are important

both for export and domestic market, not only for the nutritive value but also as income and employment in Colombia. Studies on nematode biodiversity focusing at species level are needed since wide distributions of plant-parasitic nematodes have been recorded in these crops, resulting in significant economic losses (see chapter 2).

Biodiversity concepts considered here are those defined at the summit of the United Nations Earth in Rio de Janeiro, 1992: “Biodiversity is the totality of genes, species and ecosystems in a region. Biodiversity can be divided into three hierarchical categories -genes, species, and ecosystems- that describe quite different aspects of living systems and that scientists measure in different ways”.

According to Noss (1990): "Biodiversity is not simply the number of genes, species, ecosystems, or any other group of things in a defined area". A definition of biodiversity that is altogether simple, comprehensive, and fully operational (i.e. responsive to real- life management and regulatory questions) is unlikely to be found. More useful than a definition, perhaps, would be a characterization of biodiversity that identifies the major components at several levels of organization. Composition, structure, and function determine, and in fact constitute, the biodiversity of an area. Composition has to do with the identity and variety of elements in a collection, and includes species lists and measures of species diversity and genetic diversity. Structure is the physical organization or pattern of a system, from habitat complexity as measured within communities to the pattern of patches and other elements at a landscape scale. Function involves ecological and evolutionary processes, including gene flow, disturbances, and nutrient cycling”. Systematics assesses biodiversity using the species as unit.

Based upon former concepts, this thesis focuses mainly on two composition components (species and genes) of biodiversity and as contribution to know the biodiversity of the nematofauna in Colombia. Because of its location and variety of ecosystems, Colombia ranks among the world’s top five countries for biodiversity. Despite of the lack of complete inventories of flora and fauna, the country ranks first in species of birds and amphibians, second in vascular plants, and third in mammals worldwide; in average, one out of ten species of flora and fauna occurs in Colombia

(Alexander von Humboldt Institute, 2008*). Studies on biodiversity represent a long-term multidisciplinary task.

Thus, the aim of this research is to study the biodiversity of plant parasitic nematodes associated with plantain and fruit crops in Colombia, to acquire the knowledge and skills for the application of different identification methods, to interpret the results based on a multidisciplinary approach in order to provide an accurate identification and understand relationships between species. Previous studies on nematodes in Colombia (see chapter two) show that *Meloidogyne, Pratylenchus* and *Radopholus* are the most important taxa (more prevalent and abundant) of plant parasite nematodes in Colombia and therefore have been selected for this study.

Specific objectives are:

To characterize morphologically, morphometrically and molecularly Colombian *Meloidogyne* populations associated to five crops: *Solanum quitoense* Lam., *Cyphomandra betacea* (Cav.) Sendt. , *Passiflora ligularis* Juss., *Rubus glaucus* Benth. and plantain.

To characterize morphologically, morphometrically and molecularly Colombian *Pratylenchus* populations associated to plantain.

To characterize morphologically, morphometrically and molecularly Colombian *Radopholus* populations associated to plantain.

* http://www.humboldt.org.co/ingles/en-biodiversidad.htm

Chapter 2

Previous studies on nematodes associated with fruit crops in Colombia

Introduction

The four major fruit crops grown in temperate climate (50.000 has.) in Colombia are *Solanum quitoense* Lam. (lulo or naranjilla); *Cyphomandra betacea* (Cav.) Sendt. (syn. *Solanum betaceum* Cav.) (tomate de árbol, tamarillo or tree tomato); *Passiflora ligularis* Juss. (granadilla) and *Rubus glaucus* Benth. (mora). Another important crop is plantain (*Musa* ABB), cultivated on 400.000 hectares, with different cultivars (hartón, dominico, and dominico hartón) grown at different altitudes. These five crops are important both for export and domestic market, not only for the nutritive value but also as income and employment in Colombia.

Production of those fruit crops is affected by many factors. Although plant parasitic nematodes are often considered economically less important than some other biotic and non-biotic constraints on crop production, they can nevertheless cause extensive damage and substantial yield losses. Colombian nematological research has been focused mainly on root-knot nematodes. *Meloidogyne* is a cosmopolitan pest genus and a lot of Colombian phytopathologists participated in the largest nematological research project, International *Meloidogyne* Project led by the North Carolina University (USA).

Studies on nematodes associated with the four fruits mentioned above and target in this thesis have been carried out by different institutions, mainly official institutions responsible for national agricultural research, ICA and CORPOICA and through theses at bachelor level at different universities. A recent project "Integrated Management Pest for the improvement of the sustainable production of fruits in the Andean Zone"[1] represented the first inter-institutional effort for the study of nematodes associated with *S. quitoense*, *C. betacea* and *P. ligularis* from different regions in Colombia. Moreover, most of the nematodes included in this thesis come from fields and in vivo collections obtained during this project. Some results presented here have not been published yet. The

1 Project coordinated by the Instituto Interamericano de Cooperación para la Agricultura IICA and sponsored by Fondo Regional de Tecnología Agropecuaria -FONTAGRO and some government and private institutions of Colombia (CORPOICA), Ecuador (INIAP) and Venezuela (INIA).

purpose of this chapter is to give a general overview of previous nematological research associated with the fruit crops dealt with in this thesis. Therefore, materials and methods, some results and discussion are not treated in detail here.

Nematological research on S. quitoense, C. betacea, P. ligularis *and* R. glaucus

Grey literature associated with fruit crops

Grey literature produced in Colombia and associated with *S. quitoense*, *C. betacea*, *P. ligularis* and *R. glaucus* was collected. From 436 references (collected until year 2002), 280 dealt with pests associated with the fruit crops. The main problems addressed were *Colletotrichum* sp. and *Meloidogyne* spp. associated with *C. betacea* and *S. quitoense*, respectively. Topics on nematodes most frequently dealt with were: identification to genus level of nematodes associated with those fruit crops, genetic resistance, chemical and biological control, yield losses and interaction with other organisms. Most studies are on genetic resistance as strategy for control (Fig. 2.1) (Muñoz *et al*., 2003).

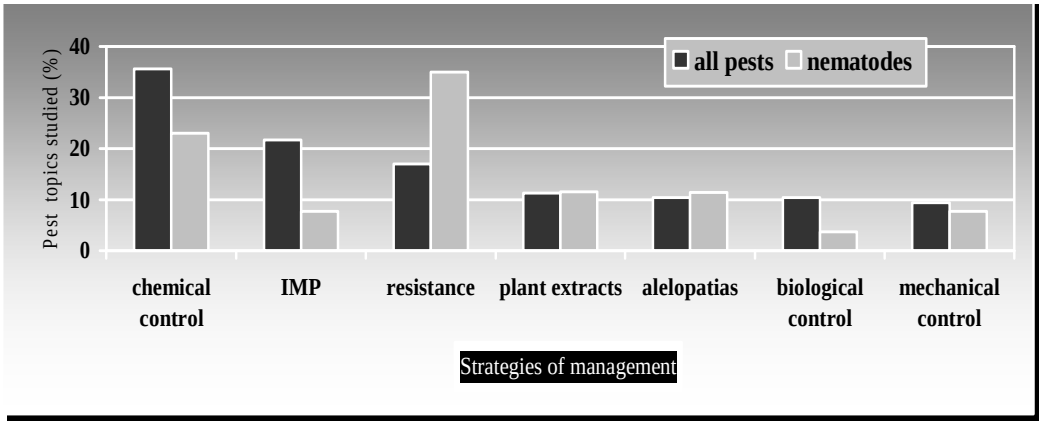


Fig. 2.1 Management strategies of pest problems associated with Colombian fruit crops based on grey literature.

Characterization of production system

A thousand farms from different Colombia regions were included in a study about characterization of production systems of *S. quitoense*, *C. betacea* and *P. ligularis*. A

questionnaire to obtain information on biophysical and technological data, knowledge of farmers about nematode damage and pests in general was distributed and responses analyzed as a first step in building a "base-line"[2] for nematological research. It showed that *C. betacea* had a more diverse production system; *P. ligularis* the most homogeneous system with highest technology and *S. quitoense* showed a homogenous system with the lowest technology. In *C. betacea* and *S. quitoense* the monoculture was predominant. Data about seeds, prunes, fertilizers, spatial and temporal distribution of crops, pesticides, pest control and other agronomical practices, were obtained (Table 2.1, 2.2; Fig. 2.2) (Díaz & Múnera, 2002, 2003).

Table 2.1 Biophysical characteristics of the production systems of *Cyphomandra betacea*, *Rubus glaucus*, *Solanum quitoense* and *Passiflora ligularis* in Colombia.

Characteristics	***C. betacea***	***R. glaucus***	***S. quitoense***	***P. ligularis***
altitude (meters)	1600 a 2600	1600 a 2600	1400 a 2400	1800 a 2400
temperature (ºC)	14 a 18	16 a 18	16 a 24	15 a 18
rainfall (mm/year)	1000 a 2850	1000 a 2650	1000 a 3000	2200 a 3200
relative humidity (%)	75 a 85			80 a 90
area (has)	7500	5500	4500	3500
zone with largest area of fruit crops	Antioquia	Cundinamarca	Huila	Viejo Caldas
zone with highest yield (t/ha/year)	Norte Antioquia	Cundinamarca	Cundinamarca	Caldas
yield (ton/ha/year)	20	8	7.8	12

Plant parasitic nematodes associated with Colombian fruit crops in field

Soil and root samples were collected from 1000 fields in 9 states belonging to various agro-ecological regions. Twelve genera of phytoparasitic nematodes were found associated with the rhizosphere of *S. quitoense*, *C. betacea* and *P. ligularis* in different regions of Colombia. The genus *Meloidogyne* was both most prevalent and abundant in all the zones sampled. *M. incognita, M. javanica*, *M. hapla* and *M. arenaria* were, identified in the rhizosphere of *S. quitoense* and *C. betacea,* whereas *M. incognita M. javanica* and *M. hapla* were found in the rhizosphere of *P. ligularis*. In general

2 basic knowledge on production systems of those fruit crops.

Fig. 2.2 *Solanum quitoense*, *Cyphomandra betacea* and *Passiflora ligularis* grown in field as polyculture with other fruit crops: *C. betacea* X *Rubus glaucus* (1), *C. betacea* X *S. quitoense* (2), *C. betacea* X bean (3), *C. betacea* X cauliflower (4), *C. betacea* X *Arracacia xanthorriza* (5), *S. quitoense* X coffee (6); *P. ligularis* monoculture using high technology (7); *S. quitoense* monoculture using low technology (8).

Table 2.2 Percentage of mixed, surrounding and previous crops associated with *Cyphomandra betacea*, *Passiflora ligularis* and *Solanum quitoense* in Colombia.

	C. betacea			***P. ligularis***			***S. quitoense***		
	Mixed crops	Surrounding crops	Previous crops	Mixed crops	Surrounding crops	Previous crops	Mixed crops	Surrounding crops	Previous crops
C. betacea	-	-	8	14	12	3	14	16	3
P. ligularis	9	6	1	-	-	4	-	3	1
S. quitoense	10	6	1	12	6	2	-	-	3
R. glaucus	15	11	3	8	8	2	10	10	3
coffee	7	4	3	29	15	20	17	8	11
grasses	-	8	26	5	6	18	4	10	20
bean	7	11	13	5	14	18	7	10	11
Pisum sativum	3	6	6	5	5	4	7	10	10
maize	7	4	6	3	5	5	10	4	6
plantain	2	2	-	5	5	4	7	10	-
Arracacia xanthorriza	4	3	2	5	5	4	-	1	7
stubble		1	11		-	2		1	15
potato	5	3	8	-	3	5	-	2	3
others	31	35	12	9	16	9	24	15	7

phytoparasitic nematode populations associated with those crops were composed mainly of *Meloidogyne, Pratylenchus* and *Helicotylenchus* genera (70%). *Scutellonema* was found associated with plants of the genus *Passiflora* in high population in one locality (Table 2.3) (Corrales, 1996; Lozada. *et al*., 2002; Múnera, 2000, 2002a, 2002c, 2003a, 2004a; Múnera & Navarro, 2000a, 2002; Navarro, 1986, 1990; Vargas *et al*., 2002; Varón, 1994; Volcy, 1998).

Subsequently to field studies, pot experiment on nematodes associated with *R. glaucus* and parasitism tests were conduced. Results showed that *Trichodorus sp.*[3], *Hemicycliophora* sp. and *Pratylenchus* sp. reproduce on *R. glaucus* plants. *M. incognita*, and *M. javanica*, *Helicotylenchus* sp. and *Paratylenchus* sp. do not reproduce on *R. glaucus* plants (Table 2.3) (Múnera & Navarro, 2000b; Navarro & Múnera, 2000).

[3] One trichodorid nematode was identified as *Trichodorus* sp. but its taxonomical status is not clear yet.

Table 2.3 Populations of plant parasitic nematodes associated with *Solanum quitoense*, C*yphomandra betacea*, *Passiflora ligularis* and *Rubus glaucus* from different Colombian regions.

Género	*S. quitoense*		*C. betacea*		*P. ligularis*		*R. glaucus*	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Meloidogyne	4679 53-27100	429 11-3444	500 49-6378	83 1-496	77 53-7868	23 11-255	99 38-200	-
Helicotylenchus	27 49-811	19 3-96	95 46-5789	16 2-76	99 53-2492	13 3-58	217 71-460	-
Pratylenchus	20 22-200	6 2-44	5 22-103	9 7-32	58 53-240	14 2-62	85 59-113	11 1-36
Trichodorus	63 22-182	-	63 46-124	-	65 53-280	-	83 40-112	-
Xiphinema	-	-	52 7-58	-	63 21-343	-	-	-
Paratylenchus	-	-	53	-	64 53-159	-	7 7-27	-
Tylenchorhynchus	-	-	26	-	50 7-100	7 1-19	-	-
Scutellonema	-	-	-	-	256 53-4641	7 3-66	-	-
Criconemella	7 7-10	-	-	-	47 7-52	-	-	-
Hemicycliophora	-	-	54 51-341	-	-	-	46 7-85	-
Macroposthonia	-	-	87 7-124	-	-	-		-
Rotylenchulus	-	-	-	-	-	8	-	-

* S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively. Data in form: mean and below range (minimum-maximum).

Meloidogyne species associated with Colombian fruit crops in field

Samples of roots of *S. quitoense, C. betacea* and *P. ligularis* were collected from 9 Colombian states for identification of the associated *Meloidogyne* species. The highest number of samples was collected at Antioquia state. *Meloidogyne* identification was based on perineal pattern of females. From 319 samples, 233 showed presence of root-knot nematode (egg mass and adult females). Most of the samples come from *C. betacea* (59%) followed by *S. quitoense* (26%) and *P. ligularis* (13%). (Table 2.4). (Gaviria & Navarro, 2003).

Four *Meloidogyne* species were clearly identified: *M. incognita, M. javanica, M. hapla* and *M. arenaria*, with the first three species being predominant at different regions. Additionally, perineal patterns close to those described for *M. exigua* were

Table 2.4 Species of *Meloidogyne* associated with *Solanum quitoense*, C*yphomandra betacea* and *Passiflora ligularis* at different Colombia regions (identified based on perineal pattern of adult females) (adapted from Gaviria & Navarro, 2003).

State	**samples collected (%)**	***S. quitoense*** (59%)	***C. betacea*** (26%)	***P. ligularis*** (13%)
Antioquia	59	*Mi, Mj, Mh, Ma**	*Mi, Mj, Mh, Ma*	*Mi, Mj, Mh*
Caldas	15	*Mi, Me**	-	*Mi*
Cundinamarca	10	*Mi, Mj, Mh, Ma*	*Mi, Mj, Mh, Ma*	*Mi*
Cauca	3	*Mi, Mj, Ma*	-	-
Quindío	3	*Mj*	*Mi, Mj*	*Mj*
Risaralda	3	*Mi, Me**	*Mi*	*Mi, Mj*
Tolima	1	*Mi*	*Mi, Mh, Ma**	-

Mi: M. incognita; Mj: M. javanica; Mh: M. hapla; Ma: *M. arenaria* and *Me: M. exigua*; * doubtful pattern.

obtained from females of root knot nematode associated with *C. betacea* from two localities. Because some perineal pattern showed features that did not match with a specific-species group of *Meloidogyne*, they could not be identified. Mixed species populations were frequently observed, i.e. *C. betacea* showed mixed species, thus *M. incognita* and *M. javanica* (4%); *M. incognita* and *M. hapla* (3%); *M. javanica* and *M. hapla* (2%). (Table 2.5).

Table 2.5 Percentage of *Meloidogyne* species associated with *Solanum quitoense*, C*yphomandra betacea* and *Passiflora ligularis* in Colombia (adapted from Gaviria & Navarro, 2003).

Fruit crop	***M. incognita***	***M. javanica***	***M. hapla***	***M. arenaria***	***M. exigua***	**undefined**
S. quitoense	28	25	33	3	3	15
C. betacea	42	21	28	4	-	19
P. ligularis	46	8	46	-	-	-

Nematodes associated with germplasm of Colombian fruit crops

Cyphomandra germplasm

Associated with the rhizosphere of *Cyphomandra* spp. (in situ- germoplasm collection at Research Center La Selva - CORPOICA) we found five and three genera of phytoparasitic nematodes on resp. soil and roots. The genus *Meloidogyne* was both most prevalent and abundant. The root knot nematode was found in 70% of the samples. The

species identified based on perineal pattern of females were *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria.* All *C. betacea* accessions showed galls on roots, second juvenile stage were present on soil but were not present on roots except one *C. materna* accession which did not show galls nor nematodes (Table 2.6 and 2.7). Nematode populations varied depending on sampling period during the year (Múnera, 2002b).

Table 2.6 Number of *Cyphomandra* accessions associated with five genera of plant parasitic nematodes.

Cyphomandra species	Genera of nematodes								
	A	Meloi		Heli		Praty		Tricho*	Hemic
		S	R	S	R	S	R	S	S
C. betacea	42	42	28	26	15	1	2	4	2
C. amotapensis	1	1	1	1	10	-	-	-	-
C. corymbiflora	1	1	1	1	-	-	-	-	-
C. diversifolia	1	1	1	1	-	-	-	-	-
C. hartwegii	4	2	4	3	3	-	-	-	-
C. materna	2	1	1	1	1	-	-	-	-
C. uniloba	2	-	2	2	1	-	-	-	-

A: Total number of *Cyphomandra* accessions; g: presence of galls on root; S and R: presence of nematodes respectively in soil and roots. * One trichodorid nematode was identified as *Trichodorus* sp. but its taxonomical status is not clear yet. *Meloidogyne* (Meloi), *Helicotylenchus* (Heli), *Pratylenchus* (Praty), *Trichodorus* (Trico), *Hemicycliophora* (Hemic).

Table 2.7 Population of nematodes associates with seven species of *Cyphomandra*.

Cyphomandra species	Genera of nematodes							
	Meloi		Heli		Praty		Tricho*	Hemic
	S	R	S	R	S	R	S	S
C. betacea	228 49-1946	28 1-279	294 46-5789	24 2-76	103	20 7-32	52 46-54	196 51-341
C. amotapensis	108	70 22-118	541	-	-	-	-	-
C. corymbiflora	162	95 75-114	162	-	-	-	-	-
C. diversifolia	551	59 2-116	114	-	-	-	-	-
C. hartwegii	240 114-576	20 2-82	2044 51-5789	16 3-26	-	-	-	-
C. materna	446	17 3-31	1014	-	-	-	-	-
C. uniloba	-	126 47-261	132 110-154	-	-	-	-	-

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively. * One trichodorid nematode was identified as *Trichodorus* sp. but taxonomical status is not clear yet. Data in form: mean and below range (minimum-maximum). *Meloidogyne* (Meloi), *Helicotylenchus* (Heli), *Pratylenchus* (Praty), *Trichodorus* (Trico), *Hemicycliophora* (Hemic).

Passiflora germplasm

Associated with the rhizosphere of *Passiflora* spp. (in situ- germplasm collection composed of 61 accessions divided over 21 *Passiflora* species at Research Center La Selva – CORPOICA) seven and five genera of phytoparasitic nematodes were found in soil and roots, respectively. The genera *Helicotylenchus*, *Scutellonema* and *Meloidogyne* were most prevalent and abundant. 25% of *Passiflora ligularis* and *P. edulis* accessions were susceptible to *M. incognita* which was confirmed by the presence of all developmental stages of this root knot nematode. Variation in susceptibility to *Meloidogyne* was observed within and between *Passiflora* species (Table 2.8 and 2.9) (Múnera & Navarro, 2001b).

Solanum (lulo) germplasm

ICA-CORPOICA produced a plant of *Solanum* ("lulo La Selva") resistant to *Meloidoyne* spp. by crossing of *S. quitoense* (commercial species with desirable agronomical features) and *S. hirtum* (a wild species resistant to *Meloidogyne* spp. but with some non desirable agronomic features). However in the field some galls were observed in clones of the new material. The three clones that composed the new material were evaluated on their response to inoculation with 5000 eggs of three different *Meloidogyne* species.

Plant development parameters and nematode population, reproduction factor and galls index are shown in Table 2.10. All clones showed absence of *M. incognita* which corroborated resistance to this species of nematode. However, the three clones showed low nematode populations, in general, except for clone1 which showed the highest population of *M. hapla*. Plants inoculated with *M. hapla* showed best development but at the same time, this nematode species was unique showing final population on soil. Thus, no correlations between development parameter of plants and nematode populations were found (Múnera, 2003c).

Yield losses in field caused by nematodes

Fruit orchards infected with high populations of *Meloidogyne* spp. (*M. incognita*, *M. javanica* and *M. hapla* were found mixed) were selected to assess the effect of a

Table 2.8 Number of accessions of *Passiflora* spp. associated with seven genera of plant parasitic nematodes.

Passiflora		***Meloidogyne***		***Helicotylenchus***		***Scutellonema***		***Pratylenchus***		***Paratylenchus***		***Trichodorus****		***Xiphinema***	
species	A	S	R	S	R	S	R	S	R	S	R	S	R	S	R
P. ligularis	12	4	3	12	4	8	4	3	2	2	-	2	-	2	-
P. edulis	11	1	3	11	6	11	8	-	-	-	-	2	-	1	-
P. mollisima	5	1	3	5	4	4	1	1	-	-	-	-	-	-	-
P. maliformis	5	3	4	2	1	3	-	-	-	-	-	2	-	-	-
P. india	4	1	2	4	3	3	2	-	-	-	-	-	-	-	-
P. alnifolia	3	2	2	3	-	3	2	-	-	-	-	1	-	-	-
P. caerulea	3	1	1	3	2	3	2	2	2	-	-	-	-	-	-
P. manicata	3	2	2	3	1	-	1	-	-	-	-	1	-	1	-
P. cincinata	2	2	-	-	2	-	1	-	-	-	-	-	-	-	-
P. tripartita	2	2	1	-	2	-	2	-	-	-	-	-	-	-	-
P. alata	1	-	-	1	1	1	-	-	-	-	-	-	-		-
P. ambigua	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
P. apoda	1	-	-	1	-	1	-	-	-	-	-	1	-	1	-
P. capsularis	1	-	-	1	1	1	-	-	-	-	-	-	-	-	-
P. hanni	1	1	1	1	-	1	-	-	-	-	-	1	-	-	-
P. pinnatistipula	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-
P. quadrangularis	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
P. sanguinolenta	1	1	-	1	1	-	-	-	-	-	-	-	-	-	-
P. subpeltata	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-
P. tilaefolia	1	1	1	1	-	1	-	-	-	-	-	-	-	2	-
P. vitifolia	1	1	-	1	1	1	-	-	-	-	-	-	-	-	-
Total accesions	61	22	27	59	47	27	-	-	-	-	-	10	-	7	-
Total species	21	15	12	20	18	14	-	-	-	-	-	9	-	6	-

A: Total number of *Passiflora* accessions; S and R: presence of nematodes in soil and roots, respectively. * One Trichodoridae nematode was identified as *Trichodorus* sp. but its taxonomical status is not clear yet.

Table 2.9 Population of plant parasitic nematodes associated with 21 species of *Passiflora.*

Passiflora		***Meloidogyne***		***Helicotylenchus***		***Scutellonema***		***Pratylenchus***		***Paratylenchus***		***Trichodorus****		***Xiphinema***	
species	A	S	R	S	R	S	R	S	R	S	R	S	R	S	R
P. ligularis	12	225	71	912	14	507	9	71	30	159	-	163	-	43	-
P. edulis	11	185	31	658	8	729	23	-	-	-	-	59	-	63	-
P. mollisima	5	514	80	2067	30	342	12	53	-	-	-	-	-	-	-
P. maliformis	5	375	28	526	5	167	-	-	-	-	-	52	-	-	-
P. india	4	100	49	1014	23	154	26	-	-	-	-		-	-	-
P. alnifolia	3	1741	179	597	-	4641	45	-	-	-	-	91	-	-	-
P. caerulea	3	741	225	2492	12	1691	17	214	13	-	-	-	-	-	-
P. manicata	3	97	46	136	3	-	5	-	-	-	-	24	-	24	-
P. cincinata	2	-	-	547	-	53	-	-	-	-	-	-	-	-	-
P. tripartita	2	106	-	371	-	662	-	-		-	-	106	-	-	-
P. alata	1	-	-	216	31	162	39	-	-	-	-	-	-	-	-
P. ambigua	1	106	79	476	4	476	26	-	-	-	-	-	-	53	-
P. apoda	1	-	-	106	-	2700	66	-	-	-	-	106	-	-	-
P. capsularis	1	-	-	1059	3	476	22	-	-	-	-	-	-	-	-
P. hanni	1	212	11	265	-	476	15	-	-	-	-	53	-	-	-
P. pinnatistipula	1	-	-	1271	-	159	-	-	-	-	-	-	-	-	-
P. quadrangularis	1	476	46	-	-	-	-	-	-	-	-	-	-	-	-
P. sanguinolenta	1	618	-	185	10	680	-	-	-	-	-	-	-	-	-
P. subpeltata	1	-	-	1305	18	54	-	-	-	-	-	-	-	343	-
P. tilaefolia	1	53	26	53	5	-	5	-	-	-	-	-	-	-	-
P. vitifolia	1	53	-	371	-	318	26	-	-	-	-	-	-	-	-

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively. * One trichodorid nematode was identified as *Trichodorus* sp. but taxonomical status is not clear yet.

Table 2.10 Development and growth parameters of *Solanum* (*S. quitoense* X *S. hirtum*) plants and nematode populations, reproduction factor and root-knot index after inoculation with 5000 eggs of *Meloidogyne incognita, M. javanica* and *M. hapla*.

	Height of plants (cm.)			Diameter (cm.)			number of leaves		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
control 1	42 cd	42 cd	42 cd	0,95 ab	0,95 ab	0,95 ab	10 c	10 c	10 c
clone 1	37 d	42 cd	47 bc	0,94 ab	0,91 b	1,06 ab	11 bc	12 bc	12 bc
control 2	44 bcd	44 bcd	44 bcd	0,94 ab	0,94 ab	0,94 ab	15 a	15 a	15 a
clone 2	44 bcd	45 bcd	59 a	1,00 ab	0,96 ab	0,94 ab	13 ab	11 bc	12 bc
control 3	47 bcd	47 bcd	47 bc	0,94 ab	0,94 ab	0,94 ab	11 bc	11 bc	11 bc
clone 3	50 bcd	51 ab	52 ab	0,97 ab	0,94 ab	0,96 ab	13 ab	12 bc	12 bc

	Fresh root weight (g)			Fresh shoot weight (g.)			Dry shoot weight (g.)		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
control 1	42 ab	42 ab	42 ab	61 ab	61 ab	61 ab	14,6 bcd	14,6 bcd	14,6 bcd
clone 1	39 bc	30 c	51 ab	45 c	51 bc	64 ab	13,7 d	12,6 d	16,4 abcd
control 2	40 abc	40 abc	40 abc	66 a	66 a	66 a	16,0 abcd	16,0 abcd	16,0 abcd
clone 2	37 bc	44 ab	51 a	58 abc	59 ab	61 ab	13,9 cd	14,1 cd	16,3 abcd
control 3	48 ab	48 ab	48 ab	67 a	67 a	67 a	18,1 abc	18,1 abc	18,1 abc
clone 3	45 ab	42 ab	52 a	68 a	66 a	66 a	20,3 a	19,4 ab	18,9 ab

	root-knot index *			Total juveniles (roots plus soil)			Reproduction factor		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
control 1	0	0	0	0	0	0	0	0	0
clone 1	0	5	5	0	50	7653	0	0,01	1,53
control 2	0	0	0	0	0	0	0	0	0
clone 2	0	5	5	0	20	280	0	0,01	0,06
control 3	0	0	0	0	0	0	0	0	0
clone 3	0	5	5	0	20	239	0	0,01	0,05

Clone1: HFG/PR22, clone2: HOF+G, clone3: HO/HO585024; *Mi*, *Mj* and *Mh*: *M. incognita*, *M. javanica* and *M. hapla*, respectively. Similar letters mean no significative differences according Duncan's multiple-range test. * The arbitrary root-knot index describes extent of infection as follows: 0-5 scale: 0 = no galling, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = >100 galls per root system. Control 1, 2 and 3 are *Solanum* plants non- inoculated. Tomato plants were also inoculated for check viability of eggs used for inoculation of plants (data no showed).

granular nematicide (furadan 5G^{TM} –carbofuran-, 15g/plant) and a fumigant (telone II^{TM}, 90L/ha) on population level of nematodes and determine yield losses due to nematodes. Yield and nematodes population were calculated. Data of number of fruits (per plant, plot and treatment); mean weight of fruit (per unit, plot and treatment); yield (per plot and treatment); number of plants (per plot and treatment) were considered but those are not included here. Results are shown in figure 2.3 (Múnera, 2003b). Phytotoxicity was observed in plots of *S. quitoense* treated with fumigant. It could explain the inferior yield obtained on those plots compared with granular nematicide.

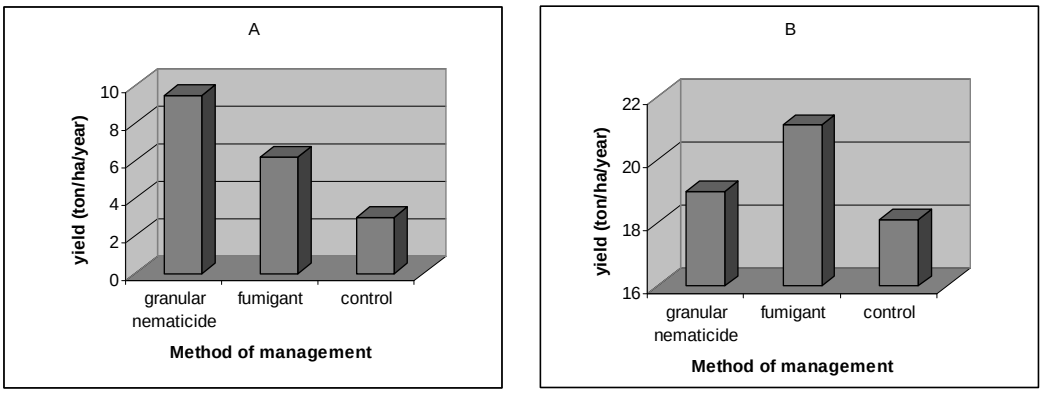


Fig. 2.3 Total yield (tons/ha/year) of *Solanum quitoense* (A) and *Cyphomandra betacea* (B) orchards after application of a granular nematicide (furadan 5G^{TM} –carbofuran-, 15g/plant) and a fumigant (telone II^{TM}, 90L/ha) for nematode control.

Meloidogyne and *Fusarium* interaction

50 plants of granadilla grown in plastic pots that contained sterile soil received the following treatments*: Fusarium solani*, *M. incognita* , *M. incognita* and *F. solani* applied simultaneously, *F. solani* applied 15 days after *M. incognita* and control. Doses of nematode and fungi applied were 5.000 eggs of *M. incognita* and 1x10^6 conidia of *F. solani*. After 120 days, the root-gall index and the reproduction factor were calculated for the nematodes and development and growth parameters for the plants. Similar experiments were conducted with *M. javanica* and *M. hapla* (Table 2.11).

In *M. incognita x F. solani* interaction treatments, the development and growth parameters of the granadilla plants were smaller compared to the control and in higher proportion when *F. solani* was applied 15 days after *M. incognita*, following by the treatments *M. incognita* and *F. solani* applied simultaneously, *M. incognita* alone and *F.*

Table 2.11 Development and growth parameters of *Passiflora ligularis* plants and nematode populations, reproduction factor and root-knot index after inoculation with *Meloidogyne incognita, M. javanica* and *M. hapla* and *Fusarium solani* in different treatments.

Treatment	Height of plants (cm.)			Diameter (cm.)			number of leaves		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
Control	33 a	33 a	33 a	0.51 a	0.51 a	0.51 a	12 a	12 a	12 a
F. solani	36 a	36 a	36 a	0.48 ab	0.48 ab	0.48 ab	11 ab	11 ab	11 ab
M	26 ab	26 ab	20 abc	0.42 abc	0.47 ab	0.42 abc	10 abc	10 abc	10 abc
M x F	13 c	28 a	13 c	0.33 c	0.49 ab	0.34 c	6 c	10 abc	6 bc
M → F	16 bc	24 ab	22 abc	0.38 bc	0.42 abc	0.45 ab	7 bc	9 abc	10 abc

	Fresh root weight (g)			Fresh shoot weight (g.)			Dry shoot weight (g.)		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
Control	5 a	5 a	5 a	23 a	23 a	23 a	5.5 a	5.5 a	5.5 a
F. solani	4 b	4 b	4 b	18 a	18 a	18 a	5.0 ab	5.0 ab	5.0 ab
M	9 ab	5 ab	5 ab	14 abc	17 a	13 abc	2.7 cd	3.7 abc	2.7 bcd
M x F	4 b	5 ab	4 b	6 c	18 a	8 bc	1.2 e	4.4 ab	1.6 de
M → F	6 ba	5 ab	7 ab	8 bc	14 ab	16 abc	1.4 de	3.4 abc	4.1 abc

	root-knot index *			Total juveniles (roots plus soil)			Reproduction factor		
	Mi	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*	*Mi*	*Mj*	*Mh*
Control	0	0	0	0	0	0	0	0	0
F. solani	0	0	0	0	0	0	0	0	0
M	5	5	5	156	124	363	0.03	0.03	0.07
M x F	5	5	5	84	11	455	0.02	0	0.09
M → F	5	5	5	93	26	153	0.02	0.01	0.03

Treatments: *F. solani* applied alone*;* M: *M. incognita* applied alone, MxF: *M. incognita* and *F. solani* applied simultaneously; M→F: *F. solani* applied 15 days after *M. incognita* and control. Doses of nematode and fungi applied were 5.000 eggs of *M. incognita* and $1x10^6$ conidia of *F. solani* (similar treatments with *M. javanica* and *M. hapla*). *Mi*, *Mj* and *Mh*: *M. incognita*, *M. javanica* and *M. hapla*, respectively. Similar letters mean no significative differences according Duncan's multiple-range test. * The arbitrary root-knot index describes extent of infection as follows: 0-5 scale: 0 = no galling, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = >100 galls per root system. Control is *P. ligularis* plants non-inoculated.

solani alone. Similar results were obtained with *M. javanica* and *M. hapla* (Múnera, 2001, Múnera & Saldarriaga, 2003). These results show the damaging effect of both nematodes and fungi.

Nematological research on plantain (Musa spp.)

Nematological information on nematodes associated with plantain is dispersed and focused on nematode genera and their populations associated with different cultivars of plantain at different Colombian regions. Genera of plant parasitic nematodes recordedon plantain are: *Radopholus, Helicotylenchus, Meloidogyne, Pratylenchus, Hoplolaimus, Rotylenchulus* (Volcy, 2003). Additionally, specimens of Criconematidae and Trichodoridae have been recorded. Data on genera and population at both different regions and different cultivars are shown (Table 2.12 to 2.16).

Table 2.12 Population of nematodes associated with plantain at Palestina, Caldas (adapted from Guzmán & Castaño, 2004).

Musa cultivar	***Radopholus similis***		***Helicotylenchus***		***Meloidogyne***		***Pratylenchus***	
	S	R	S	R	S	R	S	R
Dominico hartón	412	26	204	12	412	7	412	7
africa	0	0	3136	192	3704	32	285	8
FHIA 20	0	0	312	202	2580	38	860	7
FHIA 21	1168	31	584	31	2336	40	1752	15

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively.

Table 2.13 Population of nematodes associated with plantain (*Musa* AAB) at Montenegro, Quindío (Múnera, laboratory data, 1999, unpublished).

Musa cultivar	*Radopholus similis*		*Helicotylenchus*		*Meloidogyne*	
	S	R	S	R	S	R
dominico hartón	210 34-760	27 4-157	77 47-137	29 1-130	223 43-583	27 6-99

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively. Data in form: mean and below range (minimum-maximum).

Table 2.14 Population of nematodes associated with plantain (*Musa* AAB) at C.I. Tulenapa, Carepa, Urabá Region (Múnera, laboratory data, 2003, unpublished).

Musa cultivar	*Radopholus similis*		*Helicotylenchus*		*Meloidogyne*		*Hoplolaimus*	
	S	R	S	R	S	R	S	R
hartón	310	70	346	156	43	7	34	0

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively.

Table 2.15 Population of nematodes associated with plantain (*Musa* AAB) at Arauca, Los Llanos Orientales region (Múnera, laboratory data, 2003, unpublished).

Musa cultivar	*Radopholus similis*		*Helicotylenchus*		*Meloidogyne*		*Pratylenchus*		Trichodoridae	
	S	R	S	R	S	R	S	R	S	R
hartón	70	56	185	103	160	7	70	80	67	0

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively.

Table 2.16 Population of nematodes associated with *Musa* spp. at Guapi, Cauca (Múnera, 2004b).

Musa	Rado		Heli		Meloi		Praty		Roty		Crico		Tri	
Cultivar	S	R	S	R	S	R	S	R	S	R	S	R	S	R
cera	19	2	54	2	64	2	17	1	95	5	0	0	0	0
bocadillo	240	12	130	15	59	7	19	1	102	45	181	0	0	0
FHIA	45	0	181	18	90	31	11	0	105	27	15	0	15	0
hartón	12	4	379	46	107	33	205	40	91	50	55	0	0	0
gros michel	17	5	69	10	370	108	7	0	105	25	15	1	0	0
dominico	11	5	535	135	0	0	9	0	107	214	0	0	0	0
manzano	5	0	0	0	114	19	13	0	0	4	0	0	57	0

S and R: nematodes in 100 cm^3 of soil and 1 g. of roots, respectively. Abbreviations: Rado: *Radopholus*, Heli: *Helicotylenchus*, Meloi: *Meloidogyne*, Praty: *Pratylenchus*, Roty: *Rotylenchulus*, Crico: *Criconema*, Tri: *Trichodorus*.

Conclusions

Twelve genera of phytoparasitic nematodes were found associated with the rhizosphere of *S. quitoense*, *C. betacea*, *P. ligularis* and *R. glaucus* at different regions in Colombia. *Meloidogyne, Pratylenchus* and *Helicotylenchus* were found in 70% of the

samples. The genus *Meloidogyne* was both most prevalent and abundant in all regions sampled.

Six genera of plant parasitic nematodes were recorded on plantain. *Radopholus, Helicotylenchus* and *Meloidogyne* genera were present in all regions. Higher populations of nematodes associated with plantain were recorded from Palestina, Caldas and are comparable with those reported for banana plants in Urabá region.

Although *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* (*Radopholus* only on plantain) were found in most of localities, their populations (size, composition) changed according to the crop species and variety, region and sampling season. Data about nematode populations and their distribution, resistance/susceptibility of germplasm to plant parasitic nematodes, interaction and yield losses give support to proposals of integrated management of nematodes.

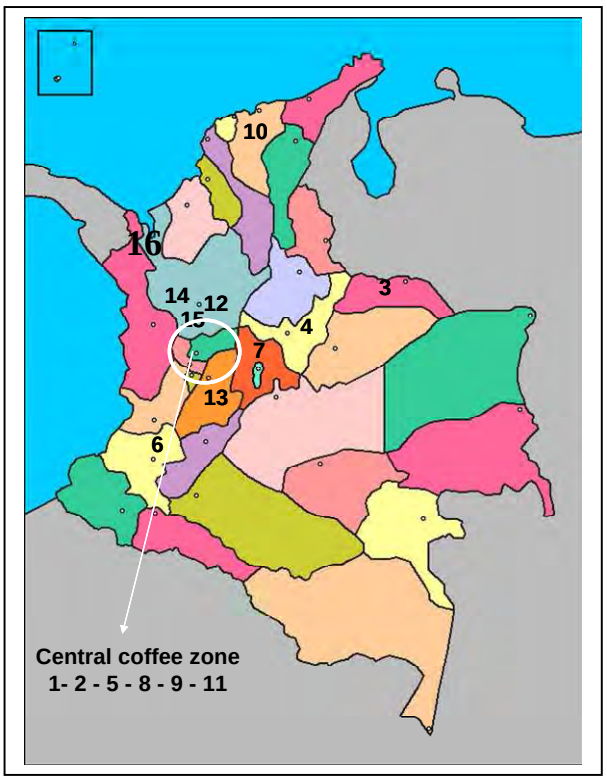
Because the previous studies have demonstrated that nematodes of genera *Meloidogyne, Pratylenchus* and *Radopholus* are important pests in Colombia and their correct species identification is crucial, next chapters (4, 5 and 6) will include the combination of methods to obtain accurate identification of nematodes at species level.

Chapter 3

General materials and methods

Nematode populations

Roots and soil from *Solanum quitoense* Lam., *Cyphomandra betacea* (Cav.) Sendt. (syn. *Solanum betaceum* Cav.), *Passiflora ligularis* Juss., *Rubus glaucus* Benth. and *Musa* AAB ("dominico hartón" cultivar) were collected from different localities of Colombia (Fig. 3.1). Details of all regions included in sampling are shown in chapter 2. One sample per field was taken. Each soil-roots sample of 1 kg was made by mixing up 30 cores of soil plus roots which were taken at first 30 cm. in the rhizosphere of the plants, at the site a subsample of soil (250g.) containing roots was taken to the laboratory. Samples were packed in plastic bag and stored at 4°C until used. The bags were labeled with details of location, host crop, soil type and date of sampling. From the colletected, 100 g was used for extraction of nematodes (100 g.) and to stablish an in-vivo collection an additional 150 g was used at the agriculture research institute CORPOICA (Colombia).



1. Aguadas
2. Aranzazu
3. Arauca
4. Boyacá
5. Caldas
6. Cauca
7. Cundinamarca
8. Génova
9. La Tebaida
10. Magdalena
11. Manizales
12. Rionegro
13. Tolima
14. Urrao
15. Venecia
16. Carepa

Fig. 3.1 Sampling areas from Andean Natural Region, except for Magdalena and Carepa- Urabá Zone (Caribbean Region, Northwest of Colombia) and Arauca (Orinoquía Region, Eastern Colombia).

At the start, only Colombian nematode specimens in suspension received authorization for import to Belgium. A total of 100 samples were collected from fruit crops, mainly in Andes Region and all were used to increase nematode populations in tomato plants at ILVO, Merelbeke. Since *Pratylenchus* and *Radopholus* field population densities were limited in most of the samples (see tables 2.3, 2.14 and 2.15) and their populations in suspension decreased more quickly than *Meloidogyne*, only one *Pratylenchus* population and two *Radopholus* populations, containing at least 80 specimens were studied. *Pratylenchus* and *Radopholus* populations were not increased and specimens from original hosts were directly used for different analyses. An attempt to increase these two genera on carrots discs failed since few specimens used has been stored in suspension for more than three months.

Meloidogyne populations were brought in culture in a greenhouse at ILVO-Merelbeke (Belgium) at 25-30°C (day temperature) and 15-20°C (night temperature), during one year. To increase populations, plastic pots of 1 kg filled with sterilized soil and containing tomato plants (*Lycopersicon esculentum* L. cultivar Money Maker) were inoculated with specimens in suspension from original hosts for each population of *Meloidogyne*. The tomato plants were watered at alternate days except for month six when watering was performed twice a week in order to obtain numerous males. Fertilization was carried out once a month.

After two months, egg masses appeared in 24 out of the 100 inoculated plants only; then, pieces of roots were taken with care cut for the purification of populations (mixture of species based on the perineal pattern was found). Plastic recipients of 150 cm^3 were filled with sterilized soil and tomato plants (*Lycopersicon esculentum* L. cultivar Money Maker) which were inoculated with a single egg mass of *Meloidogyne*; five replicates from each population. Three populations were successfully purified. Measurements of second-stage juveniles from a single egg mass were analysed separately in both cases, pure and not pure populations.

Pratylenchus and *Radopholus* specimens from original Colombian hosts and specimens from *Meloidogyne* populations cultured on tomato plants in greenhouse at ILVO were used for morphological, morphometric, biochemical, molecular and phylogenetic studies.

Extraction of nematodes

The nematode suspensions of *Pratylenchus*, *Radopholus* and *Meloidogyne* populations prepared in Colombia were obtained by extraction of specimens from soil by Cobb's decanting and sieving method (Cobb, 1917) and from roots using maceration (blender) and sieving methods.

For the populations of *Meloidogyne* at ILVO greenhouse, adult females with attached egg mass were dissected directly from the root galls. They were placed separately in Petri dishes containing distilled water or an isotonic solution (0.9% NaCl) prior to preparation of perineal pattern or protein analysis. Second-stage juveniles hatched from the egg masses in the Petri dishes were used for different analyses. Males of root knot nematodes were sorted by Cobb's decanting and sieving method and kept in suspension or collected directly from the roots (always around egg mass) with a dissection needle.

Morphological observations

The specimens were collected in a very small drop of water in an embryo dish. Formalin (4 % with 1 % glycerol) was heated to 70° C and an excess (4-5 ml), was quickly added to the specimens to fix and kill the nematodes in one process (Seinhorst, 1966). The fixed nematodes were processed to anhydrous glycerol following the glycerol-ethanol method (Seinhorst, 1959 as modified by De Grisse, 1969) and mounted on aluminium slides with double coverslips (Cobb, 1917).

Measurements were prepared manually with a camera lucida on an Olympus BX 51 DIC microscope (Olympus optical, Tokyo, Japan) which was equipped with Olympus C5060Wz camera for photographs.

Nematodes were identified to species level or species group using original descriptions, identification keys and comparative studies of different authors (see chapters 4, 5 and 6).

For scanning electron microscopy (SEM), specimens preserved in anhydrous glycerin were transferred to a drop of 4% formalin. A subsequent ultrasonic treatment (10 min) removed particles adhering on the body surface of the specimen. The specimens

were dehydrated by passing them through a gradual ethanol gradient of 25 (overnight), 50, 75, 95 (3 h each) and 100% (overnight) at 25±C. They were critical point dried with liquid CO_2, mounted on stubs and coated with gold-palladium (25 nm) before observation with a JEOL LSM-840 at 15 kV.

Molecular analyses

The molecular analyses were focused in rDNA on D2D3, 18S and ITS1-5.8-ITS2 regions (Fig. 3.2). Juveniles hatched from a single egg mass attached to a female specimen were used for DNA extraction and specific primer. The same female was used for protein analysis or morphological analysis. Additionally and prior to DNA extraction, males/females from 5 populations (P3b, P5b, P14b, P19c and P24c) were kept in 0.5 ml of acetone (one specimen for each eppendorf tube) and used only for sequences analyses (Table 3.1).

For *Pratylenchus,* six specimens from Arauca population and for *Radopholus,* five nematodes per each population (Los Llanos and Urabá) were included in the molecular analyses.

Because molecular analyses were made from a single nematode specimen, some samples did not contain enough DNA for the analysis of all DNA regions previously mentioned. Furthermore in some cases the reactions failed. Thus, the number of sequences successfully obtained for *Meloidogyne*, *Pratylenchus* and *Radopholus,* was variable: 28, 6, and 10 resp. from D2D3; 27, 1, 8 resp. from 18S and 10, 1 and 5 resp. from ITS1-5.8-ITS2 (Table 3.1).

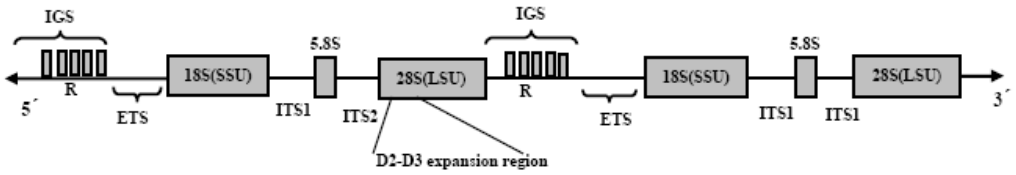


Fig. 3.2 Structure of the ribosomal DNA gene family in nematodes. Coding regions of the 18S small subunit (SSU), 5.8S, and 28S large subunit (LSU), transcription unit, and spacers (ITS1 and ITS2), and the short repeats (R) in the intergenic spacer (IGS) are indicated. ETS=external transcribed spacer, ITS=internal transcribed spacer (from: http://users.ugent.be/~avierstr/).

Preparation of DNA

A single nematode specimen was transferred into 25 µl worm lysis buffer (50 mM KCl; 10 mM Tris pH 8.3; 2.5 mM $MgCl_2$; 0.45 % NP 40 (Tergitol Sigma); 0.45 % Tween 20; 60 µg/ml proteinase K), cut into pieces and transferred into a 0.5 ml tube. The tubes were incubated at -80 °C (10 min), 65°C (1 h) and 95°C (10 min) consecutively. After centrifugation (1 min; 16000g), 5 µl of the DNA suspension was added to the PCR mixture *(Taq DNA Polymerase*, Qiagen Germany) with the D2A (5' - ACAAGTACC GTGAGGGAAAGTTG - 3') and D3B (3' - TCCTCGGAAGGAACCAGCTACTA -3') primers for D2D3 region; G18S4 (5' - GCT TGT CTC AAA GAT TAA GCC - 3') and 18P (5' - TGA TCC WMC RGC AGG TTC AC -3') primers for 18S region or Vrain2F (5’ - CTTTGTACACACCGCCCGTCGCT - 3’) and Vrain2R (5’ - TTTCACTCGCCGTTACTAAGGGAATC - 3’) for the spacer region. The PCR conditions were 30 sec at 94 °C, 30 sec at 54 °C and 1 min (D2D3, ITS) or 2 min (18S) at 72 °C for 40 cycles.

Sequence analyses *

Sequencing was performed using an Applied Biosystems ABI 3130XL Genetic Analyser. PCR product was treated with shrimp alkaline phosphatase (1 U/µl, Amersham E70092Y) and exonuclease I (20 U/µl, Epicentre Technologies X40505K) for 15 minutes at 37 °C, followed by 15 minutes at 80 °C to inactivate the enzymes. This material was then used for cycle sequencing without any further purification, using the ABI Prism BigDye V 3.1 Terminator Cycle Sequencing kit. The sequencing conditions were 30 sec at 96 °C, 15 sec at 50 °C and 1 min at 60 °C for 27 cycles.

Primers used for sequencing were the same as for PCR in D2D3 and ITS cases. For 18S, G18S4, 18P, 2FX (5'- GGA AGG GCA CCA CCA GGA GTG G -3'), 23R (5'- TCT CGC TCG TTA TCG GAA T -3'), 13R (5'- GGG CAT CAC AGA CCT GTT A -3'), 23F (5'- ATT CCG ATA ACG AGC GAG A -3'), 9FX (5'- AAG TCT GGT GCC AGC AGC CGC -3'), 9R (5' -AGC TGG AAT TAC CGC GGC TG -3'), 26R (5'- CAT TCT TGG CAA ATG CTT TCG -3') and 22R (5'- GCC TGC TGC CTT CCT TGG A -3') primers were used for sequencing. Cycle sequence products were precipitated by

* Protocolo suggested by Applied Biosystem for BigDye Terminator v.3.1 Cycle Sequencing kit. 2002.

adding 25 µl of 95 % ethanol and 1 µl 3 M sodium acetate, pH 4.6 to each cycle sequencing reaction (10 µl). The samples were placed at room temperature for 15 minutes and centrifuged at 14.000 rpm for 15 minutes.

After precipitation, an additional wash of the pellet was performed with 125 µl of 70 % ethanol and centrifugated at 14.000 rpm for 5 minutes. The pellet was dried in a Speedvac concentrator, re-dissolved in formamide and run on 50 cm capillaries with POP7 polymer. Sequences were edited and assembled with Seqman 7.0 (DNASTAR Lasergene).

The above DNA extraction and sequencing methods were modified from Tandingan De Ley *et al*. (2002).

Phylogenetic analyses

Apart from our study, sequences of *Meloidogyne, Pratylenchus* and *Radopholus* for phylogenetic analysis were also obtained from GenBank. Only homologous sequences of similar size (short sequences were discarded) to the Colombian populations were used for different comparisons. Short sequences were discarded. Number of sequences analyzed for each rDNA region and outgroups used for phylogenetic trees are shown in Table 3.1.

The sequences were aligned with Clustal W (Thompson *et al.*, 1994) and visually checked. Differences between sequences were estimated using the DNA distance option provided by BioEdit sequence alignment editor (Hall, 1999).

Phylogenetic analyses were restricted to Bayesian inference (BI), since this method has several advantages compared with other phylogenetic methods (Ronquist & Huelsenbeck, 2003) and several arguments indicate the superiority of model-based methods in general over maximum parsimony and distance analyses (Swofford *et al.* 2001). BI was performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) with a general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + Γ), as estimated by PAUP/Mr Modeltest 1.0b (Nylander, *et al.*, 2004). Analyses were run for 3 million generations and trees were generated using the last 2 million generations well beyond the burn-in value.

Table 3.1 Number of sequences analyzed per genus/rDNA region and outgroups used for phylogenetic trees.

	source	**D2D3**	**18S**	**ITS1-5.8-ITS2**
Meloidogyne spp.	This study	28	27	10
	Genbank	18	18	42
outgroups		*P. dunensis* AJ890461	*P. goodeyi* AJ966498	*P. goodeyi* AB053485
		Pratylenchus sp. Pra34 from Col.*	*Pratylenchus* sp. GM16 from Col.	*Pratylenchus* sp. GM17 from Col.
Pratylenchus spp.	This study	6	1	1
	Genbank	31	6	2
outgroups		*Radopholus similis* DQ328712	*Radopholus similis* AJ966502	*Radopholus similis* AY903311
		Helicotylenchus multicinctus DQ328745	*Helicotylenchus dihystera* AJ966486	*Helicotylenchus dihystera* DQ309585
Radopholus spp.	This study	10	8	5
	Genbank	1	1	5
outgroups		*Helicotylenchus vulgaris* DQ328759	*Helicotylenchus varicaudatus* EU306354	*Heterodera schachtii* AF498389
		Heterodera zeae DQ328695	*Helicotylenchus dihystera* AJ966486	*Helicotylenchus dihystera* DQ309585

* Col.: Colombia

Abbreviations used in this study

Abbreviation	**Character**
L	Total body length
m.b.w.	maximum body width
a	Total body length/maximum body width
b	Total body length/distance from anterior end to pharyngo-intestinal junction
b’	Total body length/distance from anterior end to posterior end of pharyngeal gland
c	Total body length/tail length
c’	Tail length/body width at anus
V	Distance from head end to vulva/body length x 100
DGO	Dorsal pharyngeal gland opening
ph. length	Pharynx length
ph. gland length	Pharyngeal gland length
metac.	Distance from head end to metacorpus
J	Distance from head end to pharyngeal - intestinal junction
hem.	Distance from head end to hemizonid
S-E pore	Distance from head end to secretory- excretory pore
spic.	Spicula length along of the arc
gub.	Gubernaculum length
PUS	Post uterine sac

Chapter 4

Meloidogyne *from Colombia*

Introduction

Although root-knot or galls on plants have been reported several times before 1855, the cause was unknown or related with other organisms than nematodes. It was the Englishman, Berkeley (1855) who correlated, for the first time, galls on glasshouse cucumber roots with nematodes. In 1887 (published in 1892) Göldi described different diseases and pest organisms from coffee plants in Brazil, including a root-knot nematode. He named the nematode *Meloidogyne exigua* and briefly described and illustrated it. This publication however, was until 1949 ignored or unknown to most nematologists (Karssen, 2002).

At the end of the 19th century, root-knot nematodes were known from all over the world and their polyphagous behaviour was recognized. However, no type material was deposited, and type localities were often vaguely indicated. Until 1949, the classification of root-knot nematodes was confusing with species being described either under the genus *Heterodera* or *Anguillula*, rarely as *Tylenchus* (Hirschmann, 1985; Siddiqi, 2000). In 1949, Chitwood published a revision of the genus *Meloidogyne* Göldi, 1887. He separated the root-knot nematodes from the cyst nematode genus *Heterodera*, re-erected the genus *Meloidogyne*, and described the generic diagnosis. Karssen (2002) gave a historical review of the genus *Meloidogyne* and discussed the authorship of the genus since in the literature different dates of publication of the type species *M. exigua* and spelling of the author name can be found. Based on the International Code of Zoological Nomenclature (ICZN: article 51), he concluded that Göldi (1892) is the correct author and date of publication of the type species *Meloidogyne exigua*.

Up to 2008, 111 *Meloidogyne* species have been described, comprising 95 valid species, 11 synonymized and five *species inquirendae* (Table 4.1). 55% of the *species* were described after 1980, 14 of those during the last decade. The genus is easily recognized but identification to species level is difficult due to relatively small inter-specific morphological variation.

Table 4.1 List of nominal *Meloidogyne* species based on Siddiqi (2000) and Karssen & Moens (2006) and supplemented with species more recently described. Junior synonym, *species inquirenda* and *nomen nudum* are indicated.

1. *M. acronea* Coetzee, 1956
2. *M. acrita* (Chitwood, 1949) Esser, Perry & Taylor, 1976 (junior synonym of *M. incognita*)
3. *M. actinidae* Li & Yu, 1991
4. *M. africana* Whitehead, 1960
5. *M. aquatilis* Ebsary & Eveleigh, 1983
6. *M. arabicida* Lopez & Salazar, 1989
7. *M. ardenensis* Santos, 1968
8. *M. arenaria* (Neal, 1889) Chitwood, 1949
9. *M. artiellia* Franklin, 1961
10. *M. baetica* Castillo, Vovlas, Subbotin & Troccoli, 2003
11. *M. bauruensis* (Lordello, 1956) Esser, Perry & Taylor, 1976 (junior synonym of *M. javanica*)
12. *M. brasilensis* Charchar & Eisenback, 2002
13. *M. brevicauda* Loos, 1953
14. *M. californiensis* Abdel-Rahman & Maggenti, 1987 (*nomen dubium* in Abdel Rahman, 1981)
15. *M. camelliae* Golden, 1979
16. *M. caraganae* Shagalina, Ivanova & Krall, 1985
17. *M. carolinensis* Eisenback, 1982(*nomen dubium* in Fox, 1967)
18. *M. chitwoodi* Golden, O'Bannon, Santo & Finley, 1980
19. *M. christiei* Golden & Kaplan, 1986
20. *M. cirricauda* Zhang & Weng, 1991
21. *M. citri* Zhang, Gao & Weng, 1990
22. *M. coffeicola* Lordello & Zamith, 1960
23. *M. cruciani* Garcia-Martinez, Taylor & Smart, 1982
24. *M. cynariensis* Bihn, 1990
25. *M. decalineata* Whitehead, 1968
26. *M. deconincki* Elmiligy, 1968 (junior synonym of *M. ardenensis*)
27. *M. donghaiensis* Zheng, Lin & Zheng, 1990
28. *M. dunensis* Palomares-Rius, Vovlas, Troccoli, Liebanas, Landa & Castillo, 2007
29. *M. duytsi* Karssen, van Aelst & van der Putten, 1998
30. *M. elegans* da Ponte, 1977 (junior synonym of *M. incognita*)
31. *M. enterolobii* Yang & Eisenback, 1983
32. *M. ethiopica* Whitehead, 1968
33. *M. exigua* Göldi, 1892
34. *M. fallax* Karssen, 1996
35. *M. fanzhiensis* Chen, Peng & Zheng, 1990
36. *M. floridensis* Handoo, Nyczepir, Esmenjaud, van der Beek, Castagnone-Sereno, Carta, Skantar & Higgins, 2004
37. *M. fujianensis* Pan, 1985

38. *M. grahami* Golden & Slana, 1978 (junior synonym of *M. incognita*)
39. *M. graminicola* Golden & Birchfield, 1965
40. *M. graminis* (Sledge & Golden, 1964) Whitehead, 1968
41. *M. goeldi* Lordello, 1951 *species inquirenda*
42. *M. hainanensis* Liao& Feng, 1995
43. *M. hapla* Chitwood, 1949
44. *M. haplanaria* Eisenback, Bernard, Starr, Lee & Tomaszewski, 2003
45. *M. hispanica* Hirschmann, 1986
46. *M. ichinohei* Araki, 1992
47. *M. incognita* (Kofoid & White, 1919) Chitwood, 1949
48. *M. inornata* Lordello, 1956
49. *M. indica* Whitehead, 1968
50. *M. javanica* (Treub, 1885) Chitwood, 1949
51. *M. jianyangensis* Yang, Hu, Chen & Zhu, 1990
52. *M. jinanensis* Zhang & Su, 1986
53. *M. kikuyensis* de Grisse, 1960
54. *M. kirjanovae* Terenteva, 1965 (junior synonym of *M. incognita*)
55. *M. konaensis* Eisenback, Bernard & Schmitt, 1994
56. *M. kongi* Yang, Weng & Feng, 1988
57. *M. kralli* Jepson, 1984
58. *M. lini* Yang, Hu & Zhu, 1988
59. *M. litoralis* Elmiligy, 1968 (junior synonym of *M. ardenensis*)
60. *M. lordelloi* da Ponte, 1969 (junior synonym of *M. javanica*)
61. *M. lucknowica* Singh, 1969 (junior synonym of *M. javanica*)
62. *M. lusitanica* Abrantes & Santos, 1991
63. *M. mali* Itoh, Ohshima & Ichinohe, 1969
64. *M. marioni* (Cornu, 1879) Chitwood & Oteifa, 1952 *species inquirenda*
65. *M. maritima* (Jepson, 1987) Karssen, van Aelst & Cook, 1998
66. *M. marylandi* Jepson & Golden, 1987
67. *M. mayaguensis* Rammah & Hirschmann, 1988
68. *M. megadora* Whitehead, 1968
69. *M. megatyla* Baldwin & Sasser, 1979
70. *M. megriensis* (Poghossian, 1971) Esser, Perry & Taylor, 1976 *species inquirenda*
71. *M. mersa* Siddiqi & Booth, 1991
72. *M. microcephalus* Cliff & Hirschmann, 1984 (original spelling *microcephala*)
73. *M. microtyla* Mulvey, Townshend & Potter, 1975
74. *M. mingnanica* Zhang, 1993
75. *M. minor* Karssen, Bolk, van Aelst, van den Beld, Kox, Korthals, Molendijk, Zijlstra, Van Hoof & Cook, 2004
76. *M. morocciensis* Rammah & Hirschmann, 1990
77. *M. naasi* Franklin, 1965
78. *M. nataliei* Golden, Rose & Bird, 1981
79. *M. oryzae* Maas, Sanders & Dede, 1978
80. *M. oteifai* Elmiligy, 1968 (original spelling *oteifae*)
81. *M. ottersoni* (Thorne, 1969) Franklin, 1971

82. *M. ovalis* Riffle, 1963
83. *M. panyuensis* Liao, Yang, Feng & Karssen, 2005
84. *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996
85. *M. partityla* Kleynhans, 1986
86. *M. petuniae* Charchar, Eisenback & Hirschmann, 1999
87. *M. phaseoli* Charchar, Eisenback, Charchar & Boiteux, 2008
88. *M. pini* Eisenback, Yang & Hartman, 1985
89. *M. piperi* Sahoo, Ganguly & Eapen, 2000
90. *M. pisi* Charchar, Eisenback, Charchar & Boiteux, 2008
91. *M. platani* Hirschmann, 1982
92. *M. poghossianae* Kirjanova, 1963 *species inquirenda*
93. *M. propora* Spaull, 1977
94. *M. querciana* Golden, 1979
95. *M. salasi* Lopez, 1984
96. *M. sasseri* Handoo, Huettel & Golden, 1993
97. *M. sewelli* Mulvey & Anderson, 1980
98. *M. sinensis* Zhang, 1983
99. *M. spartinae* (Rau & Fassuliotis, 1965) Whitehead, 1968
100. *M. subarctica* Bernard, 1981
101. *M. suginamiensis* Toida & Yaegashi, 1984
102. *M. tadshikistanica* Kirjanova & Ivanova, 1965
103. *M. thailandica* Handoo, Skantar, Carta & Erbe, 2005
104. *M. thamesi* (Chitwood, 1952) Goodey, 1963 (junior synonym of *M. arenaria*)
105. *M. trifoliophila* Bernard & Eisenback, 1997
106. *M. triticoryzae* Gaur, Saha & Khan, 1993
107. *M. turkestanica* Shagalina, Ivanova & Krall, 1985
108. *M. ulmi* Palmisano & Ambriogioni, 2001
109. *M. vandervegtei* Kleynhans, 1988
110. *M. vialae* (Lavergne, 1901) Chitwood & Oteifa, 1952 *species inquirenda*
111. *M. wartellei* Golden & Birchfield, 1978 (junior synonym of *M. incognita*)

The aim of this study was to identify the *Meloidogyne* species on plantain and four fruit crops from Colombia, and to study the diversity of populations by using morphological features (LM), scanning electron microscopy, esterase and malate dehydrogenase phenotypes, species-specific primers and analysis of rDNA sequences.

Materials and Methods

Nematode populations

Roots and soil from *Solanum quitoense* Lam., *Cyphomandra betacea* (Cav.) Sendt. (syn. *Solanum betaceum* Cav.), *Passiflora ligularis* Juss., *Rubus glaucus* Benth.

and *Musa* AAB were collected (see general material and methods) from different localities of Colombia (Table 4.2). Twenty four *Meloidogyne* populations were brought in culture in the greenhouse at ILVO- Merelbeke (Belgium). Three populations were successfully purified.

Based on the perineal pattern, a mixture of species was observed in the samples. Therefore, measurements of second-stage juveniles from a single egg mass were dealt with separately (Table 4.8). Nematodes from original material from Colombia and from populations cultured on tomato plants (*Lycopersicon esculentum* L. cultivar Money Maker) in greenhouse/laboratory were used for morphological, morphometric, biochemical, molecular and phylogenetic studies.

Morphological observations

The specimens were collected, killed, fixed, mounted and measured as indicated in chapter 2, general materials and methods. Perineal patterns for observation were prepared following method described by Hartman & Sasser (1985).

Nematodes were identified to species level or species group using original descriptions, identification of *Meloidogyne* species diagnostic keys from Chitwood (1949), Eisenback (1985b), Hewlett & Tarjan (1983), Jepson (1983, 1987), Karssen (2002) and Whitehead (1968) were used. For females, the differentiation to species level by the perineal pattern was based on the species groups recognized by Jepson (1987). Also morphological comparative studies by Eisenback (1985a, 1993), Eisenback & Hirschmann (1980, 1981), Eisenback *et al.* (1980), Hirschmann (1985) Karssen (2002) and Triantaphyllou & Sasser (1960) using LM and SEM were considered. Orton Williams (1972, 1973, 1974 and 1975) descriptions of the four most common species were also included.

Morphometrics of males and second- stage juveniles

Morphometrics of adult males and second-stage juveniles from 13 and 22 *Meloidogyne* populations respectively were included in this study. Populations of which few specimens (1-2) were obtained or most of them not in lateral position were not included in the analyses of morphometric data. Only the best differentiating characters

Table 4.2 Code, localities and original host associated with *Meloidogyne* populations from Colombia.

Population	Localities	Host	Notes
P1	Aguadas	*C. betacea*	
P2	Aranzazu	*C. betacea*	
P3a	Arauca	*Musa* AAB plantain	
P3b	Arauca	*Musa* AAB plantain	only sequence analysis of male
P4	Boyacá	*C. betacea*	
P5a	Caldas-Villamaria	*C. betacea*	
P5b	Caldas-Villamaria	*C. betacea*	only sequence analysis of **male**
P6	Caldas-Villamaria	*S. quitoense*	
P7	Cauca	*S. quitoense*	
P8	Cundinamarca	*P. ligularis*	
P9	Génova	*C. betacea*	
P10	La Tebaida	*Musa* AAA banana	Gros Michel cultivar
P11	La Tebaida	*Musa* AAB-plantain	FHIA21 cultivar
P12	Magdalena	*Musa* AAB plantain	
P13	Manizales	*C. betacea*	
P14a	Manizales	*S. quitoense*	
P14b	Manizales	*S. quitoense*	only sequence analysis of female
P15	Rionegro-bodega	*R. glaucus*	
P16	Rionegro-bodega	*C. betacea*	
P17	Rionegro-correa	*S. quitoense*	
P18	Rionegro-vega	*S. quitoense*	
P19a	**Tolima-eggmass1**	***S. quitoense***	**pure culture from egg-mass1**
P19b	**Tolima-eggmass2**	***S. quitoense***	**pure culture from egg-mass2**
P19c	Tolima-eggmass2	*S. quitoense*	only sequence analysis of female
P20	Urrao-Guapantal	*P. ligularis*	
P21a	**Urrao-Guapantal-eggmass1**	***S. quitoense***	**pure culture from egg-mass1**
P21b	**Urrao-Guapantal-eggmass2**	***S. quitoense***	**pure culture from egg-mass2**
P22	Urrao-Chuscal	*C. betacea*	
P23	Urrao-Chuscal	*S. quitoense*	
P24a	**Venecia-eggmass1**	***S. quitoense***	**pure culture from egg-mass1**
P24b	**Venecia-eggmass2**	***S. quitoense***	**pure culture from egg-mass2**
P24c	Venecia-eggmass2	*S. quitoense*	only sequence analysis of female
P25	Belgium	*L. esculentum*	control population, *M. javanica*

We refer to 25 populations (24 from Colombia plus one reference or control population of *M. javanica* from ILVO-Belgium). However 3 pure populations were each split into two populations (P19a, P19b, P21a, P21b, P24a and P24b, each one from different egg-mass) as indicated in table above. Additionally, 5 specimens (P3b, P5b, P14b, P19c and P24c) each one from different populations and different developmental stage were included only for sequence analysis, and are considered repetition of their respective populations. Pure populations are indicated in bold.

based on their broad range within the genus and low coefficient of variability were considered for analysis. Thus, for juveniles, resp. two taxonomically important quantitative features (tail and hyaline tail terminus length) while for male resp. three (stylet length, cone length and DGO) were used.

Canonical Discriminant Analysis (CDA)

Morphometrics of adult males and juveniles from the same populations included in morphometric analysis were used in a Canonical discriminant analysis. For juveniles, resp. two taxonomically important quantitative features (tail and hyaline tail terminus length) and 15 quantitative characters were used in the analyses while for male resp. three (stylet length, cone length and DGO) and 11 characters were used.

Isozyme - Electrophoresis

To carry out rapid and reliable species identification of *Meloidogyne* specimens, an electrophoretic method modified and adapted by Karssen *et al*. (1995) for an automated electrophoresis system (PhastSystem, Pharmacia) was used. Proteins of young single *Meloidogyne* females were separated using polyacrylamide gradient gels (8-25) and stained for the isozymes esterase and malate dehydrogenase.

M. javanica from tomato plants of ILVO-Merelbeke was used as a reference for esterase and malate dehydrogenase phenotypes. As indicated by Esbenshade & Triantaphyllou (1985b) each phenotype is designed by a letter suggestive of the species it specifies (H= *hapla*, I=incognita, J=*javanica* and A= *arenaria*) and a number indicative of the number of bands present of esterase (Est or E) and malate dehydrogenase (Mdh or N). Phenotypes of *Meloidogyne* populations from Colombia and their relative rate of migration (Rm) were compared with those reported in the literature.

Molecular characterization and analyses

The molecular analyses were focused on rDNA: D2D3, 18S and ITS1-5.8-ITS2 regions. Single egg mass from each Colombian nematode population that was attached to the females used for protein analysis were selected. After hatching, DNA extraction from single juvenile was made for each Colombian population. These samples were used for both species-specific primers and sequence analysis. Additionally and prior to DNA extraction, males or females from 5 populations resp. P3b, P5b and P14b, P19c, P24c)

were kept in 0.5 ml of acetone (one specimen for each eppendorf tube) and used only for sequence analysis (Table 4.2).

Preparation of DNA, sequence analyses, sequence comparison and phylogenetic analyses were conducted as explained in chapter 2, general materials and methods. Only some details concerning to the outgroup used for phylogenetic analyses/trees are mentioned here.

Pratylenchus dunensis AJ890461 accession number from de la Peña, Moens, van Aelst & Karssen (2006) and one *Pratylenchus* sp., Pra34, sequence from Colombia, were selected as outgroup taxa for D2D3 analyses based on hypotheses of their affinities with *Meloidogyne* found in the literature (Subottin *et al*., 2006). For 18S analysis, outgroup taxa selected were *P. goodeyi* accession number AJ966498 from Meldal *et al.* (2007) and *Pratylenchus* sp., GM16, sequence from Colombia. *P. coffeae* from Saeki *et al.* (2003) accession number AB053485 and one *Pratylenchus* sp., GM17, sequence from Colombia, were outgroup taxa for ITS analyses.

Specific primers

After preparation of DNA, D2D3 primers were used in the reaction to amplify the D2D3 extension region of the 28S rDNA gene to check DNA presence. 5 µl of the DNA suspension were added to a polymerized chain reaction (PCR) master mix (5 µl 10X DNA Taq polymerase incubation buffer, 2 µl $MgCl_2$, 1 µl DNTP mixture (200 µM each), 0.3 µl of each primer, 0.4 µl Taq-polymerase in 36 µl ddH_2O) (Table 4.3).

The obtained PCR product from each population was also used in other PCR reactions using species-specific primers to detect the presence of any of the four common *Meloidogyne* species: *M. incognita* (Finc/Rinc), *M. javanica* (Fjav/Rjav), *M. arenaria* (Far/Rar) and *M. hapla* (JMV1/JMVhapla) as well as *M. chitwoodi* (JMV1/JMV2) and *M. fallax* (JMV1/JMV2) (Wishart *et al.*, 2002; Zijlstra, 2000; Zijlstra *et al.*, 1995, 2000). Photographs were made of agarose gels after electrophoresis of the PCR amplified from different species-specific primers.

Table 4.3 Nucleotide sequences of primers used for amplification of D2D3 region species-specific PCR.

PRIMER *	SEQUENCE (5' TO 3')	SIZE (*bp*)	REFERENCE
PCR of D2D3 region			
primer D2A	5'-ACA AGTACC GTG AGG GAA AGT TG-3'		De Ley *et al*., 1999
Primer D3B	3'-TCC TCG GAA GGA ACC AGC TAC TA-5'		De Ley *et al*., 1999
Species-specific PCR			
Finc	ctctgcccAATGAGCTGTCC	1200	Zijlstra *et al*., 2000
Rinc	ctctgcccTCACATTAAG		Zijlstra *et al*., 2000
Fjav	GGTGCGCGATTGAACTGAGC	670	Zijlstra *et al*., 2000
Rjav	caggcccttcAGTGGAACTATAC		Zijlstra *et al*., 2000
Far	ctctgcccAATGAGCTGTCC	420	Zijlstra *et al*., 2000
Rar	ctctgcccACACTACAACT		Zijlstra *et al*., 2000
JMV1	5'-GGATGGCGTGCTTTCAAC-3'		Wishart *et al*., 2002
JMV2	5'-TTTCCCCTTATGATGTTTACCC-3'	540/670	Wishart *et al*., 2002
JMVhapla	5'-AAAAATCCCCTCGAAAAATCCACC-3'	440	Wishart *et al*., 2002

* Pairs shown (Finc/Rinc; Fjav/Rjav; Far/Rar; JMV1/JMVhapla; JMV1/JMV2; JMV1/JMV2) were used in PCR reactions using two primers to specifically detect a single species of *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi* and *M. fallax*, respectively. Lower case letters represent (part of) the sequence of the progenitor primer (Zijlstra *et al*., 2000; Wishart *et al*., 2002).

Results

Morphological identification of the populations

The differential importance of qualitative and quantitative characters differs between the sexes and the developmental stage J2 (Jepson, 1983, 1987; Eisenback, 1985a, 1985b; Eisenback & Hirschmann (1980, 1981), Karsssen, 2002; Karssen & Moens, 2006). For females, we focussed on the perineal pattern to distinguish species while in males, we have used both qualitative features (head shape) and quantitative (length of stylet, length of stylet cone and DGO). In second stage juveniles, qualitative differences between species are not easy to define in practise and therefore species differentiation will be based mainly on quantitative features (tail length and length of hyaline tail length) and one qualitative feature (tail shape). Because quantitative features of males and juveniles showed overlap between populations their analysis was complemented with CDA analyses.

Females

Perineal pattern

In general, the perineal pattern has been used as main diagnostic feature for species identification despite considerable intraspecific variation may be observed. However, some of the features of the pattern remain relatively constant (Eisenback, 1985b; Jepson, 1987; Karssen, 2002).

Jepson (1987) grouped *Meloidogyne* species with similar morphology and distinguished six morphogroups, with or without further subdivision and illustrated by diagrams. The information shown in the diagrams is (1) the general shape of the pattern, (2) some indication of coarseness of striae, (3) the general nature of the striae (straight, wavy,..), (4) presence of features that form the dorsal arch, lateral fields, phasmids.

Most of the *Meloidogyne* perineal patterns from Colombian populations fitted to the description of the major economically important species of the genus (Chitwood, 1949; Orton Williams, 1972, 1973, 1974, 1975; Jepson, 1983, 1987; Eisenback, 1985a; Karssen, 2002). Perineal patterns commonly found in females of Colombian populations fit groups 1, 4, 5 and 6 described by Jepson (1987) which matches with *M. hapla* (39%), *M. arenaria* (13%), *M. javanica* (48%) and *M. incognita* (61%), respectively (Table 4.16). However, some specimens showed patterns that were placed into group 3 of Jepson. Group 3 is a more complex group, has the largest numbers of species and is further subdivided. It is characterized by round/oval patterns without marked lateral lines.

M. incognita-group pattern

Populations P3a, P4, P5a, P8, P11, P16, P18, P19a, P19b, P20, P21a, P21b, P22, and P23 agreed with group 6 or *M. incognita* group (Fig. 4.1 A, B, C, D). They are characterized by the presence of a high, squarish dorsal arch that often contains a distinct whorl in the tail terminal area. The striae are smooth to wavy, sometimes zigzagged. Distinct lateral lines are absent, but sometimes the lateral field is marked by breaks and forks in the striae. Often some striae bend toward the vulval edges.

Most specimens of these populations showed the pattern of *M. incognita sensu lato*: the striae are distinct, moderately fine to coarse and fairly closely spaced (they may

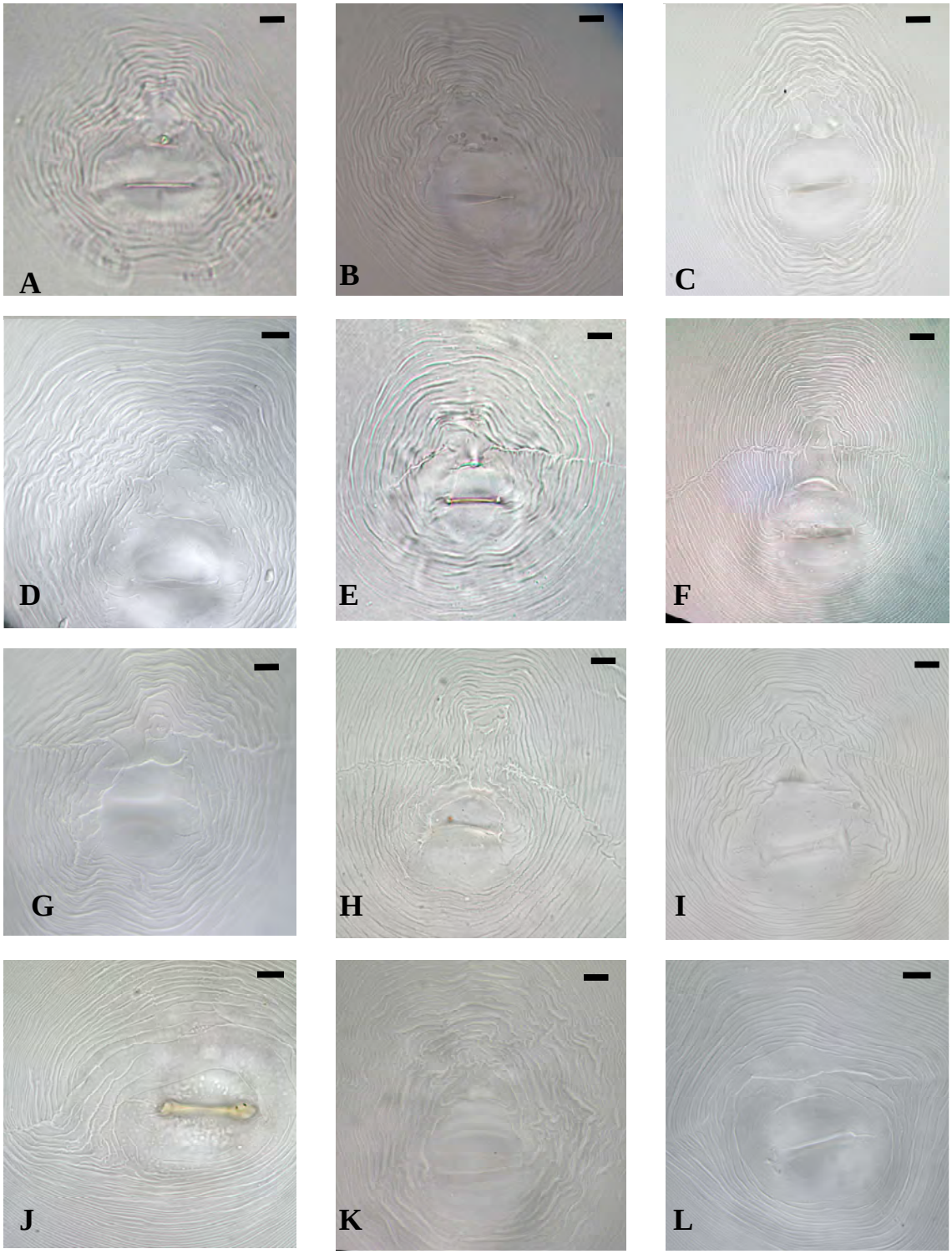


Fig. 4.1 LM photographs of perineal pattern of Colombian *Meloidogyne* populations showing variation within the genus. *M. incognita* group (A=P16, B=P20, C=P8, D=P3a); *M. javanica* group (E, F=P25, G=P24a, H=P13, I=P14a); *M. hapla* (J=P19a); *M. arenaria* (K=P5a) and other groups (L=P21a). Scale bars: 10µm.

be straight, wavy, single or paired, continuous or broken). The dorsal arch is usually high and squared; although in some it may be more rounded. The lateral field is indistinct, and the dorsal and ventral striae are generally not interrupted at this point. The perivulval area is free of striae except for some individuals where it extends towards each end of the vulva. Rarely, there are cross-striae between the vulva and anus. One of the most characteristic features of the pattern is the ʌ -shape formed by striae in the region just dorsal to the tail. Phasmids are not prominent and are about the same distance apart as the width of the vulva.

***M. javanica*-group pattern**

The *M. javanica* group perineal pattern was present in populations P6, P13, P14a, P16, P18, P19a, P19b, P21a, P21b, P24a, P24b and P25 (Fig. 4.1 E, F, G, H, I). Those perineal patterns are easily recognized because of the lateral ridges that divide the dorsal and ventral striae. Generally, the ridges run the entire width of the pattern, but gradually disappear near the tail terminus. The dorsal arch is low and rounded to high and squarish and often contains a whorl in the tail terminal area. The striae are smooth to slightly wavy, and some striae may bend toward the vulval edges.

Most specimens of these populations showed the typical pattern of *M. javanica*, i.e. dorso-ventrally ovoid and composed of moderately fine, continuous and closely spaced striae. The striae are interrupted by conspicuous double incisures forming the lateral field. The incisures fade out dorsally towards the anus, where the transverse striae form a small tail whorl. The dorsal arch may be low to moderately high. The perivulval region is generally free of striae; however, in some populations individuals may show transverse striae between the vulva and anus forming a pattern in which the centre of the origin is on the tail. The phasmids are closer than or as widely separated as the vulva width.

***M. hapla*-group pattern**

Populations P9, P16, P17, P18, P19a, P19b, P21a, P21b and P23 matched to group 1 or *M. hapla* group (Fig. 4.1 J). This unique pattern is characterized by the overall rounded hexagonal to flattened ovoidal shape, very fine striae, and subcuticular punctations in the smooth tail terminal area. The dorsal arch is usually low and rounded,

but may be high and squarish. Lateral ridges are absent but the lateral fields are marked by irregularities in the striae. The dorsal and ventral striae often meet at an angle, and the striae are smooth to slightly wavy. Some patterns may form wings on one or both lateral sides. According to Jepson (1987), the most characteristic feature of the pattern is the punctation concentrated in an area enclosed by striae located between the anus and tail terminus. The pattern is rounded with smooth, fine, closely spaced striae. The dorsal arch is flat and the lateral field usually clearly marked and leading outward from the punctations. The perivulval area is free from striae. Lateral striae, on one or both sides, ventral to the lateral field may form a wing, leading outward to meet the lateral field horizontally.

***M. arenaria*-group pattern**

Most perineal patterns obtained from populations P5a, P10 and P12 agreed with group 4 or *M. arenaria* group (Fig. 4.1 K). The pattern is transversely ovoid with fine closely spaced striae, dorsal arch low, sometimes flat with dorsal striae curving abruptly to the lateral field and forming "shoulders". The lateral lines are clear and marked by forking of the dorsal and ventral striae at their junction. The area immediately dorsal to the anus is free of striae as is the perivulval region.

Other patterns observed

Additional to the four previously described groups, we observed some perineal patterns showing combine features of different species in unique patterns (e.g. population P21a, Fig. 4.1 L) which were not included in a specific group (see Table 4.16)

Males

Males were present in all populations, however, when few specimens (1-2) were found or specimens were not in lateral position, they were not included in the analysis of morphometric data. Measurements of males from 13 Colombian populations of *Meloidogyne* are shown in Table 4.5. Morphology comparison of *Meloidogyne* male structures using LM microscopy and SEM reported by Eisenback (1985a, 1985b) and Eisenback & Hirschmann (1980, 1981) were also considered.

Although stylet shape was not considered for the analysis, a special feature (2 to 10 projections protruding from the shaft) was observed in populations P3a, P5a, P8, P16, P18 and P22 (Table 4.16, Fig. 4.2 C).

Head shape of males using LM

Three distinct groups were differentiated using head shape of males. Those were compared with grouping of *Meloidogyne* species (Table 4.4) based upon head morphology in males according Jepson (groups was named with the same number used by Jepson, 1987). From 15 populations analyzed, 73% showed features of *M. incognita*; 33% of *M. hapla* and 27% of *M. javanica.* Additionally, 44% of populations showed a head shape pattern which was not specific to any species (Table 4.16).

Table 4.4 Summary of main four *Meloidogyne* species for measurements of males stylet length, DGO and head shape (adapted from Jepson, 1987).

Species			**Stylet length**				**DGO**			
	head group	**pop.**	**Species-range**	average	min	max	**Species-range**	average	min	max
M. incognita	**6**	P1	**24-26**	**24,8**	24,3	26,1	2-3	**2,9**	2,7	3,2
		P2			23,0	26,0		2,5	1,7	3,5
		P3		25,0	23,0	32,7		2,1	1,4	2,5
M. javanica	**3**	P1	**20-22**	**20,3**	18,9	23,4	3-4	**3,9**	2,7	5,4
		P2			20,0	21,0			3,0	3,0
		P3		21,2	20,0	23,0		2,9	2,2	4,7
M hapla	**7**	P1	**20-22**	**20,4**	19,4	21,6	4-5	**4,1**	2,7	5,4
		P2			17,0	18,0			4,0	6,0
		P3		20,0	17,3	22,7		2,9	2,5	3,2
M. arenaria	**7**	P1	**20-22**	**21,6**	20,7	23,4	7-8	**7,1**	5,0	11,3
		P2			20,0	24,0			4,0	7,0

P1, P2 and P3 are different populations; min.,and max. minimum and maximum, respectively

***M. incognita*-group of male head (LM)**

Males of the populations P3a, P5a, P8, P16, P18, P19a, P19b, P20, P21a, P21b, and P22 (Fig 4.1 A, B, C) showed similarities with the characteristics of group 6 in Jepson (1987). In species group 6 or *M. incognita* group, males are characterized by a large, round labial disc, raised above of the median lips. The labial disc is concave to flat, and the high head cap is nearly as wide as the head region in lateral view. The head region is usually marked by 2-5 annulations but may be completely smooth. The head region is not distinctly from the rest of the body.

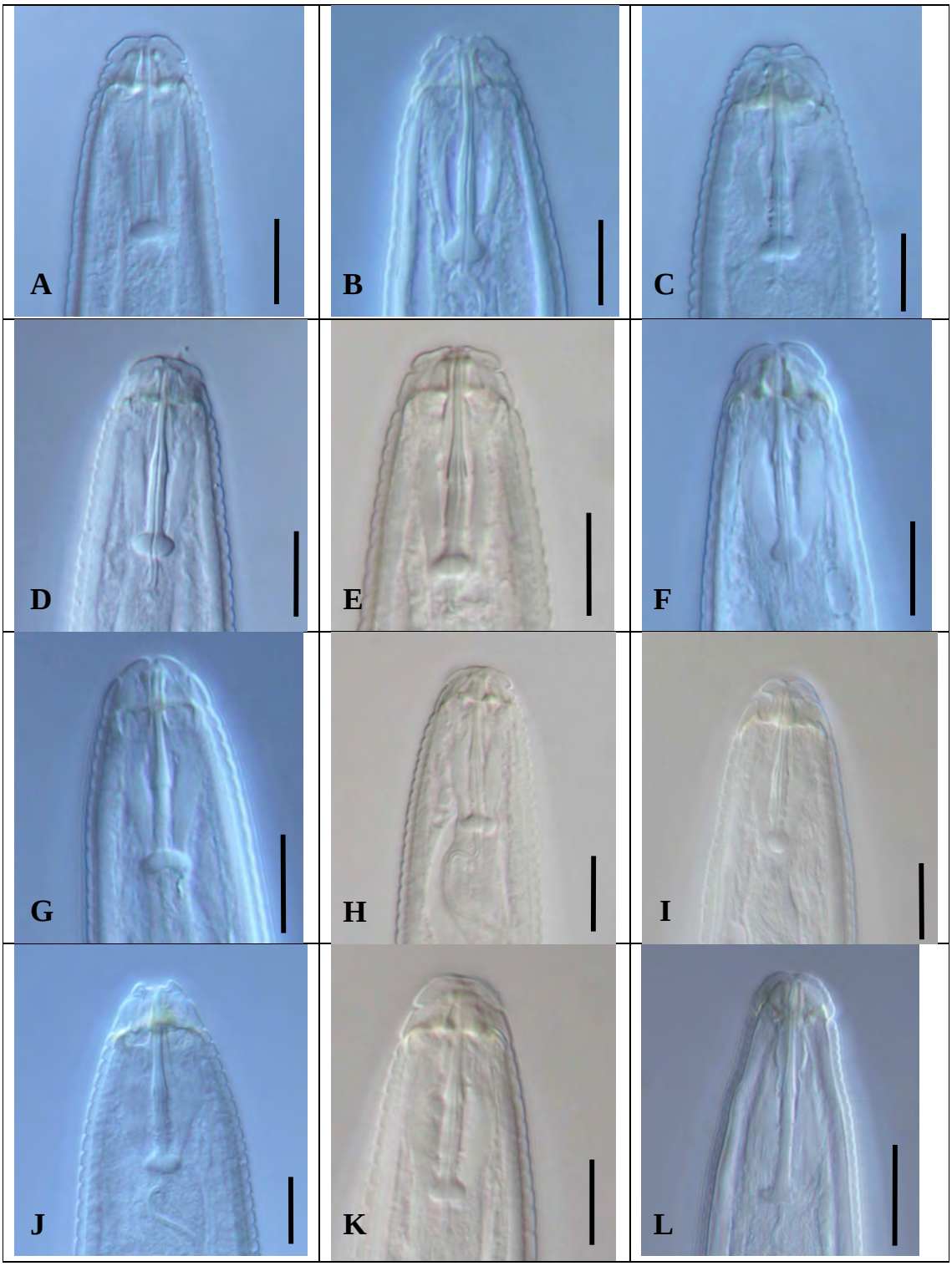


Fig. 4.2 LM photographs of male heads of Colombian *Meloidogyne* populations showing variation within the genus. *M. incognita* group (A=P16, B=P8, C=P3a); *M. javanica* group (D=P25, E=P24a); *M. hapla* (F=P19a, G=P17); other groups (H=P21a, I=P5a, J=P18, K=P22); *Meloidogyne* sp. (L=European population from dunes). Scale bars: 10 µm.

***M. javanica*-group of male head (LM)**

Males of the populations P21a, P21b, P24a, P24b and P25 belong to group 3 or *M. javanica* group, characterized by a high, rounded head cap, distinctly set off from the head region. The labial disc and medial lips are fused and form one smooth, continuous structure which is almost as wide as the head region in lateral view. The head region may be smooth or marked by 2-3 incomplete head annulations. The head region is not distinctly set off from the rest of the body (Fig 4.2 D, E).

***M. hapla* and *M. arenaria*-group of male head (LM)**

Males of the populations P16, P17, P18, P19a and P19b shared those features (Fig 4.2 F, G) belong to group 7 or *M. hapla* and *M. arenaria* group: They are characterized by a high and narrow head cap and set-off head region. The labial disc and median lips are fused and form one smooth, continuous structure that is much narrower than the head region. The head region is smooth and distinctly set off from the body annulations. The body annules increase in width and diameter posteriorly.

Head shape of males using SEM

Accurate details of different groups are shown in scanning electron micrographs of *Meloidogyne* males (Fig 4.3). Scanning only was used for populations P3a, P8, P16, P17, P18, P19a, P24a and P24b. In general male head features using SEM fitted the three groups mentioned before. Details of median lip in face view were distinct for each group.

***M. incognita*-group of male head (SEM)**

Group 6 (*M. incognita* group, P3a, P8, P16 and P18): nematodes possess a round labial disc that is distinctly raised above the medial lips and depressed for some distance around the prestomatal opening. The medial lips are crescent-shaped in face view and extend for some distance onto the head region. The lateral lips are generally not visible, although remnants may be present on the head region .The head region usually bears either two or three irregular incomplete annulations (Fig 4.3 A-G).

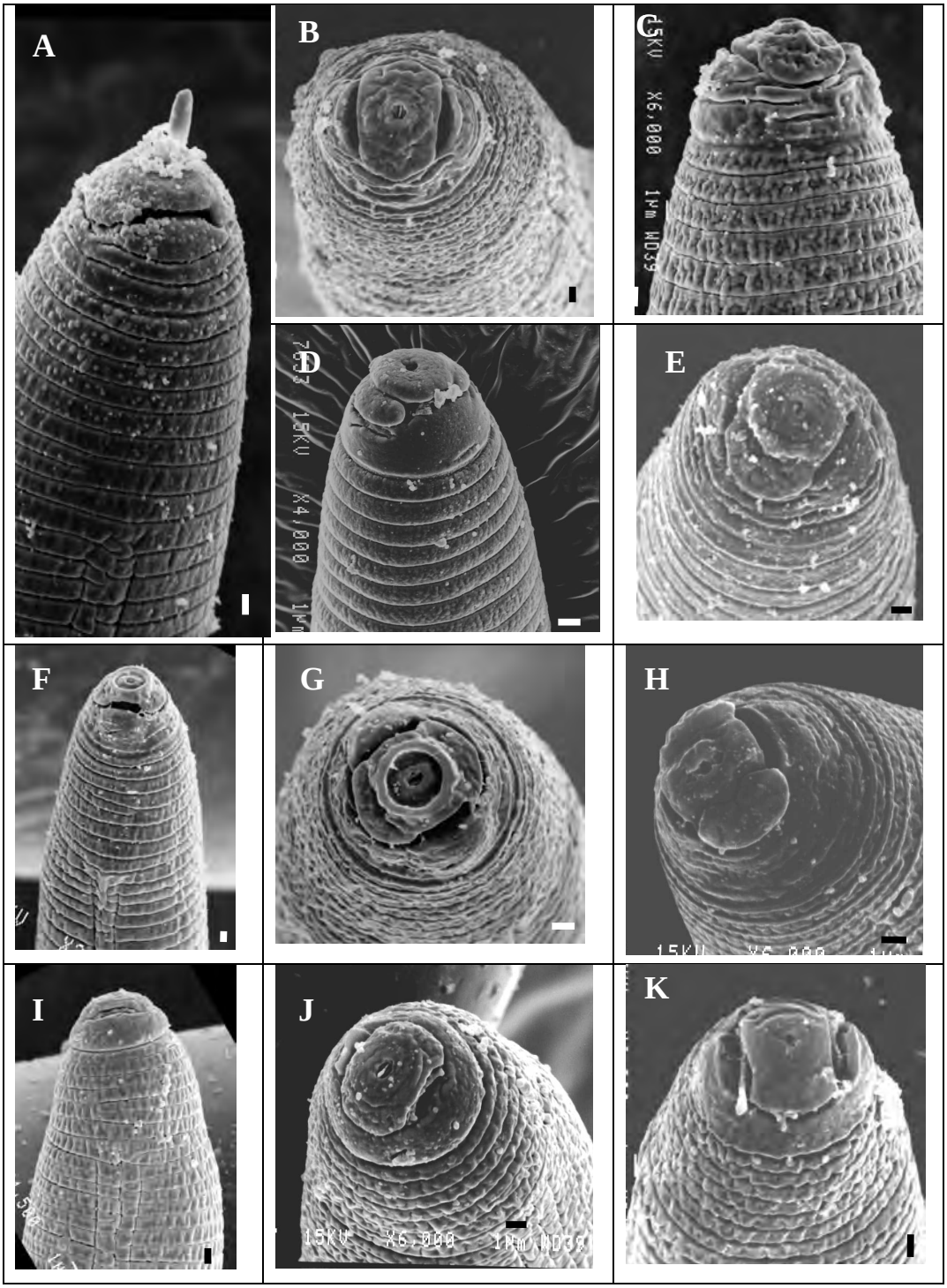


Fig. 4.3 SEM lateral and face view photographs of male head region of Colombian *Meloidogyne* populations. *M. incognita* group (A=P3a; B=P18; C=P16; D=P3a; E=P8; F, G =P16); *M. javanica* group (H=P24a); *M. hapla* (I, =P19a; J=P19b; K=P17). Scale bars: 1 µm.

***M. javanica*-group of male head (SEM)**

In Group 3 (*M. javanica* group, P24a, P24b): labial disc and medial lips are in the same contour and fuse to form a continuous elongate structure. The lateral edges of the labial disc are almost straight in face view, so that the large amphid openings are exposed. The medial lips are wider than the labial disc, and lateral lips are absent. Cephalic sensilla are not expressed externally, and the head region is not annulated (Fig 4.3 H).

***M. hapla*-group of male head (SEM)**

Group 7 (*M. hapla* group, P17, P19a and P19b): The labial disc is not raised above the medial lips, and the lip structures are the same width and form a rectangle in face view. Lateral lips are not present in these populations. The head region is not annulated, except that a remnant of a short annulation is occasionally present laterally on one or both sides. In most specimens, the head region is set off from the body. The head region is set off from the body annules (Fig 4.3 I, J, K).

Morphometrics of males

Measurements of adult males from 13 *Meloidogyne* populations are shown in Table 4.5. Only the best differentiating characters based on their broad range within the genus and low coefficient of variability were considered for analyses: stylet length, cone length and DGO.

Because measurements of stylet length, cone length and DGO overlapped between the four common *Meloidogyne* species and most Colombian populations matched to those species, these features were not helpful to distinguish between species (Table 4.4 and Table 4.5). Exceptions were P5a and P17 which felt into *M. hapla* based on stylet length; and P8 and P22 which felt into *M. incognita* based on DGO. However, note that this assertion, based on a unique quantitative feature could be wrong (based on the combination of different identification methods, see discussion). Cone length was slightly greater than, or equal to, half total stylet length in all Colombian populations, thus this feature could not be used to separate our populations at species level.

Table 4.5 Morphometric features of *Meloidogyne* spp. males from different localities of Colombia. Measurements are in µm.

Pop	L	m.b.w	cone	stylet	group*	DGO	group*	S-E pore	metac	J	tail	spic.	gub.	hem.	a	b	c
P2	1579	47,9	10,2	21,4	j,h,a	3,4	i,h,a	164	89	110	10,2	25,9	7,3	-	32,9	14,4	157
P3a	1298	31,1	11,4	20,5	j,h,a	2,3	i,h,a	129	79	96	10,7	27,3	7,3	113	41,6	11,5	123
P5a	1413	34,1	10,7	18,6	h	2,3	i,h,a	139	73	116	7,9	36,7	8,5	-	41,7	12,3	180
P8	1805	30,1	12,8	23,0	i,h,a	1,5	i	160	85	114	11,1	34,4	8,7	-	62,2	16,3	176
P13	1198		10,2	20,0	j,h,a	3,4	i,h,a	149	-	-	-	-	-	-	-	-	-
P16	1834	29,9	12,1	22,7	j,h,a	3,0	i,h,a	157	84	115	9,0	-	-	-	61,9	15,3	209
P17	1154	27,9	10,2	17,7	h	2,8	i,h,a	119	63	95	10,2	25,1	7,3	106	42,0	12,3	120
P18	1912	28,8	12,1	23,1	i,h,a	2,3	i,j	171	93	127	11,6	-	-	-	66,5	15,0	-
P20	1745	27,3	12,3	21,4	j,h,a	2,7	i,h,a	154	81	114	11,7	28,2	7,6	-	66,7	16,0	155
P21b	1071	31,8	10,6	19,8	j,h,a	3,3	i,h,a	136	74	114	10,9	25,6	6,2	127	34,0	9,6	116
P22	1420	34,1	11,8	21,7	j,h,a	1,7	i	138	75	109	11,3	-	-	-	40,8	13,0	131
P24a	1312	33,5	11,8	20,9	j,h,a	3,5	i,h,a	158	91	120	12,6	31,0	7,1	-	39,4	11,7	112
P25	1266	33,6	11,0	20,1	j,h,a	2,6	i,h,a	136	80	111	11,5	27,9	7,7	133	38,1	11,6	116

*Jepson group based on measurements of stylet and DGO. Letters i, j, h , a, mean *M. incognita*, *M. javanica*, *M. hapla*, *M. arenaria*, respectively. For relations between code-localities-host see table 4.2. Pop: population. Pure population are indicated in bold.

Canonical discriminant analysis (CDA) of males

Using a combination of the three most common features selected for differentiation of *Meloidogyne* species based on males (stylet, cone and DGO), the CDA did not allow sharp separation between populations; however, populations with features of *M. incognita* (P3a, P8, P16, P18 and P20), *M. javanica* (P21b, P24a and P25) and *M. hapla* (P17) appear in the CDA plot (Fig. 4.4) above, in the middle and in the bottom, respectively. Stylet length (85.3 % variation in root1- value 1.11) was the best feature for the separation of populations (Table 4.6, case 1)

Table 4.6 Standardized coefficients for canonical variates of males from Colombian *Meloidogyne* species.

	Root 1	**Root 2**
Case 1 (Tx hyaline area)		
% of variation	**85.29**	11.16
Selected characters	Vector loadings	
Stylet	1.1125	0.3936
Cone	0.1422	-0.0952
DGO	-0.6442	1.4806
Case 2 (11 features)		
% of variation	**80.56**	14.41
Selected characters	Vector loadings	
DGO	4.229	-3.711
m.b.w.	1.296	2.049
J	0.206	-1.227
L	-0.057	0.057
S-E pore	-0.074	0.079
a	0.217	1.318
b	2.805	-9.721
C	0.052	-0.021
Cone	-0.086	-2.046
Metacorpus	-0.211	0.191
Stylet	0.592	-2.529

Populations 1, 7, 26 and 29 do not have data for all features.

Tendency to separate species in clusters similar as case 1 was observed when eleven features based on males (DGO, GBD, J, L, S-E pore, a, b, c, cone, metacorpus and stylet) were combined for differentiation of *Meloidogyne* species. The percentage of variation for roots 1 obtained was 80.56% (Table 4.6, case 2). Three morphological traits (*viz.* DGO, b and m.b.w.) provided the most useful features for discrimination of *Meloidogyne* populations.

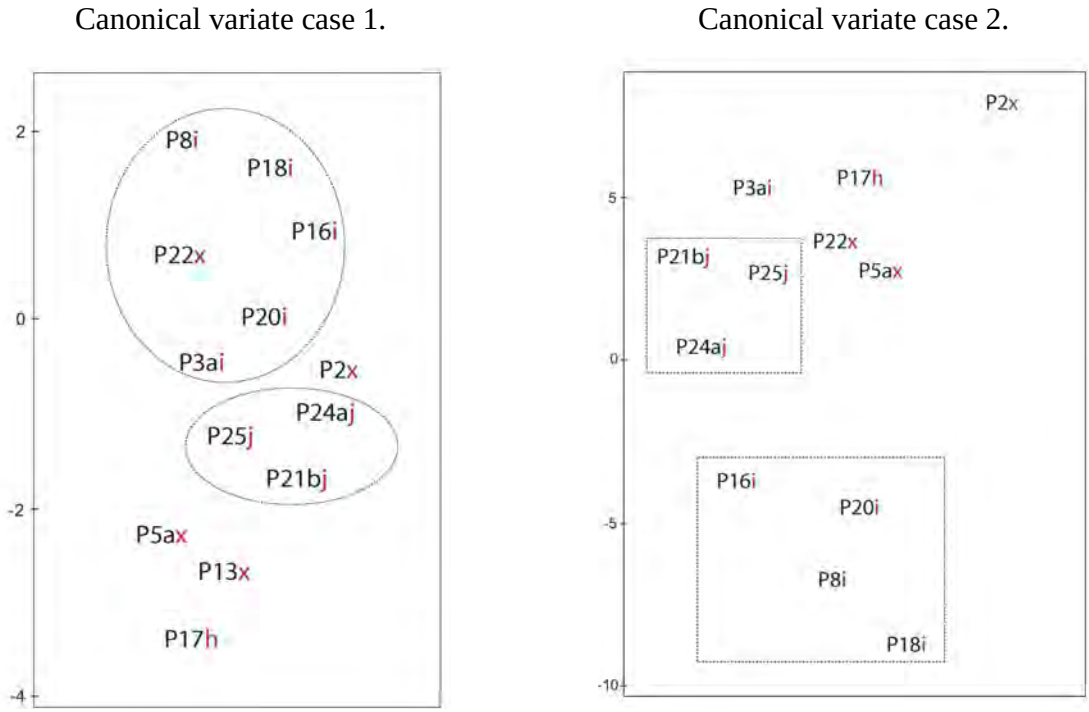


Fig. 4.4 Canonical Discriminant Analysis of 13 *Meloidogyne* populations from Colombia for males performed with 3 morphometric variables: stylet, cone and DGO (case1) and performed with 11 morphometric variables: DGO, m.b.w., J, L, S-E pore, a, b, c, cone, metacorpus and stylet (case2). Red letters mean: i, *M .incognita*; j, *M. javanica*; h, *M. hapla* and x, indefinite species. Circles and squares group same species, except P22x.

Second-stage juveniles

Jepson (1987) divided *Meloidogyne* second-stage juveniles into species groups on the basis of similarity in tail shape and within these morphogroups, species differentiations depend on differences in measurements of tail length and length of hyaline terminus (Table 4.7). She recognized 12 species groups and stated that the groups 4 to7 are more similar and contain more than half of the species (known at that

time) and have (apart from one exception) a very narrow range (40-60 µm) of tail lengths.

Tail shape

J2 of four populations (P3a, P8, P16 and P18) of *Meloidogyne* possess a tail shape with the hyaline terminus broadly rounded as described for *M. incognita* or group nr 6 in Jepson (1987) (Fig. 4.5 A, B). Juveniles of populations P10, P12, P14a, P21a, P21b, P24a, P24b and P25 had narrow tapering tail terminus ending in finely rounded tip*;* there are often cuticular constrictions mid-way along the hyaline terminus. These features matched with *M. javanica* or *M. arenaria,* i.e. group nr 7 in Jepson (Fig. 4.5 C, D). Finally, populations P17 and P19b showed a tapering tail terminus but with a broadly rounded tip and the hyaline terminus of which the sides are more or less parallel. This fits to *M. hapla* or group 8.

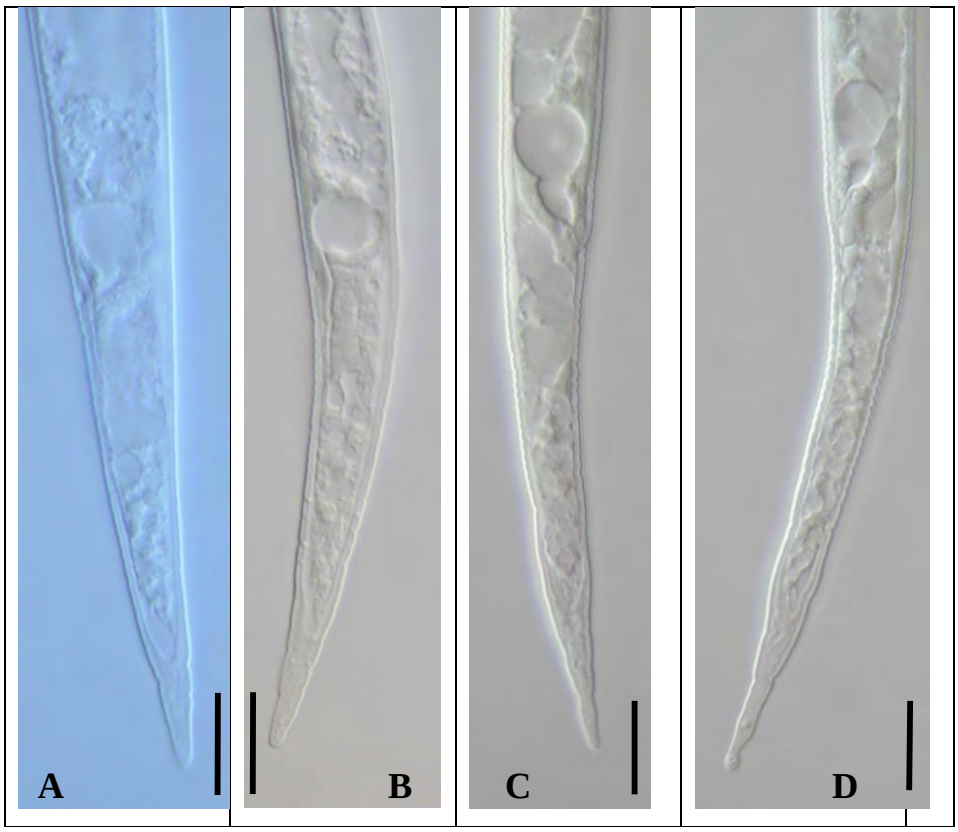


Fig. 4.5 LM photographs of second-stages juvenile tails of *Meloidogyne spp.* from Colombia: P8 (A); P16 (B); P24a and 24b (C); P21a and P21b (D). Scale bars: 10 µm.

Morphometrics of second-stages juveniles

Measurements of second-stages juveniles from 22 *Meloidogyne* populations are shown in Table 4.8. Only the best differentiating characters based on their broad range within the genus and low coefficient of variability were considered for analyses: tail and hyaline tail terminus length (Table 4.7).

Table 4.7 Summary of main four *Meloidogyne* species measurements of second-stage juvenile tails and hyaline tail terminus (adapted from Jepson, 1983, 1987).

Species				**Tail length**			**Hyaline tail terminus length**		
	Species group	**pop**	**Species range**	**average**	**min.**	**max.**	**average**	**min.**	**max.**
M. incognita	**6**	P1	**38-55**	**48,7**	45,0	52,2	**8,9**	6,3	13,5
		P2		46,0	38,0	55,0			
M. javanica	**7**	P1	**36-60**	**54,3**	46,8	59,8	**13,7**	9,0	18,0
		P2		49,0	36,0	56,0			
M. arenaria	**7**		**52-60**	**55,8**	52,2	59,9	**14,8**	10,8	19,8
M. hapla	**8**	P1	**33-70**	**59,1**	48,2	69,8	**15,7**	11,7	18,9
		P2		43,0	33,0	48,0			

P1 and P2 are different populations. Tail length values are given for each population but hyaline terminus values are given for each species. Tail length in J2 shows only a small coefficient of variation (5% on 50 µm tail).

Tail length

Average tail length in the 22 populations of *Meloidogyne* juveniles was 45.4µm. The range of mean tail lengths for the populations of juveniles was large from 37µm to 53µm (Table 4.8). Populations with short tails (< 40 µm) were P5a, P6 and P16; the long tail populations (tail >50 µm) are P10, P21a, P21b and P25. "Short" and "long" terms were arbitrarily assigned.

Hyaline tail terminus length

The range of mean length tail terminus for the populations of juveniles was large from 8.9µm to 14.6µm. Average tail terminus length for the 22 populations of *Meloidogyne* juveniles was 12µm. Populations with short tail terminus (< 9.8µm) were

Table 4.8 Morphometric features of *Meloidogyne* spp. second stage juveniles from different localities of Colombia. Measurements are in µm.

Pop	L	m.b.d.	Stylet ant. end	stylet	cone	DGO	met.	J	S-E pore	tail	group*	hyaline	group*	anus width	a	b	c
P1	332	10,7	12,3	8,6	4,4	2,7	48	64	72	37	i,j,h	12,2	i,j,h,a	7,4	31	5,3	9,2
P3a	350	14,0	13,0	9,1	4,9	2,2	47	64	69	46	i,j,h	12,0	i,j,h,a	11,4	25	5,4	7,6
P4	399	12,2	13,6	9,9	5,2	2,7	52	68	75	47	i,j,h	12,1	i,j,h,a	9,0	33	5,8	8,5
P5a	344	11,8	12,3	8,9	4,7	2,8	45	57	66	38	i,j,h	8,9	i	9,1	29	6,1	9,3
P6	341	12,0	12,5	8,8	4,1	2,3	48	65	68	39	i,j,h	10,6	i,j,h,a	8,5	29	5,3	8,8
P8	370	12,4	13,7	9,3	4,9	2,3	52	67	72	47	i,j,h	10,8	i,j,h,a	9,3	30	5,5	7,9
P9	320	11,8	13,1	8,9	3,9	1,7	48	63	66	42	i,j,h	12,6	i,j,h,a	8,5	27	5,1	8,2
P10	416	11,1	13,5	9,3	4,7	2,3	53	70	79	51	i,j,h	14,4	i,j,h,a	8,3	37	5,9	8,1
P11	350	11,4	13,3	9,4	4,9	2,7	47	61	69	42	i,j,h	10,7	i,j,h,a	8,6	31	5,8	8,4
P14a	415	11,5	13,6	12,7	5,2	2,3	54	71	76	50	i,j,h	12,3	i,j,h,a	8,1	36	5,9	8,2
P15	345	11,8	12,2	8,6	3,9	1,1	45	59	64	40	i,j,h	10,4	i,j,h,a		29	5,9	8.6
P16	339	10,9	12,5	9,1	4,8	2,5	46	58	66	39	i,j,h	9,3	i,j,h,a	8,4	29	5,5	8,4
P17	359	11,3	13,3	9,3	4,7	2,9	49	63	69	44	i,j,h	12,5	i,j,h,a	8,4	32	5,7	8,2
P18	392	11,4	13,4	9,8	5,3	2,0	50	66	75	47	i,j,h	11,5	i,j,h,a	9,2	34	5,9	8,4
P19a	317	12,1	13,1	9,3	4,4	2,3	46	62	65	41	i,j,h	11,0	i,j,h,a	8,5	27	5,3	7,7
P19b	338	11,8	13,9	10,3	4,9	2,8	49	69	72	46	i,j,h	11,6	i,j,h,a	9,5	29	5,1	7,4
P21a	435	11,4	14,3	10,6	5,6	2,2	53	71	79	53	i,j,h,a	14,6	j,h,a	8,1	39	6,2	8,3
P21b	436	11,6	14,2	11,4	5,4	2,6	54	71	80	53	i,j,h,a	13,2	i,j,h,a		38	6,1	8,2
P22	362	11,7	13,0	9,5	4,7	1,9	48	65	69	40	i,j,h	10,7	i,j,h,a	9,4	31	5,6	9,1
P24a	387	11,5	13,8	10,0	5,2	2,8	52	68	75	44	i,j,h	11,0	i,j,h,a	7,5	34	5,7	8,7
P24b	428	12,2	14,1	10,1	5,2	2,8	55	72	80	51	i,j,h	13,2	i,j,h,a	8,5	35	6,0	8,5
P25	427	11,4	14,2	10,8	5,6	2,7	53	71	78	51	i,j,h	13,8	j,h,a	7,9	38	6,1	8,4

*Jepson group based on measurements of tail and terminus length. Letters i, j, h, a mean *M. incognita, M. javanica, M. hapla*, *M. arenaria*, respectively. For relations between code-localities-host see table 4.2. hyaline: hyaline area of tail. Pure population are indicated in bold. Pop: population.

P5a and P16; populations with long tail terminus (>13.6µm) were P10, P21a and P25. "Short" and "long" terms were arbitrarily assigned.

Combinations of tail shape, tail and hyaline tail terminus length of juvenile nematodes from Colombia matched with descriptions of group 6, 7 and 8 of Jepson (1987). However, because measurements of tail and hyaline tail terminus length overlapped between the four common *Meloidogyne* species and most of Colombian populations matched to those species, these features were not helpful to distinguish between species (Table 4.7 and Table 4.8). Unique exception was the short hyaline tail terminus length present in population P5a which fitted into *M. incognita*. As mentioned for males, after a combinations of different methods used for species identification (see discussion), this assertion based on a single quantitative feature could be wrong.

Canonical discriminant analysis (CDA) of second-stage juveniles

Using the two main features selected for differentiation of *Meloidogyne* species in J2 (tail length and hyaline tail terminus length) the Canonical discriminant analysis (CDA) clearly separated three main groups (clusters) (Fig. 4.6) with percentage of variation for root 1 of 89.86% (Table 4.9, case 1). Group 1 included P10, P14a, P21a, P21b, P24b and P25; group 2 included P3a, P4, P8, P9, P11, P16, P17, P18, P19a, P19b and P24a; group 3 included P1, P5, P6, P15 and P22. Tail length (89.8 % variation in root1- value 0.34) was the best feature for separation of the populations.

CDA using a combination of 15 features: L, stylet, tail, hyaline area, DGO, GBD, a, b and c, junction, diameter at anus, S-E pore, cone, stylet to anterior end, metacorpus and stylet (Table 4.9 case 2) allowed only one separate group (Fig.4.6), similar to group 1 in the previous analysis and no clear cluster of other populations were observed. This can be explained by the low percentage of variation obtained (53.17%); no features were considered for separation of populations.

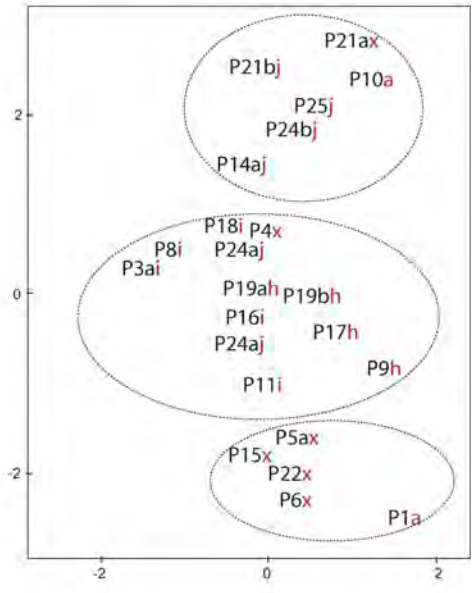
Isozyme - Electrophoresis

Nineteen phenotypes showing esterase and malate dehydrogenase activity were detected among 17 populations of *Meloidogyne* spp. from plantain and fruit crops (Table 4.10; Fig. 4.7). Because most of electrophoresis was made before purification of populations more than one phenotype was associated to each population. Thus, 35% of

Table 4.9 Standardized coefficients for canonical variates of second juvenile stages of Colombian *Meloidogyne* populations.

	Root 1	Root 2
Case 1 (2 features)		
% of variation	**89.86**	10.14
Selected characters	Vector loadings	
Tail length	0.3479	-0.1716
Hyaline tail terminus length	0.1824	0.7045
Case 2 (15 features)		
% of variation	**53.17**	15.06
Selected characters	Vector loadings	
DGO	0.963	0.797
m.b.w	2.481	-2.637
J	2.237	3.040
L	-0.527	-0.533
S-E pore	-0.112	0.023
Tail	-0.013	0.634
Diameter at anus	0.421	-0.613
a	0.747	-0.641
b	26.424	37.094
c	0.986	3.110
Cone length	-0.033	-0.192
Hyaline tail end	-0.128	0.060
metacorpus	0.045	0.138
Stylet to ant. end	-0.103	-0.509
stylet	0.187	0.106

Canonical variate case 1



Canonical variate case 2

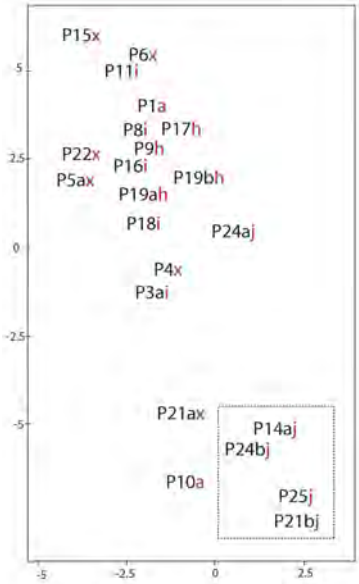


Fig. 4.6 Canonical discriminant analysis of 22 *Meloidogyne* populations from Colombia for juveniles performed with 2 morphometric variables: tail length and hyaline tail terminus length (case1) and performed with 15 morphometric variables: tail, L, hyaline area, stylet, DGO, m.b.w, a, b , c, metacarpus, S-E pore, J, anal body width, cone, stylet to ant. end distance (case2). Red letters mean: i, *M .incognita*; j, *M. javanica*; h, *M. hapla*; a, *M. arenaria* and x, indefinite species.

populations yield one phenotype; 53% showed two different phenotypes and 12% showed three different phenotypes (Table 4.16). Because only few lines were successful in gel 2, 7, 8, 9, 11, 15, 23 and 25, those are not show in figure 4.7.

M. javanica from tomato plants with characteristic phenotype J3N1 (Est-Rm 0.46, 0.55, 0.59; Mdh-Rm: 0.24) was used as reference (P25). The reference population showed between 3 to 5 esterase bands and 0 to 3 malate dehydrogenase bands (Table 4.10) in the different gels.

M. incognita phenotypes

53% of Colombian populations yielded *M. incognita* phenotypes (Table 4.16). I1N1 phenotype as reported for *M. incognita* by Esbenshade and Trianphyllou (1990) was observed in five populations: P3a, P8, P11, P16 and P18 Rm values for esterase and malate dehydrogenase were 0.46 and 0.24, respectively.

The I2N1 phenotype was displayed by two populations: P16 and P18 (Est-Rm: 0.46, 0.48; Mdh-Rm: 0.24). This phenotype is linked to *M. incognita*. Seven populations (P3a, P5a, P8, P16, P18, P20 and P22 showed I2N2a phenotype with Est-Rm: 0.46, 0.48 and Mdh-Rm: 0.24 - 0.26. Esterase bands of this phenotype matched to *M. incognita* but no two bands had been reported for this species. One additional phenotype I2N2b with two esterase bands, but Mdh-bands faster than I2N2a was observed in population P23.

Females of P8 (I1N0), P18 (I2N0) and P20 (I2N0) showed one or two esterase bands but no malate dehydrogenase bands. These phenotypes could be linked to *M. incognita* based on their respective Est-Rm: 0.46; 0.46, 0.48.

M. javanica phenotypes

Typical *M. javanica* phenotypes (J3N1) were observed in 6 Colombian populations (P13, P14a, P16, P18, P19a and P24b) which yielded Rm averages similar to that of the reference phenotype. P6, P16 and P18 (J3N0) gave an esterase pattern similar to *M. javanica* control but no malate dehydrogenase bands were observed.

In gel 12, females of population P16 and P18 showed extremely slow esterase and malate dehydrogenase migration rates (Fig. 4.7). Because the reference *M. javanica* showed similar result, problems during gel running could be expected.

Table 4.10 Esterase (Est) and malate dehydrogenase (Mdh) phenotypes observed in a study of 17 populations of *Meloidogyne* spp. from Colombia.

	M. hapla	***M. incognita***						***M. javanica***					***M. arenaria***		**Rare phenotypes**				
Phenotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15*	16	17	18	19
Est Mdh	**H1 H1**	**I1 N0**	**I1 N1**	**I2 N0**	**I2 N1**	**I2 N2a**	**I2 N2b**	**J3 N0**	**J3 N1**	**J3 N2**	**J5 N2**	**J3 N3**	**A1 N2**	**A4 N2**	**E1a N1a**	**E1b N1b**	**E3 N1**	**E3 N2**	**E2 N2**
Population																			
P3a			X_{24}			X_{14}													$X_{15,24}$
P5a						X_{9}								X_{19}				X_{1}	
P6								X_{19}											
P8		X_{16}	X_{26}			X_{16}							X_{16}						
P10																			X_{21}
P11			X_{26}																$X_{21,26}$
P13									X_{27}										
P14a									X_{5}							X_{5}			
P16			$X_{4,19,24}$		X_{4}	X_{25}		X_{24}	$X_{12,13}$		X_{13}								
P18			X_{4}	X_{24}	X_{4}	X_{19}		X_{24}	$X_{12,13}$										
P19a	X_{11}								X_{11}								X_{3}		
P19b	$X_{3,11}$																X_{3}		
P20				X_{6}		$X_{6,22}$									X_{22}				
P22						X_{25}													
P23	X_{23}						X_{23}												
P24a											X_{10}								
P24b									X_{10}										
P25 control								$X_{16,24}$	$X_{3,4,7,8,}$12,13,26	$X_{6,7,9,14,19,23}$	$X_{10,22,25}$	X_{5}							

As indicated by Esbenshade & Triantaphyllou (1985) each phenotype is designed by a letter suggestive of the species it specifies (H= *hapla*, I=*incognita*, J=*javanica* and A= *arenaria*) and a number indicative of the number of bands present. Numbers marked in subscript indicate number of the gel. E and N mean esterase and malate dehydrogenase patterns unknown, respectively. Because mistakes happened during phastsystem process , no successful results were obtained for populations P1, P2, P4, P7, P9, P12, P15, P17, P21a and P21b. *E1aN1a fit to *M. paranaensis*/ *M. konaensis phenotype*.

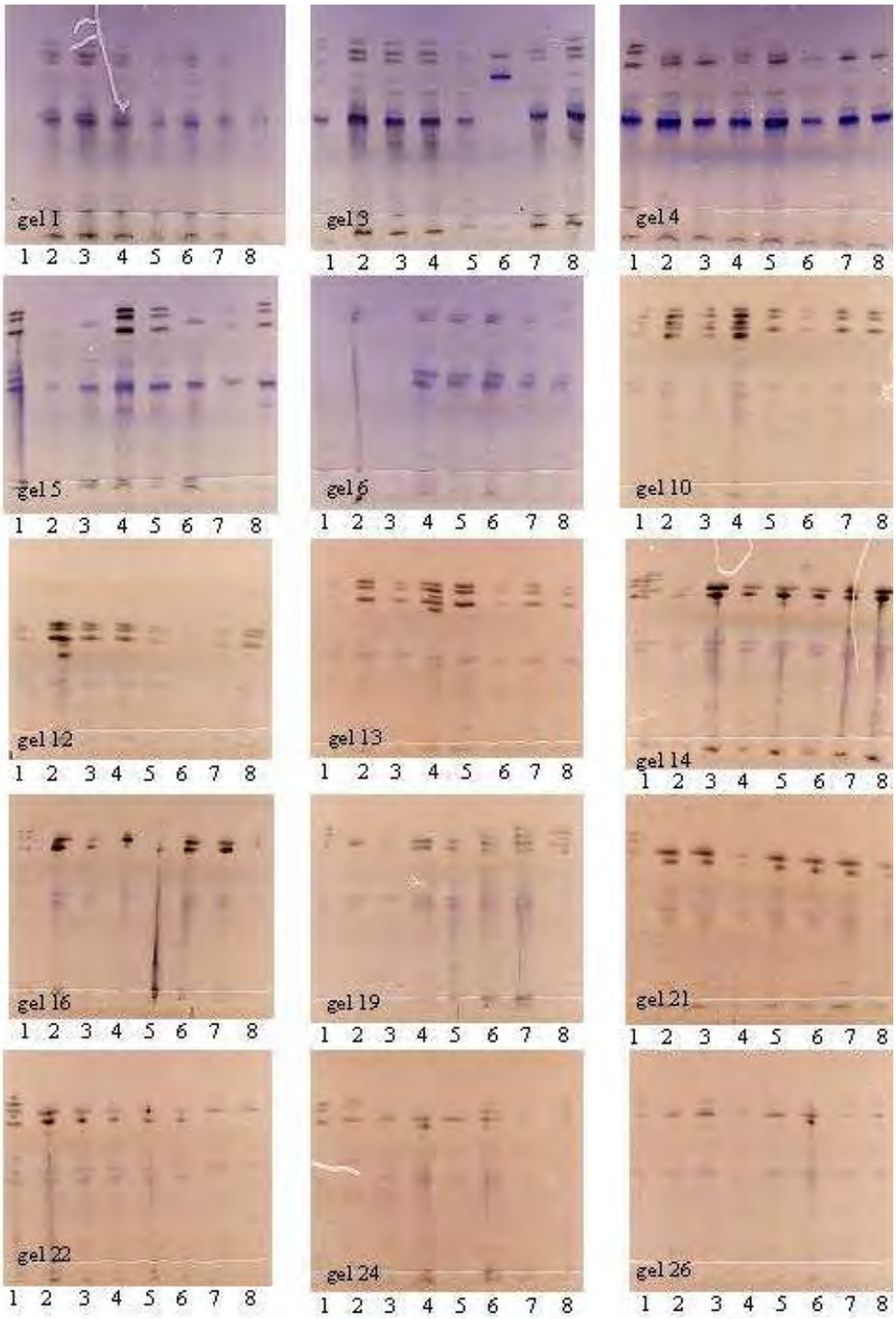


Fig. 4.7 Photograph of a polyacrymlamide gel slab illustrating some of esterase and malate dehydrogenase phenotypes detected in 18 *Meloidogyne* populations from Colombia. For explanations see text (isozyme electrophoresis). Because only few lines were successful in gel 2, 7, 8, 9, 11, 15, 23 and 25, those are not show here. In gels, negative and positive charges match to bottom and top, respectively.

The phenotypes J5N2 (P16 and P24a) was similar to J3N2 based on its Rm-esterase bands but it showed two additional esterase bands: one band between bands 2-3 and one band slight slower than band 1. However, problems of the phastsystem apparatus that result in the production of double bands cannot be excluded. The same potential problem is possible for population P25 (reference) which showed three Mdh bands (J3N3) in gel 5. 47% of Colombian populations showed as minimum one *M. javanica* phenotype.

M. arenaria phenotypes

Phenotypes A1N2 (Rm-esterase 0.53; Rm-Mdh 0.24, 0.26) and A4N2 (Rm-esterase 0.47, 0.50, 0.53, 0.56; Rm-Mdh 0.24, 0.26) were detected in populations P8 and P5a, respectively. Esterase patterns of those populations matched to *M. arenaria.* 12% of Colombian populations showed as minimum one *M. arenaria* phenotype.

M. hapla phenotypes

H1N1 as reported for *M. hapla* by Esbenshade & Trianphyllou (1990) were observed in three populations: P19a, P19b and P23. Rm values for esterase and malate dehydrogenase were 0.50 and 0.37, respectively. 18% of Colombian populations showed as minimum one *M. hapla* phenotype.

M. paranaensis / M. konaensis phenotypes

Phenotype **E1aN1a** (named phenotype 15 in tables 4.10, 4.16 and 4.17) (Fig. 4.7, gel 22) with Est-Rm 0.57 and Mdh-Rm: 0.26 was detected in population P20. Esterase pattern of this population matched to *M. paranaensis* / *M. konaensis* phenotypes.

Rare phenotypes

The remaining phenotypes were not species specific and were designated, for convenience, by the letter E for esterase, and N, standing for "non-specific phenotype" of malate dehydrogenase and a number indicating the number of bands of activity.

Four rare phenotypes were found in following populations: **E1bN1b** (named phenotype 16 in tables 4.10, 4.16 and 4.17) in population P14a (Fig. 4.7, gel 5) with Est-Rm 0.50; Mdh-Rm: 0.24; **E2N2** (named phenotype 19 in tables 4.10, 4.16 and 4.17) in

populations P3a, P10 and P11 (Fig. 4.7, gel 24, 26) with Est-Rm 0.44, 0.46 and Mdh-Rm: 0.22, 0.24. Phenotype **E3N1** (named phenotype 17 in tables 4.10, 4.16 and 4.17) with Est-Rm 0.46, 0.50, 0.59 and Mdh-Rm: 0.24; and phenotype **E3N2** (named phenotype 18 in tables 4.10, 4.16 and 4.17) with Est-Rm 0.46, 0.50, 0.59 and Mdh-Rm: 0.24, 0.26 were detected in populations P19a and P19b (Fig. 4.7, gel 3) and population P5a (Fig. 4.7, gel 1), respectively. Those four rare phenotypes (47% in Table 4.16) were not characteristic of any particular species.

Species specific PCR

Prior to use specific primer, DNA presence was checked in samples with primers described by De Ley *et al.* (1999) (Fig. 4.8). No amplification could be observed when *Meloidogyne* populations P5a, P16 and P25 were used as template DNA.

Using species-specific primers designed from RAPD fragments (Zijlstra *et al.*, 2000) and from IGS regions (Wishart *et al*., 2002) the PCR amplified DNA products of different size for 16 of the 29 *Meloidogyne* populations tested.

The Finc/Rinc primer set (used to detect *M. incognita*) amplified a 1200 bp fragment when populations P3a, P8, P11 and P20 were used as template (Fig. 4.9). Similarly, the primer Fjav/Rjav primer set (used to detect *M. javanica*) resulted in a670 bp amplicon when populations P14a, P21b, P24a and P24b were used as template (Fig. 4.10); the primer JMV1/JMV2hapla primer set (used to detect *M. hapla*) resulted in a 440 bp amplicon when populations P9, P15, P17, P19a and P19b were used as template (Fig. 4.11); the specific primer pair Far/Rar amplified a 420bp fragment when populations P1, P10 and P12 were used as template (Fig. 4.12).

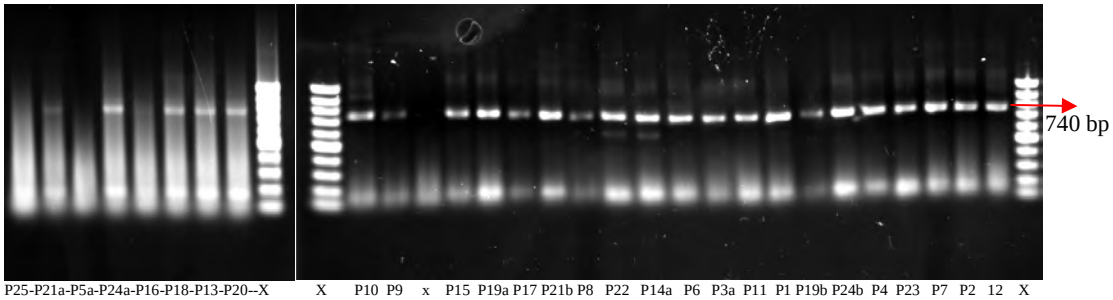


Fig. 4.8 PCR products (≈740bp) of *Meloidogyne* spp. obtained after using primers and PCR conditions for D2D3 amplifications as described by De Ley *et al.* (1999) (X = DNA ladder, Invitrogen). P25 to P12 are *Meloidogyne* populations (see table 4.2).

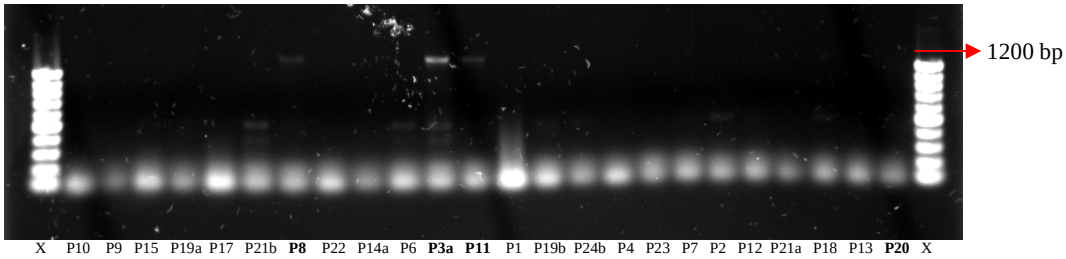


Fig. 4.9 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair Finc/Rinc for *M. incognita* populations. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen).

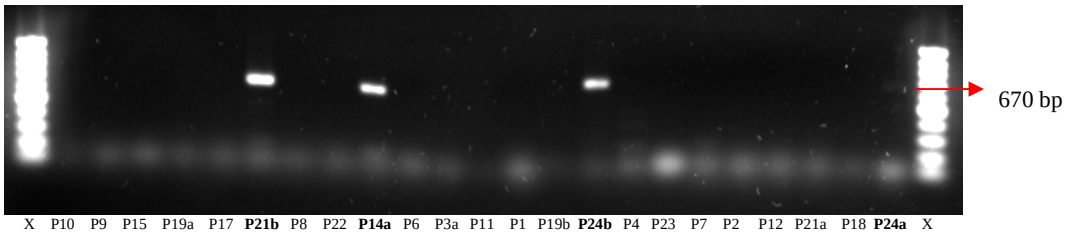


Fig. 4.10 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair Fjav/Rjav for *M. javanica* populations. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen).

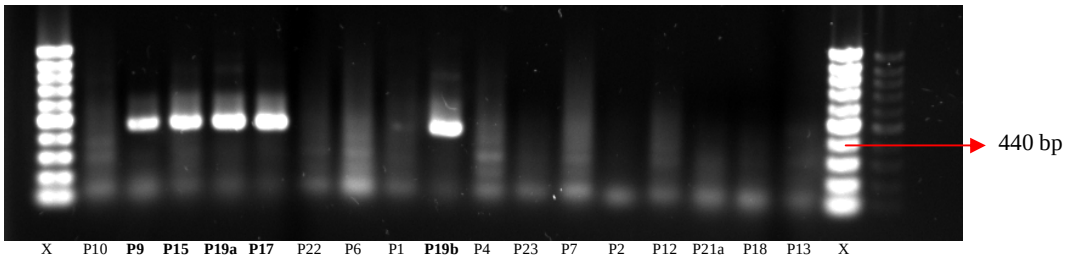


Fig. 4.11 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair JMV1/JMVhapla for *M. hapla* populations. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen).

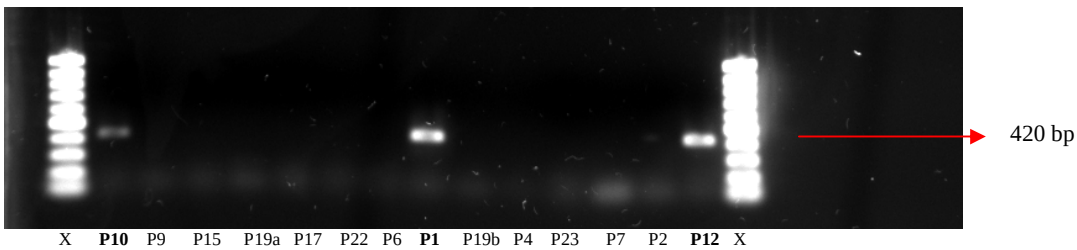


Fig. 4.12 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair Far/Rar for *M. arenaria* populations. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen).

No amplification could be observed when *Meloidogyne* populations P2, P4, P6, P7, P13, P18, P21a, P22 and P23 were used as template DNA.

No bands were obtained using the specific primer: JMV1 52-GGATGGCGTGCTTTCAAC-32 JMV2 52 TTTCCCCTTATGATGTTTACCC-32 which yield a 540 bp amplicon for *M. chitwoodi* and a 670 bp for *M. fallax* (Wishart *et al*., 2002) (Fig. 4.13).

Finc/Rinc, Fjav/Rjav, Far/Rar; JMV1/JMV2hapla primer set yielded patterns with clear bands that based on its size clearly distinguished one species from the others. DNA products of 1200bp, 670 bp, 420 bp and 440 bp were associated with *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, respectively (Fig. 4.14).

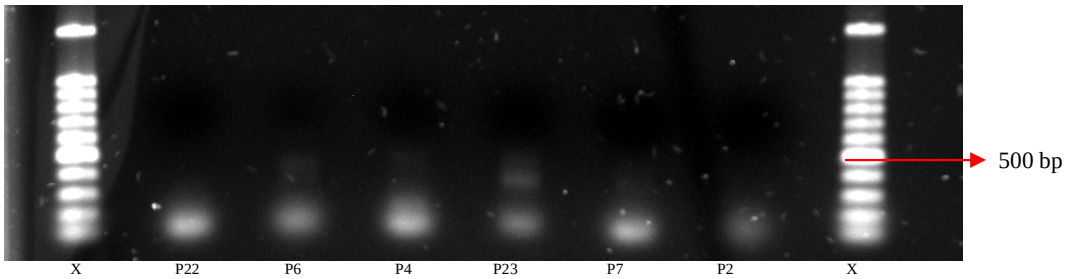


Fig. 4.13 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair JMV1/JMV2 for *M. chitwoodi* and *M. fallax* populations. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen).

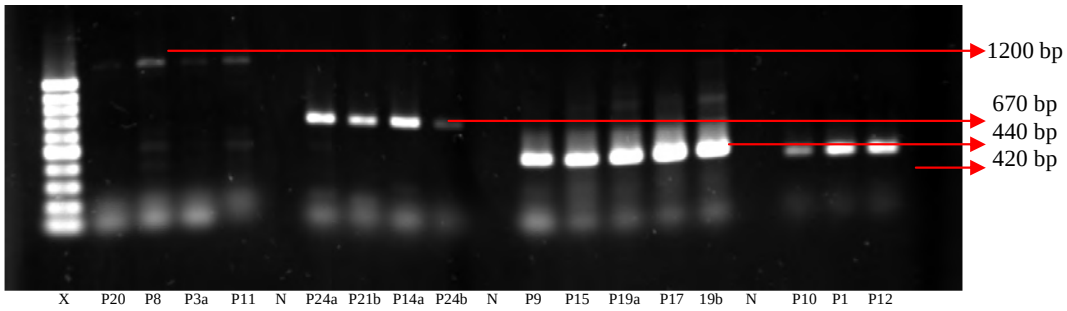


Fig. 4.14 Agarose gel electrophoresis of the polymerase chain reaction amplified fragment with the specific primer pair Finc/Rinc; Fjav/Rjav, Far/Rar and JMV1/JMV2hapla for *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, respectively. Numeric codes are listed in table 4.2. X: 1kb DNA marker (Invitrogen). Negative control without DNA template: lane N.

Molecular characterization

All obtained sequences of *Meloidogyne spp.* Colombian populations were deposited in EMBL (Table 4.11). Because the DNA quantity extracted from some samples was not enough or the reaction failed, not all populations could be analyzed for the three different regions of rDNA.

Table 4.11 Sequences size of *Meloidogyne* populations from Colombia.

Population code	D2D3 bp	18S bp	ITS-bp	Population code	D2D3 bp	18S bp	ITS bp
P1	736	1747	732	**P14b**	738	1780	-
P2	729	1756	727	**P15**	732	1749	675
P3a	736	1759	-	**P16**	738	1741	-
P3b	738	1934	-	**P17**	649	1751	721
P4	729	1749	-	**P19a**	738	1760	-
P5a	735	1738	-	**P19b**	740	1750	-
P5b	753	1733	-	**P19c**	740	1785	-
P6	738	1752	-	**P21b**	737	1758	592
P7	737	1750	669	**P22**	742	1737	-
P8	731	1747	-	**P23**	743	1750	727
P9	-	1741	-	**P24a**	740	-	-
P10	737	1740	-	**P24b**	730	1752	684
P11	738	1755	-	**P24c**	735	1957	-
P12	730	1757	676	**P25**	740	-	-
P14a	733	1750	579				

X: Populations with unsuccessful reaction because of insufficient DNA or contamination. bp: number of base pairs (sequence size).

Based on phylogenetic analysis of rDNA sequences, Colombian populations were grouped in three clear clades: clade 1 included the meiotic and mitotic parthenogenetic species *M. hapla* A and B, respectively; clade 2 comprised one *M. hapla* population (P15) and one *M. arenaria* (P1) as well as five unidentified species (P5a, P22, P21a6, P2 and P5B; unknown reproductive strategy); and clade 3 comprised three mitotic parthenogenetic species (*M. incognita, M. arenaria,* and *M. javanica*). See figures 4.15, 4.16 and 4.17.

28S rDNA

The polymerase chain reaction amplified a single DNA product of about 735 bp (649-753) for each of the 28 *Meloidogyne* populations. D2D3 rDNA region sequences obtained were subsequently used for phylogenetic analysis. Additionally, comparisons with 18 GenBank sequences of *Meloidogyne* spp. were included.

In GenBank 76 sequences from the *Meloidogyne* 28S rDNA gene were available. They were submitted by 19 authors and concerned 17 species: *M. incognita* (12), *M. javanica* (6), *M. arenaria* (15), *M. hapla* (17), *M. chitwoodi* (7), *M. exigua* (3), *M. paranaensis* (3), *M. thailandica* (3), *M. fallax* (2), *M. artiellia* (1), *M. baetica* (1), *M. graminicola* (1), *M. konaensis* (1), *M. minor* (1), *M. naasi* (1), *M. panyuensis* (1), *M. tripholiophila* (1). Only sequences longer than 600 bp and homologous with Colombian populations were used for comparison and phylogenetic trees, i.e. 18 accessions of 11 species (Table 4.12). Sequences of *M. javanica, M. thailandica* (296bp), *M. fallax* (325bp), *M. minor* (709bp), *M. naasi* (824bp), *M. panyuensis* (870bp) were not homologous with Colombian *Meloidogyne* sequences or did not present the full length of the D2D3 region. So, no sequences of *M. javanica* were used since the accession of this species from GenBank did not align with Colombian sequences.

Comparison of the D2D3-LSU sequences at inter-populations level of *Meloidogyne* spp. from Colombia showed intra-generic divergence between 0 and 12.4% (Table 4.13). Percentages of divergence ranging between 0.5 and 2.1% were obtained within populations in clade 1; 0.4 and 1.2% for clade 2 and 0 to 3.5% within population in clade 3. The interspecific variation was much greater with conspicuous divergences when *Meloidogyne* sequences from GenBank were included in the analysis (0-30.5%).

The relationship of the detected populations with other *Meloidogyne* species was measured through Bayesian inference (BI) analysis. The obtained alignment presented 651 characters of which 263 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, three well supported clades that contain Colombian populations could be appointed (Fig. 4.15). Clade 1 (PP=1.00) included P17, 19a, P19b, P19c and two GenBank accessions of *M. hapla*; clade 2 (PP=0.98) included P1, P2, P5a, P5b, P15, P22 and P23. Populations P3a, P3b, P6, P7, P8, P10, P11, P12, P14a, P14b, P16, P21b, P24a, P24b, P24c and P25, seven GenBank accession

Table 4.12 Accession number of *Meloidogyne* species from GenBank used for comparison with *Meloidogyne* spp. sequences from Colombia.

Species	28S	author	18S Accession	author	ITS Accession	author
M. arabicida			AY942625	Tigano *et al.*, 2005		
M. ardenensis			AY593894	Helder *et al.* **		
M. arenaria	U42342	Georgi & Abbott, 1998	U42342	Georgi & Abbott, 1998	AF077086	Hugall *et al.*, 1999
	U42339	Georgi & Abbott, 1998	AY942623	Tigano *et al.*, 2005	AF077087	Hugall *et al.*, 1999
	AF435803	De Ley *et al*, 2005	AY268118	Lee & Williamson *	U96301	Powers *et al.*, 1997
					AY438554	Liao *
					AF387092	Sui *et al.* *
					AF077083	Hugall *et al.*, 1999
					AF510061	Quader & Rilley *
					AF510062	Quader & Rilley *
					AF510063	Quader & Rilley *
M. artiellia	AT150369	Castillo *et al.*, 2003	AF248477	De Giorgi *et.al.*, 2002		
M. baetica	AY150367	Castillo *et al.*, 2003				
M. chiwoodi	AF435802	Tenente *et al.*, 2004	AY593883	Helder *et al.* **	AY281852	Karssen *et al.*, 2004
			AY593884	Helder *et al.* **	U96302	Powers *et al.*, 1997
			AY593885	Helder *et al.* **	AF077090	Hugall *et al.*, 1999
			AY593886	Helder *et al.* **		
			AY593887	Helder *et al.* **		
			AY593888	Helder *et al.* **		
			AY593889	Helder *et al.* **		
M. ethiopica			AY942630	Tigano *et al.*, 2005		
M. exigua	AF435795	Tenente *et al.*, 2004	AY942627	Tigano *et al.*, 2005		
	AF435796	Tenente *et al.*, 2004				
	AF435804	Tenente *et al.*, 2004				
M. fallax			AY593895	Helder *et al.* **	AY281853	Karssen *et al.*, 2004
M. floridensis			AY949621	Tigano *et al.*, 2005		
M. graminicola	AF435793	Tenente *et al.*, 2004				
M. hapla	DQ145641	Nadler *et al*, 2006	AY593892	Helder *et al.* **	AF516722	Quader & Rilley *
	DQ328685	Subbotin *et al*, 2006	AY942628	Tigano *et al*, 2005	AF007089	Hugall *et al.*, 1999
			AY268119	Lee & Williamson *	AY281854	Karssen *et al.*, 2004
			AT593893	Helder *et al.* **	AY268108	Lee & Williamson *
			AY593898	Helder *et al.* **	AF516721	Quader & Rilley *

Species	28S	author	18S Accession	author	ITS Accession	author
					U96303	Powers *et al.*, 1997
					AF077088	Hugall *et al.*, 1999
M. incognita	AF435794	De Ley *et al*, 2005	U81578	Frisse *et.al* *	U96304	Powers *et al.*, 1997
			AY942624	Tigano *et al.*, 2005	AY438556	Liao *
			AY284621	Helder *et al.* **	AF516723	Quader & Rilley *
			AY268120	Lee & Williamson *	AF510064	Quader & Rilley *
					AF387093	Sui *et al.* *
					AF077084	Hugall *et al.*, 1999
M. javanica			AY942626	Tigano *et al.*, 2005	AF387095	Sui *et al.* *
			AY268121	Lee & Williamson *	AF077085	Hugall *et al.*, 1999
					U96305	Powers *et al.*, 1997
					AY829374	Vovlas *et.al.*, 2005
					AY829375	Vovlas *et.al.*, 2005
					AY438555	Liao *
					AF510057	Quader & Rilley *
					AF510058	Quader & Rilley *
					AF510059	Quader & Rilley *
					AF510060	Quader & Rilley *
					AF387094	Sui *et al.* *
					AF077082	Hugall *et al.*, 1999
M. konaensis	AF435797	De Ley *et al*, 2005				
M. mayaguensis			AY942629	Tigano *et al.*, 2005		
M. minor			AY593899	Helder *et al.* **	AY281855	Karssen *et al.*, 2004
M. morocciensis			AY942632	Tigano *et al.*, 2005		
M. naasi			AY593900	Helder *et al.* **	AY302249	Karssen *et al.*, 2004
			AY593901	Helder *et al.* **		
			AY593902	Helder *et al.* **		
M. oryzae			AY952631	Tigano *et al.*, 2005		
M. panyuensis					AY394719	Liao *
M. paranaensis	AF735798	De Ley *et al*, 2005	AY942622	Tigano *et al.*, 2005		
	AF735799	De Ley *et al*, 2005				
	AF735800	De Ley *et al*, 2005				
M. trifoliophila	AF435801	De Ley *et al*, 2005			AF077091	Hugall *et al.*, 1999

* unpublished; ** patent No. PCT/NL2004/000247)-A 01-Apr-2004

of *M. incognita, M. arenaria, M. konaensis* and *M. paranaensis* composed a clade 3 on their own (PP=0.99). Clade 0 included only Genbank sequences.

18S rDNA

The polymerase chain reaction amplified a single DNA product of nearly 1765 bp (1733-1957) for each of the 27 *Meloidogyne* populations when the 18S rDNA region were analyzed (Table 4.11). Additionally, comparisons with 36 GenBank sequences of *Meloidogyne* spp. were established.

In GenBank 185 sequences from the *Meloidogyne* 18S rDNA gene were available. They were submitted by 10 authors and concerned 25 species: *M. incognita* (8), *M. javanica* (12), *M. arenaria* (12), *M. hapla* (44), *M. chitwoodi* (26), *M. graminicola* (33), *M. mayaguensis* (12), *M. graminis* (4), *M. arabicida* (1), *M. ardenensis* (1), *M. artiellia* (2), *M. enterolobii* (1), *M. ethiopica* (1), *M. exigua* (1), *M. fallax* (5), *M. floridensis* (2), *M. haplanaria* (2), *M. minor* (2), *M. morocciensis* (2), *M. naasi* (4), *M. oryzae* (1), *M. paranaensis* (2), *panyuensis* (1), *M. partytila* (2), *M. thailandica* (2). Only sequences longer than 1600 bp and homologous with Colombian populations were used for comparison and phylogenetic trees, i.e. 36 accessions of 18 species (Table 4.12).

The comparison of the 18S-SSU sequences at inter-populations level of *Meloidogyne* spp. from Colombia showed intra-generic divergence ranging between 0 to 4.9% (Table 4.14). Percentages of divergence within populations ranged between 0.4 and 0.5% in clade 1; 0.4 and 1.3% for clade 2 and 0.1 to 1.4% within population in clade 3. The interpopulation variation was much greater with conspicuous divergences when *Meloidogyne* sequences from GenBank were included in the analysis (0-11.2%).

The relationship within *Meloidogyne* species was measured through Bayesian inference (BI) analysis. The obtained alignment presented 1690 characters of which 202 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, three well supported clades that contain Colombian populations could be appointed (Fig. 4.16). Clade 1 (PP=1.00) included P9, P17, P19a, P19b and P19c and four GenBank records of *M. hapla* and *M. ardenensis*; clade 2 (PP=0.98) included P1, P2, P5a, P5b, P15, P22 and P23 and one GenBank record of *M. ethiopica*. Populations P3a, P3b, P4, P6, P7, P8, P10, P11, P12, P14a, P14b, P16, P21b, P24b and P24c and 13

Table 4.13 Pairwise D2D3 sequences (651bp) alignment of *Meloidogyne* Colombian populations

% Similarity															
	Clade 1								Clade 2				Clade 3		
Populations	P3b,10, 12, 14b ;21b, 24, 24c	P7= P25	P3a=11=24a	P4	P14a	P16	P8	P6	P2= P22	P1= P5a	P5b= P23	P15	P19b= P19c	P17	P19a
P3b=P10= P12= P14b=P21b= P24b= P24c=	100	99.8	99.5	99.3	98.8	98.2	97.7	97.6	95.5	95.2	94.9	94.7	89.2	88.8	87.6
P7= P25		100	99.3	99.5	98.7	98.4	97.9	97.4	95.7	95.7	95.0	94.9	89.4	88.9	87.8
P3a=P11= P24a			100	99.2	98.4	97.7	97.7	97.4	95.0	94.7	94.4	94.6	98.1	88.9	87.5
P4				100	98.2	97.9	97.6	97.3	95.2	94.9	94.6	94.4	88.9	88.5	87.3
P14a					100	97.1	96.6	96.5	94.4	94.1	93.8	93.6	88.1	87.8	86.5
P16						100	97.4	96.9	96.3	96.0	95.7	95.5	89.4	88.9	87.8
P8							100	97.4	96.0	95.7	95.4	95.2	89.4	89.6	87.8
P6								100	96.7	96.1	95.8	95.7	89.7	89.6	88.1
P2=P22									100	99.6	99.3	99.2	89.7	89.2	88.1
P1=P5a										100	99.3	99.2	89.4	88.9	87.8
P5b= P23											100	98.8	89.1	88.6	87.5
P15												100	89.2	88.8	87.8
P19b=P19c													100	99.5	98.4
P17														100	97.9
P19a															100

Table 4.14 Pairwise 18S sequences (1690bp) alignment of *Meloidogyne* Colombian populations

	% Similarity														
	Clade 1								Clade 2				Clade 3		
Populations	P12=	P3b	P3a=P10	P7	P14a=P21b	P4	P6	P8=	P1=P2=	P23	P5a	P15	P9=P17	P19a	P19c
P12=P14b	100	99.9	99.8	99.7	99.6	99.3	99.0	98.9	98.7	98.5	98.4	97.9	95.7	95.4	95.2
P3b		100	99.8	99.6	99.6	99.4	99.1	99.0	98.8	98.5	98.4	98.0	95.8	95.4	95.3
P3a=P10= P11			100	99.6	99.5	99.2	99.1	98.9	98.7	98.5	98.4	97.8	95.7	95.3	95.2
P7=P24c				100	99.4	99.1	98.9	98.9	98.6	98.4	98.4	97.9	95.5	95.2	95.0
P14a=P21b= P24b					100	99.0	98.7	98.6	98.4	98.2	98.1	97.6	95.4	95.1	94.9
P4						100	99.2	98.7	99.0	98.7	98.5	98.1	95.9	95.6	95.4
P6							100	99.0	99.4	99.2	99.1	98.7	95.9	95.5	95.4
P8= P16								100	98.7	98.5	98.5	98.6	95.5	95.2	95.0
P1=P2= P5b= P22									100	99.6	99.5	99.0	95.9	95.5	95.4
P23										100	99.5	98.7	95.6	95.2	95.1
P5a											100	9.88	95.6	95.3	95.1
P15												100	95.1	94.9	94.7
P9=P17=P19b													100	99.6	99.5
P19a														100	99.5
P19c															100

GenBank records a of *M. incognita, M. javanica, M. arenaria, M. konaensis* and *M. paranaensis, M. floridensis, M. arabicida* and *M. morocciensis* composed a separate clade 3 (PP=0.96). Additionally, GenBank accessions of *Meloidogyne* containing mainly temperate species were consistently placed as a separate clade which was named clade 0.

ITS1-5.8-ITS2 rDNA

The polymerase chain reaction amplified a single DNA product of nearly 678 bp (579-732) for each of the 10 *Meloidogyne* populations of which ITS rDNA regions were analyzed (Table 4.11). The complete sequence included ITS1-5.8-ITS2 regions. Additionally, comparisons with 42 GenBank sequences of *Meloidogyne* spp. were established.

In GenBank almost one thousand *Meloidogyne* ITS sequences were found, derived from 15 species: *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi, M. thailandica, M. fallax, M. artiellia, M. graminicola, M. minor, M. naasi, M. panyuensis, M. tripholiophila, M. mayaguensis* and *M. dunensis.* Only sequences longer than 650 bp and homologous with Colombian populations were used for comparison and phylogenetic analysis, or 42 accessions of 10 species (Table 4.12). Because some sequences of *M. arenaria, M. hapla, M. thailandica, M. artiellia, M. graminicola, M. mayaguensis* and *M. dunensis* were not comparable or shorter than 660 bp they were not used for comparison with Colombian *Meloidogyne* sequences.

The comparison of the ITS sequences at inter-populations level of *Meloidogyne* spp. from Colombia showed a divergence that ranged between 0 and 13.4% (Table 4.15). Since P17 was the unique Colombian population in clade 1 (however this clade included also seven accessions from GenBank), no divergence was considered. Percentages of divergence range between 0 and 0.5% for clade 2 and 0.3 to 1.3% within population in clade 3. The interspecific variation was much greater with conspicuous divergences when *Meloidogyne* sequences from GenBank were included in the analysis (0 - 43.7%).
The obtained alignment presented 669 characters of which 309 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, three well supported clades that contain Colombian populations could be appointed (Fig. 4.17). Clade 1 (PP=1.00) contained P17 and seven GenBank accessions of *M. hapla* and one of

M. javanica; clade 2 (PP=0.84) included P1, P2, P15 and P23 and two GenBank sequences of *M. arenaria* and *M. javanica*, respectively. Populations P7, P12, P14a, P21b and P24b and 23 GenBank of *M. incognita, M. javanica, M. arenaria* composed a separate clade 3 (PP=0.99). Additionally, GenBank accessions of *Meloidogyne* containing mainly temperate species were consistently placed as a separate clade which was named clade 0.

Additional to miajor *Meloidogyne* species, some GenBank sequences clustered with *Meloidogyne* populations from Colombia. For 28S, *M. paranaensis* (accessions AF735798, AF735799, AF735800) resolved as sister taxa of Colombian population P4; and *M. konaensis* (AF435797) with *M. incognita, M. javanica, M. arenaria* sequences from Colombia within clade 3. For 18S, *M. ardenensis* (AY593894) was positioned in clade 1 together with *M. hapla; M. ethiopica* (AY492630) was located with Colombian *Meloidogyne* populations included in clade 2. For clade 3, *M. arabicida* AY492625 and *M. paranaensis* AY492622 were resolved as sister taxa, but these species were placed separate from Colombian populations in the phylogenetic tree. Additionally *M. morocciensis* (AY492632) and *M. floridensis* (AY 949621) were resolved as sister taxa to some Colombian populations (P3a, P3b, P7 and P11).

Table 4.15 Pairwise ITS sequences (669bp) alignment of *Meloidogyne* Colombian populations

% Similarity							
	Clade 1				Clade 2		Clade 3
Populations	P14a=P24b	P12	P21b	P7	P1=P2	P15=P23	P17
P14a=P24b	100	99.7	99.5	98.9	86.8	86.6	71.2
P12		100	99.5	99.1	86.8	86.6	71.0
P21b			100	98.7	86.6	86.4	70.8
P7				100	86.2	86.0	70.4
P1=P2					100	99.5	74.1
P15=P23						100	74.3
P17							100

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Sequence divergences as showed in tables 4.13, 4.14, 4.15 agreed with the topology of the trees from the three DNA regions for all Colombian populations studied. Some GenBank accessions mentioned in this paragraph did not show divergence with some Colombian populations (data not shown).

Discussion

Combination of results using different methods, before and after purification are shown in table 4.16 and 4.17.

Morphological identification

Female: perineal pattern

Observations of perineal pattern showed unique patterns in 30% of 23 Colombian populations, while 35%, 17% and 17% of populations showed two, three and four different patterns, respectively. However in purified populations (P19a, P19b, P21a, P21b, P24a and P24b) 100% of perineal patterns showed the main features of their respective species: *M. hapla* pattern for populations P19a and P19b and *M. javanica* pattern for populations P21a, P21b, P24a and P24b.

Most specimens of each one of Colombian populations showed a similar perineal pattern, especially in pure populations. These observations are in agreement with Dropkin (1953) and Sasser (1954). Both authors observed intraspecific variation, though the perineal pattern remained relatively constant within a species and was not changed by host influence. Former results are different from the observations by Allen (1952) and Netscher (1978) who noticed morphological pattern variation within a single egg-population of *Meloidogyne* spp.

Since Chitwood (1949) re-established the genus *Meloidogyne* and described five new species, the structure of the perineal pattern has become the major character used in species identification; although often this has been supplemented by up to 140 other characters from the different life stages (Esser & Perry, 1974; Esser *et al*., 1976; Franklin, 1979; Karssen & Van Aelst, 2001; Taylor & Sasser, 1978; Whitehead, 1968). About half of the *Meloidogyne* species identification has been based on the female perineal pattern. However, the number of new species increased rapidly so that it

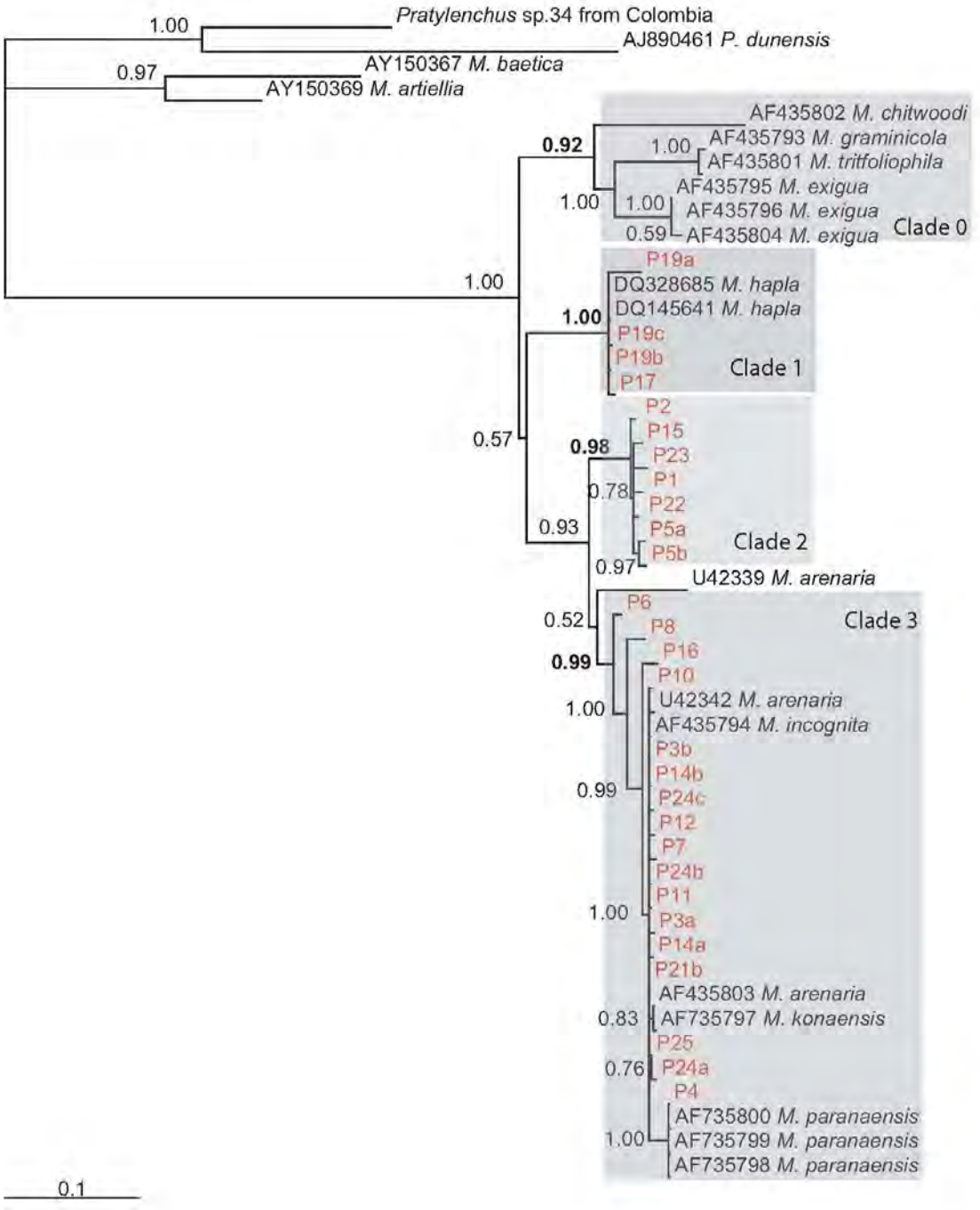


Fig. 4.15 Summary cladogram for 28 Colombian populations and 18 *Meloidogyne* GenBank accession-28S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *P. dunensis* AJ890461 and *Pratylenchus* sp., Pra34, sequence from Colombia. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP). Colombian population are indicated in red color.

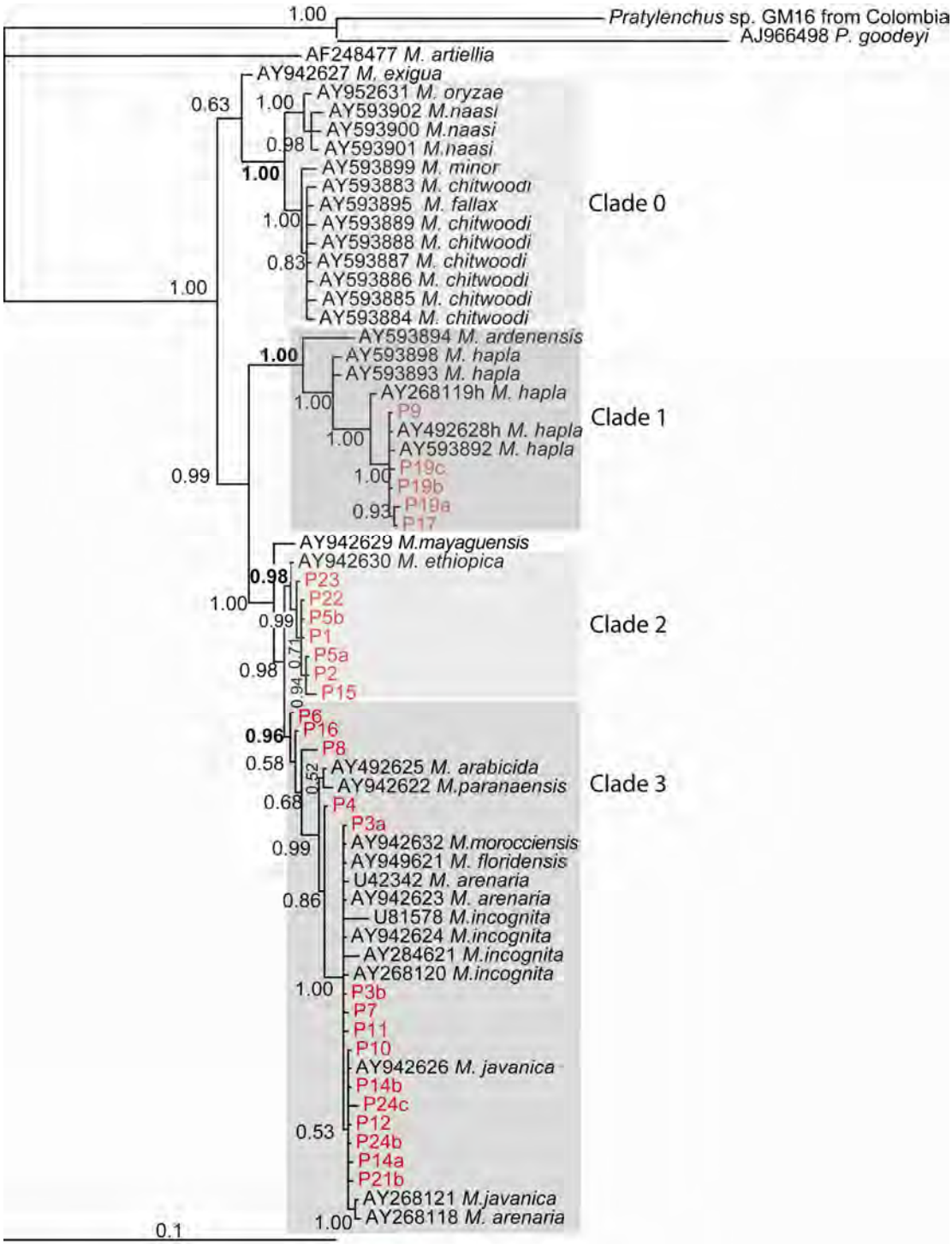


Fig. 4.16 Summary cladogram for 28 Colombian populations and 18 *Meloidogyne* GenBank accession-18S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *P. goodeyi* AJ966498 and *Pratylenchus* sp., GM16, sequence from Colombia. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP). Colombian population are indicated in red color.

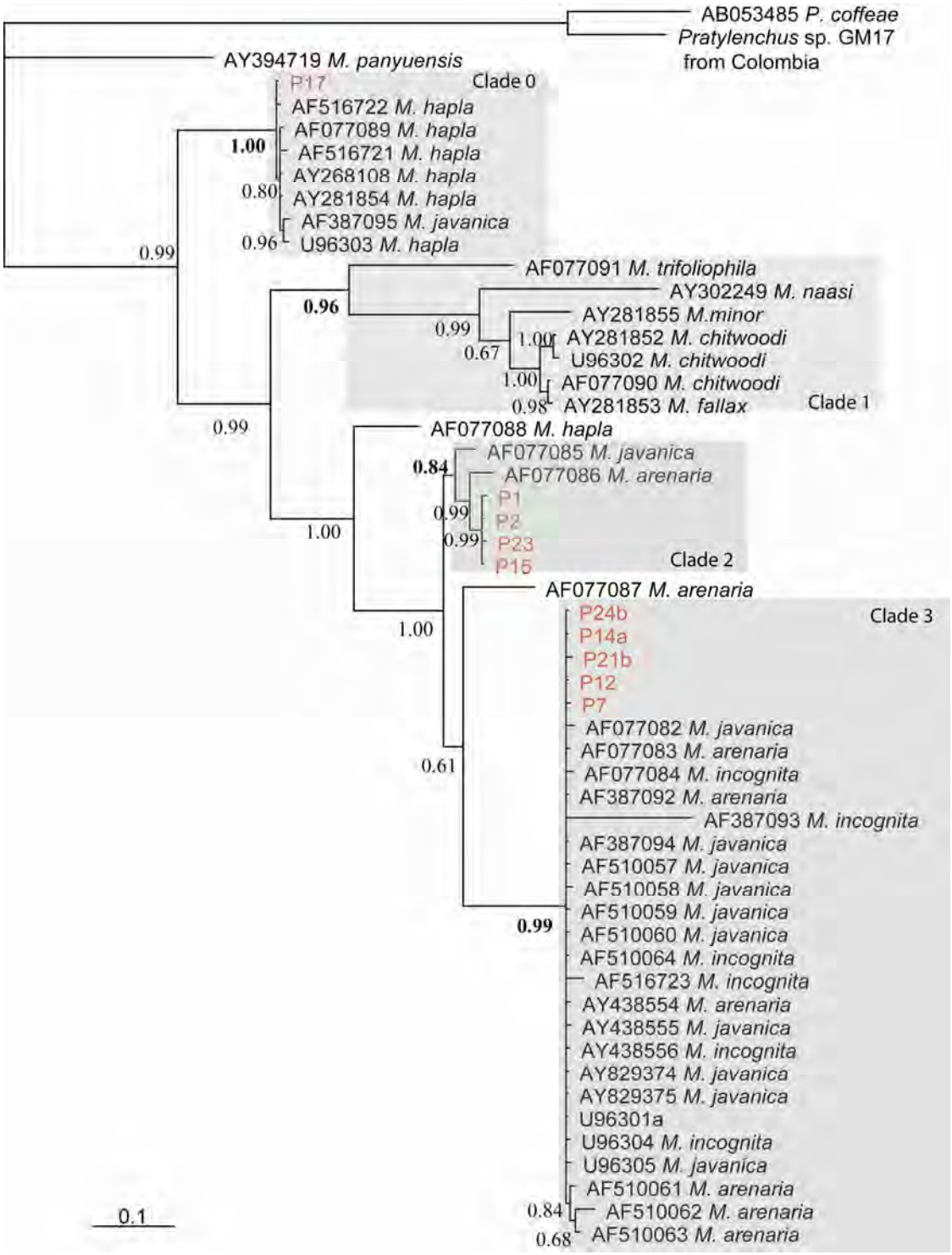


Fig. 4.17 Summary cladogram for 28 Colombian populations and 18 *Meloidogyne* GenBank accession-ITS1-5.5-ITS2 rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *P. goodeyi* AB053485 and *Pratylenchus* sp., GM17, sequence from Colombia. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP). Colombian population are indicated in red color.

became more and more difficult to differentiate species upon perineal patterns alone.

Researchers soon realized that the perineal patterns were quite variable, and many aberrant and intermediate forms were found. Variation is especially prominent in species with putative advanced characteristics, such as *M. incognita,* which has broad host ranges and reproduces solely by parthenogenesis (Triantaphyllou, 1963, 1966, 1985 Triantaphyllou & Hirschmann, 1980).

Karssen (2002) compared the eight most important descriptions of *M. hapla*. He concluded that although taxonomists mentioned the most important features as a low dorsal arch, presence of wings and punctuations, a lateral field and fine striae, the differences (human factor) in these descriptions are striking. He agreed with Franklin (1972) who indirectly recommended to reduce the human factor by using photographs instead of drawings. However, according to observations made during this study the combination of photographs and drawings provide the most reliable result because features are illustrated at different depth levels. In the future, this could be complemented with video capture pictures (De Ley & Bert, 2002).

A number of species have perineal patterns of a similar type (e.g. *M. incognita* and related species) and may be grouped on this basis, while the patterns of other species are unique. Thus, based on general pattern morphology, Jepson (1987) divided 50 species into 6 distinct morphogroups. Jepson's grouping was very useful in our research as a first step to separate/group Colombian populations.

Male: head shape

Based on the male head shape three species groups were recognized using LM and SEM. Most of the male heads fitted the description of four common species (Eisenback, 1985a, 1985b; Eisenback & Hirschmann, 1980, 1981; Hirschmann, 1985; Jepson, 1983, 1987) which differ in head morphology with respect to shape and size of the head cap, presence or absence of annulation in the head region, and the way the head region fuses with the body region.

Our observations in SEM were in agreement with those from Eisenback & Hirschmann (1980) who stated that *M. incognita* is similar to *M. arenaria* but *M. incognita* is characterized by a very prominent labial disc that is centrally concave and

Table 4.16 Overwiew results of identification of *Meloidogyne* Colombian population using combined methods, also before purification.

			female-perineal					male-head						SEM			phastsystem										
Pop	**Localities**	**Host**	**i**	**J**	**H**	**A**		**i**	**J**	**H**	**A**	**k-p**		**i**	**J**	**H**	**i**	**J**	**H**	**A**	15	16	17	18	19	**spPr**	**clade**
P1	Aguadas	*C. betacea*																								**A**	**2**
P2	Aranzazu	*C. betacea*																								**X**	**2**
P3a	Arauca	*Musa* AAB plantain	**i**				**X**	**i**				**K**		**i**			**i**								**X**	**i**	**3**
P3b	Arauca	*Musa* AAB plantain																									**3**
P4	Boyacá	*C. betacea*	**i**																							**X**	**3**
P5a	Caldas-Villamaria	*C. betacea*	**i**			**A**	**X**	**i**				**K**	**X**				**i**			**A**				**X**		noDNA	**2**
P5b	Caldas-Villamaria	*C. betacea*																									**2**
P6	Caldas-Villamaria	*S. quitoense*		**J**			**X**						**X**					**J**								**X**	**3**
P7	Cauca	*S. quitoense*																								**X**	**3**
P8	Cundinamarca	*P. ligularis*	**i**				**X**	**i**				**K**		**i**			**i**			**A**						**i**	**3**
P9	Génova	*C. betacea*			**H**		**X**																			**H**	**1**
P10	La Tebaida	*Musa* AAA banana				**A**	**X**																		**X**	**A**	**3**
P11	La Tebaida	*Musa* AAB-plantain	**i**				**X**										**i**								**X**	**i**	**3**
P12	Magdalena	*Musa* AAB plantain				**A**																				**A**	**3**
P13	Manizales	*C. betacea*		**J**														**J**								**X**	**no**
P14a	Manizales	*S. quitoense*		**J**			**X**											**J**				**X**				**J**	**3**
P14b	Manizales	*S. quitoense*																									**3**
P15	Rionegro-bodega	*R. glaucus*																								**H**	**2**
P16	Rionegro-bodega	*C. betacea*	**i**	**J**	**H**		**X**	**i**		**H**		**K**	**X**	**i**			**i**	**J**								noDNA	**3**
P17	Rionegro-correa	*S. quitoense*			**H**					**H**						**H**										**H**	**1**
P18	Rionegro-vega	*S. quitoense*	**i**	**J**	**H**		**X**	**i**		**H**		**K**	**X**	**i**			**i**	**J**								**X**	**no**
P19a	**Tolima-eggmass1**	***S. quitoense***	**i**	**J**	**H**		**X**	**i**		**H**						**H**		**J**	**H**				**X**			**H**	**1**
P19b	**Tolima-eggmass2**	***S. quitoense***	**i**	**J**	**H**		**X**	**i**		**H**						**H**			**H**				**X**			**H**	**1**
P19c	Tolima-eggmass2	*S. quitoense*																									**1**
P20	Urrao-Guapantal	*P. ligularis*	**i**					**i**									**i**				**P,K**					**i**	**no**
P21a	**Urrao-Guapantal-**	***S. quitoense***	**i**	**J**	**H**		**X**	**i**	**J**				**X**													**no**	**no**
P21b	**Urrao-Guapantal-**	***S. quitoense***	**i**	**J**	**H**		**X**	**i**	**J**																	**J**	**3**
P22	Urrao-Chuscal	*C. betacea*	**i**					**i**				**K**	**X**				**i**									**X**	**2**
P23	Urrao-Chuscal	*S. quitoense*	**i**		**H**		**X**										**i**		**H**							**X**	**2**
P24a	**Venecia-eggmass1**	***S. quitoense***		**J**			**X**		**J**						**J**			**J**								**J**	**3**
P24b	**Venecia-eggmass2**	***S. quitoense***		**J**					**J**						**J**			**J**								**J**	**3**
P24c	Venecia-eggmass2	*S. quitoense*																									**3**
P25	Belgium	*L. esculentum*		**J**		**A**			**J**									**J**								noDNA	**3**

Letters i, J, H, A, P, K and X, mean *M. incognita, M. javanica, M. hapla, M. arenaria*, *M. paranaensis, M. konaensis* and not identified species, respectively. For 15, 16, 17, 18 and 19 see text result of specific primer. Gray shading: analysis was not made. Fem-pp: perineal pattern of females; spPr: specific primer; cla: clade. Pop: population.

Table 4.17 Summary of *Meloidogyne* Colombian population identification after using combined methods*.

	Localities	Host	fem-pp					male-head						SEM			phastsystem									spPr	cla	Comb. results
			i	J	H	A	X	i	J	H	A	X	sty	i	J	H	i	J	H	A	P/K	16	17	18	19			
P1	Aguadas	*C. betacea*																								**A**	**2**	X
P2	Aranzazu	*C. betacea*																								**X**	**2**	X
P3a	Arauca	*Musa* AAB plantain	**i**					**i**						**i**			**i**									**i**	**3**	i
P3b	Arauca	*Musa* AAB plantain																									**3**	i
P3													**p/ k**															p/k
P3							**X**																		**X**			new phen.
P4	Boyacá	*C. betacea*	**i**																							**X**	**3**	P
P5a	Caldas-Villamaria	*C. betacea*					**X**					**X**												**X**		noDNA	**2**	n. sp.
P5b	Caldas-Villamaria	*C. betacea*																									**2**	n. sp.
P5			**i**					**i**									**i**											i
P5													**p/ k**															p/k
P5						**A**														**A**								A
P6	Caldas-Villamaria	*S. quitoense*		**J**														**J**									**3**	J
P6							**X**					**X**														**X**		X
P7	Cauca	*S. quitoense*																								**X**	**3**	X
P8	Cundinamarca	*P. ligularis*	**i**					**i**						**i**			**i**									**i**	**3**	i

	Localities	Host	fem-pp					male-head						SEM			phastsystem									spPr	cla	Comb. results
			i	J	H	A	X	i	J	H	A	X	sty	i	J	H	i	J	H	A	P/K	16	17	18	19			
P8													**p/k**															p/k
P8							**X**													**A**								A
P9	Génova	*C. betacea*			**H**																					**H**	**1**	H
P9							**X**																					X
P10	La Tebaida	*Musa* AAA banana				**A**																				**A**	**3**	A
P11	La Tebaida	*Musa* AAB-plantain	**i**														**i**									**i**	**3**	i
P11							**X**																		**x**			new phen.
P12	Magdalena	*Musa* AAB plantain				**A**																				**A**	**3**	A
P13	Manizales	*C. betacea*		**J**														**J**								**X**	**no**	J
P14a	Manizales	*S. quitoense*		**J**														**J**								**J**	**3**	J
P14b	Manizales	*S. quitoense*																									**3**	J
P14							**X**															**x**						new phen.
P15	Rionegro-bodega	*R. glaucus*																								**H**	**2**	X
P16	Rionegro-bodega	*C. betacea*	**i**					**i**						**i**			**i**									No DNA	**3**	i
P16				**J**														**J**										J
P16					**H**					**H**																		H
P16													**p/k**															K

	Localities	Host	fem-pp					male-head						SEM			phastsystem									spPr	cla	Comb. results
			i	J	H	A	X	i	J	H	A	X	sty	i	J	H	i	J	H	A	P/K	16	17	18	19			
P16							**X**					**X**																X
P17	Rionegro-correa	*S. quitoense*			**H**					**H**						**H**										**H**	**1**	H
P18	Rionegro-vega	*S. quitoense*	**i**					**i**						**i**			**i**									**X**	**no**	i
P18				**J**														**J**										J
P18					**H**					**H**																		H
P18													**p/k**															K
P18							**X**					**X**																X
P19a	**Tolima-eggmass1**	***S. quitoense***			**H**					**H**						**H**			**H**							**H**	**1**	H
P19b	**Tolima-eggmass2**	***S. quitoense***			**H**		**X**			**H**						**H**			**H**							**H**	**1**	H
P19c	Tolima-eggmass2	*S. quitoense*																									**1**	H
P19			**i**																									i
P19				**J**														**J**										J
P19							**X**																**X**					n. sp.
P20	Urrao-Guapantal	*P. ligularis*	**i**					**i**									**i**									**i**	**no**	i
P20																					**p/k**							P
P21a	**Urrao-Guapantal-**	***S. quitoense***		**J**					**J**																	**no**	**no**	J
P21b	**Urrao-Guapantal-**	***S. quitoense***		**J**					**J**																	**J**	**3**	J

	Localities	Host	fem-pp					male-head						SEM			phastsystem									spPr	cla	Comb. results
			i	J	H	A	X	i	J	H	A	X	sty	i	J	H	i	J	H	A	P/K	16	17	18	19			
P21			**i**					**i**																				i
P21					**H**																							H
P21							**X**					**X**																X
P22	Urrao-Chuscal	*C. betacea*										**X**														**x**	**2**	X
P22			**i**					**i**									**i**											i
P22													**p/ k**															p/k
P23	Urrao-Chuscal	*S. quitoense*					**X**																			**x**	**2**	X
P23			**i**														**i**											i
P23					**H**														**H**									H
P24a	**Venecia-eggmass1**	***S. quitoense***		**J**					**J**						**J**			**J**								**J**	**3**	J
P24b	**Venecia-eggmass2**	***S. quitoense***		**J**					**J**						**J**			**J**								**J**	**3**	J
P24c	Venecia-eggmass2	*S. quitoense*																									**3**	J
P24							**X**																					X
P25	Belgium	*L. esculentum*		**J**					**J**									**J**								No DNA	**3**	J
P25						**A**																						A

* Included data before and after purification. When morphological data matched to minimum one molecular result we considered it enough criteria to identify the population. i: *M. incognita*; J: *M. javanica*; H: *M. hapla*; A: *M. arenaria*; P/K: *M. paranaensis* or *M. konaensis*; X: *Meloidogyne* sp.; fem-pp: perineal pattern of female; sty: stylet of male; spPr: specific primer; cla: clade; noDNA/no : result failed during DNA extraction or sequentiation; numbers 17 and 18 in phastystem columns fit to E1N1 and E1N2 phenotypes of a new species of *Meloidogyne* (see discussion). Fem-pp: perineal pattern of females; spPr: specific primer; cla: clade. Pop: populaton. Comb.results: combined results. New phen: new phenotype.

the medial lips are crescent-shaped in face view. *M. javanica* can be differentiated in *en face* view from *M. arenaria* and *M. incognita* by the elongated labial disc and medial lips and the nonannulated head region. *M. hapla* can be distinguished from the other species by the small rectangular labial disc and medial lips.

Although *M. arenaria* populations from Colombia were not analyzed by SEM, observations in LM were enough to separate this species based on head shape. Most of the Colombian populations matched to four common *Meloidogyne* species. Thus, those were easily separated/identified based on LM when specimens were in appropriate position (lateral). The combination of LM and SEM observations however, would provide even more accurate results. Additional morphological details could be of special importance to distinguish closely related species as e.g. *M. incognita, M. konaensis* and *M. paranaensis*.

Male: stylet and cone

Stylet length was found to be the best differentiating character because of its rather broad range within the genus (16-27 µ) and low coefficient of variation (4%) (Mulvey quoted by Hirschmann, 1985). Stylet cone length, which is correlated with stylet length (i.e. about half of stylet length) can be helpful in those species with a much shorter cone. Although DGO exhibits more variation in general, some species can be distinguished on the basis of DGO distance (Jepson, 1987). Because stylet and cone length overlap between common species according Jepson criteria (Table 4.7) and because most Colombian populations matched one of the four common species, those criteria were not helpful to separate populations at species level.

The number of projections protruding from the stylet shaft of males was a helpful tool to separate some Colombian populations at species level. It is the most useful diagnostic character in *M. konaensis* (6-12 large projections) (Eisenback *et al*, 1994) and also have been observed in *M. paranaensis* and *M. brasilensis* (1-2 projections) (Carneiro *et al.,* 1996b; Charchar & Eisenback, 2002).

Second-stage juveniles: tail shape

The tail shapes of second-stage juveniles in most of Colombian populations fitted into three species groups as described by Jepson which include the four most common *Meloidogyne* species. As all qualitative criteria used for separation at species group level, it was helpful used in combination with other ones in our studies.

Second-stage juveniles: Tail and hyaline tail terminus length

For the Colombian populations different ranges for tail and hyaline tail terminus length with respect to the Jepson classification were found (see Table 4.7 and 4.8). According to Jepson (1987) only *M. hapla* juveniles are relatively distinct by combination of the longest tail (59.2 µm) and longest hyaline tail terminus lengths (15.7µm). However, in the Colombian populations, specimens with the longest tails fitted populations of *M. javanica* (identified after combination of different methods): P14a, P21b, P24b which measured 53, 50 and 51 µm, respectively. The range of mean hyaline tail terminus of Colombian populations failed within the range 9.3µm – 14.6µm with almost half of the populations 11-12µm; thus, clear distinction between species using this feature was not possible.

Additionally, relationship between origin of the samples and either tail and hyaline tail terminus measurements were not observed, e.g. specimens from same locality, host and single egg-mass had either similar or different measurements.

Note that, several differences compared with the results of Jepson could possibly have a significant influence on the morphometrical comparison: the fixation procedure was different (De Grisse *vs* TAF), origin of populations (subtropical and temperate climate *vs* tropical), different age at moment of analysis (less than one week vs after hatching) and impact of culture.

CDA of second-stage juveniles and males

CDA analyses were made to provide additional, objective approaches in differentiation of species. CDA allowed to differentiate one clear cluster using combinations of tail and hyaline tail terminus lengths and both of them combined with other features. This cluster contained 5 populations (P14a, P21b, P24a, P24b and P25) which fitted to *M. javanica*. This result agreed with other morphometric analyses.

Morphometrics of tail and hyaline tail terminus from second-stage juveniles and stylet, cone length and DGO from males respectively were able to discriminate species useful only in a supplementary way. The CDA for juveniles and males features did not allow clear separation between all species (except *M. javanica* juveniles); however, two traits (*viz.* tail length for juveniles; stylet length for males) were found to be helpful for grouping most populations. Additionally, DGO, b and GBD were useful features for discrimination of *Meloidogyne* male populations when 11 features were combined.

Protein analysis

Our results showed that the combined use of isozyme esterase and malate dehydrogenase provide a helpful tool for differentiating Colombian populations of *Meloidogyne*. This result agreed with biochemical studies which demonstrated that the main species of *Meloidogyne* can be differentiated by species-specific enzyme phenotypes, especially esterase (Bergé & Dalmasso, 1975; Carneiro *et al.*, 1996a, 1996b, 1998, 2000, 2004a, 2004b, 2005, 2007, 2008; Cofcewicz *et al.*, 2004, 2005; Esbenshade & Triantaphyllou, 1985a, 1985b, 1985c, 1986, 1987a, 1990; Fargette, 1987a, 1987b; Flores-Romero & Navas, 2005; Hernandez *et al.*, 2004a, 2004b; Hussey, 1985; Janati *et al*., 1982; Karssen *et al.*, 1995).

M. incognita phenotypes

Populations identified by morphology and specific primers as *M. incognita* (P3a, P8, P11, P16, P18 and P20) had five isozyme phenotypes: one EST band plus one MDH band (I1N1); two EST band plus one MDH band (I2N1); two EST band plus two MDH band (I2N2), one (I1N0) or two (I2N0) esterase band but no MDH bands, therefore showing some variability among the isolates (Table 4.10).

Phenotype I1N1 associated with *M. incognita* from Santa Marta (Colombia) had been already reported by Esbenshade & Triantaphyllou (1985c). They considered it as one of the most prevalent phenotypes in their research which included 65 countries and 16 *Meloidogyne* species. Carneiro *et al.* (1996a) found the same phenotype associated with coffee, soybean, tomato, lettuce, pepper, carrot, peach tree, cotton, beans and mulberry tree in Brazil. Populations of *M. incognita* having esterase phenotypes I1 or I2 were observed in cotton and coffee by Carneiro *et al.* (1996a, 2004) and Randig *et al.*

(2002). The EST I2 was the most frequently found phenotype of *M. incognita* in banana plantations (Cofcewicz *et al.*, 2004). Similar results were observed by Castro *et al.* (2003) in a soybean survey made in Brazil.

Populations of *M. incognita, M. javanica* and *M. arenaria* showing MDH phenotype N2 were reported from Spain by Flores-Romero & Navas (2005). Dalmasso & Bergé (1978) reported two MDH bands (one strong and one weak band) in *M. javanica* and *M. arenaria*. No other reports of MDH phenotypes showing two bands are known. However, the I2N2a phenotype was the most frequently found phenotype of *M. incognita* in Colombian populations, following by I1N1. Phenotype I2N2b was not reported before but it was only displayed in gel 23 and its Mdh bands were not really clear. Phenotypes characteristic to *M. incognita* (I2N2a,b) were also found in populations P5a, P22 and P23 prior to purification. Those populations showed more than one *Meloidogyne* species and this is exemplary for the presence of mixed populations of *Meloidogyne* in the field.

Although electrophoretic profiles do not provide enough information to separate the four races of *M. incognita* (Esbenshde & Triantaphyllou, 1990). Carneiro *et al.* (2000) found that *M. incognita* races 2 and 3 had esterase phenotype I1 and race 1 and 4 had esterase phenotype I2.

M. javanica phenotypes

The species specific phenotype J3 was detected in eight *M. javanica* populations from different localities (Table 4.10) and was also used as standard control in each gel. The phenotype J3N1 associated with *M. javanica* from Colombia was reported by Esbenshade & Triantaphyllou (1985c). This is one of the most prevalent esterase phenotype around the world (Esbenshade & Triantaphyllou, 1985c; Carneiro *et al.*, 1996a, 2004b; Cofcewicz *et al.*, 2004; Flores-Romero& Navas, 2005).

The EST phenotype J2 (data not show in table 4.10) was found in control populations of *M. javanica* from ILVO (P25). According Cofcewicz *et al.* (2004) it is probable that some individual females isolated from plants were not in a good physiological condition and these females presented a lack of band resolution thus producing the atypical phenotypes J2 and J2a. The phenotype J2 was also detected by

Tomaszewski *et al.* (1994) and Carneiro *et al.* (1996a). The atypical phenotype J2(a) was reported first by Castro (2001) and Cofcewicz *et al.* (2004).

Two atypical phenotypes were linked to *M. javanica* based on their rate of migration of esterase bands: J5N2, for which 3 bands are in the same position of J3 and 2 bands between them, was associated to populations P16 and P24a; and phenotype J3N3 displayed by population P25 (control). Esterase bands of the phenotype J5N2 in gel number13 appeared as double and short bands but esterase bands appear both complete and clear in gel number 10; no similar phenotypes have been recorded in literature, however morphological characterization of those populations fitted *M. javanica.*

Zero to two bands were associated with MDH phenotypes of *M. javanica* from Colombia. However, the number of MDH bands reported in literature for *M. javanica* populations has been one (Esbenshade & Triantaphyllou, 1985c; Carneiro *et al.*, 1996a); two (Flores-Romero & Navas, 2005) or three (Carneiro *et al.*, 2000). The latter authors found that populations with malate dehydrogenase phenotypes N3 (3 bands) induced four or five times more galls and eggs on soybean than MDH phenotype N1 (one band).

M. arenaria phenotypes

Esterase phenotype A4 associated with two MDH bands were found in populations P5a and P8. Similar esterase pattern (M3F1) was encountered in a single atypical *M. arenaria* population in El Salvador (Esbenshade & Triantaphyllou, 1985c).

M. hapla phenotypes

The characterization of Colombian populations of *M. hapla* based on EST phenotype H1 and MDH phenotype H1 matched with morphological features of the populations. Similar phenotypes for *M. hapla* were reported from different countries associated with coffee (Hernandez *et al.*, 2004a, 2004b), rosemary, strawberry, borao potato, lettuce, tomato and kiwi (Carneiro *et al.*, 1996a, 2000). This phenotype was firstly designated H1 for "M. *hapla* phenotype," by Esbenshade & Triantaphyllou (1985c). It had the highest rate of migration of all MDH bands observed.

M. paranaensis / M. konaensis phenotypes

A single atypical esterase phenotype characterized by one fast band was found in population P20 (**E1aN1a**). This phenotype was first observed by Bergé & Dalmaso (1975) in two populations of *M. incognita acrita* from Germany. Later, Janati *et al.* (1982) reported a closely related phenotype from coffee trees from Brazil (3 populations) and one population from Suriname (no host mentioned in latter country) showing two esterase bands. A similar phenotype to Colombian E1aN1a was associated with six populations of an undescribed species (F1) isolated from coffee in Brazil, Peru and Surinam and one population of *M. querciana* from USA (Esbenshade & Triantaphyllou, 1985c). Fargette (1987b) found the same esterase phenotype (pIII) with one esterase band in natural populations from West Africa; no host was indicated but it was reproduced in tomato, coffee trees, soybean, lettuce, *Amaranthus viridis* and *Sesbania rostrata* using host test.

Carneiro *et al.* (1996b) reported the same phenotype (firstly named F1 and later P1 by them) associated with the species *M. konaensis* and *M. paranaensis* in coffee plants. Four years later, they found differences between *M. konaensis* phenotype (named K3) and *M. paranaensis* phenotype (named P1), based on the number of esterase bands and their migration rates which are 1.1, 1.3, 1.4, and 1.4, respectively (Carneiro *et al.*, 1996b, 2000). The last study of the authors about diversity of root knot nematodes on coffee from Brazil, Central America and Hawaii recorded one new esterase phenotype P2 (Rm: 0.9, 1.32) in two populations of *M. paranaensis* from coffee trees in Guatemala. However, in order to confirm the identification of the species they needed features of the males (Carneiro *et al.*, 2004). Further, the isolate of *M. konaensis* from Hawaii did not show the typical esterase profile K3 earlier reported by Carneiro *et al.* in year 2000 but one esterase P1 phenotype that clustered together with the Brazilian populations of *M. paranaensis* in the molecular analysis

Based on the number of esterase bands and its rate of migration (Rm) our Colombian phenotype E1aN1a fits to the phenotype P1 described for *M. paranaensis* and *M. konaensis* (Carneiro *et al.* , 1996a, 1996b, 2000, 2004). This result combined with morphological analysis, mainly number of projections protruding from the shaft in stylet of males, confirms the presence of *M. paranaensis* and *M. konaensis* in Colombia.

However, more research is needed to define geographical distribution and host range of both *Meloidogyne* species in Colombia but additional information about clear differentiation of these species using isozymes phenotypes is required too.

Rare phenotypes

Esterase band of phenotype **E1bN1b** (population P14a) matched with *M. hapla* but the Mdh band was really slow. This phenotype was unrecorded before. Morphological features of this population felt into *M. javanica*. This phenotype probably represents a new species, however additional specimens are needed for accurate morphological and morphometrical studies.

E3N1, E3N2 phenotypes were found in Colombian *Meloidogyne* populations: P5a, P19a and P19b. Because the migration rate of esterase bands was identical for both phenotypes, the second Mdh band in E3N2 probably is a double band obtained by mistake during sample processing, therefore we do not have two but one phenotype. This phenotype represents a new species which will be described from Iran, Africa, Chile and Brazil populations by Karssen, Carneiro and other researchers (personal communication; December 10, 2007). A similar phenotype (named M3) with three bands in positions 8, 10, and 14 and Rm media was observed by Esbenshade & Triantaphyllou (1985c) in three populations from South America (from Argentina, Bolivia and Ecuador) and one population from Turkey (E923). It could not be identified to species level.

Populations P3a, P10 and P11 yielded two esterase bands (**E2N2**): one slow band, whereas the fast band had a migration rate approximately the same as the I1 band of the *M. incognita* phenotype (Fig. 4.7, gel 21, 24, 26). The last two populations were sampled on *Musa* plants (Gros Michel and FHIA cultivars) in the same locality (La Tebaida-Andean Region at East of Colombia) but morphological characterization of P10 fitted *M. arenaria* while P11 matched with *M. incognita*. P3a was found in *Musa* plants (hartón cultivar) in Arauca, West of Colombia. This phenotype was firstly detected by Esbenshade & Triantaphyllou (1985c) who named it S1M1. They observed it in six atypical populations of *M. arenaria* (from Nigeria, Ivory Coast, Philippines and Samoa) and one population (U.S.A., AR-E944) that could not be identified to species level but probably representing a new form. Cofcewicz *et al*. (2004) found the same phenotype

(named B2) associated with *Musa* AAA, Grande Naine cultivar in Brazil but it could not identified it to the species level. The authors agreed with Esbenshade & Triantaphyllou (1985c) that it probably represents a new form. They lost this population during the purification process on tomato plants.

General remarks on protein analyses

Because enzyme phenotypes were made before purification of species some populations showed more than one isozyme phenotype; however, most of them matched with morphological identification. Additionally, these results confirm that *Meloidogyne* species are frequently mixed in field.

Esbenshade & Triantaphyllou (1985c) stated that for routine identification of the various phenotypes, one should not rely heavily on the numerical expression of the migration rate of the various bands. The rate of migration (Rm) of proteins in a gel is highly dependent upon the conditions of protein extraction, sample storage, and electrophoretic conditions of separations. Such factors or conditions may vary among laboratories and from one electrophoretic run to the other in the same laboratory. For reliable identification of the phenotype of an unknown population, protein extracts from a pure population of a known species should always be included in the same gel where they can be compared directly. They recommend that pure populations of *M. hapla* and *M. javanica* be used as standard controls in each gel. Especially with reference to esterase, the phenotypes of these two species will serve as efficient guides for identification of all other phenotypes, often by simple visual evaluation of the gels (without actual measurement of Rm values).

Because the number of described species have increased and populations with similar esterase patterns showed different MDH patterns (more than one bands or different Rm), combination of esterase with malate dehydrogenase on a single gel is more appropriate to use than esterase alone. This was confirmed in our study.

Specific primers

Sixteen Colombian populations of *Meloidogyne* produced the expected band sizes for *M. incognita, M. javanica, M. hapla* and *M. arenaria* using species specific primers (Table 4.16). Those results agreed with the identification based on

morphological features and isozyme electrophoresis. Only two exceptions were the populations P1 and P15 which produced the expected band sizes for *M. arenaria* and *M. hapla*, respectively; however, the latter one did not show the typical parsimony informative characters of *M. hapla* in the different phylogenetic trees and both of them were included in clade 2 with unknown species in phylogenetic trees. Only morphological data of juvenile stages were obtained (no perineal pattern, no isozymes results) from populations P1 and P15 which clustered with unknown species in CDA (Fig. 4.6); thus, it confirms the position of these populations in clade 2 and therefore not closely related to *M. hapla*. For population P15 a wrong result from PCR with specific primers is possible or an alternative reproductive strategy separates this population from other *M. hapla* from Colombia, as suggested in the next paragraph.

The specific primers used in our case were built based on a gene pool from different origins (Holand, Queensland and Cyprus). The gene pool contained both *M. hapla* races, a diploid, facultative meiotic parthenogenetic species that reproduces either sexually or, in the absence of male gametes, parthenogenetically, and *M. hapla*, race B, which is an obligate mitotic parthenogenetic species and is usually triploid. In this respect, Wishar *et al*. (2002) found intraspecific molecular variability in *M. hapla* and this species was more closely related to the tropical species than to *M. chitwoodi* or *M. fallax*, mainly the race B isolates shows extra variability due to polyploidy.

Concerning the host relation of population P15 (*M. hapla* ?) it was associated with *Rubus glaucus* (Andean blackberry - Rosaceae family). This host has been reported as resistant to *M. incognita* and *M. javanica* in preliminary studies; however, no research about resistance/ susceptibility of *R. glaucus* to *M. hapla* was conducted (Navarro & Múnera, 2000). Because *Meloidogyne* populations from Andean blackberry were increased in tomato plants using root pieces, no egg mass (see Chapter 2: general material and methods) and no records of *Meloidogyne* spp. in *Rubus glaucus* are known, additional research about status of host of *R. glaucus* to *Meloidogyne* species is needed; especially an improved molecular/morphological characterizaction of the aberrant population P15. *Rubus glaucus* is widespread in Latin America (Colombia, Ecuador, Peru, Guatemala, Costa Rica, Panamá and Mexico) and Hawaii. In Colombia and

Ecuador, Andean blackberry is widely cultivated and is an economic important crop (higher price compared with other fruits) mainly for local markets. In Hawaii, *R. glaucus* is considered a potentially-invasive weeds.

No bands were obtained when *M. fallax* and *M. chitwoodi* species-specific primers were used. This is in accordance with the expected absence of this species in tropical conditions (Karssen, 2002; De Waele & Elsen, 2007).

Other studies confirmed that PCR with species-specific primers is a rapid and useful tool for differentiating mixtures of root-knot nematodes (Williamson *et al.*, 1997; Wishart *et al*., 2002; Zijlstra, 2000). However, Adam *et al.* (2005) reported inconsistent results with lines of *M. incognita* (from Massa) "when DNA of single females was used, but a band of the expected size was produced with DNA extracted from a bulk sample of nematodes suggesting that variation in the primer site(s) or sensitivity to DNA concentration may occur with the Finc and Rinc primers".

PCR methods for distinguishing some species of root-knot nematodes have also been reported using mitochondrial DNA (Blok *et al.*, 2002; Harris *et al.*, 1990; Hugall *et al.*, 1997; Powers & Sandal, 1988; Powers *et al*., 1986a, 1986b); satellite DNA (Castagnone-Sereno *et al*., 1999), and ribosomal DNA region (Adam *et al.*, 2005, 2007; Blok *et al*., 1997a, 1997b, 2007; De Ley *et al.*, 2002; Guirao *et al*., 1995; Handoo *et al*., 2004, 2005; Hugall *et al*.,1999; Peterson & Vrain, 1996; Peterson *et al*., 1997; Powers & Harris, 1993; Powers *et al.*, 1997, 2005; Tandingan De Ley *et al.* 2002; Tenente *et al.*, 2004, Zijlstra *et al.*, 1995, 2000) .

Phylogenetic analysis

Analysis of Colombian *Meloidogyne* populations at different rDNA regions (D2D3, 18S and ITS1-5.8-ITS2) yielded phylogenetic trees with similar topologies. All of the generated trees contained three identical clades which were strongly supported by high PP values. Additionally, GenBank accessions of *Meloidogyne* containing mainly temperate species were consistently placed as a separate clade (clade 0). Our studies agreed with van der Beek *et al.* (1997) who found molecular evidence that *M. hapla* and *M. fallax* are distinct biological entities.

Clade 1

Meloidogyne Colombian populations in clade 1 fitted *M. hapla* according to our previous results (morphological, biochemical and species-specifics PCR identification). All sequences from this species showed many parsimony informative characters compared to the other three species and thus could be clearly separated from other species, the only exception was population P15 (see clade 2 in this section and specific primer discussion). Our studies agreed with van der Beek *et al.,* 1997 who found molecular evidences that *M. hapla, M. chitwoodi* and *M. fallax* are distinct biological entities.

Clade 2

Meloidogyne populations P1, P2, P5a, P15, P22 and P23 were separated in clade 2 in all phylogenetic trees (18S, 28S, ITS1-5.8-ITS2 trees). Using species-specific PCR it was not possible to identify those populations at species level which suggests that those populations did not fit to the four major *Meloidogyne* species. Most of the cases of CDA based on morphometrics of males and juveniles of these populations clustered separate from the common *Meloidogyne* species. However, the purification of these populations is necessary to obtain their accurate identification because an insufficient number of individuals did not allow accurate analysis of e.g. perineal pattern and isozymes. Additionally, consistent results of three different rDNA regions reinforced species-specific PCR results that those populations do not fit to four common *Meloidogyne* species.

Clade 3

Meloidogyne Colombian populations in clade 3 fitted *M. incognita, M. javanica, M. arenaria* according to our previous results (morphological, biochemical and species-specific PCR identification).

Phylogenetic analysis using both Colombian sequences and GenBank sequences demonstrated that *M. incognita, M. javanica* and *M. arenaria* always were included in the same clade using D2D3, 18S as well as ITS1-5.8-ITS2 analysis and a clear separation at species level based only on these sequences was not possible. Thus, our results confirmed that sequences from different rDNA regions (28S, 18S, ITS1-5.8-

ITS2) are useful to separate certain *Meloidogyne* species groups but suggest that these techniques cannot be used for the study of interspecific variation within main cosmopolitan *Meloidogyne* species (*M. incognita, M. javanica, M. arenaria*).

Integration of major methods

Analysis of morphological (stylet protuberances in populations P3a, P5a, P8, P16, P18 and P22), biochemical (E1N1a phenotype associated to population P20) and molecular data (in 28S rDNA, position of P4 resolved in clade 3 together with three *M. paranaensis* accessions reported in GenBank and 100% similarity with two *M. paranaensis* accessions- data no included here) suggest that in addition to the four main *Meloidogyne* species, *M. paranaensis* and *M. konaensis* are present in Colombia. However, special attention should be paid to the low divergence of nucleotides between *M. paranaensis* AY492622 (18S), AF735798 (28S), AF735799 (28S), AF735800 (28S), *M. konaensis*, AF435797 (28S) and some *Meloidogyne* accessions from Colombia and GenBank. Our results agree with Tenente *et al.* (2004) who stated that "relationships between *M. arenaria*, *M. konaensis*, *M. incognita* and *M. paranaensis* were not resolved consistently across different alignments and algorithms" and "the mitotic parthenogenetic species *M. arenaria* (race 2), *M. incognita*, *M. konaensis*, *M. paranaensis* are actually all extremely similar to one another in D2/D3 sequence (as is e.g. evident from the branch lengths). It is therefore possible that at least some of these are synonymous species, but we consider it more likely that the D2/D3 region is simply too conserved for the phylogenetic analysis of mitotic parthenogenetic *Meloidogyne* species".

According Tandingan De Ley *et al*., 2002 "only mitochondrial DNA data (Powers and Sandall, 1988) placed *M. arenaria* in apposition to *M. incognita* and *M. javanica.* Hyman and Powers (1991) subsequently revised some of the identifications in the previous study but retained *M. arenaria* as the more divergent species, and hypothesized that it could actually represent a hybrid lineage. Discordance between the mitochondrial results and those obtained with other data may be due to some or all of the methodological differences in types of characters (e.g., fragment lengths vs. chromosome numbers or nucleotides), sources of data (e.g., rapidly evolving mitochondrial loci vs. a highly conserved ribosomal locus), numbers of included taxa, and tree construction methods. If hybridization did indeed occur in *Meloidogyne,* then this would be probable

the most important cause of disagreement between mitochondrial and ribosomal trees (Hugall *et al.*, 1999)".

The evolutionary relationships of *Meloidogyne* have been inferred from several types of data starting with Triantaphyllou (1966, 1985) who inferred the phylogeny of root-knot nematodes from cytogenetic data. Our results placed the ameiotic species on a separate clade that is distantly related to either the obligate amphimictic or meiotic species. This agrees with previous phylogenies of Castagnone-Sereno *et al*. (1994, 2006), Esbenshade & Triantaphyllou (1987), Tandingan De Ley *et al.* (2002), Tigano *et al.*, (2005), van der Beek & Karssen (1997) and van der Beek *et al.* (1998).

The phylogenetic trees from all three rDNA regions (18S, D2D3 and ITS1-5.8-ITS2) result in a similar topology for the Colombian populations (see figures 4.15, 4.16 and 4.17); the same clades could be appointed independent of the analyzed sequence, thus: clade 1 contained *M. hapla* populations (exception was population P15, see species-specific PCR for explanation); clade 2 grouped six populations not identified at species level and therefore their reproductive strategies are unknown, and clade 3 contained *M. incognita, M. javanica* and *M. arenaria*. These topologies agreed to cytogenetic data as indicated above from different authors.

Colombian populations containing *M. paranaensis* and *M. konaensis* were taken in orchards where fruit trees grow together with coffee plants as mixed crops. *M. paranaensis, M. konaensis, M. arabicida* have coffee plants as type host and their type localities are respectively Brazil, Hawaii and Puerto Rico. Because *M. paranaensis* and *M. konaensis* have been reported as dangerous pest organisms to coffee production in other countries and coffee is one of the most economically important crops in Colombia priority should be given to determine presence/absence, distribution and losses due to these species. The fruit crops included in this study are also important in local and export markets no only in Colombia but also in some Latin American countries. Similar studies based on different identification techniques but not only major *Meloidogyne* species should be considered.

M. incognita was identified in this study from *Passiflora ligularis* in two localities, Urrao and Bogotá, and from *Musa* plants, FHIA and hartón cultivars, from La Tebaida and Arauca, respectively. However, *M. incognita* has already been reported in

the first host in different Colombian regions and studies about damage, interaction between *M. incognita* and *Fusarium solani* and preliminary studies about resistance in passiflora germoplasm collections have been performed. *M. incognita* has also been recorded from *S. quitoense* and *C. betacea* in Colombia (Corrales, 1996; Lozada *et al.*, 2002; Múnera 2000, 2002; Múnera & Navarro, 2000; Navarro, 1990; Vargas *et al.*, 2001; see Chapter 3).

M. javanica was identified from *Solanum quitoense* in three localities, Urrao, Manizales and Venecia and *M. hapla* from Tolima and Rionegro. These two *Meloidogyne* species have been reported also from *P. ligularis* and *C. betacea* (Corrales, 1996; Lozada *et al.*, 2002; Múnera 2002; Múnera & Navarro, 2000; Vargas *et al.* , 2001; see Chapter3). Studies about resistance of different clones of "lulo La Selva" (*S. quitoence* x *S. hirtum*) to *M. incognita, M javanica* and *M. hapla* were conduced by Múnera (2003) in Colombia

M. arenaria was identified from *Musa* plants, Gros Michel and hartón cultivars from La Tebaida and Magdalena, respectively. The same species was identified from *Cyphomandra betacea* from Aguadas. Records of *M. arenaria* from C. *betacea* in Colombia are known (Lozada *et al.*, 2002; Múnera & Navarro, 2000; Navarro, 1990; see Chapter 3).

M. paranaensis was identified from *P. ligularis* (from Bogotá); *Musa* plants, hartón cultivar (from Arauca); *C. betacea* (from Villamaria and Rionegro) and *S. quitoense* (from Rionegro and Urrao). In the former study in Colombia, a root-knot nematode population was found to exhibit a perineal pattern similar to that of *M. incognita* but produced a response to the North Carolina differential host test similar to that reported for *M. paranaensis* (Campos, V. 2005, quoted by De Waele and Elsen, 2007). However, this is a first register of this species in Colombia. Because its widespread ocurrence; wide host range and observed mixed populations in field, this species needs special attention in Colombia and South America. *P. incognita P. paranaensis* and *P. konaensis* are morphologically similar, making identification difficult for the non-specialist, but distinguishing them is important for utilizing appropriate crop rotations, managing resistance effectively and for plant quarantine requirements and for integrated management of the crops.

One locality (Rionegro) showed a remarkably larger number of *Meloidogyne* species that are mixed in the field. This could be associated with the introduction of different fruit materials (seeds and vegetative material) from different geographical areas of the country.

Conclusions

Perineal patterns to group *Meloidogyne* populations sensu Jepson (1987) were useful in our research as a first step to separate/group populations from the field. From the combination of several analyses it turned out that several species occur together in the field. Thus, the purification of populations (from single egg mass) is recommended as second step.

Qualitative characters (juvenile tail shape and male head shape) showed to be more useful than morphometric values, especially SEM of head region of *Meloidogyne* males appeared to show a high species resolution. The head morphology of males, mainly in *face* view, was able to differentiate species within the five populations studied. Three typical patterns were found and these fitted resp. to *M. incognita, M. javanica,* and *M. hapla* SEM descriptions. Other differences detected in the light microscope (LM) were also helpful for identification e.g. head shape in lateral view. Males of Colombian populations showed that head shape morphology using LM is a reasonably reliable taxonomic character for the identification of the four common root-knot nematode species.

Combinations of measurements are useful as supporting data but are never unequivocal. The quantitative characters proposed by Jepson (1987) (stylet length, cone and DGO for males and tail and tail terminus length for juveniles) are those of highest practical value in terms of intraspecific variation and differentiation between species group. Qualitative characters (perineal pattern for females, male head shape and juvenile tail shape) are frequently species-specific.

Biochemical studies have demonstrated that the main species of *Meloidogyne* can be differentiated by species-specific enzyme phenotypes, mainly esterase. The esterase phenotypes combined with malate dehydrogenase phenotypes can be used as a rapid and

efficient method to carry out extensive field surveys to determine the frequency and relative distribution of *Meloidogyne* spp., to characterize *Meloidogyne* species and detect atypical phenotypes; and to detect mixed species isolates for purification prior to conducting other studies which need purification.

Additionally, species-specific PCR is a rapid and useful tool for differentiating mixtures of root-knot nematodes species. The method has potential to be optimized for routine practical diagnostic tests facilitating the control of these economically important pest organisms.

At this time, the resolution of the routine sequenced loci is too low and no simple, a single-step PCR capable of amplifying from and specifically identifying single specimens of all three important tropical species (*M. incognita, M. javanica, M. arenaria*) has been described. Other loci must therefore be investigated to be useful to discriminate species and unravel the evolutionary history of these species.

Meloidogyne populations included in clade 2 of the phylogenetic trees (P1, P2, P5a, P5b, P15, P22 and P23) did not fit to known *Meloidogyne* species based on all analyses; however, studies based on purified populations are necessary for their accurate characterization and to understand their diversity.

In conclusion, six *Meloidogyne* species: *M. incognita, M. javanica, M. arenaria, M. hapla, M. paranaensis and/or M. konaensis* were found associated with four fruit crops and plantain in Colombia. Because last two species show morphological features of *M. incognita* they were probably misidentified in the past; thus, only the use of combined methods allowed accurate identification of species. This is necessary to select more appropriate management methods to control nematode populations.

Chapter 5

Pratylenchus *from Colombia*

Introduction

Plant parasitic nematodes belonging to the genus *Pratylenchus* are mobile endoparasites. They usually live in roots, rhizomes or tubers, but occasionally they are found in above-ground parts like stems or fruits. The root parts where these nematodes multiply often assume a dark red or brown color, caused by necrosis of the invaded cells and invasion of secondary pathogens like fungi or bacteria. These discolored patches (lesions) have earned *Pratylenchus* species the vernacular name of root lesion nematode and are a fairly good diagnostic (Loof, 1991).

Pratylenchus species are among the economically most damaging plant-parasitic nematodes and are found in a wide variety of crops. *Pratylenchus coffeae* is a species of world quarantine importance (Ryss, 2002). Although *Pratylenchus* species are polyphagous, there are clear differences in host preference among the species (Al-Banna, *et. al*., 2004). Most of the better known species have a wide distribution which is zonal in character. Some species occur throughout the northern temperate zone but also occur in mountainous regions of the tropics. Other species occur throughout the tropics but may maintain themselves in temperate zones in greenhouses. Pratylenchids multiply to very large numbers (10.000 – 35.000 specimens per 10 g of roots). It is not unusual to find two or three species together in a soil sample (Loof, 1991).

de Man is credited with describing the first root-lesion nematode as *Tylenchus pratensis* in 1880. In 1884 de Man redescribed and illustrated this species. The genus *Pratylenchus* was erected by Filipjev in 1936 with *P. pratensis* as the type species. The name had already been published in 1934 by Filipjev, but at that time no generic diagnosis was given; only the type species was indicated (Handoo & Golden, 1989).

The genus *Pratylenchus* at present comprises 139 original descriptions among them four subspecies (Table 5.1) but the number of valid species is variable according to different authors. Some species have been synonymized or considered *species inquirida* or *nomina nuda*.

Table 5.1 List of nominal *Pratylenchus* species based on Sher & Allen (1953), Loof (1960, 1978, 1991) Corbett (1969, 1983), Café Fihlo & Huang (1989), Frederick & Tarjan (1989), Handoo & Golden (1989), Siddiqi, 2000 and Ryss (2002) and supplemented with species more recently described. Junior synonym, *species inquirenda* and *nomen nudum* are indicated (valid species in bold according Siddiqi, 2000 and recently descriptions of new species).

- *P. aberrans* (Thorne, 1935) Filipjev, 1936 (transferred to *Nacobbus* by Thorne & Allen, 1944)
- ***P. acuticaudatus*** Braasch & Decker, 1989
- ***P. agilis*** Thorne & Malek, 1968 (sp. inq. for Frederick & Tarjan, 1989)
- ***P. alleni*** Ferris, 1961
- ***P. allius*** Shahina & Maqbool, 1996
- ***P. andinus*** Lordello, Zamith & Boock, 1961
- *P. angelicae* Kapoor, 1983 (nomina nuda for Siddiqi, 2000)
- ***P. angulatus*** Siddiqi, 1994
- ***P. arlingtoni*** Handoo, Carta & Skantar, 2001
- ***P. artemisiae*** Sheng & Chen, 1994
- ***P. australis*** Valenzuela & Raski, 1985 (syn. of *P. bolivianus* for Frederick & Tarjan, 1989)
- ***P. barkati*** Das & Sultana, 1979 (sp. inq. for Loof, 1991)
- ***P. bhattii*** Siddiqi, Dabur & Bajaj, 1991
- *P. bicaudatus* Meyl, 1954 (Meyl, 1961 syn. of *P. penetrans bicaudatus* Meyl, 1954; sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. bolivianus*** Corbett, 1983
- ***P. brachyurus*** (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941 (syn. *P. leiocephalus* Steiner, 1949; syn. of *P. steineri* Lordello, Zamith & Boock, 1954)
- ***P. brassicae*** Shahina & Maqbool, 1996
- *P. brevicercus* Das, 1960 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. brzseskii*** Karssen, Waeyenberge & Moens, 2000
- *P. capitatus* Das & Sultana, 1979 nec *P. capitatus* Ivanova, 1968 (syn. *P. dasi* and *P. neglectus*; sp. inq. for Handoo & Golden, 1989)
- ***P. cerealis*** Haque, 1965 (sp. inq. for Loof, 1978, 1991)
- ***P. chrysanthus*** Edward, Misra, Rai & Peter, 1969(sp. inq. for Loof, 1978, 1991; for Frederick & Tarjan, 1989; for Handoo & Golden, 1989)
- *P. clavicaudatus* Baranovskaya & Haque, 1968 (sp. inq. for Handoo & Golden, 1989; for Siddiqi, 2000; valid species for Frederick & Tarjan, 1989;)
- ***P. codiaei*** Singh & Jain, 1984 (sp. inq. for Loof, 1991)
- ***P. coffeae*** (Zimmerman, 1898) Filipjev & Schuurmans Stekhoven, 1941 (syn. *P. musicola* (Cobb, 1919) Filipjev, 1936; syn. *P. mahogany* (Cobb, 1920) Filipjev, 1936)
- *P. coffeae brasiliensis* Lordello, 1956 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- *P. coffeae brevicauda* Rahm, 1928(sp. inq. for Loof, 1960)
- ***P. convallariae*** Seinhorst, 1959
- ***P. crassi*** Das & Sultana, 1979 (sp. inq. for Loof, 1991)
- ***P. crenatus*** Loof, 1960 (syn. *P. pratensis* apud Thorne, 1949; syn. *P. clavicaudatus* in Loof, 1991)
- ***P. crossandrae*** Subramaniyan & Sivakumar, 1991
- ***P. cruciferus*** Bajaj & Bhatti, 1984

- *P. cubensis* Razjivin & O'Relly, 1976 (syn. *P. zeae*; sp. inq. for Handoo & Golden, 1989)
- ***P. curvicauda*** Siddiq, Dabur & Bajaj, 1991
- ***P. dasi*** Fortuner, 1985 (=nom.nov. for *P. capitatus* Das & Sultana, 1979; syn. *P. hyderbadensis* Singh & Gill, 1986; sp. inq. for Loof, 1991)
- ***P. delattrei*** Luc, 1958 (syn. *P. singhi* Das & Sultana, 1979; syn. *P. portulacus* Zarina & Maqbool, 1998)
- *P. dendrophilus* (Marcinowski, 1909) Filipjev, 1936
- ***P. dioscoreae*** Yang & Zhao, 1992
- ***P. dunensis*** de la Peña, Moens, van Aelst & Karssen, 2006
- ***P. ekrami*** Bajaj & Bhatti, 1984
- ***P. elamini*** Zeidan & Geraert, 1991
- ***P. emarginatus*** Eroshenko, 1978 (sp. inq. for Loof, 1991)
- ***P. estoniensis*** Ryss, 1982
- ***P. exilis*** Das & Sultan, 1979 (sp. inq. for Loof, 1991)
- ***P. fallax*** Seinhorst, 1968 (syn. of *P. cerealis* for Frederick & Tarjan, 1989)
- ***P. flakkensis*** Seinhorst, 1968
- ***P. gibbicaudatus*** Minagawa, 1982
- ***P. globulicola*** Romaniko, 1960 (syn. of *P. penetrans* for Loof, 1978, 1991; sp. inq. for Handoo & Golden, 1989)
- ***P. gongjuensis*** Choi, Lee, Park, Han & Choi, 2006
- ***P. goodeyi*** Sher & Allen, 1953
- ***P. gotohi*** Mizukubo & Minagawa, 1991
- ***P. graminis*** Subramaniyan & Sivakumar, 1991
- *P. graminophilus* (Goodey, 1933) Filipjev, 1936
- *P. gulosus* (Kuhn, 1890) Filipjev & Schuurmans Stekhoven, 1941 (syn. *P. penetrans*)
- *P. gutierrezi* Golden, López & Vílchez, 1992 (syn. *P. panamaensis*)
- *P. helophilus* Seinhorst, 1959 (syn. *P. pratensis*)
- *P. heterocercus* Kreis, 1930, syn. of *P. penetrans* for Andrassy, 1960; sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. hexincisus*** Taylor & Jenkins, 1957
- *P. hyderbadensis* Singh & Gill, 1986 (syn. *P. capitatus* and *P. dasi*)
- *P. himalayensis* Kapoor, 1983 (nomina nuda for Siddiqi, 2000)
- ***P. hippeastri*** Inserra, Troccoli, Gozel, Bernard, Dunn & Duncan, 2007
- *P. impar* Khan & Singh, 1975 (syn. *P. zeae*; valid species for Frederick & Tarjan, 1989; for Handoo & Golden, 1989)
- *P. indicus* Das, 1960 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- *P. irregularis* Loof, 1960 (syn. *P. pratensis*)
- ***P. jaehni*** Inserra, Duncan, Troccoli, Dunn, Maia Dos Santos, Kaplan & Vovlas, 2001
- ***P. japonicus*** Ryss, 1998 (syn. of *P. macrostylus japonicus* Ryss, 1988 (Mizukubo *et al.* (1997) elevated it to species level)
- ***P. jordanensis*** Hashim, 1984
- ***P. kasari*** Ryss, 1982
- *P. kolourus* Fortuner, 1985 (sp. inq. for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. kralli*** Ryss, 1982 (syn. of *P. ventroprojectus* for Frederick & Tarjan, 1989;
- ***P. kumaoensis*** Lal & Khan, 1989
- *P. leiocephalus* Steiner, 1949 (syn. *P. brachyurus*)
- ***P. loofi*** Singh & Jain, 1984 (sp. inq. for Loof, 1991)
- ***P. loosi*** Loof, 1960 (syn. *P. coffeae* apud Loos, 1953)
- ***P. macrostylus*** Wu, 1971

- *P. macrostylus japonicus* Ryss, 1988 (syn. *P. japonicus*)
- *P. mahogani* (Cobb, 1920) Filipjev, 1936 (syn. *P. coffeae*)
- ***P. manaliensis*** Khan & Sharma, 1992
- ***P. manohari*** Quraishi, 1982 (syn. of *P. cerealis* for Frederick & Tarjan, 1989; sp. inq. for Loof, 1991)
- ***P. mediterraneus*** Corbett, 1983
- *P. menthae* Kapoor, 1983 (nomina nuda for Siddiqi, 2000)
- ***P. microstylu****s* Bajaj & Bhatti, 1984
- *P. minyus* Sher & Allen, 1953 (syn. *P. neglectus*)
- *P. montanus* Zyubin, 1966 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. morettoi*** Luc, Baldwin & Bell, 1986
- ***P. mulchandi*** Nandakumar & Khera, 1970 (syn. of *P. muli* Nandakumar & Khera, 1969, = nomen nudum)
- *P. muli* Nandakumar & Khera, 1969 (syn. *P. mulchandi*)
- *P. musicola* (Cobb, 1919) Filipjev, 1936 (syn. *P. coffeae*)
- ***P. neglectus*** (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941(syn. of *P. minyus* Sher & Allen, 1953; syn. of *P. capitatus* Ivanova, 1968; syn of *P. neocapitatus* Khan & Singh, 1975; syn of *P. similis* Khan & Singh, 1975)
- ***P. neobrachyurus*** Siddiqi, 1994
- *P. neocapitatus* Khan & Singh, 1975 (syn. *P. neglectus*; valid species for Handoo & Golden, 1989)
- ***P. nizamabadensis*** Maharaju & Das, 1981 (sp. inq. for Loof, 1991)
- ***P. obtusicaudatus*** Romaniko, 1977 (sp. inq. for Loof, 1991)
- *P. obtusus* (Bastian, 1865) Goodey, 1951 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. okinawaensis*** Minagawa, 1991
- ***P. panamaensis*** Siddiqi, Dabur & Bajaj, 1991 (syn. of *P. gutierrezi* Golden, López & Vílchez, 1992)
- *P. peerlari* Chawla & Prasad, 1973 (syn. *P. ranjani*)
- ***P. penetrans*** (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 (syn. of *P. gulosus* (Kuhn, 1890) Filipjev & Schuurmans Stekhoven, 1941)
- ***P. pinguicaudatus*** Corbett, 1969
- *P. portulacus* Zarina & Maqbool, 1998 (syn. *P. delattrei*)
- ***P. pratensis*** (de Man, 1880) Filipjev, 1936 apud Thorne, 1949
- *P. pratensis bicaudatus* Meyl, 1954 (syn. *P. bicaudatus*)
- *P. pratensis tenuistriatus* Meyl, 1953 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. pratensisobrinus*** Bernard, 1984(syn. of *P. pratensis* for Frederick & Tarjan, 1989)
- ***P. pseudocoffeae*** Mizukubo, 1992
- ***P. pseudofallax*** Cafe Filho & Huang, 1989
- ***P. pseudopratensis*** Seinhorst, 1968
- ***P. ranjani*** Khan & Singh, 1975 (sp. inq. for Loof, 1978; valid sp. for Handoo & Golden, 1989; syn. of *P. peelari* in Chawla & Prasad, 1973 = nomen nudum)
- *P. rhizasinus* Sher, 1948 (nomina nuda for Siddiqi, 2000)
- ***P. roseus*** Zarina & Maqbool, 1998
- *P. sacchari* (Soltwedel, 1888) Filipjev, 1936 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- ***P. scribneri*** Steiner in Sherbakoff & Stanley, 1943
- *P. sefaensis* Fortuner, 1973 (syn. *P. pseudopratensis*; valid species for Handoo & Golden, 1989)
- ***P. sensillatus*** Anderson & Townshend, 1985

- ***P. septincisus*** Chang, 1991
- ***P. silvaticus*** Brzeskii, 1998
- *P. similis* Khan & Singh, 1975 (syn. *P. neglectus*; valid species for Frederick & Tarjan, 1989; Handoo & Golden, 1989)
- *P. singhi* Das & Sultana, 1979 (syn. *P. delattrei*; valid species for Handoo & Golden, 1989)
- *P. steineri* Lordello, Zamith & Boock, 1954 (syn. *P. brachyurus*)
- ***P. stupidus*** Romaniko, 1977 (sp. inq. for Loof, 1991; sp. inq. for Frederick & Tarjan, 1989)
- ***P. subpenetrans*** Taylor & Jenkins, 1957
- ***P. subranjani*** Mizukubo, Toida, Keereewan & Yoshida, 1990
- ***P. sudanensis*** Loof & Yassin, 1971
- ***P. tenuis*** Thorne & Malek, 1968 (sp. inq. for Loof, 1978, 1991; for Frederick & Tarjan, 1989)
- ***P. teres*** Khan & Singh, 1975
- ***P. thornei*** Sher & Allen, 1953 (syn. *P. thornei*, 1948 = nom. nud.)
- ***P. tulaganovi*** Samibaeva, 1966 (sp. inq. for Loof, 1978, 1991; for Frederick & Tarjan, 1989; for Handoo & Golden, 1989)
- *P. tumidiceps* Merzheevskaya, 1951 (sp. inq. for Frederick & Tarjan, 1989; for Handoo & Golden, 1989; for Siddiqi, 2000)
- *P. tumefaciens* (Cobb, 1932) Filipjev, 1936
- ***P. typicus*** Rashid, 1974
- ***P. unzenensis*** Mizukubo, 1992
- ***P. uralensis*** Romaniko, 1966 (sp. inq. for Loof, 1991; for Frederick & Tarjan, 1989)
- ***P. variacaudatus*** Romaniko, 1977 (sp. inq. for Loof, 1991; for Frederick & Tarjan, 1989)
- ***P. ventroprojectus*** Bernard, 1984 (syn. of *P. kralli* for Frederick & Tarjan, 1989)
- ***P. vulnus*** Allen & Jensen, 1951
- ***P. wescolagricus*** Corbett, 1983
- ***P. yamagutii*** Minagawa, 1991
- ***P. yassini*** Zeidan & Geraert, 1991
- ***P. zeae*** Graham, 1951 (syn. of *P. cubensis* Razjivin & O'Relly, 1976; syn. of *P. zeae* Steiner and Clayton & McMurtrey, 1950=nomen nudum; syn. of *P. impar* Khan & Singh, 1975)

Until now, nine *Pratylenchus* identification keys have been published. Characters commonly used to diagnose and to differentiate species are number of head annules, presence/absence of males, shape of head, shape of spermatheca, length and structure of posterior uterine sac (PUS), shape of female tail and terminus, the de man ratios, body length, length of stylet, shape of stylet knobs and structure of lateral field.

Pratylenchus has been considered as a genus with many sibling species which are difficult to separate because of the small number of diagnostic characters at species level and because of the intraspecific variability of some of these characters (Luc, 1987). Several studies on intraspecific variation of morphometric characters have been published (Anderson & Townshend, 1980; Corbett & Clark, 1982; Inserra *et al*., 1996,

1998, 2001, 2007; Rashid & Khan, 1976; Roman & Hirschmann, 1969; Tarjan & Frederick, 1978; Tarté & Mai, 1976a, 1976b; Taylor & Jenkins, 1957). The general outcome of these studies is that the majority of commonly used diagnostic characters are highly variable (Loof, 1991) and overlapping of morphometric characters between species is common.

The use of molecular diagnostic tools in combination with phylogenetic analyses contribute to overcome such a problem. The 28S rDNA gene has been used quite frequently to characterize different populations and species of plant-parasitic nematodes. DNA-based techniques have been used in the last twenty years to discriminate among species of *Pratylenchus* (Al-Banna *et al*., 1997, 2004; de la Peña *et al*., 2006, 2007; De Luca *et al*., 2004; Duncan *et al*, 1999; Handoo *et al*., 2001; Inserra *et al*., 2001, 2007; Orui, 1996; Uehara *et al*., 1998; Waeyenberge *et al*., 2000). Sequence variability was also considered in a phylogenetic framework in the studies above mentioned.

The purpose of this study was to characterize one *Pratylenchus* population from Colombia using morphological, morphometric and molecular criteria.

Materials and Methods

Terminology

Tail shape: Tails tapering strongly, with terminus narrow are described as "**conoid**"; tails tapering but little and with broad terminus are indicated as "**subcylindrical**". However, these two terms suggesting the existence of two well-separated classes of tail shape are inadequate, since a continuous grading transition series from markedly conoid to nearly **cylindrical** can be observed (Loof, 1991).

Tail terminus: When the transverse cuticular striation extends around the terminus, the latter is called **crenate**; when not, the terminus is **smooth** (Loof, 1991). A variation called "clef" which combines tail annulation and bilobed tail tip shape (Frederick & Tarjan, 1989) is included here. Tail tip shape in literature described as: blunty pointed, digitale, finely pointed, hemispherical, subdigitate, subhemispherical, truncate (see Frederick & Tarjan, 1989); pointed, conically rounded, rounded, spherical,

truncate, bilobed, irregular, conical with heel-like dorsal outline, obliquely truncate (see Ryss, 2002).

En face view of head: In *Pratylenchus*, the *en face* view of head seen by SEM is characterized by the fusion of the median lip sectors to each other and the oral disc; the large lateral lip sectors remain separated in many species (Geraert, 2006). Different arrangements divide the genus into three groups. In group 1, the nematodes have a plain undivided face with no division between sub-median and lateral lip sectors. In the other two groups, the area immediately surrounding the oral aperture is similar, but the outer part of the face is sub-divided into sub-median and lateral sectors. Group 2 has sub-median segments that are fused to the oral disc and are narrower at their inner extremity but widened towards the outer edge of the face: they are separated from the larger lateral segments that complete the circular face, with amphid openings on their inner edges. Group 3 has a more distinctive dumb-bell shaped pattern of the sub-median segments with slightly smaller lateral segments to complete the circle. The amphid apertures are again on the inner edges of the lateral lip sectors (Corbett & Clark, 1982).

Morphological observations

Root and soil samples of plantain (*Musa* AAB, "dominico hartón" cultivar) were collected in fields from Arauca (Los Llanos Orientales Region) situated in the East of Colombia (see general material and methods).

Nematodes were identified to species level using both original descriptions and polytomous identification keys. *Pratylenchus* diagnostic keys used were from Brzeskii (1998), Café Fihlo & Huang (1989), Corbett (1969), Frederick & Tarjan (1989), Handoo & Golden (1989), Sher & Allen (1953), Loof (1960, 1991) and Ryss (2002). Morphological comparative studies by Corbet & Clark (1982), Mizukubo (1992), Roman & Hirschmann (1969); Tarjan & Frederick (1978), Tarté & Mai (1976a, 1976b) using LM and SEM were considered.

Molecular characterization

Six nematodes from Arauca population were included in the analysis. DNA was extracted from a single nematode previously identified morphologically and used for

analysis of three different regions of rDNA: D2D3 expansion region of 28S, 18S and ITS1-5.8-ITS2.

Preparation of DNA, sequence analyses, sequence comparison and phylogenetic analyses were conducted as explained in chapter 2, general materials and methods. Only some details concerning the outgroup used for phylogenetic analyses/trees are mentioned here.

Radopholus sp., DQ328712 accession number and one *Helicotylenchus multicinctus*, accession number DQ328745 from Subbotin *et al.* (2006, 2007) were selected as outgroup taxa for D2D3. For 18S analysis, outgroup taxa selected were *R. similis* accession AJ966502 and *H. dihystera*, accession number AJ966486 from Meldal *et al.* (2007). *Radopholus similis* accession AY903311 and one *H. dihystera*, accession number DQ309585 from Zhou *et al.* (2005, unpublished) and Chen *et al.* (2006, unpublished) were outgroup taxa for ITS analyses.

Results

Morphological identification

Nematodes samples collected in fields of plantain (*Musa* ABB) from Arauca (Los Llanos Orientales Region) in Eastern Colombia fitted morphological descriptions for a root lesion nematode species described here as *Pratylenchus* n.sp.

Pratylenchus n. sp.

(Figs 5.1 – 5.2)

Measurements

Females and males: see Table 5.2.

Description

Female

Body vermiform, slender and tapering towards both ends. Cuticle finely annulated with annules 0.9 μm wide at mid-body. Lateral field marked by four lines or three bands beginning at level of annule 7 as two lines, widening to three at following

annules and to four by level of median pharyngeal bulb and extending to phasmid. Only outer incisures extending beyond phasmid i.e. a few annules anterior to the tail tip. Lateral field with four lines non-equidistant, with middle ridge narrower than outer ridges, occupying almost half of body diam. at mid-body. Outer lines partially areolated, with alternate complete and one to three incompletely interrupted transverse striae. The internal band can be smooth or sculptured by striae, parallel to the main incisures.

Head not offset from the neck region by constriction but often showing a small discontinuity in body outline at its base; head width over three times its height. Moderate sclerotization of cephalic framework. Two annules on the labial region (second lip annulus thicker than first), but also two annules on one side and three annules on the other (LM). In scanning electron microscopy a minute and incomplete initiation of a third lip annulus visible in some specimens. Anterior surface of the oral disc may be low-conical or flat with rounded edges. Face view characterized by complete fusion of the lip sectors to each other and to oral disc, resulting in a plain and smooth face.

Stylet short and strong, with well developed round to oblong basal knobs, directed laterally. Stylet cone length equal to length of shaft plus knobs. Dorsal pharyngeal gland opening at 1.8-3.1 µm (mean *ca* 2.7 μm) behind the stylet knobs. Median bulb oval, occupying half of body width. Secretory-excretory pore and canal located slightly anterior to pharyngeal-intestinal junction. Hemizonid located just anterior to S-E pore and two body annuli long. Hemizonion distinct and located eight to ten annules posterior to hemizonid; not visible in some specimens. Pharyngeal glands overlapping intestine ventrally.

Vulva at 78 percent of total body length from anterior end and lips well-developed, often protruding. Genital tract monoprodelphic; extending forward for nearly two times the distance between vulva and anus; ovary with single row of oocytes. Spermatheca variable in size, oval to slightly round, filled with rounded sperm. Distance from vulva to anus three times the distance between vulva and spermatheca. Post-uterine sac generally short, undifferentiated. Sometimes cellular tissue is present at the distal end. Anus round to oval shaped.

Table 5. 2 Morphometrics of *Pratylenchus* n. sp. from Colombia. Measurements in µm and in form: mean ± standard deviation (range).

	Female	Male
n	40	36
L	462 ± 31 (376 - 511)	438 ± 32 (377 - 517)
a	23.9 ± 2.3 (19.6 – 29.2)	25.9 ± 2.6 (21.3 – 36.8)
b	6.2 ± 0.6 (4.8 – 7.8)	6.2 ± 0.5 (5.1 – 7.5)
b'	4.7 ± 0.4 (3.6 - 5.9)	2.0 ± 0.5 (1.5 – 4.6)
c	17.6 ± 1.4 (14.5 – 21.1)	4.8 ± 0.5 (3.8 – 5.7)
c´	2.3 ± 0.3 (1.7 - 2.9)	2.5 ± 0.4 (1.8 – 3.5)
V%	78 ± 1.2 (75 - 80)	-
m.b.w	19.5 ± 2.3 (15.3 – 23.3)	17.0 ± 1.8 (13.0 – 20.0)
Body diam. at vulva	17.0 ± 1.8 (13.5 – 20.9)	-
Body diam. at anus	11.4 ± 1.5 (8.6 – 17.2)	9.6 ± 1.4 (7.4 – 12.3)
Head diam.	7.2 ± 0.3 (6.7 – 7.4)	6.6 ± 0.4 (6.1 – 7.4)
Head height	2.5 ± 0.7 (2.0 – 4.0)	2.6 ± 0.3 (2.0 – 4.0)
Stylet length	15.3 ± 0.4 (14.7 – 15.9)	14.3 ± 0.8 (12.9 – 15.9)
DGO	2 .7 ± 0.4 (1.8 – 3.1)	2.6 ± 0.4 (1.8 – 3.4)
Anterior end to S-E pore	72 ± 5.3 (57 - 84)	70 ± 5.2 (55 - 86)
to pharyngo-intestinal junction	75 ± 5.4 (61 - 88)	71 ± 5.5 (59 - 82)
to posterior end pharyngeal gland	99 ± 8.4 (78 - 115)	93 ± 8.9 (82 - 117)
to vulva	359 ± 24.4 (298 - 401)	-
Pharyngeal gland length	24.2 ± 7.7 (10.4 – 46.6)	22.0 ± 6.3 (11.0 – 43)
Length posterior uterine sac	18.1 ± 4.0 (9.8 – 24.5)	-
Tail length	26.3 ± 2.5 (21.5 – 31.9)	23.8 ± 2.5 (19.0 – 30.7)
Tail annules	99 ± 8.4 (78 - 115)	-
Anterior genital branch	142 ± 20.5 (98 - 176)	-
Vulva – anus distance	12.1 ± 0.8 (11 – 14.5)	-
Spicula length	-	19.0 ± 2.2 (16.0 – 22.0)
Gubernaculum length	-	6.5 ± 2.4 (5.3 – 6.5)

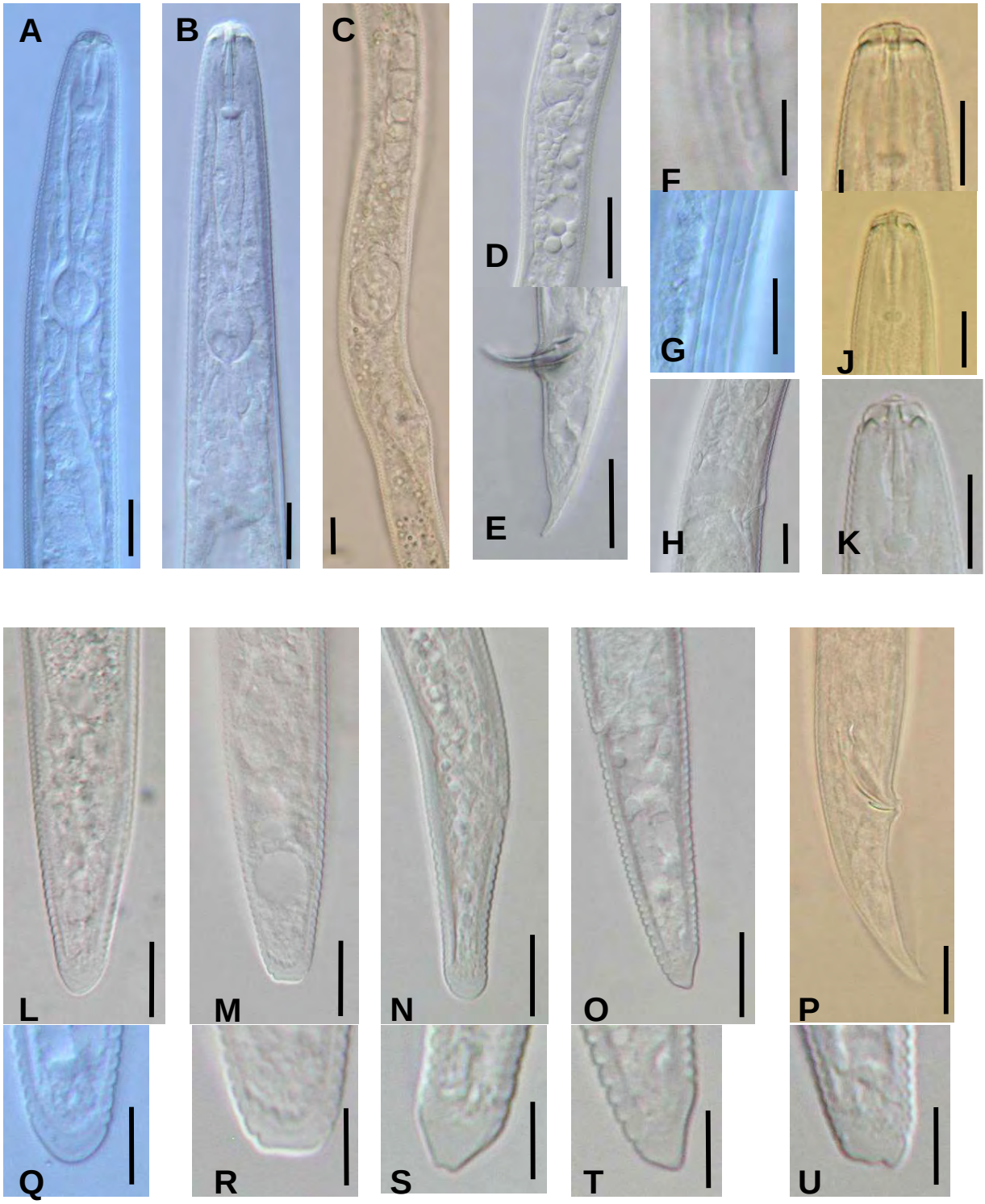


Fig. 5.1 LM photographs of *Pratylenchus* n. sp. from Colombia. Female (A, C-D, F-O, Q-U); male (E, P); anterior end (A, B); female reproductive system (C); PUS (D), spicule details (E); lateral field at mid-body (F), at vulva (G); S-E pore (H); head (I-K); tail shape of female (L-O), male (P) and tail terminus (Q-U). Scale bars: A-P: 10µm; Q-U: 5µm.

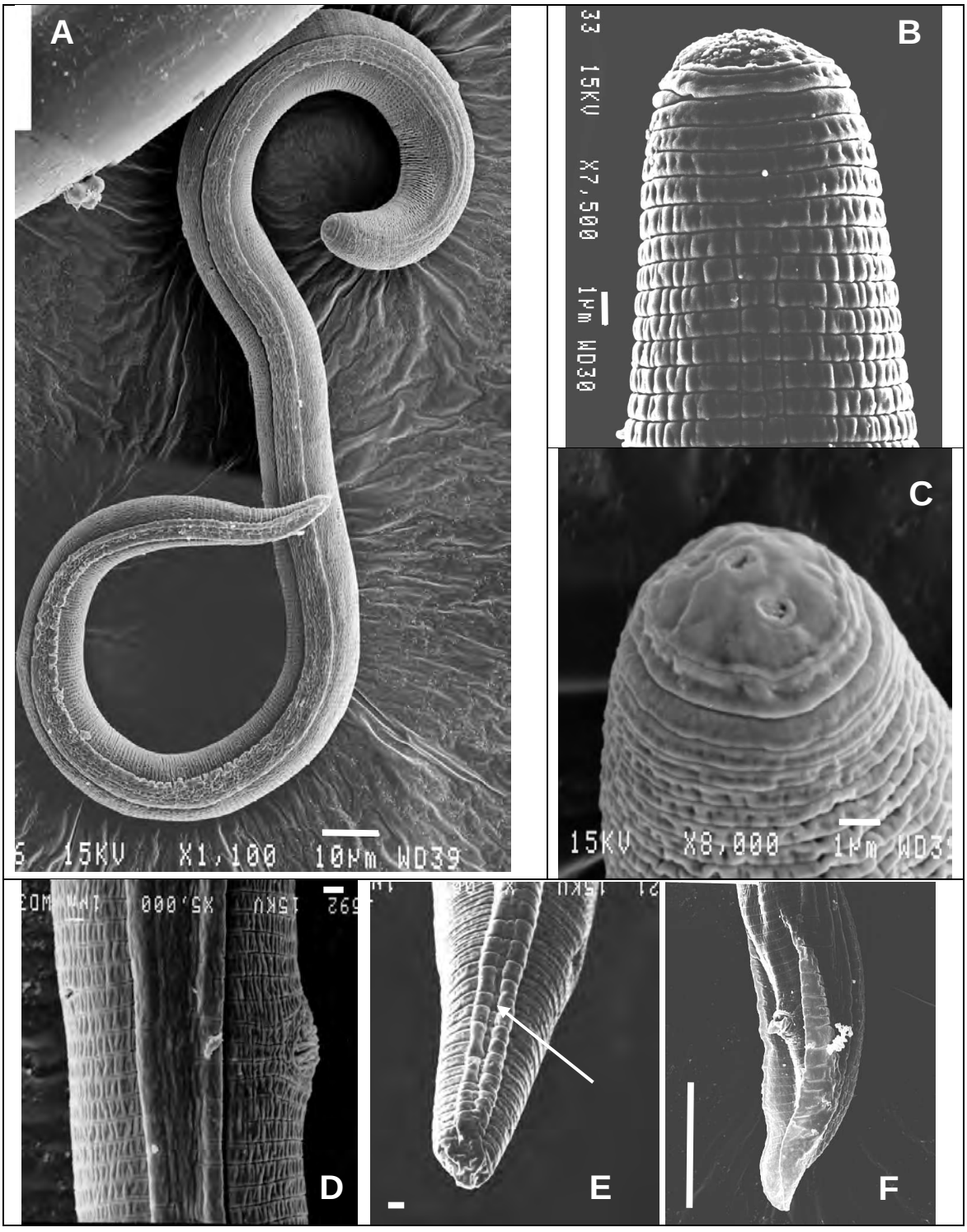


Fig. 5. 2 SEM Photographs of *Pratylenchus* n. sp. from Colombia. Female (A-E); male (F); entire body (A); anterior end (B); face view (C); lateral field at vulva (D); tail shape of female with arrow shown phasmid (E); tail shape of male with bursa details (F). Scale bars: A, F: 10µm; B, C, D and E: 1 µm.

Tail clearly conoid in half of population but subcylindrical (25%) or continuous grading from conical to subcylindrical (25%) in rest of population. Tail terminus variable in shape, mostly conical rounded or rounded but also truncate, subdigitate, bilobed, trilobed or occasional cleft tip. Twenty one (16-28) annules present on ventral side of tail; smooth tail tip. In SEM an unusual form of break in annulation just above tail tip visible; resulting in a 'tiled' appearance. Small, rounded phasmid located at mid-tail, between outer lateral field lines, 10 to 15 annules anterior to tail tip. Hyaline portion of tail terminus distinct.

Male

As abundant as female. Morphologically similar to females, but smaller for all morphometric data, except ratio a, being larger than in females. Reproductive system with a single outstretched anterior testis, not extending to pharyngeal glands. Testis length *ca* 195 µm; spicule length *ca* 15 µm. Sperm globular. Gubernaculum simple, not protruding. Male tail pointed; bursa arising from a little anterior to head of spicule and enveloping tail; bursa edge smooth or finely crenate proximally. Phasmid distinct, situated in bursa region at, or just anterior to mid-tail.

Type host and locality

Pratylenchus n. sp. was found in roots of *Musa* ABB, hartón cultivar in Caño Limón area, Municipality of Tame, Arauca State, at North East of Colombia. Latitude 6°56'N; longitude: 71°05'; altitude 140 meters above sea level; temperature 26.5°C; annual precipitation 2000 mm and tropical climate; topography: alluvial plain; soil type sandy; life zone (Holdridge, 1964): Tropical dry forest.

Diagnosis and relationships

Pratylenchus n. sp., is characterised by a combination of the following morphological features of females: slender body, flat, plain and smooth face, head with two lip annuli, with or without an incomplete third annulus and second lip annulus thicker than anteriormost, stylet ca 15.3 µm, vulva at 78% of body length from anterior end, oval to rounded spermatheca filled with round sperm, and conoid to subcylindrical tail with smooth and variable shape terminus.

As is the case for *Pratylenchus* n. sp. the following nine species all have two lip annules; a non-crenate tail and female spermatheca filled with sperm and males present, usually abundant: *P. alleni* Ferris, 1961; *P. brzeskii* Karssen, Waeyenberge & Moens, 2000; *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941; *P. dunensis* de la Peña, Moens, van Aelst & Karssen, 2006; *P. jaehni* Inserra, Duncan, Troccoli, Dunn, Maia Dos Santos, Kaplan & Vovlas, 2001; *P. loosi* Loof, 1960; *P. neobrachyurus* Siddiqi, 1994 and *P. pseudocoffeae* Mizukubo, 1992.

Morphologically *Pratylenchus alleni* differs from *Pratylenchus* n. sp. by a shorter body (330-440 *vs* 376-511µm); smaller stylet (<15 *vs* 14.7-15.9 μm); more posterior vulva position (78-83 *vs* 75-80%); spherical spermatheca (*vs* oval to rounded); tail terminus with 1-2 indentations; phasmids in the anterior half of the tail (*vs* phasmids at mid-tail). *P. pseudocoffeae* differs from *Pratylenchus* n. sp. in a generally more posterior vulva position (78-83 *vs* 75-80%), long pharyngeal overlap (61-115 *vs* 10-47 μm), long distance between the base of the pharyngeal gland lobe and head end (141-210 *vs* 78-115 μm), elongated spermatheca (*vs* oval to round) and a well differentiated post uterine branch (*vs* undifferentiated and short). *Pratylenchus neobrachyurus* is shorter than *Pratylenchus* n. sp. (310-410 *vs* 376-511 μm) with a more posterior vulva (80-83 *vs* 75-80%), anterior head annule angular, and a non-areolated lateral field. *P. brzeskii* is longer than *Pratylenchus* n. sp. (691 *vs* 462 μm), has a greater body diameter (24 *vs* 20 μm), a longer stylet (19 *vs* 15.3 µm) and tail length (48 *vs* 26 μm).

Examination of the *en face* head pattern by SEM separate other species above mentioned. *Pratylenchus* n. sp., *P. coffeae** , *P. jaehni* and *P. loosi* are the only amphimictic lesion nematodes with two lip annuli, and with the lip sectors fused together and also fused with the oral disc, presenting a smooth face. The head pattern of *P.*

* *P. jaehni* (paratypes), *P. coffeae* (K6) and *P. loosi* (T) morphometric used for our comparison were taken from Inserra *et al.* (2001) and Duncan *et al.*, 1999. Research of Inserra *et al.* (2001) provided the first evidence that the *P. coffeae* specimens from Java described by Zimmermann (1898) probably had smooth faces. They selected the isolate K6 (*P. coffeae*) from coffee in Kaliwining, Java and *P. loosi* isolate T from tea of Sri-Lanka for use as a standard for morphological comparison with other *Pratylenchus* populations included in their study. Because measurements of live specimens of *P. loosi* were reported, measurements of fixed specimens of the same isolate (T) but from other papers by the same authors (Duncan *et al.*, 1999) were considered.

dunensis (dum-bell shaped pattern) and *P. yamagutii* fits to group 3 and 2 of Corbett & Clark (1982), respectively.

P. coffeae, P. jaehni** and *P. loosi** share a tail terminus smooth, unstriated but different in shape, the latter is truncate or hemispherical in *P. coffeae* (standard populations used by Inserra, 2001), usually subhemispherical in *P. jaehni* and bluntly or finely pointed in *P. loosi*. Tail terminus of *Pratylenchus* n. sp. shows considerable variation in shape: conical rounded or rounded in most specimens but truncate, subdigitate, bilobed, trilobed or cleft tip also occurs occasionally.

Pratylenchus n. sp. shares with *P. jaehni* a generally posterior vulva position though less posterior compared to *P. coffeae* and *P. loosi* (78 *vs* 80%). Body length is shorter in *Pratylenchus* n. sp. than in *P. jaehni, P. loosi* and *P. coffeae* (462 *vs* 488, 522, 622 μm). The mean values of body width (19.5 *vs* 23.7, 21.6, 23.3 μm), width at vulva (17 *vs* 20.2, 18.1, 19.5 μm), width at anus (11.4 *vs* 13.0, 12.1, 13.2 μm), pharyngeal overlap (24.2 *vs* 33.0, 50.1, 49.8 µm), post-uterine branch (18.1 *vs* 22.9, 29.1, 28.8 μm); ratio c (17.6 *vs* 19.2, 18.3, 19.5 μm) are also smaller in *Pratylenchus* n. sp. compare to those in *P. jaehni, P. loosi* and *P. coffeae* (Table 5.3).

The mean values of tail length (26.3 *vs* 25.3, 29.0, 32.0 µm), stylet length (15.3, *vs* 14.5, 15.5, 16.4 µm), and ratio a (23.9 *vs* 20.7, 24.7, 26.0 µm) are larger in *Pratylenchus* n. sp. than in *P. jaehni*, but smaller that in *P. loosi* and *P. coffeae* (Table 5.3).

Because following the *Pratylenchus* keys the root lesion nematode specimens from Colombian (*Pratylenchus* n. sp.) result in *P. coffeae*, comparison of morphological characters of 26 *P. coffeae* populations from different hosts and localities worldwide are presented in Table 5.4. In general, *Pratylenchus* n. sp. shared maximum 30% of morphological and morphometrical character values with each one of all populations of *P. coffeae* considered.

Table 5.3 Comparison of morphometrics of *Pratylenchus* n. sp. population from Colombia with those of *P. jaehni*, *P. loosi* and *P. hippeastri* from different crops and localities around the world. Measurements in µm and in form: mean ± standard deviation (range).

	Pratylenchus n. sp. *Musa* AAB Colombia	*P. jaehni* *Citrus* Brazil 2001	*P. jaehni* *Citrus*-**C1** Brazil 1999	*P. jaehni* *Citrus*-**C2** Brazil 1999	*P. jaehni* *Coffee*-**K5** Brazil 1999	*P. loosi* bahia grass USA-**N1**	*P. loosi* b. grass * USA-**N1b**	*P. loosi* maiden.* USA-**N2**	*P. loosi* maiden. USA-**N2b**	*P. loosi** tea-**T** Sri Lanka	*P. coffeae* *Coffee*-**K6** Java	*P. hippeastri* Amarillis USA
n	40	20	20	20	20	20	20	20	20	20	20	20
L	462 ± 31 (376 - 511)	488 ± 28 (434 - 537)	517 ± 6 (462 - 553)	527 ± 6 (475 - 576)	483 ± 41 (394 - 561)	500 ± 13 (421 - 602)	507 ± 6 (458 - 549)	538 ± 9 (452 - 602)	631 ± 14 (511 - 745)	522 ± 13 (430 - 615)	622 ± 36 (536-670)	590± 22 (445 - 627)
a	23.9 ± 2.3 (19.6-29.2)	20.7 ± 2.0 (16.6- 5.8)	23.7 ± 0.4 (20.1-26.3)	25.0 ± 0.4 (22.4-28.2)	25.6 ± 1.5 (21.9-28.3)	28.2 ± 0.6 (23.9- 2.8)	25.3 ± 0.3 (21.8-27.7)	28.4 ± 0.4 (24.4- 1.4)	26.8 ± 0.5 (22.3-32.6)	24.7 ± 1.0 (19.5-29.9)	26.0 ± 1.8 (23.9-30.5)	25.5 ± 1.2 (23.2– 7.9)
b	6.2 ± 0.6 (4.8 -7.8)	6.0 ± 0.4 (5.4- 6.7)	5.9 ± 0.1 (5.3-6.7)	5.9 ± 0.1 (5.3-6.8)	5.4 ± 0.5 (4.3- 6.2)	5.4 ± 0.1 (4.8-6.5)	5.5 ± 0.1 (4.8-5.9)	5.6 ± 0.1 (4.9- 6.3)	6.1 ± 0.1 (5.3-7.0)	5.8 ± 0.2 (4.7-7.1)	6.9 ± 0.4 (6.0-7.5)	6.5 ± 0.4 (5.7-7.1)
c	17.6 ± 1.4 (14.5-21.1)	19.2 ± 1.7 (17.1-23.7)	20.4 ±0.5 (17.8-25.0)	19.5 ± 0.5 (19.5-24.6)	19.7 ± 1.5 (17.2-23.1)	15.7 ± 0.4 (12.0-18.6)	17.1 ± 0.3 (15.1-19.7)	16.7 ± 0.3 (15.2-19.4)	16.9± 0.3 (14.5-19.5)	18.3 ± 0.4 (16.2-20.5)	19.5 ± 1.1 (17.4-21.4)	16.1 ± 1.0 (14.6-18.7)
V%	78 ± 1.2 (75- 80)	78 ± 0.7 (77– 80)	79 ± 0.2 (78–81)	79 ± 0.3 (77–81)	79 ± 1.5 (77– 82)	77 ± 0.2 (76–79)	78 ± 0.2 (76–79)	77 ± 0.2 (75–79)	77± 0.2 (76–79)	80 ± 0.3 (79- 83)	80.2 ± 0.9 (79.0-82.0)	77 ± 0.8 (75-80)
m.b.w.	19.5 ± 2.3 (15.3-23.3)	23.7 ± 2.3 (18.5-28.5)	21.9 ± 0.4 (19.5-24.5)	21.2 ± 0.2 (19.0-23.0)	18.9 ± 1.8 (15.5-22.0)	17.7 ± 0.3 (16.5-20.0)	20.1 ± 0.3 (18.0-23.0)	19.0 ± 0.3 (17.0-22.5)	23.6 ± 0.5 (19.5-27.0)	21.6 ± 1.1 (16.0-31.0)	23.3 ± 2.1 (17.5-26.0)	23.2 ± 1.4 (21.0-27.0)
Stylet	15.3 ± 0.4 (14.7-15.9)	14.5 ± 0.3 (14.0-15.0)	15.2 ± 0.1 (14.5-16.0)	15.1 ± 0.1 (14.5-15.5)	15.3 ± 0.4 (15.0-15.0)	15.3 ± 0.1 (14.7-15.8)	15.0 ± 0.1 (14.0-15.5)	15.0± 0.1 (14.0-15.5)	15.1 ± 0.1 (14.0-15.5)	15.5 ± 0.1 (15.0-16.0)	16.4 ± 0.2 (16.0-16.5)	15.5 ± 0.4 (15.0-16.0)
S-E pore	72 ± 5.3 (57- 84)	73 ± 4.5 (74-91)	88 ± 1.0 (81-98)	87.0 ± 1.3 (72-96)	84 ± 5.3 (75-92)	84 ± 1.1 (78-95)	84 ± 1.2 (75-92)	87 ± 1.3 (68-95)	97± 1.6 (84-113)	75 ± 1.6 (64-87)	91 ± 4.3 (85-97)	91± 2.5 (85-95)
ph.aryngeal gland	99 ± 8.4 (78-115)	81± 5.4 (70 - 92)	87± 0.9 (82 - 95)	90 ±1.1 (81 - 98)	90 ± 3.7 (82 - 99)	92 ± 0.8 (86-96)	92± 1.1 (83 - 100)	97 ± 0.7 (90 - 103)	104 ± 1.4 (93 - 119)	92 ± 2.8 (80 - 109)	90.1 ± 3.1 (86 -96)	81± 5.4 (70 - 92)
PUS	18.1 ± 4.0 (9.8-24.5)	22.9 ± 4.5 (17.5-34.0)	23.8 ± 1.0 (16.5-31.0)	27.3 ± 0.8 (20.5-36.0)	20.5 ± 2.4 (16.5-26.0)	30.0 ± 0.8 (23.5-35.2)	28.4 ± 1.0 (22.5-40.0)	29.7 ± 1.3 (21.5-42.0)	34.8 ± 2.1 (21.5-53.5)	29.1 ± 0.9 (25.0-36.0)	28.8 ± 4.2 (21.5-35.0)	30.0 ± 4.9 (21.0-45.0)
Tail length	26.3 ± 2.5 (21.5-31.9)	25.3 ±2.0 (22.0-29.0)	25.6 ± 0.6 (20.0-30.0)	27.3 ± 0.7 (22.0-34.0)	24.6 ± 2.2 (19.5-30.0)	31.9 ± 0.6 (27.4-36.2)	29.8 ± 0.6 (24.0-36.0)	32.2± 0.5 (28.5-37.0)	37.4 ± 0.7 (31.0-41.1)	32.0 ± 2.3 (28.0-36.0)	32.0 ± 2.3 (28.0-36.0)	36.8 ± 2.2 (32.0-42.0)

* b. grass: bahia grass; madein.: madeincane.

Table 5.4a Comparison of morphometrics of *Pratylenchus* n. sp. population from Colombia with those of *P. coffeae* from different crops and localities around the world. Measurements in µm and in form: mean ± standard deviation (range).

Population	This study	Sher & Allen (1953)	Loof (1960)	Roman & Hirschmann (1969)	Van den Berg (1971)	Rashid & Khan (1976)	Rashid & Khan (1976)
Locality	Arauca Colombia	Bangor Indonesia		Florida USA	Cape South Africa	Aligarh India	Aligarh India
Host	*Musa* ABB roots	*Coffee* sp. roots	-	Chinese evergreen	vegetable	*Chrysanthemum carinatum*- roots	*Chrysanthemum carinatum*- soil
n	40	-	69	25	2	20	20
L	462 ± 31 (376 - 511)	590* (450-700)	529 (374-698)	593 ± 11 (516-721)	- (620 & 590)	590 ± 14 (500-725)	519 ± 0.0 (445-610)
a	23.9 ± 2.3 (19.6 – 29.2)	34* (25-35)	23.7 (17.7-30.5)	26.3 ± 0.6 (20.1-33.7)	- (20.3 & 14.1)	21.5 ± 0.6 (17.6-28.0)	25.6 ± 0.6 (21.7-29.1)
b	6.2 ± 0.6 (4.8 – 7.8)	6.3* (5-7)	6.8 (5.0-7.8)	6.7 ± 0.1 (5.2-7.5)	-	6.6 ± 0.2 (5.7-7.5)	6.4 ± 0.1 (5.9-7.0)
c	17.6 ± 1.4 (14.5 – 21.1)	21* (17-22)	19.0 (13.7-23.9)	18.2 ± 0.3 (14.9-20.8)	- (33.4 & 25.9)	21.1 ± 0.8 (16.6-31.0)	19.9 ± 0.6 (16.4-27.7)
V%	78 ± 1.2 (75 - 80)	81.9* (76-83)	80.1 (75.8-84.2)	78.6 ± 0.4 (74-79)	- (83.4 & 83.6)	73 ± 0.4 (74-80)	76 ± 0.7 (65-80)
m.b.w.	19.5 ± 2.3 (15.3 – 23.3)	-	-	22.5 ± 0.5 (19.2-27.0)	-	27.5 ± 0.7 (22-34)	21.0 ± 0.6 (17-26)
Stylet	15.3 ± 0.4 (14.7 – 15.9)	18* (15-18)	- (14-17.5)	15.5 ± 0.1 (14.4-16.8)	- (17.6 & 16.9)	16.3 ± 0.2 (15.0-17.0)	15.3 ± 0.2 (15.0-17.0)
S-E- pore	72 ± 5.3 (57 - 84)	-	-	87 ± 1.0 (81-104)	- (114 & 84.6)	91 ± 1.4 (78-100)	80 ± 1.6 (70-102)
Pharynx length	75 ± 5.4 (61 - 88)	-	-	87 ± 0.9 (78-97)	-	118 ± 2.0 (102-145)	115 ± 3.1 (92-145)
PUS	18.1 ± 4.0 (9.8 – 24.5)	1.5 BDV	-	-	- (23.5 & 55.9)	44.8 ± 2.6 (19-63)	31 ± 1.9 (18-52)
Tail length	26.3 ± 2.5 (21.5 – 31.9)	-	-	32 ± 0.5 (27.0-38.4)	- (18.8 & 22.8)	28.2 ± 1.0 (19-33)	27 ± 0.6 (22-32)

Table 5.4b Comparison of morphometrics of *Pratylenchus* n. sp. population from Colombia with those of *P. coffeae* from different crops and localities around the world. Measurements in µm and in form: mean ± standard deviation (range). Populations with asterisk (*) were included in phylogenetical analyses.

Population	Bajaj & Bhatti (1984)	Pourjam (1997a)	Pourjame (1997b)	Inserra *et al.* (1996)	Inserra *et al.* (1996)	Inserra *et.al* (1996)	Duncan *et al.* (1999)
Locality	Sirsa India	Lahijan & Shiraz-Iran	Honduras	Florida USA	Florida USA	Florida USA	Florida USA
Host	Citrus-soil	Citrus	-	*Citrus lemon*	*Citrus lemon*	*Citrus lemon*	*Citrus roots* - C4*
n	30	8	7	20	20	20	20
L	615 ± 44 (552-703)	515 ± 43 (460-570)	526 ± 25 (480-560)	700 ± 54 (568-788)	748 ± 59 (618-838)	731± 85 (586-907)	749 ± 13 (618-839)
a	28 ± 4.9 (23-38)	27 ± 4 (22-32)	24.7 ± 2.4 (20.4-27.0)	28.5 ± 1.9 (24.2-32.1)	29.5 ± 1.6 (25.2-32.9)	28.4 ± 2.1 (25.4-33.1)	29.6 ± 0.4 (25.2-33.0)
b	7.3 ± 0.5 (6.1-7.8)	5.5 ± 0.4 (5.1-6.3)	6.5 ± 0.4 (5.7-7.0)	7.0 ± 0.5 (6.1-8.1)	7.4 ± 0.6 (6.3-8.4)	7.0 ± 0.5 (5.9-8.0)	7.4 ± 0.1 (6.3-8.4)
c	20 ± 2.1 (17-26)	19.8 ± 1.8 (16.5-22.0)	17.7 ± 1.7 (14.6-20.0)	21.3 ± 2.2 (18.3-26.2)	22.7 ± 2.4 (18.5-28.4)	21.5 ± 2.1 (17.5-27.2)	22.8 ± 0.5 (18.6-28.4)
V%	81 ± 1.2 (79-84)	81 ± 1.3 (80.0-82.5)	79 ± 1.8 (76-80)	79.6 ± 1.9 (76-83)	80.6 ± 1.4 (78-84)	81± 1.3 (77-83)	80.6 ± 0.4 (78.3-83.5)
m.b.w.	24 ± 3.6 (15-27)	19.9 (15-25)	21.4 ± 2.1 (20-25)	-	-	-	25.3 ± 0.4 (22.5-28.4)
Stylet	15 ± 0.4 (14-17)	16.0 ± 1.1 (15.0-18.0)	15.1 ± 0.7 (14.0-16.0)	16.5 ± 0.3 (15.5-17.0)	16.5 ± 0.6 (15.5-17.5)	16.5 ± 0.4 (15.5-17.5)	16.7 ± 0.2 (15.7-17.7)
SE- pore	-	87 ± 3.9 (80-90)	85.± 4.9 (81-91)	103 ± 6.5 (89-117)	103 ± 6.0 (89-112)	103 ± 9.9 (87-127)	103 ± 1.3 (89-112)
Pharynx length	-	93 ± 4.9 (90-100)	124 ± 9 (116-138)	-	-	-	101 ± 1.8 (88-115)
PUS	27 ± 4.8 (18-37)	28 ± 5.0 (20-33)	28.6 ± 6.3 (17-36)	58 ± 22.6 (21.5-102)	62 ± 22.7 (32-100)	63 ± 23.4 (31-111)	62.4 ± 5.1 (32.3-99.9)
Tail length	30 ± 3.7 (24-39)	26.0 ± 2.2 (21-30)	30.0 ± 2.9 (26-35)	-	33 ± 2.3 (28.0-37.0)	34 ± 2.7 (29-39)	33.0 ± 0.5 (28.4-37.2)

Table 5.4c Comparison of morphometrics of *Pratylenchus* n. sp. population from Colombia with those of *P. coffeae* from different crops and localities around the world. Measurements in µm and in form: mean ± standard deviation (range).

Population	Inserra *et al.* (1998)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)
Locality	Djember - Java Indonesia	Kaliwining Indonesia	Ghana	Honduras	Costa Rica	Malaysia	Florida USA
Host	*Coffee* sp. 45-yr-old	*Coffee sp* roots – K6*	Banana roots - B1*	Banana roots - B2*	Banana roots - B3	Banana roots - B4	*Aglaonema* - M3*
n	7	20	10	10	15	16	20
L	514± 38 (467-579)	622 ± 36 (536-670)	674 ± 14 (563 - 808)	659 ± 26 (474 - 749)	626 ± 7 (576 - 672)	533 ± 9 (438 - 620)	646 ± 64 (527-763)
a	21.8 ± 2.4 (19.1-25.0)	26.0 ± 1.8 (23.9-30.5)	24.0 ± 0.4 (21.0 – 27.7)	24.1 – 0.6 (20.6 – 26.2)	23.2 ± 0.6 (19.6 – 27.3)	25.9 ± 0.5 (21.8 – 28.5)	23.4 ± 2.0 (19.7-27.3)
b	6.2 ± 0.4 (5.6-6.8)	6.9 ± 0.4 (6.0-7.5)	6.9 ± 0.2 (5.7 – 8.4)	6.8 ± 0.2 (5.6 – 7.6)	6.8 ± 0.1 (6.3 – 7.8)	6.1 ± 0.1 (5.3 – 7.2)	6.8 ± 0.4 (5.9-7.4)
c	17.7 ± 1.7 (16.0-20.3)	19.5 ± 1.1 (17.4.-21.4)	20.5 ± 0.4 (18.2 - 25.6)	21.0 ± 0.8 (17.9 – 26.7)	19.2 ± 0.3 (16.9 – 20.8)	20.0 ± 0.8 (17.2 – 34.8)	20.6 ± 1.8 (17.9-24.5)
V%	80.5 ± 1.5 (78-83)	80.2 ± 0.9 (79.0-82.0)	80.0 ± 0.4 (77 – 84)	80.1 ± 0.4 (78 – 82)	80.1 ± 0.5 (74 – 82)	80.4 ± 0.2 (79 – 82)	80.2 ± 1.4 (77.8-83.4)
m.b.w.	23.8 ± 2.5 (19.5-27)	23.3 ± 2.1 (17.5-26.0)	28.1 ± 0.5 (23.5 – 32.0)	27.4 ± 1.2 (21.5 – 32.0)	27.2 ± 0.9 (21.5 – 33.0)	20.7 ± 0.5 (17.5 – 24.5)	27.7 ± 0.6 (23.5-33.0)
Stylet	-	16.4 ± 0.2 (16.0-16.5)	16.8 ± 0.1 (16.5 - 17.5)	16.1 ± 0.2 (15.0 – 16.5)	17.2 ± 0.1 (16.5 – 18.0)	15.8 ± 0.1 (15.0 - 16.5)	16.2 ± 0.4 (15.5-17.0)
SE- pore	84 ± 3.6 (80-90)	91 ± 4.3 (85.0-97.0)	98 ± 1.6 (83 – 114)	91 ± 2.5 (73 – 101)	78 ± 2.7 (59 – 92)	86 ± 1.4 (74 – 97)	89.± 1.8 (69-103)
Pharynx length	84.7 ± 1.3 (83-86)	90.1 ± 3.1 (86.0 – 96.0)	99 ± 1.3 (85 – 110)	97 ± 3.1 (83 – 115)	93 ± 1.3 (81 – 100)	88 ± 1.2 (79 – 98)	95.7 ± 1.5 (83-108)
PUS	28.3 ± 3.4 (24.5-32.0)	28.8 ± 4.2 (21.5-35.0)	51.5 ± 1.4 (27.0 – 51.5)	38.7 ± 1.8 (31.0 – 47.0	29.6 ± 1.3 (22.5 – 40.0)	28.7 ± 1.4 (20.0 – 44.5)	37.7 ± 10.0 (21.5-58.8)
Tail length	29.6 ± 2.0 (26.5-31.5)	32.0 ± 2.3 (28.0-36.0)	33.0 ± .05 (29.0 – 38.0)	31.5 ± 0.9 (26.5 – 36.0)	32.7 ± 0.4 (30.0 – 35.0)	27.2 ± 0.8 (15.5 – 33.0)	31.7 ± 5.0 (21.5-40.0)

Table 5.4d Comparison of morphometrics of *Pratylenchus* n. sp. population from Colombia with those of *P. coffeae* from different crops and localities around the world. Measurements in μm and in form: mean ± standard deviation. (range).

Population	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Duncan *et al.* (1999)	Xiuhua (2006)	Xiuhua (2006)
Locality	Martinique	Pernambuco Brazil	Puerto Rico USA	Sao Paulo Brazil	Heibei China	Heibei China
Host	Yam – Y1*	Yam – Y2*	Yam – Y3*	Cocoyam – M1*	gaoyang	*Dioscorea sp.*
n	20	20	20	20	18	20
L	558 ± 26 (518-608)	725 ± 62 (589-821)	659 ± 63 (565-821)	551 ± 25 (501-598)	543 ± 41 (426-594)	517± 32 (449-569)
a	26.9 ± 1.9 (23.1-30.2)	24.2 ± 1.6 (19.6-27.0)	27.2 ± 2.3 (23.9-31.2)	26.2 ± 1.5 (23.8-29.1)	22.5 ± 2.6 (18.0-27.2)	22.9 ± 2.3 (20.0-27.1)
b	5.9 ± 0.3 (5.5-6.4)	7.4 ± 0.7 (6.1-8.9)	6.8 ± 0.5 (6.1-7.7)	6.2 ± 0.4 (5.4-6.9)	5.8 ± 0.4 (4.9-6.6)	6.2 ± 0.4 (5.5-6.8)
c	20.6 ± 1.0 (17.9-21.8)	20.6 ± 2.2 (17.3-24.8)	20.1 ± 2.4 (16.3-25.9)	20.9 ± 1.6 (18.3-24.2)	18.4 ± 2.2 (15.8-23.6)	18.0 ± 1.4 (16.0-20.7)
V%	80.7 ± 1.3 (78-83)	81.3 ± 1.3 (79-83)	80.7 ± 1.2 (78-83)	81.6 ± 2.2 (78-86)	77.7 ± 1.2 (75.2-79.4)	78.9± 1.0 (77.2-80.4)
m.b.w.	20.9 ± 0.4 (17.5-24.5)	30.1 ± 0.5 (27.0-35.0)	24.5 ± 0.8 (19.5-31.5)	21.1 ± 0.2 (19.5-23.5)	24.4 ± 2.8 (20.7-31.6)	22.7 ± 1.8 (20.1-25.3)
Stylet	16.1 ± 0.3 (15.5-16.5)	16.5 ± 0.4 (15.5-17.0)	16.8 ± 0.3 (16.5-17.5)	16.1 ± 0.2 (15.5-16.5)	16.4 ± 0.6 (14.9-17.2)	16.0 ± 0.5 (14.9-16.7)
SE- pore	88.5 ± 0.7 (82-94)	103 ± 1.3 (89-117)	99 ± 1.5 (81-110)	86 ± 1.0 (80-96)	84.8 ± 4.3 (75.9-91.4)	76.9 ± 3.8 (69-82.8)
Pharynx length	95 ± 0.7 (89-102)	98 ± 1.7 (87-116)	98 ± 1.2 (86-110)	89 ± 0.7 (84-96)	94 ± 1.6 (86-106)	83. 1± 3.7 (75.9-89.1)
PUS	25.2 ± 4.6 (16.5-35.0)	33.4 ± 6.7 (20.5-49.0)	37.9 ± 11.4 (26.0-77.0)	28.7 ± 4.9 (20.5-41.0)	32.8 ± 3.5 (25.9-40.8)	29.7 ± 4.2 (22.4-39.7)
Tail length	27.2 ± 1.7 (25.0-32.5)	35.4 ± 2.7 (30.0-39.0)	33.1 ± 3.4 (28.0-38.0)	26.5 ± 2.2 (22.5-31.5)	29.8 ± 3.0 (24.7-35.1)	28.8 ± 2.4 (23.6-32.8)

Molecular characterization

All obtained sequences of the *Pratylenchus* n. sp. population from Colombia were deposited in EMBL (Table 5.5).

D2D3 rDNA

The polymerase chain reaction amplified a single DNA product of about 760 bp (727-779) for each of the 6 *Pratylenchus* n. sp. specimens. D2D3 rDNA region sequences obtained were subsequently used for phylogenetic analysis. Additionally, a comparison with 31 GenBank sequences of *Pratylenchus* spp. was performed.

In GenBank, 80 sequences from the *Pratylenchus* 28S rDNA gene were available; they concerned 26 species: only sequences longer than 700 bp and homologous with Colombian populations were used for comparison and phylogenetic trees, i.e. 31 accessions of 10 species. Sequences of *P. jaehni* (2), *P. loosi* (10), *P. hippeastri* (1), *P. gutierrezi* (3), *P. pseudocoffeae* (1), *P. coffeae* (10), *P. pratensis* (1), *P. penetrans*(1) , *P. dunensis* (1) and *P. brzeskii* (1) presented the full length of the D2D3 region. *Radopholus similis* DQ328712 and *Helicotylenchus multicinctus* DQ328745 (Subbotin *et al*, 2006, 2007) were selected as outgroup taxa for D2D3 analyses.

Comparison of the D2D3-LSU sequences at intraspecific level of *Pratylenchus* n. sp. did not show divergence (0%), except for the specimen GM18 which had 1.9% divergence with respect to other Colombian specimens (Table 5.6). Interspecific variation was much greater, with conspicuous divergences when *Pratylenchus* sequences from GenBank were included in the analysis (3.1-30.8%).

The relationship of the detected populations with other *Pratylenchus* species was measured through Bayesian inference (BI) analysis. The obtained alignment presented 684 characters of which 264 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, one well supported clade (PP=1.00) that contains the 6 specimens of Colombian population could be appointed. GenBank accessions of *P. coffeae; P. loosi* from *Iran; Pratylenchus loosi* plus *P. hippeastri* from USA*; and P. jaehni* composed their own clades (PP=1.00). The two latter groups appeared most

Table 5.5 Accession number and code of *Pratylenchus* species from GenBank used for comparison with *Pratylenchus* n.sp. sequences from Colombia.

Species	**28S code**		**bp***	**Author**	**18S code**		**bp**	**author**	**ITS**code**		**bp**	**author**
Pratylenchus n.sp.	-	GM16	763	This study	-	GM16	1803	This study				
	-	GM17	779	This study					-	GM17	1056	This study
	-	GM18	727	This study								
	-	GM19	761	This study								
	-	GM20	761	This study								
	-	Pra34	766	This study								
P. brzeskii	AM231911	br	747	de la Peña *et al.*, 2007								
P. coffeae	AF170428	C4	745	Duncan *et al.*, 1999					AB053485		959	Saeki *et al.*, 2003
	AF170429	C6	735	Duncan *et al.*, 1999					AY561436		1251	Lee *et al.**
	AF170430	Y1	730	Duncan *et al.*, 1999								
	AF170431	Y2	741	Duncan *et al.*, 1999								
	AF170432	Y3	726	Duncan *et al.*, 1999								
	AF170433	B1	754	Duncan *et al.*, 1999								
	AF170434	B2	731	Duncan *et al.*, 1999								
	AF170435	M1	731	Duncan *et al.*, 1999								
	AF170436	M3	750	Duncan *et al.*, 1999								
	AF170443	K6	735	Duncan *et al.*, 1999								
P. crenatus		cr			AY284610		1729	Helder *et al.* **				
P. dunensis	AJ890459	du	745	de la Peña *et al.*, 2006								
P. goodeyi		go			AJ966498		1612	Meldal *et al.*, 2007				
P. gutierrezi	AF170440	K1	740	Duncan *et al.*, 1999								

Species	28S code		bp*	Author	18S code		bp	author	ITS**code		bp	author
	AF170441	K2	742	Duncan *et al.*, 1999								
	AF170442	K3	738	Duncan *et al.*, 1999								
P. hippeastri	DQ498831	PSP3	698	Inserra *et al*, 2007								
P. jaehni	AF170426	C1	750	Duncan *et al.*, 1999								
	AF170427	C2	756	Duncan *et al.*, 1999								
P. loosi	AF170437	N1	738	Duncan *et al.*, 1999								
	AF170438	N2	740	Duncan *et al.*, 1999								
	AF170439	T	737	Duncan *et al.*, 1999								
	EF446991	P2	760	Hajieghrari *et al.*, 2007								
	EF446992	P4	760	Hajieghrari *et al.*, 2007								
	EF446993	P6	760	Hajieghrari *et al*., 2007.								
	EF446994	P7	760	Hajieghrari *et al.*, 2007								
	EF446995	P10	760	Hajieghrari *et al.*, 2007								
	EF446996	P11	760	Hajieghrari *et al.*, 2007								
	EF446997	P9	760	Hajieghrari *et al.*, 2007								
P. penetrans	AM231936	pe	747	de la Peña *et al.*, 2007								
P. pratensis	AM231929	pr	762	de la Peña *et al.*, 2007	AY284611		1605	Helder *et al.* **				
P. pseudocoffeae	AF170444	pse	739	Duncan *et al.*, 1999								
P. thornei		th1			AJ966499		1761	Meldal *et al.*, 2007				
		th2			AY284612		1717	Helder *et al.* **				
		th3			AY284613		1745	Helder *et al.* **				

bp: number of base pairs (sequence size). **ITS=ITS1-5.8-ITS2; * unpublished; ** patent No. PCT/NL2004/000247)-A 01-Apr-2004 *

Table 5.6 Pairwise D2D3 sequences (700 bp) alignment of *Pratylenchus* spp. from Colombian and Genbank sequences.

		% Similarity																				
Populations*		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Pratylenchus n. sp. - 1**	1	100	98.1	96.9	96.4	92.0	91.9	88.6	87.8	87.5	87.4	87.3	87.2	86.8	86.7	86.6	86.4	86.3	86.1	78.0	72.1	70.8	69.2
Pratylenchus n. sp. - GM18	2		100	95.0	94.5	90.2	90.0	86.9	86.0	85.8	85.7	85.6	85.5	84.9	85.0	84.7	84.5	84.4	84.2	76.5	70.8	69.6	68.2
C2*	3			100	98.6	91.2	91.2	88.2	86.2	86.0	86.6	86.4	86.2	86.0	85.3	85.8	85.6	85.5	84.8	77.8	71.0	70.0	68.3
C1*	4				100	90.7	90.9	87.7	85.7	85.5	86.1	85.9	85.7	85.5	85.5	85.3	85.1	85.0	84.3	77.3	70.5	69.7	67.9
N2*	5					100	97.4	89.5	87.3	87.2	87.6	87.9	87.8	87.4	86.3	87.3	87.1	86.9	86.3	79.8	70.3	70.2	69.5
N1, PSP3*	6						100	88.8	86.7	86.5	86.9	87.0	86.9	86.4	85.7	86.3	86.1	85.9	85.1	79.0	70.5	70.4	68.6
P.pseudocoffeae	7							100	87.2	87.0	87.1	87.7	87.6	88.6	86.2	88.5	88.3	88.2	85.1	79.3	72.8	71.1	69.4
Y1, M2, M1, M3*	8								100	99.8	94.5	84.7	84.6	87.3	98.9	87.2	87.0	86.9	84.8	80.8	71.8	71.6	68.4
Y3*	9									100	94.3	84.6	84.4	87.2	98.7	87.0	86.8	86.7	84.6	80.6	71.6	71.5	68.2
B1*	10										100	85.3	85.1	89.3	93.5	89.1	88.9	88.8	85.4	81.6	72.3	72.5	68.4
K1,C6, Y2*	11											100	99.8	88.7	83.7	88.7	88.5	88.3	84.1	79.2	69.0	68.3	68.9
K2*	12												100	88.5	83.6	88.5	88.3	88.2	83.9	79.0	68.8	68.5	68.7
P7, K6*	13													100	86.3	99.8	99.6	99.4	89.3	82.4	73.2	71.7	70.7
C4*	14														100	86.2	86.0	85.9	83.8	80.2	70.8	71.0	67.6
P9,P10	15															100	99.8	99.6	89.1	82.2	73.2	71.7	70.7
P4, P6, P2, P11*	16																100	99.4	88.9	82.1	73.0	71.5	70.5
T*	17																	100	88.8	81.9	72.9	71.5	70.4
K3*	18																		100	79.2	70.9	69.8	68.0
P. pratensis	19																			100	71.1	71.3	69.9
P. dunensis	20																				100	87.3	69.8
P. penetrans	21																					100	68.2
P. brzeskii	22																						100

for code of *P. coffeae, P. jaehni, P. loosi, P. gutierrezi, P. hippeasti* and *Pratylenchus* n. sp. see table 5.5; **1: GM16=GM17=GM19=GM20=Pra34

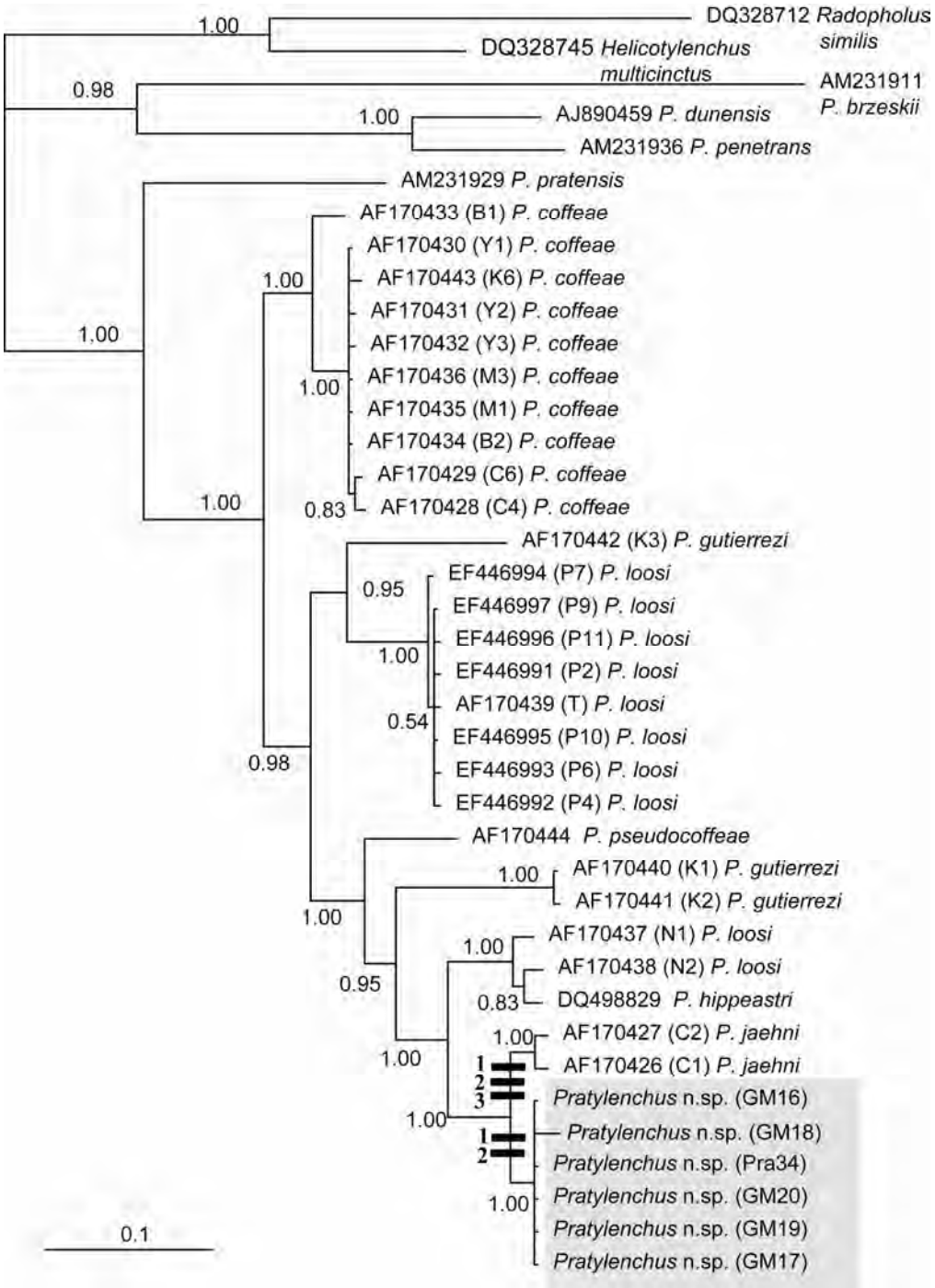


Fig. 5.3 Summary cladogram for *Pratylenchus* n. sp population (6 specimens) and 31 *Pratylenchus* GenBank accession-28S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Radopholus similis* DQ328712 and *Helicotylenchus multicinctus* DQ328745 (Subbotin *et al*, 2006, 2007). Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP). The branches with *Pratylenchus* n. sp. and *P. jaehni* are supported by two and three autapomorphies indicate with thick line.

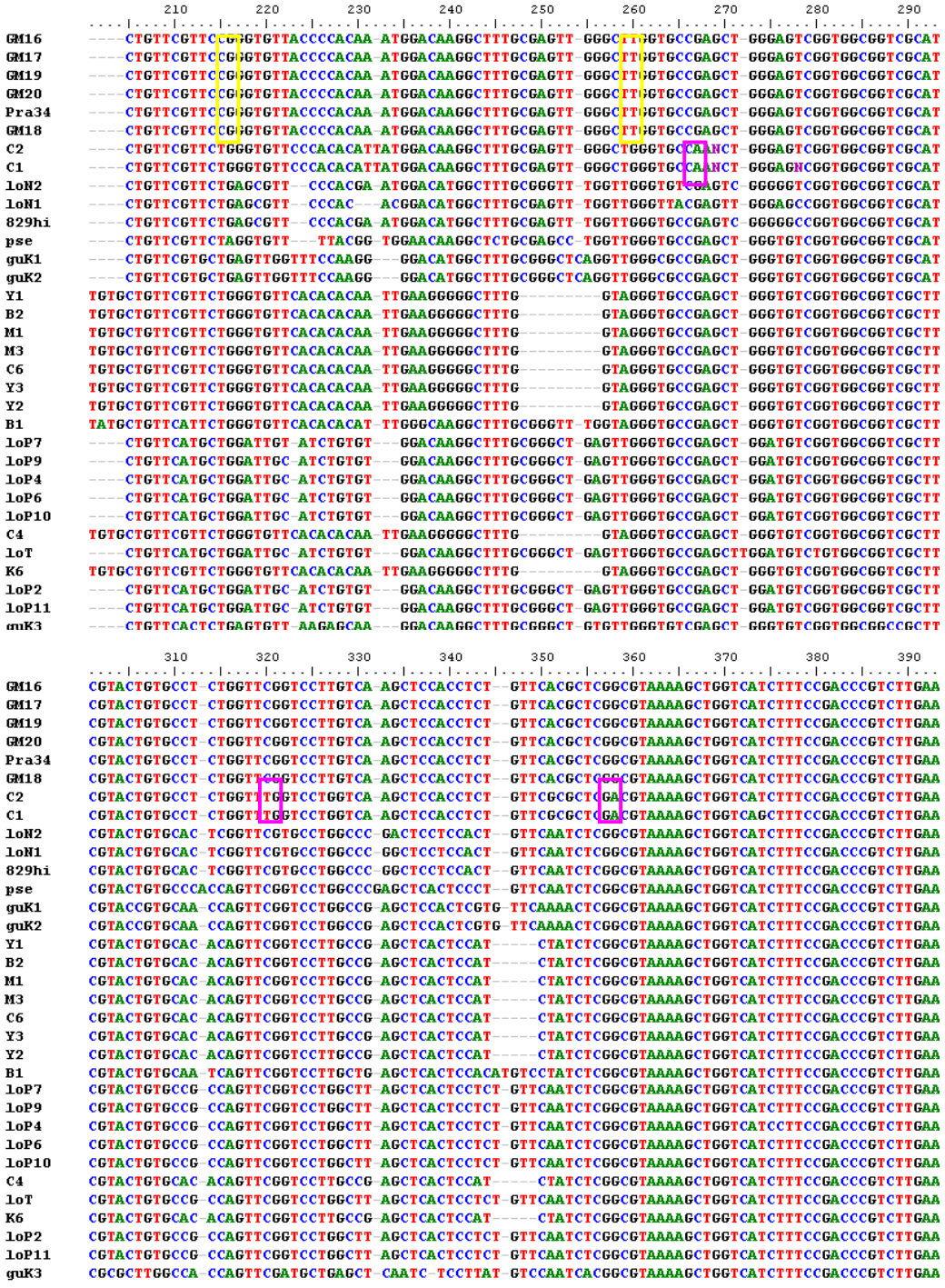


Fig. 5. 4 Clustal W (1.81) multiple sequences nucleotide alignment of segment of the LSU 28S RDNA for *Pratylenchus* n. sp. (GM16, GM17, GM18, GM19and Pra34) and related nematodes over 700 alignment positions. Taxa include species most close to *Pratylenchus* n. sp.: *P. jaehni* (C1, C2), *P. loosi* (N1, N2, P2, P4, P6, P7, P9, P10, P11 and T), *P. hippeastri* (829hip), *P. gutierrezi* (K1, K2 and K3), *P. pseudocoffeae* (pse), *P. coffeae* (M1, M3, B1, B2, C4, C6 and K6) (see codes in Table 5.5). Autapomorphic characters within Colombian populations of *Pratylenchus* n.sp. and *P. jaehni* are shown in yellow and pink boxes.

closely related to *Pratylenchus* n. sp. (Fig. 5.3). *P. jaehni* was found to have a sister relations with *Pratylenchus* n. sp. and *P. jaehni.* The branches with *Pratylenchus* n. sp. and *P. jaehni* were supported by two and three autapomorphies, respectively (Fig. 5.3).

18S rDNA

The polymerase chain reaction amplified a single DNA product of nearly 1803 bp for a single specimen of *Pratylenchus* n. sp. when the 18S rDNA region was analyzed (Table 5.5). Additionally, comparisons with 6 GenBank sequences of *Pratylenchus* spp. were established.

In GenBank 57 sequences from the *Pratylenchus* 18S rDNA gene were available. They concerned 11 species. Only sequences longer than 1700 bp and homologous with Colombian populations were used for comparison and phylogenetic trees, *i.e*. 6 accessions of 4 species: *P. goodeyi* (1), *P. pratensis* (1), *P. crenatus* (1) and *P. thornei* (3). *Helicotylenchus dihystera* AJ966486 and *Radopholus similis*, AJ966502 (Meldal *et al.*, 2007) were selected as outgroup taxa for 18S rDNA analyses.

The comparison of the 18S-SSU sequences at inter-species level showed divergence ranging between 0.9 to 24.1% when *Pratylenchus* sequences from GenBank were included in the analysis (Table 5.7).

The relationship within *Pratylenchus* species was measured through Bayesian inference (BI) analysis. The obtained alignment presented 1747 characters of which 178 were parsimony informative. The topology of the majority rule 50 consensus tree relatively weakly supported *Pratylenchus* n. sp. as a sister to *P. crenatus* (only 0.79 PP) (Fig.5.5).

Table 5.7 Pairwise 18S sequences (1700 bp) alignment of *Pratylenchus* spp. from Colombian and Genbank sequences.

		% Similarity						
***Pratylenchus* species**		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Pratylenchus n. sp. GM16	1	100	91.0	90.6	90.3	86.4	81.9	75.9
P. crenatus	2		100	92.2	91.9	87.1	81.7	77.3
P. thornei 1	3			100	99.7	87.9	83.4	83.8
P. thornei 2	4				100	87.4	83.2	83.7
P. pratensis	5					100	87.2	72.6
P. goodeyi	6						100	69.2
P. thornei 3	7							100

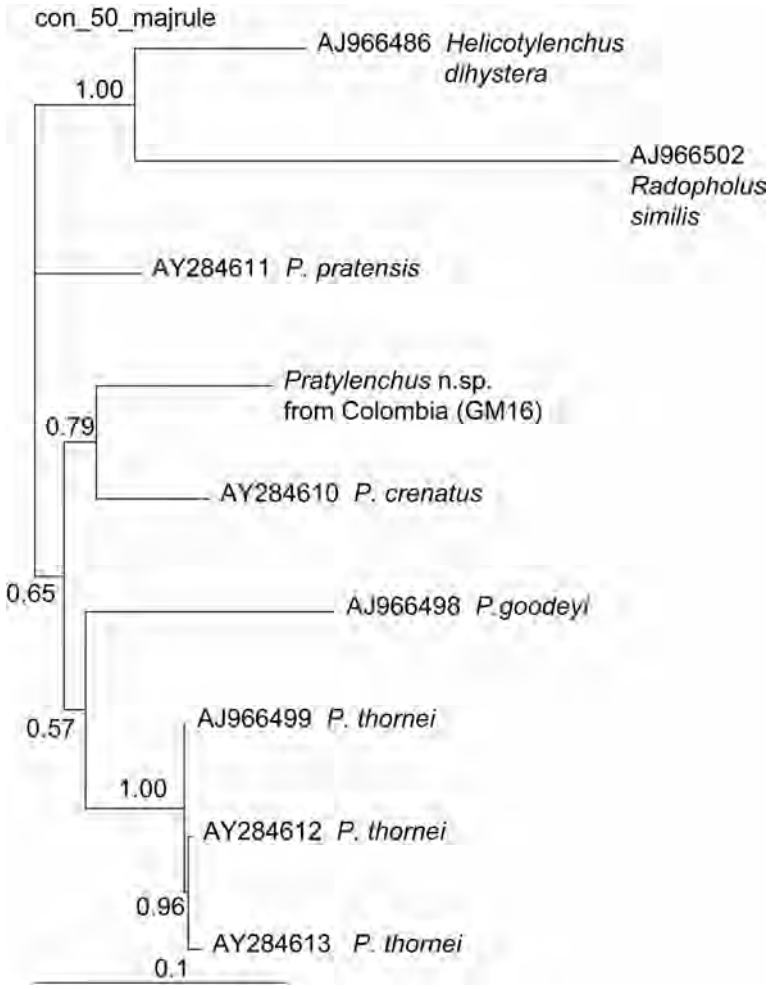


Fig. 5.5 Summary cladogram for one *Pratylenchus* n. sp specimen and 6 *Pratylenchus* GenBank accession-18S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Helicotylenchus dihystera* AJ966486 and *Radopholus similis*, AJ966502 (Meldal *et al.*, 2007). Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP).

ITS1-5.8-ITS2 rDNA

The polymerase chain reaction amplified a single DNA product of nearly 1056 bp for a single specimen of *Pratylenchus* n. sp. when ITS-5.8-ITS2 rDNA region was analyzed (Table 5.6). Additionally, comparisons with 2 available GenBank sequences of *Pratylenchus coffeae* were established. They were used for comparison and phylogenetic trees.

The comparison of the ITS-5.8-ITS2 rDNA sequences at inter-species level showed divergence ranging between 29.9 to 30.5% when *Pratylenchus* sequences from GenBank were included in the analysis (Table 5.8).

The relationship with the very few molecular comparable *Pratylenchus* species was measured through Bayesian inference (BI) analysis. The obtained alignment presented 1020 characters of which 337 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, only the sister relation of two *P. coffeae* populations could be confirmed (Fig. 5.6).

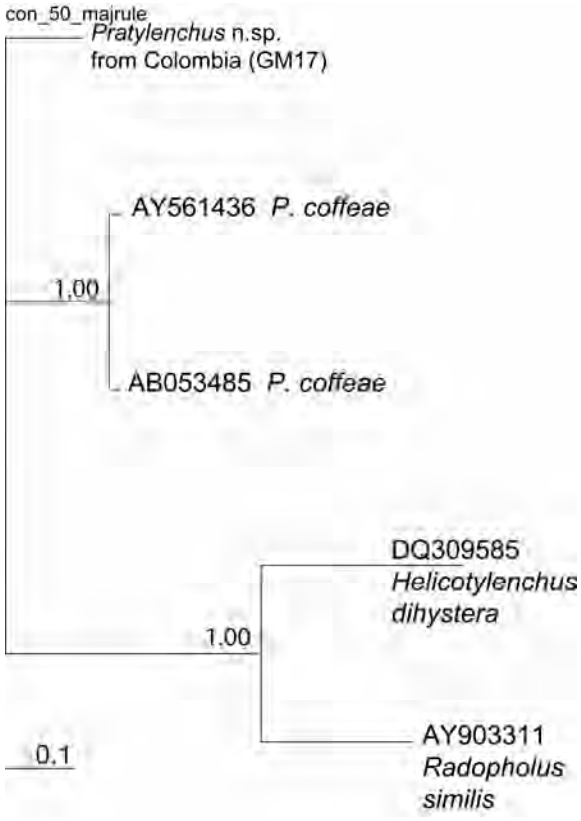


Fig. 5.6 Summary cladogram for one *Pratylenchus* n. sp. specimen and 2 *Meloidogyne* GenBank accession-ITS1-5.5-ITS2 rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Helicotylenchus dihystera* DQ309585 (Chen *et al.*, 2007) and *Radopholus similis*, AY903311 (Zhou *et al.*, 2007). Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP).

Table 5. 8 Pairwise ITS1-5.8-ITS2 sequences (1056 bp) alignment of *Pratylenchus* spp. from Colombian and Genbank sequences.

	% Similarity		
***Pratylenchus* species**	***Pratylenchus* n. sp. GM17**	***P. coffeae* AB053436**	***P. coffeae* AY561485**
Pratylenchus n. sp. GM17	100	69.5	70.1
P. coffeae AB053436		100	95.2
P. coffeae AY561485			100

Discussion

The population described here as *Pratylenchus* sp. n. resulted as *P. coffeae* following different *Pratylenchus* keys. However, comparison of morphological characters of 26 *P. coffeae* populations from different hosts and localities worldwide showed that the root lesion nematode from Colombia (*Pratylenchus* n. sp.) shared maximum 30% of morphological characters with each one of all population of *P. coffeae* considered.

Pratylenchus n. sp. showed morphometrical differences with respect to standard populations of *P. coffeae* (K6) and *P. loosi* (T) and paratypes of *P. jaehni* (Inserra, 2001), i.e. the mean values of body length, maximum body width, body width at vulva and anus, pharyngeal overlap, post-uterine sac; ratio c were smaller in *Pratylenchus* n. sp. compared to those in *P. jaehni, P. loosi* and *P. coffeae*. The mean values of tail length, stylet length and ratio a were larger in *Pratylenchus* n. sp. than in *P. jaehni,* but smaller that in *P. loosi* and *P. coffeae*. However, when other populations of those three species were considered (Table5.10), this study provided clear evidence of the difficulty of discriminating *Pratylenchus* n. sp. from *P. jaehni* and *P. loosi* based only on morphometrical characters.

Pratylenchus n. sp., *P. coffeae, P. loosi* and *P. jaehni* are the only amphimictic lesion nematodes with two lip annuli, and with the fused lip sectors also fused with the oral disc resulting in a smooth face. The separation of *Pratylenchus* n. sp. from the latter two species is difficult because these two species share similar morphometric parameters

(Tables 5.4). Molecular distinctions between those species are more clearcut than are morphological differences. A similar opinion about *P. coffeae, P. loosi* and *P. jaehni* was given by Duncan *et al.* (1999).

P. hippeastri also has a plain and smooth face and two head annules. It was used for comparison and phylogenetic trees but its morphology (mainly empty spermatheca) and morphometric characters separate it clearly from *Pratylenchus* n. sp., *P. coffeae, P. jaehni* and *P. loosi.* Correspondence between molecular tree clades and groups 1-3 based on SEM head patterns proposed by Corbett & Clark (1982) has been found (Carta *et al*., 2001; Duncan *et al*., 1999; Inserra e*t al*., 2001).

The taxonomic separation of the various species of root lesion nematodes (reared under standard conditions) is difficult since they exhibit little interspecific morphological diversity, and morphological and morphometric intraspecific comparisons show a high degree of variability (Corbet & Clark, 1983; Mizukubo, 1992; Roman & Hirschmann, 1969; Tarté & Mai, 1976a,1976b) with few species-specific diagnostic characteristics available (Loof, 1978; Orui, 1996). Single species subjected to different environmental conditions and different host plants differ also in many morphometric relationships (Roman & Hirschmann, 1969). Doucet *et al.* (2001) reported that temperature significantly influenced the morphometrics of individuals derived from a single isolate of *P. vulnus*. Together, these findings indicate that morphometric characters are not always reliable as primary characters for *Pratylenchus* species identification. Additionally, when comparison is established between populations of one species, the differences found by different authors in morphometric data may also be influenced by fixation procedures, origin of populations (different hosts and localities), different age at moment of analysis and impact of culture.

The description of a new nematode species or taxon should be based on a consensus of all available data. The term polyphasic taxonomy has been coined for this type of integrated taxonomy, which is based not only on phenotypic and genotypic differences, and phylogenic relationships, but also on differences in the ability of populations to infect host plants (De Waele & Elsen, 2007).

For *Pratylenchus* n. sp. as for *P. jaehni, P. loosi* and *P. coffeae* (Inserra *at al*., 2001) type host associate are *Musa* ABB (plantain); *Citrus aurantium* L.; *Paspalum*

notatum Flugge and *Panicum hemitomon* and coffee, respectively. *P. jaehni* is associated also with coffee plants and *P. loosi* (Inserra *at al*., 2001) with tea; *P. coffeae* has been reported from a wide range of hosts. *P. jaehni* and *P. loosi* have been not reported on *Musa* plants at present. Although *Pratylenchus* species are polyphagous, there are clear differences in host preference among the species. For example, whereas *P. vulnus* and *P. penetrans* are commonly found on a range of perennial fruit crops in California, *P. brachyurus* is mainly associated with cotton (McKenry & Roberts, 1985 quoted by Al-Banna, 2004). Thus, to predict risks of crop damage based on pre-plant nematode levels, proper identification of *Pratylenchus* populations to species is essential.

In addition to the morphology and morphometrics, the sequence comparison of the D2D3 rDNA expansion region clearly separates the new species from *P. jaehni, P. loosi* and *P. coffeae.* Although about 200 *Pratylenchus* spp. sequences are available on Genbank, most of them from LSU rDNA region, only sequences of similar size and homologous with Colombian population were used for comparison and phylogenetic trees; thus, this discussion (molecular and phylogenetic) is based on results of D2D3 rDNA region. Nucleotide divergences of *Pratylenchus* n. sp. ranged between 3.1-3.6%; 7.9-8.0% and 11.2-11.3% for respect to *P. jaehni, P. loosi* and *P. coffeae,* respectively. Molecular (D2D3 DNA sequences) dissimilarities among *P. jaehni* sp. n., *P. coffeae* and *P. loosi* were documented in two previous studies (Duncan e*t al*., 1999; Inserra e*t al*., 2001). This rDNA region has been widely used to separate different groups of nematodes at species level, including pratylenchids (Carta *et al*., 2001; de la Peña *et al.*, 2006, 2007; De Luca *et al*., 2004; Duncan *et al.*, 1999; Handoo *et al*., 2001; Inserra *et al*., 2001, 2007). In the most recent descriptions of new species of *Pratylenchus*, i.e. *P. arlingtoni*, *P. jaehni*, *P. dunensis* and *P. hippeastri*, molecular distinctions had higher weight than morphological and morphometric comparison (de la Peña *et al.*, 2006, 2007; Handoo *et al.*, 2001; Inserra *et al*., 2001, 2007).

Al-Banna (1977) found that the D3 expansion region for *Pratylenchus* is specific and does not vary among populations of conspecifics. Intra-specific variability of the *Pratylenchus* genome has been demonstrated in *P. coffeae* by others (Duncan *et al*., 1999; Waeyenberge *et al*., 2000) and evidence has been produced that several species closely related to *P. coffeae* are species complexes (Duncan *et al.*, 1999). The concept of

species complex (syngameon), indicate a large number of species linked by frequent and occasional hybridization in nature (Grant, 1957). The recent availability of sequence data has allowed the identification in other taxa of cryptic species which are morphological identical, yet genetically distinct (van Oppen *et al*., 2001).

The phylogenetic position based on molecular characters indicates that the genus *Pratylenchus* is paraphyletic, suggesting that lineage sorting is incomplete (Al-Banna *et al*., 1997; Duncan *et al*., 1999; Inserra *et al*., 2001, 2007). The existence of cryptic nematode species suggesting the existence of species complexes has recently been reported in other nematode genera (Courtright *et al*., 2000). Therefore, further molecular studies are needed to revise the species that currently are included in the genus *Pratylenchu*s (De Luca *et al.*, 2004).

Thus, numerous studies suggest that nematode isolates described as *P. coffeae* must be a complex of several species, many of which are still undescribed. However, the type of species concept used is the main problem in taxonomical studies. Numerous species concepts have been proposed and advocated for general use in species delimitation. Of these, the Linnean or typological morphospecies concept, biological (BSC) evolutionary (ESC), and phylogenetic (PSC) species concepts figure most prominently in the substantial literature on the subject. A review of the taxonomic literature suggests that, in practice, most nematode taxonomists are operating within the Linnean system (Adams, 1998). However, recent descriptions include biological, evolutionary, and phylogenetic species concepts. For most of the 26 *Pratylenchus* species described since 1985 only the morphological description is available, sometimes supplemented with mostly DNA-based comparisons with related *Pratylenchus* species (De Waele & Elsen, 2007).

Adams (1998) stated that “according to the objectives of taxonomy, the best species concept for use in nematology is an amalgamation of the ESC and the PSC” and “fundamental to any description of species is the underlying concept used to delimit them, and I encourage nematologists of all persuasions to make explicit their methods of delimitation”.

In our molecular analysis of sequences especially attention was given to the resulting tree and to the autapomorphic characters present in all specimens studied on

D2D3 rDNA (see figure 5.4). Thus, our results fill requirements of evolutionary species concept (a species forms a separate lineage or separate clade) and phylogenetic species concept (a lineage can be characterized by autapomorphies; an autapomorphy is a derived trait unique to any given taxon). Important for a trust full "an amalgam of the evolutionary and phylogenetic species concept we need autapomorphies in both lineages" (Adams, 1998, 2001; Coomans, 1988; Nadler, 2002); those could be observed in figure 5.4 for *Pratylenchus* n. sp. and *P. jaehni*, two and three autapomorphies, respectively.

Results of mating experiments showed that *P. jaehni* is reproductively isolated from *P. coffeae* (Inserra e*t al*., 2001). Similar experiments will be carry out in the future with *Pratylenchus* n. sp., *P. coffeae, P. jaehni* and *P. loosi*. Limited case studies have shown that the molecular based species concepts are more in agreement with the biological species concept than with the typological species concept (Eyualem and Blaxter, 2003). Our study provides arguments based on typological morphospecies concept, evolutionary and phylogenetic species concepts but biological species concept was not included. However, we consider previous concepts valid to characterize and introduce a new species.

Conclusions

The population described here as *Pratylenchus* sp. n. is closely related to three *Pratylenchus* species but clearly different from other species within the genus. It shares with *P. coffeae, P. loosi* and *P. jaehni* some morphology features (they are the only amphimictic lesion nematodes with two lip annuli, and with the lip sectors fused together and also fused with the oral disc to present a smooth face).

Pratylenchus sp. n. shares maximum 30% of morphological characters with *P. coffeae* populations. However, the separation of *Pratylenchus* n. sp. from *P. loosi* and *P. jaehni* is difficult because these species share similar morphometric parameters.

Although *Pratylenchus* species are polyphagous, there are differences in host preference among the species. Type host for *Pratylenchus* n. sp. is *Musa* ABB (plantain, hartón cultivar) which had been also reported for *P. coffeae*. *P. jaehni* and *P. loosi* have been not yet recorded on *Musa* plants.

The sequence comparison of the D2D3 rDNA expansion region clearly separates the new species from *P. jaehni, P. loosi* and *P. coffeae*. *Pratylenchus* n. sp., *P. coffeae, P. jaehni* and *P. loosi* were positioned in different clades, and clear autapomorphic characters were present in all specimens studied on D2D3 expansion region of 28S rDNA of *Pratylenchus* n. sp. and its sister species *P. jaehni.*

Molecular distinctions between *Pratylenchus* n. sp., *P. coffeae, P. jaehni* and *P. loosi* are more clearcut than are morphological differences. Similar conclusions were put forward for close morphometrical and morphological species in the more recently descriptions of new species of *Pratylenchus*.

Our results fill the requeriments of the evolutionary and phylogenetic species concept in agreement with Adams (1998). Additionally our new species is a typological morphospecies compared with most of species included in the genus; morphometric characters overlap only with *P. jaehni* and *P. loosi.*

Chapter 6

Radopholus similis *from Colombia and additional notes on taxonomical status of* **Radopholus** *species*

Introduction

Colombia is one of the major producers in the world of plantain and banana, with more than 450,000 hectares for local consumption and export. Among the biotic factors affecting banana yield, plant parasitic nematodes rank second after black sigatoka (*Mycosphaerella fijiensis*), reducing bunch weight and longevity and increasing the crop cycle duration. Destruction of root and corm tissues also results in a tendency for plants to uproot or topple, with a high economic impact. *Radopholus similis* (Cobb, 1893) Thorne, 1949 is considered the most important nematode associated with *Musa* plants worldwide. In Colombia, highest population densities of these nematodes were found at low altitude (Urabá Region, 50,000 nematodes per 100g of fresh roots). The burrowing nematode has been reported in different Colombian regions, including all important yield area of banana and plantain; however, so far no morphological, morphometric and molecular studies have been carried out (Sher, 1968b; Gómez, 1980; Barriga & Cubillos, 1981; Sarah *et al*., 1993).

Radopholus similis is a migratory endoparasite spending its adult vermiform life in the root, but capable of emerging in adverse conditions. All juvenile stages and females are infective. Its vernacular name, burrowing nematode, is related to the symptoms produced by it: on entering the root the nematode occupies an intercellular position in the cortical parenchyma that coalesce and are continually enlarged laterally and towards the endodermis by the nematodes feeding and tunneling, producing the characteristic reddish brown lesions throughout the cortex. 3-4 weeks after infection, when extensive cavities have formed, one or more deep cracks with raised margins appear on the surface roots. The endodermis seems to act as a barrier to the nematode; the stele is not invaded (Orton Willliams & Siddiqi, 1973).

R. similis is widespread in tropical and subtropical regions of the world and present in glasshouses in Europe. In Florida, *R. similis* causes spreading decline of citrus. In Europe, the USA and the Caribbean *R. similis* is also a pest for indoors decorative plants like *Anthurium, Calathea* sp. and *Marantha* (O´Bannon *et al*., 1976; Elbadri, 2000). Furthermore, this nematode is a major pest for the black pepper industry in Indonesia (Holdeman, 1986). Other important hosts are sugarcane, avocado, coffee,

maize, vegetables and ornamental plants (Ortom Willliams & Siddiqi, 1973). Two groups of burrowing nematodes can be differentiated, *R. similis* "banana pathotype" (called banana race) attacking banana but not citrus, and "citrus pathotype" (called citrus race or *R. citrophilu*s) pathogenic to both (DuCharme & Birchfield, 1956; Kaplan *et al.*, 2000).

The purpose of this study was to characterize two *Radopholus similis* populations from Colombia using morphological, morphometric and molecular criteria and to make a review on the taxonomical status of *Radopholus* species.

Materials and Methods

Morphological observation

Root and soil samples of plantain (*Musa* AAB, "dominico hartón" cultivar) were collected in fields from two Colombian localities: Arauca (Los Llanos Orientales Region) and Carepa (Urabá Region) situated at East and North West of Colombia, respectively (see general material and methods). Nematodes were identified to species level using both identification keys and original species descriptions. *Radopholus* diagnostic keys used were Sher (1968b), Colbran (1970), Ryss & Wouts (1997) and Ryss (2003).

Molecular characterization

Five nematodes from each field population (Los Llanos and Urabá) were included in the analysis. DNA was extracted from a single nematode previously morphologically identified. Although, the DNA was extracted from 10 specimens in total, five from Los Llanos population and five from Urabá, only for the 28S region results were successful for all ten specimens. For molecular analysis of the 18S and ITS1-5.8-ITS2 regions some analyses failed, results were only obtained from 8 and 5 nematodes, respectively (see Table 6.3).

Preparation of DNA, sequence analyses, sequence comparisons and phylogenetic analyses were conducted as explained in chapter 2, general materials and methods. Only some details concerning to the outgroup used for phylogenetic analyses/trees are mentioned here.

Heterodera zeae, accession number DQ328695 and *Helicotylenchus vulgaris*, DQ328759 from Subbotin *et al.* (2006, 2007a) were selected as outgroup taxa for D2D3. For 18S analysis, outgroup taxa selected were *Helicotylenchus varicaudatus* accession EU306354 and *Helicotylenchus dihystera*, accession number AJ966486 from Bert & Vierstraete (2008) and Meldal *et al.* (2007). *Heterodera schachtii* accession AF498389 and *Helicotylenchus dihystera*, accession number DQ309585 from Tanha Maafi *et al.* (2003) and Chen *et al.* (2006) were outgroup taxa for ITS analyses.

Results

Morphological identification of the populations

The morphological and morphometric data of 59 females and 17 males from two different Colombian localities fitted *Radopholus similis* descriptions (Table 6.1). However, some characters showed variation with respect to *Radopholus* keys and descriptions mentioned in the literature. Female average length was 13 percent shorter than populations described by different authors, but males were slightly longer. No morphometric differences between Urabá and Llanos Orientales populations were found. Differences in morphometric data between *R. similis* from different localities worldwide and *Radopholus* species that resemble *R. similis* are shown in Table 6.2.

***Radopholus similis* (Cobb, 1893) Thorne, 1949**

(Fig 6.1, LM; 6.2, SEM))

Measurements

Females and males: see Table 6.1.

Description

Female

Body straight to slightly arcuate ventrally; cuticle distinctly annulated. Lateral field with four incisures; as viewed by light microscopy (LM) outer lines areolated, extending nearly along the entire body length; inner incisures coalescing near middle of tail, posterior to the phasmid; central bands equal to outer bands in width.

Lip region hemispherical, clearly off set from body, with 3-4 annules; labial framework highly sclerotized. Stylet moderately strong with well developed round basal knobs which are occasionally indented anteriorly; dorsal and ventrosublateral knobs of equal size; length of stylet cone equal to length of shaft plus knobs.

Dorsal gland opening near stylet base (3.1µm). Hemizonid three annules long, just anterior to secretory-excretory pore located at level or posterior to pharyngeal-intestinal junction. Oval median pharyngeal bulb well developed; valvular apparatus prominent. Three pharyngeal glands, in separate lobes, overlapping intestine dorsally and dorso-laterally. Some specimens showing one or two extended gland lobes.

Reproductive system didelphic, amphidelphic, outstretched; two genital branches equally developed; oocytes in two rows; vulva prominent, just postequatorial (58% of the body length); spermathecae round and of equal size, filled with small rod-shapes sperm. Tail elongate conoid, with variously shaped tip without mucro but frequently with a narrow rounded or indented and annulated terminus. Hyaline part of tail with 4-10 annules. Phasmids distinct, located in anterior third of tail.

Male

Occurring abundantly (nearly 30% of adults). Generally males more slender and longer than females, body slightly arcuate ventrally. Strongly dimorphic relative to females. Elevated lip region set off by a distinct constriction; cephalic framework not strongly sclerotized; head cuticle smooth or sometimes with 4-5 annules. Stylet weak relative to that of female, with knobs amalgamated in a single round base. Pharynx degenerated; oval median bulb and valvular apparatus reduced. Hemizonid and secretory-excretory pore similar to females.

Reproductive system monorchic, outstretched anterior testis. Testis with 3-4 rows of spermatocytes; posteriorly with rod-shaped spermatids. Postrectal intestinal sac absent. Bursa leptoderan, frequently almost reaching the tail tip but sometimes evolving about 2/3 of tail. Spicules strongly cephalated, with pointed distal ends. Gubernaculum rod-like, protrusible. Males as viewed by light microscopy (LM) with two genital papilla-like projections on the anterior lip of cloacal aperture. Tail tapering, rounded to almost pointed terminus, with 49 (42-61) annules. Hyaline part of tail narrow and short with 2-6 annules.

Table 6.1 Morphometrics of *R. similis* population from Colombia. Measurements in μm and in form: mean ± standard deviation (range).

	Females		**Males**	
Population	**Urabá**	**Llanos**	**Urabá**	**Llanos**
n	27	22	9	8
L	594 ± 53 (504 - 722)	599 ± 44 (511 - 654)	597 ± 41 (541 - 656)	609 ± 29 (574 - 654)
a	31 ± 3.8 (20 – 36)	30 ± 4.4 (23 – 37)	33 ± 3.5 (28 – 37)	30 ± 3.9 (27 – 37)
b	8.2 ± 0.5 (7.4 – 9.2)	8.4 ± 0.7 (7.1 – 9.8)	7.7 ± 0.5 (7.2 – 8.4)	8.2 ± 0.9 (6.9 – 9.3)
b'	4.6 ± 0.5 (3.7 – 5.4)	4.4 ± 0.4 (3.1 – 5.3)	5.2 ± 0.5 (4.8 – 5.8)	4.9 ± 0.0 (4.9 – 4.9)
c	9.1 ± 0.6 (8.1 – 10.3)	8.8 ± 0.7 (7.0 – 10.3)	7.9 ± 0.5 (6.9 – 8.6)	8.2 ± 0.5 (7.8 – 9.2)
c’	4.5 ± 0.4 (3.8 – 5.3)	4.5 ± 0.8 (3.3 – 5.8)	6.0 ± 0.6 (5.2 – 7.2)	5.9 ± 0.6 (5.2 – 6.9)
m.b.w.	19.7 ± 3.5 (15.3 – 33.7)	20.3 ± 3.3 (15.3 – 24.5)	18.5 ± 1.7 (15.3 – 20.9)	20.3 ± 2.2 (16.6 – 23.3)
Body diam. at anus	14.7 ± 2.0 (11.7 – 18.4)	15.7 ± 3.0 (10.4 – 20.9)	12.7 ± 1.1 (11.0 – 14.7)	12.6 ± 0.9 (11.7 – 14.1)
Head diam.	9.2 ± 0.7 (8.0 – 11)	9.1 ± 0.9 (7.4– 11)	7.5 ± 1.0 (6.1 – 8.6)	7.6 ± 1.0 (6.7 – 9.2)
Head height	3.7 ± 0.6 (3.1 – 4.9)	4.0 ± 0.4 (3.1 – 4.9)	5.4 ± 0.7 (4.3 – 6.1)	5.8 ± 0.9 (4.3 – 7.4)
Stylet length	18.5 ± 1.1 (15.9 - 21.5)	19.0 ± 0.7 (17.2 - 20.2)	12.8 ± 1.9 (9.0 – 15.3)	13.9 ± 0.8 (13.5 – 15.3)
metac.	54 ± 3.4 (49 - 63)	56 ± 4.0 (48 - 67)	52 ± 2.2 (49 - 55)	52 ± 0.0 (52 - 52)
S-E-pore	84 ± 6.2 (73 - 99)	85 ± 4.0 (76 - 94)	84 ± 6.7 (69 - 90)	84 ± 4.9 (79 - 93)
ph. gland length	130 ± 11.6 (112 – 155)	138 ± 15.6 (115 – 186)	113 ± 8.3 (106 – 125)	126 ± 0.0 (126 – 126)
ph. length	73 ± 4.9 (64 - 82)	72 ± 4.4 (63 - 83)	76 ± 4.9 (70 - 86)	72 ± 6.9 (66 - 85)
Ant. end to anus	533 ± 46 (449 – 648)	530 ± 41 (453 – 582)	519 ± 37 (466 – 573)	531 ± 25 (492 – 564)
Tail length	66 ± 6.0 (55 - 77)	69 ± 6.2 (54 - 84)	76 ± 6.4 (67 - 85)	75 ± 5.3 (64 - 81)
Tail length/ stylet length	3.5 ± 0.4 (2.8- 4.2)	3.6 ± 0.4 (3.0- 4.6)	6.2 ± 0.8 (5.2 - 7.7)	5.4 ± 0.5 (4.7 – 6.0)
Hyaline part	9.8 ± 1.4 (6.1- 12.3)	10.0 ± 2.0 (6.1- 13.5)	9.8 ± 1.5 (8.0- 11.7)	7.5 ± 1.6 (4.9- 9.2)
V (%)	57 ± 1.6 (51 – 59)	58 ± 1.3 (55 – 62)	-	-
Ant. gonad length	121 ± 32.3 (64 - 215)	118 ± 18.0 (89 - 172)	-	-
Post. gonad length	110 ± 28.6 (64- 199)	112 ± 15.0 (75- 141)	-	-
spic.	-	-	18.8 ± 0.5 (18.3- 19.5)	18.3 ± 1.0 (17.2- 20.0)
gub.	-	-	9.4 ± 0.4 (8.6- 9.7)	8.5 ± 0.6 (7.4- 9.2)

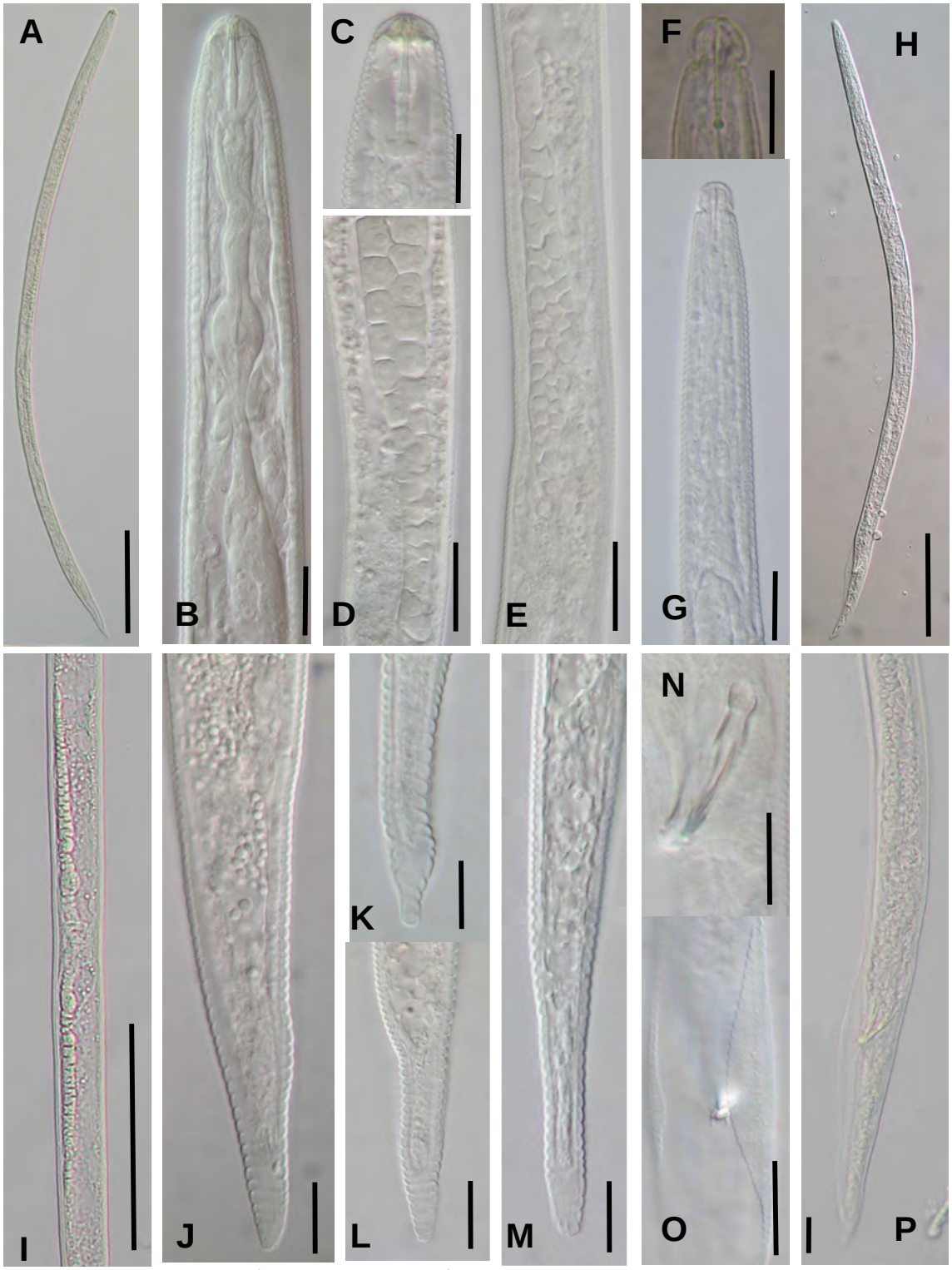


Fig. 6.1 LM photographs of *Radopholus similis* from Colombia. Female (A-D, I-M); male (E-H, N-P); entire body (A,H); neck region (B,G); head region (C,F); reproductive system (D,E,I,N); spicule details (N); bursa (O); tail shape of female (J-M) and male, posterior body region (P). Scale bars: A, H and I: 100µm; B-G and J-P: 10µm.

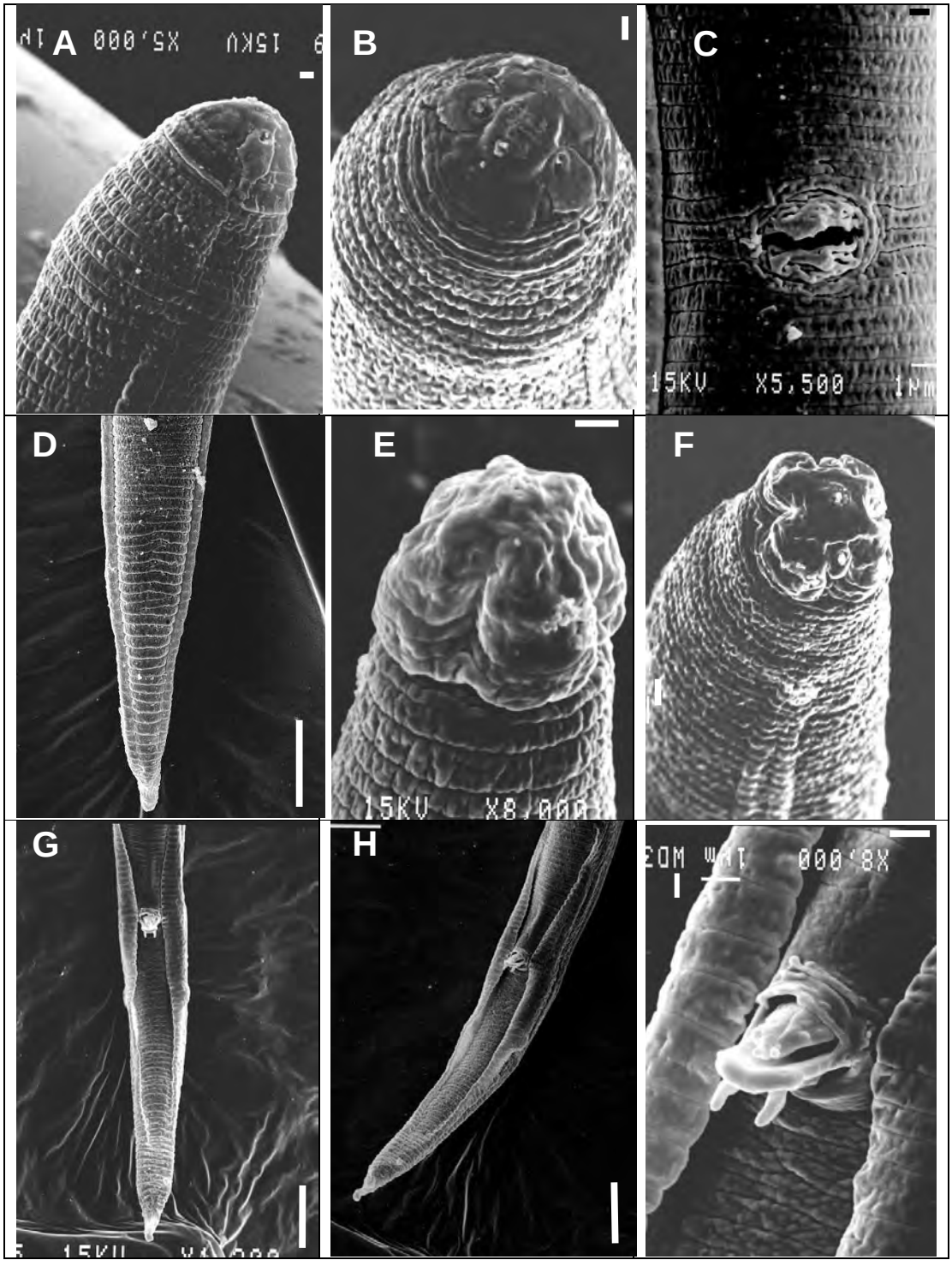


Fig. 6.2 SEM Photographs of *Radopholus similis*. from Colombia. A. female (A-D); male (E-I); anterior end female (A) and male (E); face view female (B) and male (F); tail shape of female (D) and male (G, H) (D); spicule details (I). Scale bars: A, B, C, F and I: 1µm; D, G and H: 10 µm.

Table 6.2 Morphometrics of *R. similis* populations from Colombia compared with those of *R. similis* from different crops and localities around the world and three new species described recently. Measurements in µm and in form: mean ± standard deviation (range)

Population		This study	Elbadri (2000)	Ryss &Wouts (1997)	Orton W. & Siddiqi (1973)	Sher (1968)	Van Weerdt (1958)	Stanton *et al.* (2001)	Trinh *et al.* (2004)	Nguyen *et al.* (2003)
Species		*R. similis*	*R. similis*	*R. similis*	*R. similis*	*R. similis*	*R. similis*	*R. musicola*	*R. arabocoffea*	*R. duriophilus*
Localities		Colombia	13 countries	Island-India	Fiji	Fiji		Australia	Vietnam	Vietnam
Hosts		*Musa* ABB	*Musa* and other crops	*Ginger*	*Musa* AAA	*Musa* AAA	*Citrus.* and *Musa* ABB	*Musa acuminata*	*Coffea arabica*	*Durio zibetinus*
N (n) *		2 (49)	18 (540)	x (34)	x(18)	2(25)	3 (46)	x (18)	x (20)	3(90)
L **	1	594-599	601-725	680	690	680-690	596-690	696	615	588-669
	2	504 - 722	510-860	554-792	520-880	520-880	498-785	604-781	590-682	490-840
a		30.0-31.0 20.0 –	24.5-30.5 20.2-47.5	26.4 17.8-36.0	27.0 22.0-30.0	26.0-27.0 22.0-35.0	25.7-30.0 20.0-34.3	28.9 24.0-33.3	29.9 23.6-33.6	26.3-30.5 22.5-42.0
b		8.2-8.4 7.1– 9.8	7.6-8.5 6.2-9.6	7.9 6.0-9.3	6.5 4.7-7.4	6.5-6.9 4.7-7.8	9.3-9.4 8.2-10.5	8.0 6.9-9.0	8.0 7.5-8.8	7.8-8.0 6.5-9.0
b'		4.4-4.6 3.1– 5.4	4.0-5.0 3.3-5.9	4.4 3.2-5.2	4.5 3.5-5.2	4.5-4.6 3.5-6.5	-	4.8 4.1-5.6	4.4 4.2-4.8	4.1-4.5 3.7-5.5
c		8.8-9.1 7.0-10.3	8.0-10.2 6.4-16.4	9.15 6.8-11.7	10.6 8.0-13.0	9.7-10.6 8.0-13.0	9.0-11.2 8.5-12.8	9.8 7.8-12.1	8.5 8.0-9.0	8.3-8.7 6.0-10.0
c’		4.5-4.5 3.3 – 5.8	3.7-4.5 2.3-6.2	4.2 2.4-6.3	3.4 2.9-4.0	3.4-3.6 2.4-4.1	-	4.5 3.8-5.8	4.5 3.0-5.2	4.5-4.7 3.3-6.8
m.b.w.		19.7-20.3 15.3 –	21-27.5 14.0-34.0	26.8 18.0-40.0	-	-	19-9-24.6 16.0-33.0	24.2 18.4-28.8	21.0 18.0-25.0	19.0-23.0 16.0-31.0
Body width at anus		14.7-15.7 10.4-20.9	16.0-21.0 12.5-26.0	18.8 12.0-28.0	-	-	15.0-18.0 12.0-21.0	15.5 12.0-20.8	16.0 14.0-23.0	14.9-17.3 12.0-24.0
Head height (HH)		3.7-4.0 3.1 – 4.9	3.4-5.4 2.5-6.0	3.8 2.5-4.5	-	-	-	-	3.2 2.6-3.4	4.0-4.0 3.0-6.0
Head width (HW)		9.1-9.2 7.4 – 11.0	9.0-10.5 8.0-12.0	8.9 7.5-10.0	-	-	-	-	9.1 7.7-10.3	9.4-10.3 8.0-11.5

Table 6.2 Morphometrics of *R. similis* populations from Colombia compared with those of *R. similis* from different crops and localities around the world and three new species described recently. Measurements in µm and in form: mean ± standard deviation (range)

Population	This study	Elbadri (2000)	Ryss &Wouts (1997)	Orton W. & Siddiqi (1973)	Sher (1968)	Van Weerdt (1958)	Stanton *et al.* (2001)	Trinh *et al.* (2004)	Nguyen *et al.* (2003)
Stylet length	18.5-19.0 15.9 - 21.5	17.6-20.4 15.0-23.0	18.0 16.5-20.0	19.0 17.0-20.0	19.0-19.0 17.0-20.0	17.9-18.5 16.0-20.0	17.8 15.2-20.8	15.9 14.6-17.2	17.4-18.0 16.5-19.0
DGO	3.1-3.3 2.5 – 3.7	4.0-5.5 2.0-8.5	4.0 2.0-5.5	-	-	-	3.0 2.0-4.8	3.1 2.6-4.3	4.4-4.6 3.0-5.5
J	72-73 63-83	79-94 65-120	87 73-132	-	-	-	88 74-108	77 72-84	76-82 64-92
ph. gland length	56-63 41 - 107	-	67 50-118	-	-	-		80 70-90	79-83 57-98
Ant. end to end of pharyng. gland	130-138 112 - 168	141-167 115-204	-	-	-	-	145 114-159	-	-
Ant. end to excretory-pore	84-85 73 - 99	83-100 59-116	91.5 86-100	-	-	89-97 62-107	-	84 74-90	86-92 73-110
Tail length	66-69 54- 84	63.5-86 40-104	76 50-100	-	-	62-63 49-75	72 57-91	72 67-79	70-77 48-94
Tail length/ stylet length	3.5-3.6 2.8- 4.6	-	4.2 2.9-5.5	-	-	-	-	4.6 4.1-4.9	4.0-4.2 2.8-5.5
Hyaline part	9.8-10.0 6.1- 13.5	-	14.7 7.5-23.0	-	12.0-13.0 9.0-17.0	-	7.8 5.6-13.6	8.4 6.9-11.2	-
V	57-58 51 – 62	56-58 51-68	71 53-88	56 55-61	56-56 52-61	57-59 54-63	55 53-57	55 51-58	-
Ant. gonad length	118-121 64 - 215	150-225.2 112-351	-	-	-	-	166 101-246	159 114-209	-
Post. gonad length	110-112 64- 199	137-209 105-345	-	-	-	-	153 93-236	139 103-176	-

* N: number of populations; n: number of individuals; x: number of populations were not indicated by author; ** 1: minimal and maximal average; 2: minimal and maximal measured value.

Molecular characterization

All obtained sequences of *Radopholus similis* from Colombia were deposited in EMBL – Genbank (Table 6.3). Codes of all sequences obtained per population are shown in Table 6.3.

D2D3 rDNA

PCR of D2D3 (expansion region of 28S LSU rDNA region) amplified a single DNA product of nearly 740 bp (672-754) for each of the two *Radopholus* populations when D2D3 rDNA region was analyzed (Fig.6.3). Five sequences were obtained per population (Table 6.3).

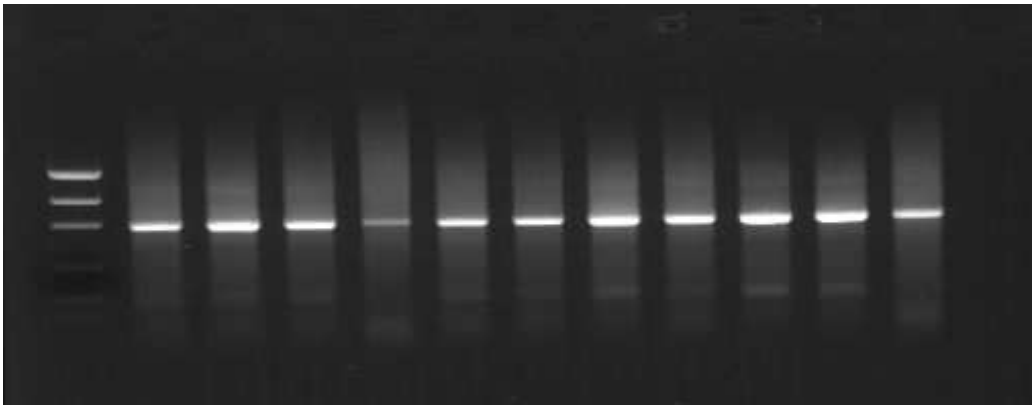
Sequences of different populations of *R. similis* (Elbadri *et al.*, 2002; Zhou *et al.*, 2005*; Xie *et al.*, 2006*, Peng *et al.*, 2007*; Al-Banna, 1996 and Long *et al.*, 2007*), one population of *R. arabocoffeae* (Trinh *et al.*, 2004) and one population of *Radopholus* sp. (Subbotin *et al.*, 2006) from Genbank were aligned and compared with the sequences of Colombian populations of *R. similis*. Since most sequences from Genbank did not match with Colombian sequences of *R. similis* and U47558 accession did not present the full length of the D2D3 region, only a 652 bp sequence of the D2D3 expansion region, accession number DQ328712 from Vietnam (Subbotin *et al.*, 2006) was used for comparisons and phylogenetic tree.

Table 6.3 Genbank accession number for *R. similis* samples from two Colombian localities; *Radopholus* spp. sequences from Genbank and outgroups included in the molecular analysis.

Localities/origen	**Samples**[1]	**D2D3 bp**	**18S bp**	**ITS**[2] **bp**
Los LLanos	Rs1	754	833	836
	Rs2	697	832	842
	Rs3	752	832	789
	Rs4	742	818	
	Rs6	672		
Urabá	Rs7	754		
	Rs8	850	832	
	Rs9	751	826	830
	Rs10	752	831	834
	Rs11	708	832	

[1] Different *R. similis* populations from Colombia are named Rs1 to Rs11; [2] ITS: ITS1-5.8-ITS2; bp: base pairs.

* unpublished



X Rs1 Rs2 Rs3 Rs4 Rs6 Rs7 Rs8 Rs9 Rs10 Rs11 *P.b*

Fig. 6.3 PCR products of *R. similis* from Colombia obtained after using primers and PCR conditions for D2D3 amplifications were as described by De Ley *et al.* (1999). *P.b*: *Pratylenchus brzeskii* (control) (X = DNA ladder, 740 bp, Promega).

The comparison of the D2D3-LSU sequences at intra-populations level showed no divergence (0%) between Rs7, Rs8, Rs9 and Rs10 (Urabá population) and between Rs2 and Rs3 of *R. similis* (Llanos population); very low sequence divergences, ranging between 0.9% and 2.3 % were observed for the other *R. similis* (Table 6.4). *Radopholus* sp. accession number DQ328712 (Subbotin *et al.,* 2006) showed a clearly larger nucleotide divergence (1.8-3.5%, average 2.0%) compared with all (Rs1 to Rs11) Colombian populations of *R. similis* (Table 6.4). Specimens Rs4 of Llanos populations showed largest nucleotide divergence (2.3%) for the Colombian sequences compared.

The phylogenetic relationship detected between *Radopholus* populations and specimens was measured through Bayesian inference (BI) analysis. A partial sequence of *Helicotylenchus vulgaris,* accession number DQ328759 and a sequence of *Heterodera zeae,* accession number DQ328695 (Subbotin *et. al*., 2006) were used as outgroups based on hypotheses of their affinities with *Radopholus* found in the literature (Subbotin *et al*., 2007a). The obtained alignment represented 640 characters of which only 76 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, the clade that contains Colombian populations (Fig. 6.4) formed a well supported clade (PP=0.98). Although *Radopholus* sp., accession number DQ328712 from Genbank was not included in the same clade, only few informative parsimony characters (10

different nucleotides) separate it from Colombian populations of *R. similis*. Sequence divergences as shown in Table 6.4 agree with topology of tree from D2D3 rDNA region (Fig. 6.4) for all Colombian populations studied.

Table 6.4 Pairwise D2D3 sequence alignment similarity (%) of *R. similis* from two Colombian localities.

% Similarity												
		Llanos					Urabá					
Locality	Specimens/ species	Rs1	Rs2	Rs3	Rs4	Rs6	Rs7	Rs8	Rs9	Rs10	Rs11	*Radopholus* DQ328712
LLanos	Rs1	100	99.1	99.1	97.4	98.9	99.2	99.2	99.2	99.2	98.9	97.5
	Rs2	-	100	100	97.9	99.5	99.8	99.8	99.8	99.8	99.8	98.3
	Rs3	-	-	100	97.9	99.5	99.8	99.8	99.8	99.8	99.8	98.3
	Rs4	-	-	-	100	97.6	97.9	97.9	97.9	97.9	97.7	96.3
	Rs6	-	-	-	-	100	99.7	99.7	99.7	99.7	99.4	98.0
Urabá	Rs7	-	-	-	-	-	100	100	100	100	99.7	98.3
	Rs8	-	-	-	-	-	-	100	100	100	99.7	98.3
	Rs9	-	-	-	-	-	-	-	100	100	99.7	98.3
	Rs10	-	-	-	-	-	-	-	-	100	99.7	98.3
	Rs11	-	-	-	-	-	-	-	-	-	100	98.2
	Radopholus DQ328712	-	-	-	-	-	-	-	-	-	-	100

18S rDNA

For the analysis of 18S rDNA region, PCR was divided in two parts, each one of 900 bp. For the first part of all sequences and second part of sequences 6 and 7 no results were obtained. The PCR amplified a single DNA product of only about 830 bp for each of the 2 *Radopholus* populations (Fig. 6.5). Eight sequences were obtained from two populations (Table 6.3). All sequences were subsequently used for the molecular analysis of these regions.

Only one homologous sequence close (similar size) to *Radopholus* - Colombian populations, accession AJ966502 (isolated in United Kingdom see Meldal *et al.*, 2007) was used for different comparisons. The comparison of the 18S sequences at intra-population level showed no divergence (0%) between both Colombian populations except for specimen Rs10 which showed very low sequence divergence (0.2%). The *R.*

similis population of Meldal *et al.* (2007), accession number AJ966502 showed only a nucleotide divergence of 0.3-0.4% compared to the Colombian populations (Table 6.5).

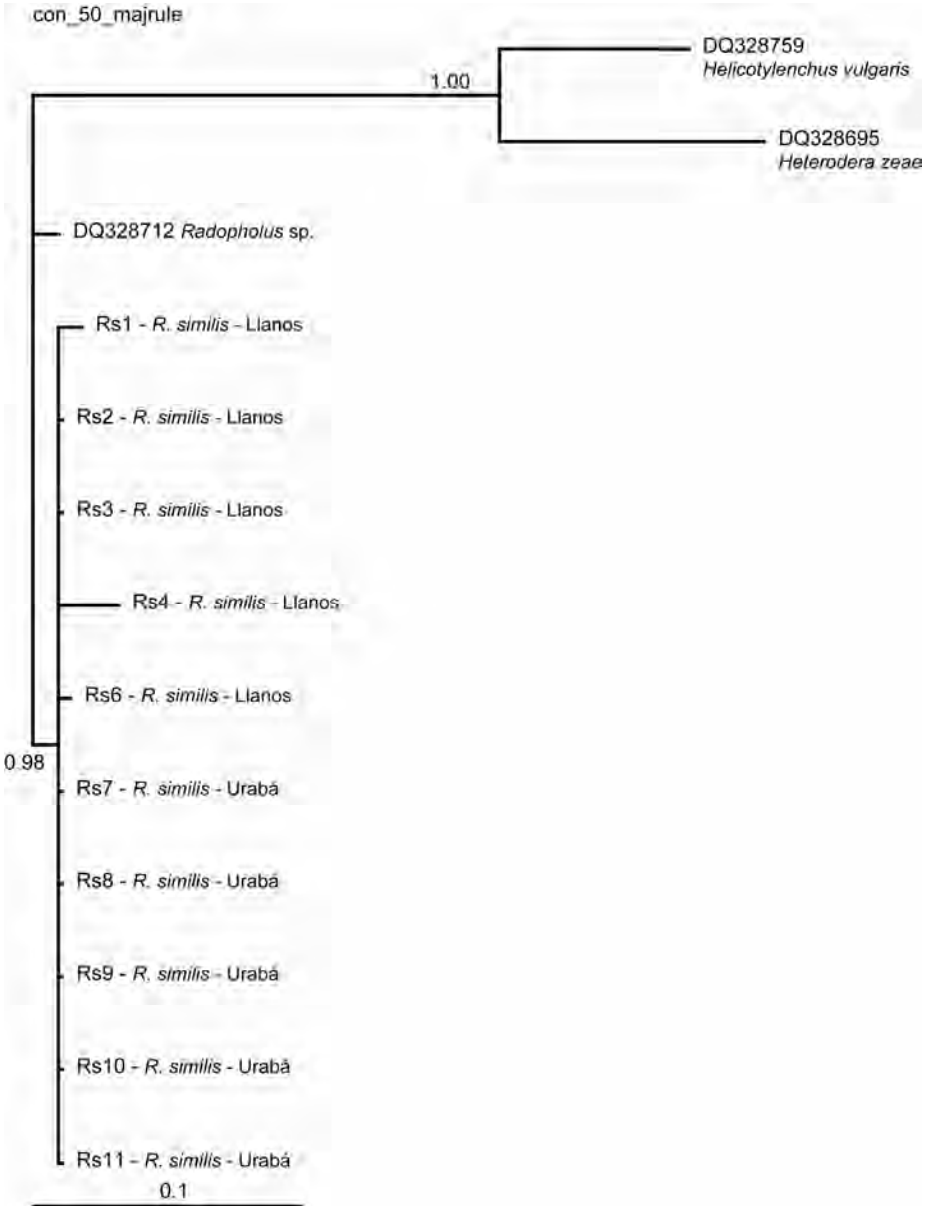
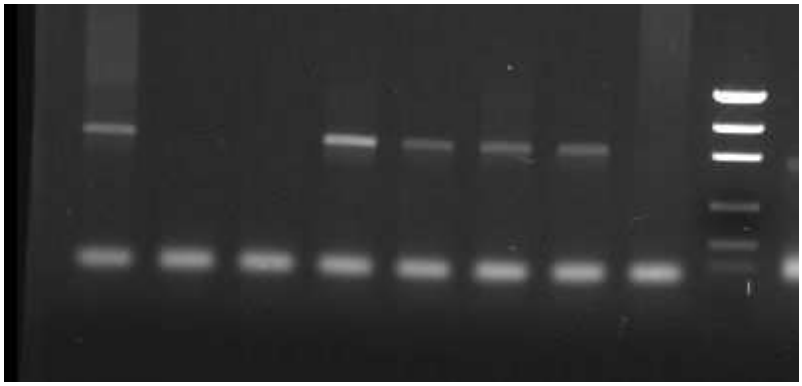


Fig. 6.4 Summary cladogram for D2D3 sequence from ten Colombian specimens of *R. similis* (Rs1 to Rs11) from two localities and one *Radopholus* sp. from GenBank accession (DQ328712). D2D3 expansion region of LSU-28S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Helicotylenchus vulgaris* DQ328759 and *Heterodera zeae* DQ328695. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP).



Rs1 Rs2 Rs3 Rs4 Rs8 Rs9 Rs10 Rs11 X

Fig. 6.5 PCR products obtained from *R. similis* from Colombia after using primers and PCR conditions for 18S amplifications were as described by De Ley et al. (1999) (X = DNA ladder, 830 bp, Promega).

The relationship detected between and within *Radopholus* populations was measured through Bayesian inference (BI) analysis. A partial sequence of *Helicotylenchus varicaudatus,* accession number EU306354 (from Genbank, deposited by Bert *et al*., 2008) and a sequence of *Helicotylenchus dihystera* accession number AJ966486 (Meldal *et. al*., 2007) were used as outgroups. The obtained alignment presented 820 characters of which 82 were parsimony informative. The topology of the majority rule 50 consensus tree was completely unresolved (Fig. 6.6). *R. similis*, accession number AJ966502 (PP=1.00) from Genbank showed only two different nucleotides. Sequence divergences as shown in Table 6.5 agreed with topology of tree from 18S rDNA region (Fig. 6.6) for all Colombian populations and specimens studied.

ITS1- 5.8- ITS2

PCR of ITS1-5.8-ITS2 amplified a single DNA product of nearly 800 bp (789-842) for each of the two *Radopholus* populations of which ITS1-5.8-ITS2 rDNA region were analyzed. Three and two sequences were obtained from populations Los Llanos and Urabá, respectively (Table 6.3).

Sequences from Genbank of 42 different populations: 39 populations of *R. similis* (most of them from Elbadri, 2000), *R. arabocoffeae* (one population; Trinh *et al.*, 2004) and *R. duriophilus* (two populations; Nguyen *et al*., 2003) were aligned and compared with the sequences of the Colombian populations of *R. similis*.

Table 6.5 Pairwise 18S sequence alignment similarity (%) of *R. similis* from two Colombian localities.

%Similarity										
		Llanos				Urabá				
Locality	Specimens/ species	Rs1	Rs2	Rs3	Rs4	Rs8	Rs9	Rs10	Rs11	*R. similis* AJ966502
LLanos	Rs1	100	100	100	100	100	100	99.8	100	99.7
	Rs2	-	100	100	100	100	100	99.8	100	99.7
	Rs3	-	-	100	100	100	100	99.8	100	99.7
	Rs4	-	-	-	100	100	100	99.8	100	99.7
Urabá	Rs8	-	-	-	-	100	100	99.8	100	99.7
	Rs9	-	-	-	-	-	100	99.8	100	99.7
	Rs10	-	-	-	-	-	-	100	99.8	99.6
	Rs11	-	-	-	-	-	-	-	100	99.7
	R. similis AJ966502	-	-	-	-	-	-	-	-	100

The comparison of the ITS1-5.8-ITS2 sequences at intra-population level showed no divergence (0%) between Rs1, Rs2, Rs3, Rs9 and a sequence from Genbank, accession number AY903311 recorded from ornamental plants from China (Zhou *et al*., 2007, unpublished). The Colombian population of *R. similis* Rs10 and 27 sequences of *R. similis* from Genbank reported from different host and localities showed very low sequence divergences (≤ 0.5). Sequence divergences, ranging between 1.8% and 2.7 % were obtained of 17 banana populations of *R. similis* from Sudan (Fig. 6.7).

One population of *R. arabocoffeae*; two population of *R. duriophilus* and 12 populations of *R. similis* from different hosts and localities showed a much larger nucleotide divergence (3.6-9.5%) compared with Colombian populations of *R. similis.* Additionally, six banana populations of *R. similis* from Sudan (5) and Costa Rica (1), were most different with 11.7-23.3% nucleotide divergence compared with Colombian sequences (Table 6.5). Divergence percentages with Colombian populations are shown in the phylogenetic tree (Fig. 6.4)

The relationship detected between *Radopholus* populations was measured through Bayesian inference (BI) analysis. A partial sequence of *Heterodera schachtii*, accession number AF498389 and a sequence of *Helicotylenchus dihystera*, accession number

DQ309585 (Chen *et al*., 2006) were used as outgroups. The obtained alignment presented 674 characters of which 138 were parsimony informative. On the basis of the topology of the majority rule 50 consensus tree, a single clade that contains only *R. similis* sequences from Colombia (5), China (7), Sudan (1), Uganda (1), Germany (1), Costa Rica (1) and Cuba (1) could be appointed (Fig. 6.7). *R. similis* sequence number AF275378 from Sudan was sister to this clade. Sequence divergences as shown in Table 6.5 differed considerably with the topology of the tree based on ITS1-5.8-ITS2 rDNA region (Fig. 6.7).

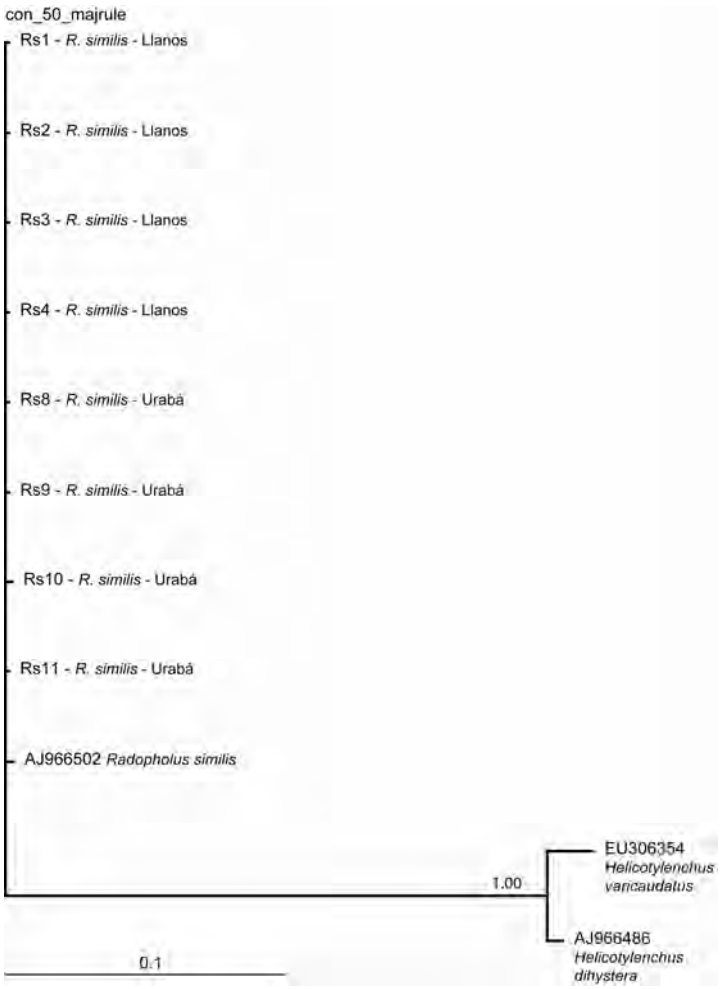


Fig. 6.6 Summary cladogram for 18S sequence from eight Colombian specimens of *R. similis* (Rs1 to Rs11) from two localities and one *Radopholus* sp. from GenBank accession (AJ966502). 18S rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Helicotylenchus varicaudatus* EU306354 and *Helicotylenchus dihystera* AJ966486. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP).

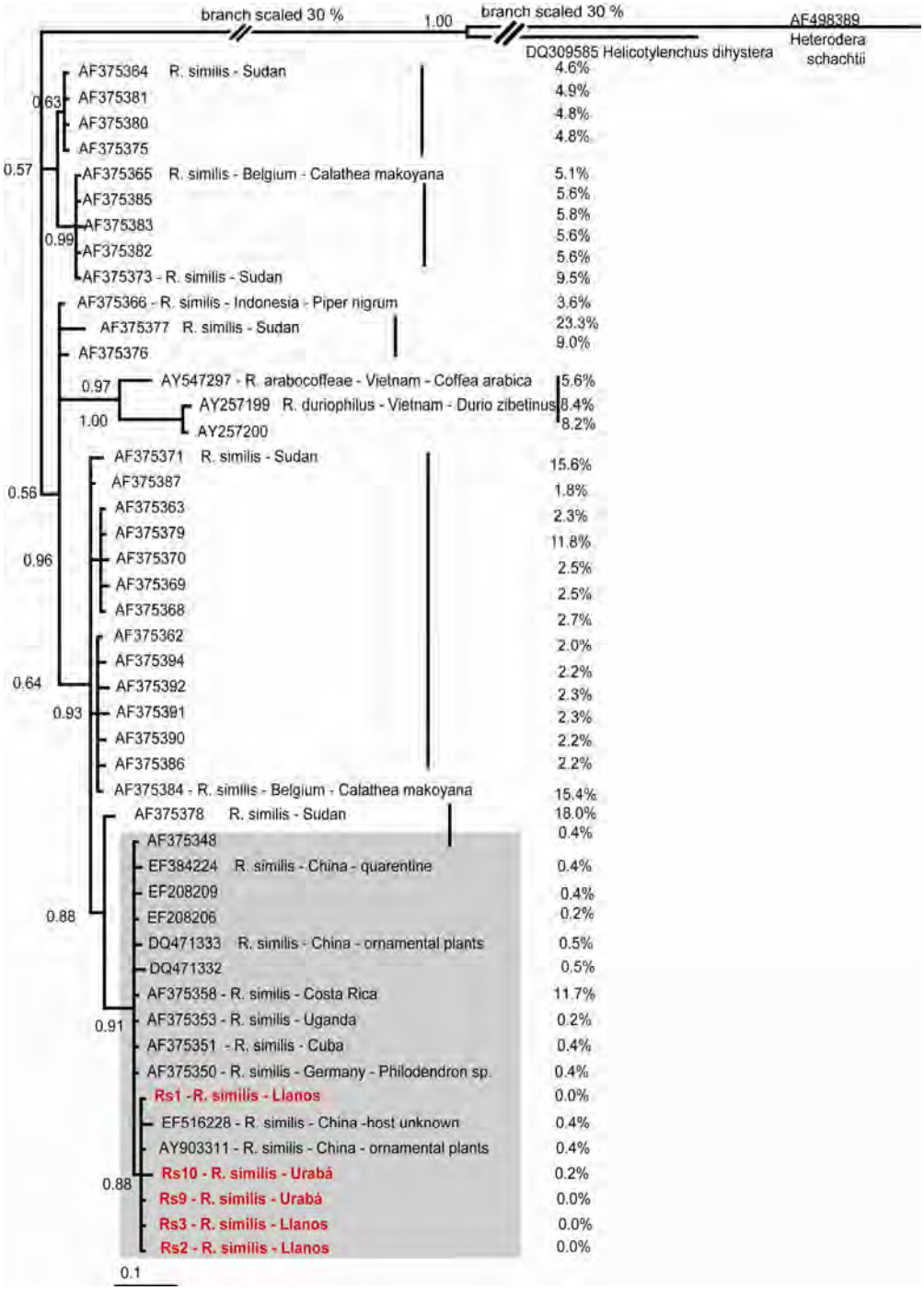


Fig. 6.7 Summary cladogram for ITS sequence from five Colombian specimens of *R. similis* (Rs1, Rs2, Rs3, Rs9 and Rs10) from two localities and 42 *Radopholus* sp. GenBank accessions. ITS1-5.8ITS2 rDNA obtained from Bayesian inference (BI) performed with MrBayes v3.1.2 using a GTR + I + Γ model, as estimated by PAUP/Mr Modeltest 1.0b. Outgroup *Heterodera schachtii* AF498389 and *Helicotylenchus dihystera* DQ309585. Branch support is indicated with PP; high (≥95% PP)/ medium (<95% to ≥50% PP)/ low (<50%% PP). Host of *R. similis* no mentioned in the tree are banana plants. Divergence percentages are shown in the last column.

Discussion on Colombian populations of R. similis

Morphological observations

With respect to morphological characters and morphometric data, Colombian populations of *R. similis* females only slightly extend the inferior limits of the other *R. similis* populations described from different localities around the world particularly with respect to body length, diameter at mid body and anus, length neck region, hyaline part of tail and length ovaries.

Presence of postrectal intestinal sac and smooth tail tip were included in *R. similis* descriptions by Ryss & Wouts (1997) but those characteristics were not observed in Colombian populations of *R. similis.* Later, Ryss (2003) considered two character states, presence or absence of postrectal intestinal sac to be valid for *R. similis*.

Tail length and length of hyaline part (65-70µm; 9-17 µm) were the unique criteria used to separate *R. similis* species in Colbran key (1970). In having a long female tail, *R. similis* resembles *R. arabocoffeae* Trinh, Nguyen, Waeyenberge, Subbotin, Karssen & Moens, 2004 , *R. bridgei* Siddiqi & Hahn, 1995, *R. colbrani* Kumar, 1980, *R. kahikateae* Ryss & Wouts, 1997, *R. musicola* Stanton, Mundo, Baldwin & Kaplan, 2001, and *R. duriophilus* Nguyen, Subbotin, Madani, Trinh & Moens, 2003. Differences between the Colombian populations of *R. similis* and species mentioned above are presented in the following paragraphs.

According to Trinh *et al*. (2004) and Nguyen *et al.* (2004) *R. similis* differs from *R. arabocoffeae* and *R. duriophilus* by the three incisures terminating at or just posterior to the phasmid *vs* four incisures of the lateral field terminating far posterior to the position of the phasmid in *R. arabocoffeae* and *R. duriophilus*. *R. similis* differs from *R. arabocoffeae* by the longer stylet of females 18.5-19.0 μm *vs* 15.9 μm (14.6-17.2) and males 12.8 -13.9 μm *vs* 10 μm (8.2-11.6). Rod-shaped sperm are present in *R. similis* and *R. arabocoffeae* whereas *R. duriophilus* possesses oval sperm. Colombian specimens of *R. similis* show on overlap with diagnostics characteristic of *R. arabacoffeae* and *R. duriophilus* except for sperm shape compared with the latter species; i.e. *R. similis* from Colombia has four incisures terminating at or just posterior to the phasmid; the stylet of females was 18.5 μm (15.9-21.5) and 19.5 μm (17.2-20.2) for Urabá and Llanos,

respectively; stylet of males was 12.8 μm (9-15.3) and 13.9 μm (13.5-15.3) for Urabá and Llanos, respectively.

Other characteristics used by these authors to separate *R. arabocoffeae* and *R. duriophilus* from *R. similis* were bursa in male reaching one third of tail, rarely half or never reaching tail terminus *vs* bursa reaching tail terminus; presence of a postrectal intestinal sac in *R. similis vs* absence in *R. arabocoffeae* and *R. duriophilus*; the secretory-excretory pore located at level of pharyngo-intestine junction in *R. similis vs* posterior to pharyngo-intestine junction in *R. duriophilus* (Nguyen *et al.*, 2003). However, some specimens of *R. similis* from Colombia showed secretory-excretory pore located at the level to pharyngeal-intestinal junction and the postrectal intestinal sac was absent in all specimens.

Females of *R. similis* differ from *R. kahikateae* in the rod-like sperm *(vs.* round), set off lip region (vs. continuous), length of stylet 18.5-19.0 µm (*vs.* 20.9-21.5µm) and low c value (8.8-9.1 vs. 13.2-15.7 µm). Males of *R. similis* differ from those of *R. kahikateae* by the knob-like cephalic region (*vs.* conical to cup-shaped) and by the amalgamated knobs in one round basal stylet knob (*vs.* distinctly separated) (Ryss & Wouts, 1997). Former authors considered that females of *R. similis* differ from *R. kahikateae* in the mostly smooth tail terminus (vs. annulated) however irregular annulations of tail tip were common in Colombian population of *R. similis.*

Females of *R. similis* differ from *R. colbrani* in cephalic annulation (3-5 annules *vs.* smooth cephalic region), rod-like sperm (*vs.* absent sperm cell in spermatheca), longer stylet (18.5-19.0 µm *vs.* 15 µm or less), longer hyaline tail portion (9.8-10.0 *vs.* 5-7 µm), longer male spicule length (17-22 *vs.* 15-16 µm) (Ryss, 2003).

Morphologically, *R. similis* is similar to *R. musicola* and *R. bridgei*, from which it is distinguished by a combination of the following characters. The stylet in males of *R. similis* is 13.4 µm (9.0-15.3) in Colombian population i.e. slightly shorter according to data from different authors: 13.2 µm (10-16) in Elbadri *et al.* (1999a); 14 µm (12-17) in Sher (1968b); 15 µm (13.5-16.5) in Ryss & Wouts (1997) but despite of the variation, the stylet remains longer than in *R. musicola* and *R. bridgei* 10.4 µm (8.8-12) and 11 calculated mean (10-12 µm), respectively. In *R. similis* females, the stylet knobs are anteriorly flattened (Ryss & Wouts, 1997) compared to rounded in *R. bridge.* The stylet

of female and male of *R. musicola* is unusually robust with large rounded knobs. In *R. similis* (Ryss & Wouts, 1997) males the stylet knobs are 'amalgamated into one round basal knobs' *vs* well-developed stylet knobs distinctly separated in *R. musicola* or 'three tiny often obscure knobs' in *R. bridgei* (Siddiqi & Hahn, 1995). Although the DGO range in females is variable and overlaps between the species, the mean value in *R. similis* is 4.0 µm (Ryss &Wouts, 1997; 3.2 µm in Colombian population of *R. similis*) similar to *R. bridgei* (4.0 µm, Siddiqi& Hahn, 1995) but larger than in *R. musicola* (2.8 µm).

Rod-shaped structures, previously considered to be sperm in *Radopholus* (Sher, 1968), and more recently identified as spermatids, were observed in most females and males of *R. similis* examined. Kaplan & Opperman (1997) considered that *R. similis* is a syngonic hermaphrodite. Self fertilization takes place ca. 50 to 60 days after the fourth moult in virgin females. Thus, they proposed that hermaphroditism is an alternative reproductive strategy within the genus *Radopholus*. Presence of these rod shaped structures in two freshly moulted, apparently virgin *R. similis* females of Colombian population suggests that these are hermaphrodites. In that case, Stanton *et al.* (2001) suggested what was refered to as the *female* and *ovary* would more strictly be *hermaphrodite* and *ovotestes*. Sher (1968b) commented that he could not satisfactorily explain the presence of females with sperm in several *Radopholus* species in which males are unknown; these species must be further examined for the possibility of hermaphroditism.

Molecular analysis

The sequence comparisons of the 18S, D2D3 rDNA expansion region and ITS1-5.8-ITS2 clearly grouped all Colombian population of *Radopholus similis* as revealed by the Bayesian inference (BI) analysis and the direct sequence comparisons.

Maximum identity and query coverage were 99% and 98-99% respectively for 18S sequences of Colombian population of *R. similis* when those were compared with *R. similis* AJ966502 accession (Meldal *et al.,* 2007). When Colombian- *R. similis* sequences from D2D3 region were compared with *Radopholu*s sp. accession DQ328712 (Subbotin *et al.,* 2006), maximum identity (97-98%) and query coverage (85-93%) were lower.

A sequence from 26S rRNA gene, D3 expansion domain (U47558 accession) submitted in genbank by Al-Banna *et al.* (1997) aligned and showed 0% divergence with Colombian population of *R. similis* when the latter ones were cut at its length (302bp).

Since only one sequence of *Radopholus* spp. has been reported from D2D3 and 18S, those regions are not appropriate to differentiate *R. similis* from another *Radopholus* species at this time. However, rDNA D2D3 and 18S sequences have been commonly used to separate different groups of nematodes at species level, including pratylenchids (Carta *et al*., 2001; de la Peña *et al*., 2006, 2007; De Luca *et al*., 2004; Duncan *et al*., 1999; Handoo *et al*., 2001; Inserra *et al*., 2001, 2007).

The majority of sequences available to date from *Radopholus similis* are from the ITS1-5.8-ITS2 rDNA region, and the Colombian populations of *R. similis* could only be compared with Elbadri *et al*., (2002), Nguyen *et al*. (2003), Trinh *et al*. (2004), Zhou *et al*., (2005)*, Xie *et al*., (2006)*, Peng *et al*., (2007)* and Long *et al*., (2007)* for this region. Topology of the ITS1-5.8-ITS tree positioned Colombian populations of *R. similis* in the same clade together with populations from different countries and hosts. Percentage of divergence yielded similar results except for the Costa Rican population which showed 11.7% divergence with respect to Colombian populations from the same clade. The populations of *R. similis* did not cluster together according to geographical distribution, nor host; for example, accessions from the same host (banana plants) and from the same country (Sudan) were positioned in different clades and showed the largest differences in percentage of divergence.

The worldwide distribution of *R. similis* is relatively recent (beginning of the 19th century) and is due to the transfer of infected plant material from country to country. The wide distribution of *R. similis* seems often to be correlated with the areas where banana plants of the subgroup Cavendish (AAA) were imported. It is speculated that in Latin America and the Caribbean, *R. similis* was introduced with the cv. Gros Michel and subsequently infested the more susceptible Cavendish cultivars (Marin *et. al,* 1998 data quoted by Gowen *et al.*, 2005). The Australian banana industry for instance was established from infected Fijian sets imported between 1890-1920; ornamentals from the USA transferred the nematode to France. *R. similis* has been recognized as the major

* unpublished

banana root pathogen, probably present in most banana-growing areas and of great economic importance in Australia, Central and South America, parts of Africa and the Pacific and Caribbean Islands (Orton Williams & Siddiqi, 1973). This worldwide distribution of *Radopholus* has most likely resulted in a phylogenetic tree which does not corresponds to the biogeographically history of the species.

The populations analyzed by Elbadri (2000) from Sudan, Uganda, Cuba and Germany and sharing a clade with Colombian populations in the phylogenetic tree, were found to be the most pathogenic populations towards banana among all the tested populations by this author. He stated that those findings agreed with the results obtained by Pastrick *et al.* (1995), Li *et al.* (1996), Blok *et al.* (1997b) and Thiéry *et al.* (1997) who were able to separate populations of nematode species with similar virulence using RAPD primers (Elbadri, 2000).

The phylogenetic position of *Radopholus* species in the ITS1-5.8-ITS tree, more particularly of *R. arabocoffee, P. duriophilus, R. similis* from Sudan and Belgium suggests that "*R. similis"* could be a species complex and more research is needed to unravel relationships. Additionally, it is not clear why sequence divergence within *R. similis* populations (e.g. accession number AF375377 from Sudan, 37%) were larger than *R. duriophilus* (8.2-8.4%) and *R. arabocoffeae* (5.6%) in relation to Colombian populations of *R. similis.*

Kaplan & Opperman (1997, 2000) and Kaplan *et al.* (1996, 1997b) stated that *R. similis* has a high degree of genomic conservation. However, genetic diversity of *R. similis* populations as determined by RAPD analysis was found between populations from Sri Lanka originating from different hosts (Hahn, *et al.*, 1996). The latter authors proposed three populations as *R. similis* biotypes on the basis of RAPD profiles. Kaplan *et al.* (1997) stated that diversity among burrowing nematodes may be greater in Africa than in Hawaii, Florida, Central America, Puerto Rico and Guadeloupe.

Notes on taxonomical status of Radopholus *species*

Thorne (1949) proposed the family Pratylenchidae in order to include the genera *Pratylenchus* Filipjev, 1936 and relatively close species. At present, the family Pratylenchidae Thorne, 1949 (Siddiqi, 1963) comprises four subfamilies: Hirschmanniellinae, Pratylenchinae, Nacobbinae, Radopholinae (Table 6.6) and eleven genera: *Pratylenchus* Filipjev, 1936; *Nacobbus* Thorne & Allen, 1944; *Radopholus* Thorne, 1949; *Pratylenchoides* Winslow, 1958; *Hoplotylus* s´Jacob, 1960; *Apratylenchoides* Sher, 1963; *Zygotylenchus* Siddiqi, 1963; *Hirschmanniella* Luc & Goodey, 1964; *Radopholoides* de Guiran, 1967; *Achlysiella* Hunt, Bridge & Machon, 1989 and *Zygradus* Siddiqi, 1991.

Table 6.6 Key to subfamilies of Pratylenchidae (Siddiqi, 2000)

1. Pharynx glands extending over intestine mostly ventrally and ventrolaterally; no marked sexual dimorphism in anterior region…….....**2**
   Pharynx glands extending over intestine mostly dorsally and dorsolaterally; with marked sexual dimorphism in anterior region……..…….**3**
2. Tails similar between sexes; phasmids near terminus……..…...**Hirschmanniellinae**
   Tails dissimilar between sexes; phasmids not near terminus…...… **Pratylenchinae**
3. Mature female spindle-shaped or batatiform, with numerous eggs within body, gall inciting……...…. **Nacobbinae**
   Mature female not spindle-shaped or batatiform, not with numerous eggs within body, not gall inciting……..……...… **Radopholinae**

The subfamily Radopholinae Allen & Sher, 1967 comprises seven genera (Table 6.7) of which *Radopholus* is the most economically important taxon worldwide. Originally, the genus *Radopholus* Thorne, 1949 was proposed in order to include those Pratylenchidae most closely resembling the genus *Pratylenchus* Filipjev, 1936 but possessing two ovaries. There are two different views on the taxonomy and species composition of *Radopholus*. The first point of view (Siddiqi, 1986, 2000; Ryss, 1988, 2003) is that the genus includes only didelphic species and female genital system consists of two equally sized opposite branches, while the closely related genus *Radopholoides* is characterized by monodelphic (prodelphic) females with the posterior genital branch reduced and non-functional.

According to the second view (Luc *et al*., 1987) both didelphic and monodelphic species are present in the genus *Radopholus* (Ryss, 2003). Recently LSU and SSU guided molecular phylogenetic studies indicate with high support a sister relationship of *Radopholus* and the ectoparasitic and cyst-forming endoparasitic nematodes (Hoplolaiminae and Heteroderinae) (Subbotin *et al*., 2007a; Bert *et al*. 2008). The inclusion of the burrowing endoparastic nematodes in a single family Pratylenchidae is classically defined by a context of similar morphological character sets that are likely the result of convergent evolution related to similar feeding modes.

Table 6.7 Key to genera of Radopholinae (Siddiqi, 2000)

1. Deirids present; at least one pharynx gland nucleus opposite or anterior to oesophago-intestinal junction…….....…….***Pratylenchoides***
   Deirids absent; all oesophageal gland nuclei behind oesophago-intestinal junction…….….**2**
2. Sexual dimorphism in anterior region prominent …….....…….**3**
   Sexual dimorphism in anterior region not prominent …….....…….**6**
3. Posterior ovary normally developed, functional…….....…..…….**4**
   Posterior ovary reduced or absented, non-functional.…….....…….**5**
4. Mature female swollen to become sausage-shaped; juveniles moulting to adult stage without feeding …….....…..……...…...***Achlysiella***
   Mature female not swollen; juveniles moulting to adult stage with feeding …….....…..……....***Radopholus***
5. Female stylet 21-29 µm long; basal knobs tulip-shaped…….……..….***Hoplotylus***
   Female stylet under 20 µm long; basal knobs not tulip-shaped……...….***Radopholoides***
6. Posterior ovary normally developed, functional…….……....….***Zygradus***
   Posterior ovary reduced, non-functional ……...……..… ***Apratylenchoides***

Radopholus at present comprises 51 original descriptions (Table 6.8) but the number of valid species varies according to different authors. Some species have been synonymized or transferred to other taxa. Four species have been synonymized with *R. similis* and one with *R. nativus;* two species were declared *species inquirendae* and 20 species were transferred to other genera in Pratylenchida, *Pratylenchus* (2), *Pratylenchoides* (2), *Hoplotylus* (1), *Hirschmanniella* (7), *Radopholoides* (1); *Achlysiella* (6) and *Zygradus* (1). We consider 24 valid species within the genus *Radopholus* based on literature.

Table 6.8. List of nominal *Radopholus* species (valid species in bold).

1. *R. acutocaudatus* (Zimmermann, 1898) Siddiqi, 1986 (syn. with *R. similis*)
2. *R. allius* Shahina & Maqbool, 1996 (transferred to *Pratylenchus*)
3. ***R. antoni*** van den Berge, Heyns & Tiedt, 2000
4. ***R. arabocoffeae*** Trinh, Nguyen, Waeyenberge, Subbotin, Karssen & Moens, 2004
5. *R. behningi* (Micoletzky, 1923) Allen, 1955 (transferred to *Hirschmanniella* by Luc & Goodey, 1962; sp. inq. for Luc & Goodey, 1962)
6. *R. biformis* (Cobb, 1909) Siddiqi, 1986 (syn. with *R. similis*)
7. *R. brassicae* Shahina & Maqbool, 1996 (transferred to *Pratylenchus*)
8. *R. brevicaudatus* Colbran, 1971 (transferred to *Achlysiella*)
9. ***R. bridgei*** Siddiqi & Hahn, 1995
10. ***R. capitatus*** Colbran, 1971 (transferred to *Achlysiella*)
11. *R. cavenessi* Egunjobi, 1968 (sp. inq. for Colbran, 1971 and for Ryss & Wouts, 1997; valid species for Siddiqi, 2000)
12. ***R. citri*** Machon & Bridgei, 1996
13. *R. citrophilus* Huettel, Dickson & Kaplan, 1984 (syn. with *R. similis*)
14. ***R. clarus*** Colbran, 1971
15. ***R. colbrani*** Kumar, 1980
16. ***R. crenatus*** Colbran, 1971
17. ***R. duriophilus*** Nguyen, Subbotin, Madani, Trinh & Moens, 2003
18. *R. ferax* Colbran, 1971 (syn. with *R. nativus*)
19. *R. gigas* Andrassy, 1954 (transferred to *Hirschmanniella* and syn. with *H. oryzae*)
20. *R. gracilis* (de Man, 1880) Allen, 1955 (also n. comb. by Hirschmann, 1955) (transferred to *Hirschmanniella* by Luc & Goodey, 1962)
21. *R. granulosus* (Cobb, 1893) Siddiqi, 1986 (syn. with *R. similis*)
22. ***R. inaequalis*** Sauer, 1958
23. ***R. inanis*** Colbran, 1971
24. ***R. intermedius*** Colbran, 1971
25. ***R. kahikateae*** Ryss & Wouts, 1997
26. *R. lavabri* Luc, 1957 (transferred to *Hirschmanniella* and syn. with *H. spinicaudata* by Luc & Goodey, 1962)
27. *R. litoralis* (de Guiran, 1967) Luc, 1987 (transferred to *Radopholoides*)
28. *R. magniglans* Sher, 1968 (transferred to *Achlysiella*)
29. ***R. megadorus*** Colbran, 1971
30. *R. megalobatus* (Bernard, 1984) Ryss, 1988 (transferred to *Pratylenchoides*)
31. *R. mucronatus* Das, 1960 (transferred to *Hirschmanniella* and syn. with *H. oryzae* by Luc & Goodey, 1962)
32. ***R. musicola*** Stanton,Mundo-Ocampo,Baldwin & Kaplan, 2001
33. ***R. nativus*** Sher, 1968
34. ***R. nelsonensis*** Ryss & Wouts, 1997
35. ***R. neosimilis*** Sauer, 1958
36. *R. nigeriensis* Sher, 1968 (transferred to *Zygradus*)
37. *R. oryzae* (van Breda de Haan, 1902) Thorne, 1949 (transferred to *Hirschmanniella*)

38. *R. paludosus* Whitlock, 1957 (sp. nomen nudum for Siddiqi, 1986 and for Ryss, 2003)
39. ***R. rectus*** Colbran, 1971
40. *R. ritteri* (Sher, 1970) Vovlas & Inserra, 1978 (transferred to *Pratylenchoides*)
41. ***R. rotundisemenus*** Sher, 1968
42. ***R. sanoi*** Mizukubo, 1989
43. ***R. serratus*** Colbran, 1971
44. ***R. similis*** (Cobb, 1893) Thorne, 1949 = type species
45. *R. trilineatus* Sher, 1968 (transferred to *Achlysiella* by Ebsary, 1991)
46. *R. triversus* (Minagawa, 1984) Luc, 1987 (transferred to *Hoplotylus*)
47. *R. vacuus* Colbran, 1971 (transferred to *Achlysiella*)
48. ***R. vangundyi*** Sher, 1968
49. ***R. vertexplanus*** Sher, 1968
50. *R. williamsi* Siddiqi, 1964 (transferred to *Achlysiella*)
51. *R. zostericola* (Allgén, 1934) Allen, 1955 (transferred to *Hirschmanniella* by Luc & Goodey, 1962; sp. inq. for Luc & Goodey, 1962)

Four *Radopholus* identification keys have been proposed (Table 6.9). Sher (1968) revised the genus including 11 species. A key to 22 didelphic species was given by Colbran (1970). Ryss & Wouts (1997) classified 23 species within the genus *Radopholus* while Siddiqi (2000) accepted only 20 species, placing several others in other genera. The most recent key was proposed by Ryss (2003) who recognized 25 species in the genus *Radopholus* (Table 6.9).

When Thorne (1949) introduced the genus *Radopholus,* it contained two species: the type species, *R. similis* (Cobb, 1893), Thorne, 1949 with *R. oryzae* (van Breda de Hann, 1902) Thorne, 1949. In 1954, *R. gigas* was described by Andrassy and a year later, Allen transferred three species from the genus *Tylenchorhynchus* Cobb, 1913 to the genus *Radopholus, R. gracilis* (de Man, 1880), *R. behningi* (Micoletzky, 1923), and *R. zostericola* (Allgén, 1934). In 1957, two new species were described, *R. lavabri* by Luc (1957) and *R. paludosus* by Whitlock. Sauer (1958) described two species *R. neosimilis* and *R. inaequalis,* from a native habitat in Australia. Das described *R. mucronatus* in 1960.

In 1964, Luc and Goodey proposed the genus *Hirschmannia* (now *Hirschmanniella*) for the large species in *Radopholus* with long overlapping ventral glands. Hereby, *R. gracilis* became *H. gracilis* and *R. oryzae* became *H. oryzae*. They synonymized *R. lavabri* with *Hirschmanniella spinicaudata* and *R. gigas* and *R. mucronatus* with *H. oryzae.* Further, *R. zostericola* became *H. zostericola and R.*

behningi became *H. behningi* but at the same time, the authors considered them as *species inquirendae*. This left only the type species, *R. similis* and the two species of Sauer in *Radopholus*. *R. paludosus* was not included in the genus, it was considered *nomen nudum* by Siddiqi (1986) and by Ryss (2003).

An additional species *R. williamsi* was proposed by Siddiqi (1964) for a population described from Mauritius as *R. similis* by Williams (1960). In 1967, de Guiran described *R. litoralis* which was transferred and designated type species of a new genus *Rapholoploides* proposed by the author in the same year.

Egunjobi (1968) described *R. cavenessi* from New Zealand but several characters introduced by Sher, e.g. the length of the hyaline area in the tail and sperm shape were not described nor can these features be determined from the illustration. The value of b (=3.2) appeared to be incorrect. For this reason, *R. cavenessi* was considered *species inquirenda* by Colbran (1970) and Ryss (2003).

Sher (1968b) described seven new species: *R. maniglans, R. nativus, R. nigeriensis, R. rotundisemenus, R. trilineatus, R. vangudeyi and R. verteplanus*. He suggested that the genus is indigenous to Australia. Two years latter, he described *R. ritteri* which was transferred to *Pratylenchoides*. In 1970, Colbran described 11 new species: *R. brevicaudatus, R. capitatus, R. clarus, R. crenatus, R. ferax, R. inanis, R. intermedius, R. megadorus, R. rectus, R. serratus* and *R. vacuus*.

In the 1980´s, four new species were described: *R. colbrani* Kumar, 1980; *R. citrophilus* Huetel, Dickson & Kaplan, 1984; *R. triversus* (Minagawa, 1984) Luc, 1987 and *R. sanoi* Mizukubo, 1989. Additionally, *Pratylenchoides megalobatus* Bernard, 1984 was transferred by Ryss (1988) to *Radopholus*. A study of *P. megalobatus* specimens showed that in this species the spicules of the male have a shape typical for *Pratylenchoides* (conical part of spicule short *vs*. long in *Radopholus*) and the male tail curves dorsally as in *Pratylenchoides* males, while in *Radopholus* the male tail is straight or curves ventrally (Ryss & Wouts, 1997).

R. citrophilus Huetel, Dickson & Kaplan, 1984 (Siddiqi, 1986) has been the most controversially introduced taxon in *Radopholus* differentiated on the basis relation of its host preference, biological characteristics and economical importance. Its ability to

Table 6.9 Valid *Radopholus* species at present (2008).

Sher-key	Colbran-key	Ryss & Wouts-key	Siddiqi-list	Ryss-key	Valid species at present
1968	**1970**	**1997**	**2000**	**2003**	**2008**
11	**22**	**23**	**20**	**25**	**24**
					*R. antoni**
					*R. arabocoffeae**
	R. brevicaudatus	*R. brevicaudatus*		*R. brevicaudatus*	
		R. bridgei	*R. bridgei*	*R. bridgei*	*R. bridgei**
	R. capitatus	*R. capitatus*		*R. capitatus*	*R. capitatus*
			*R. cavenessi**		
		R. citri	*R. citri*	*R. citri*	*R. citri**
	R. clarus	*R. clarus*	*R. clarus*	*R. clarus*	*R. clarus**
				R. colbrani	*R. colbrani**
	R. crenatus	*R. crenatus*	*R. crenatus*	*R. crenatus*	*R. crenatus**
					*R. duriophilus**
	*R. ferax **				
R. inaequalis	*R. inaequalis*	*R. inaequalis*	*R. inaequalis*	*R. inaequalis*	*R. inaequalis**
	R. inanis	*R. inanis*	*R. inanis*	*R. inanis*	*R. inanis**
	R. intermedius	*R. intermedius*	*R. intermedius*	*R. intermedius*	*R. intermedius*
		R. kahikateae	*R. kahikateae*	*R. kahikateae*	*R. kahikateae**
R. magniglans	*R. magniglans*	*R. magniglans*		*R. magniglans*	
	R. megadorus	*R. megadorus*	*R. megadorus*	*R. megadorus*	*R. megadorus**
				R. musicola	*R. musicola**
R. nativus	*R. nativus*	*R. nativus*	*R. nativus*	*R. nativus*	*R. nativus*
		R. nelsonensis	*R. nelsonensis*	*R. nelsonensis*	*R. nelsonensis**
R. neosimilis	*R. neosimilis*	*R. neosimilis*	*R. neosimilis*	*R. neosimilis*	*R. neosimilis**
R. nigeriensis	*R. nigeriensis*				
	R. rectus	*R. rectus*	*R. rectus*	*R. rectus*	*R. rectus**
R. rotundisemenus	*R. rotundisemenus*	*R. rotundisemenus*	*R. rotundisemenus*	*R. rotundisemenus*	*R. rotundisemenus*
			R. sanoi		*R. sanoi*
	R. serratus	*R. serratus*	*R. serratus*	*R. serratus*	*R. serratus**
R. similis	*R. similis*	*R. similis*	*R. similis*	*R. similis*	*R. similis**
R. trilineatus	*R. trilineatus*	*R. trilineatus*		*R. trilineatus*	
	*R. vacuus**	*R. vacuus*		*R. vacuus*	
R. vangundyi	*R. vangundyi*	*R. vangundyi*	*R. vangundyi*	*R. vangundyi*	*R. vangundyi**
R. vertexplanus	*R. vertexplanus*	*R. vertexplanus*	*R. vertexplanus*	*R. vertexplanus*	*R. vertexplanus*
*R. williamsi**	*R. williamsi*				

* males known (19/24 species)

parasitize citrus was not correlated with differences in karyotype, gametogenesis, or reproductive strategy (Kaplan & Opperman, 1997, 2000). Huettel & Yaegashi (1988) and Huettel *et al.* (1983) found morphological and biochemical differences between *R. similis* and *R. citrophilus,* respectively. Furthermore, morphological differences (Elbadri *et al.*, 1999b; Valette *et al,* 1998) and reproductive barriers between citrus-and non-citrus-parasitic burrowing nematodes could not be demonstrated (Kaplan *et al.*, 1997 and Kaplan & Opperman, 1997). Valette *et al.* (1998) and Elbadri e*t al.* (1999a) hypothesized that *R. citrophilus* should be considered a junior synonym of *R. similis.* Molecular, biological, morphological, and cytological findings involving a global collection of citrus and non-citrus parasitic burrowing nematodes conducted by Kaplan *et al.* (2000) brought them to the same conclusion. *R. citrophilus* is an invalid species designation and citrus-parasitic burrowing nematodes are correctly synonymized with *R. similis* and should be a pathotype.

Hunt, Bridge & Machon (1989) proposed a new genus *Achlysiella* for *R. williamsi*, a *Radopholus* species with somewhat swollen adult females. *R. nigeriensis* was transferred to a new genus *Zygradus* introduced by Siddiqi (1991).

Three species described early, *viz., Tylenchus granulosus* Cobb, 1893; *T. acutocaudatus* (Zimmermann, 1898) and *T. biformis* (Cobb, 1909) were synonymized with *R. similis*. Additionally, *R. triversus* was transferred to the genus *Hoplotylus* (Siddiqi, 2000).

Ebsary (1991) transferred *R. brevicaudatus, R. capitatus, R. maniglans, R. trilineatus* and *R. vacuus* to the genus *Achlysiella*. *P. bridgei* was described by Siddiqi & Hahn in 1995. One year later, Shahina & Maqbool described two new species of *Radopholus*, *R. allius* and *R. brassicae* both with one functional anterior ovary. No males were described. Ryss & Wouts (1997) considered the last two species to belong to *Radopholoides* because of the monoprodelphic genital system of their females. However, Siddiqi (2000) examined the paratypes of the two species and found them to belong to the genus *Pratylenchus,* to which they were transferred. At the same time *R. citri* was described by Machon & Bridgei (1996). Shahina & Maqbool (1996) described two new species from Pakistan: R; allius and R. brassicae but those have been considered invalid species for different authors.

Ryss & Wouts (1997) described two species, *R. kahikateae* and *R. nelsonensis* from native vegetation in New Zealand. The five species transferred to *Achlysyella* were considered to belong to *Radopholus* by them. *R. nelsonensis* has somewhat obese females, a characteristic of *Achlysiella*. Close examination of the *Achlysiella*-like *R. nelsonensis* revealed that the females do not swell until egg-laying starts. Due to this change of the character state during the females lifetime, the shape of the female body is unsatisfactory as a character for generic differentiation. The presence of a gelatinous egg sac and the juveniles moulting to adult stages without feeding, as characteristic for *Achlysiella* species, could not be verified. The advanced state of development of the few juveniles observed indicates that in *R. nelsonensis* moulting does occur. Therefore, until character states other than swollen female habitus are found characterizing the genus *Achlysiella*, the species should be recognized as a *Radopholus* species.

R. ferax Colbran 1970 was proposed as a junior synonym of *R. nativus* Sher, 1968 by Ryss & Wouts (1997) because the differences given by Colbran (1970) i.e. *R. ferax* with round spermatheca filled with rod-shaped sperm vs. spermatheca empty, continuous with the genital tract contour; broadly rounded tail terminus *vs.* rounded; ratio of tail length to stylet length (2.3-2.7 *vs.* 2.0), and length of the hyaline part of the tail = 2.5 µm (*vs.* 4-8) were not accepted as valid. The data found by Ryss & Wouts (1997) showed that the only difference between the species lies in the spermatheca being filled with rod-shaped sperm in *R. ferax* and empty in *R. nativus*. Further, they observed that in females of *R. nativus* populations spermathecae vary from set off and filled with sperm to continuos and empty, and males are rare or absent in most populations.

Additionally, Ryss & Wouts (1997) stated that "*R. cavenessi*, Egunjobi, 1968 closely resembles *R. clarus*, differing by its greater body width, the more anterior position of the excretory pore and the more posterior position of the vulva in female, and longer spicules and more distinct stylet knobs of male. The description of *R. cavenessi* is based on a single female and a male. The female specimen is flattened, what seems to be the cause of the large body width. It is probably also the cause of the posterior shift of the oesophagus, causing the nerve ring to line up with the excretory pore. This leaves the posterior position of the vulva (65.8%) and characters of the male as the only characters to distinguish it from *R. clarus*. As these are variable characters based on a single male,

the species cannot be identified with certainty. We hereby *R. cavenessi* considered a *species inquirenda* . However, Siddiqi (2000) listed *R. cavenessi* as a valid species.

R. colbrani described by Kumar (1980) and the five species transferred by Ebsary (1991) to *Achlysiella* were not included in Siddiqi 2000´s list. However these six species were considered valid by Ryss (2003) in his polytomous key to species of the genus *Radopholus*. In this key, he recognized 25 valid species being included in the genus but he did not include *R. sanoi* Mizukubo, 1989 and *R. antoni*, van den Berg, 2000.

Most recently, two species have been added from Vietnam *R. duriophilus* Nguyen, Subbotin, Madani, Trinh & Moens (2003), described from *Durio zibetinus* M. and *R. arabocoffeae* Trinh, Nguyen, Waeyenberge, Subbotin, Karssen & Moens (2004) associated with *Coffea Arabica* cv. Catimor.

Chapter 7

General discussion

Only general considerations are included here because an extensive discussion of the results was included in each of the chapters. Comparison between different methods used is given.

The process prior to identification: purification

The previous studies indicated *Meloidogyne, Pratylenchus, Helicotylenchus* and *Radopholus* as the most important genera (more prevalent and high populations) associated with the rhizosphere of fruit crops (*Solanum quitoense* Lam., *Cyphomandra betacea* (Cav.) Sendt. (syn. *Solanum betaceum* Cav.), *Passiflora ligularis* Juss. and *Rubus glaucus* Benth. and plantain in Colombia.

The prevalent taxa include a high species number and consequently nematode analysis at species level is time consuming. Therefore, this study has focused on three of the four world-wide most important genera found in Colombia: *Meloidogyne, Pratylenchus* and *Radopholus.* Samples of the three genera were obtained from different regions of Colombia. Most of the *Meloidogyne* populations survived initial conditions of storage and transportation (suspension for more than two month) but only one *Pratylenchus* and two *Radopholus* populations. Thus, actual diversity was better covered by the genus *Meloidogyne* (24 populations included in this study) compared to the other two genera.

The co-existence in the field of more than one species within a plant-parasitic genus has been frequently recorded for many genera by different authors. The most recent examples of mixed species associations of taxa involved in this study have been reported for *Pratylenchus* by Duncan *et al.*(1997), Inserra *et al.* (2001, 2007) and de la Peña *et al.* (2006, 2007); and for *Meloidogyne* by Handoo *et al.* (2001, 2004) and Carneiro *et al*. (2000, 2004a, 2008). All of those studies have lead to descriptions of new species. Mixed presence of *Meloidogyne* species within a sample from single root system of a single host were the first results of this study which were confirmed using perineal pattern and protein analyses. Thus, the first step for taxonomical studies is to determine if the sample is pure which implies than only one species is present. To make purification

of species before identification is compulsory when a new species is described because only in this way we have certainty that molecular results (sequence and isozyme phenotype) match with morphometric and morphological data of all life stages included in the description. It is also desirable to make purification of populations containing species that do no include combined methods in their original description, in order to obtain sequences and isozyme phenotypes that allow comparison with well established species within the genus. Nevertheless, purification is a difficult process for many genera.

Meloidogyne populations are commonly purified using a single egg mass to inoculate on a plant grown in sterile soil in small pots. This method was used in this research but only three populations were successfully increased. Several factors could be involved in failed purification of most populations, e.g.: vigor of egg mass (after storage for one month); different greenhouse conditions compared to original conditions in Colombia and development of life stages that could be affected by environmental conditions (mainly soil type, humidity and temperature). Additionally purification of populations in pot requires a lot of time and a lot of replicates. Alternative methods which allow a fast population increase and less risk of mixed species include modified tomato roots grown in special medium in petri dishes for *Meloidogyne* populations and carrot disks for *Pratylenchus* and *Radopholus* populations.

Because mixed species associations frequently occur in the field, identification of *Meloidogyne* based on a single young egg-laying female (Esbenshade & Triantaphyllou, 1985a, 1990; Karssen *et al*., 1995 is more appropriate than using various females (Carneiro *et al*., 1996a, 2000, 2004a). Further, DNA was always extracted from a single juvenile.

Ignoring possible mixed species associations from field samples results in overlapping of morphometric characters when populations are studied. However, overlapping in measurement has also been reported from offspring of a single female or a single egg mass for *Pratylenchus* (Roman & Hirschmann, 1969) and *Meloidogyne* (Jepson, 1987).

Overlapping in morphometric characters between species has been recorded in several studies of the three genera included here which made identification of

populations based only on those criteria difficult. Thus, combination of different methods is needed for accurate species identification.

Morphometric and morphological characters used for species identification

In nematology, species identification has been based primarily on light microscopy observations and morphological and morphometrical features, mainly of females and males. Many nematode genera, especially plant-parasitic nematode genera, exhibit little morphological diversity. Intraspecific variation of diagnostic features, the possibility of observational and interpretative mistakes, among other factors, make the precise and reliable identification of nematode species a challenge even for well-qualified taxonomists (Coomans, 2000, 2002).

In our case, morphometric and/or morphological features allowed to separate populations into small species groups as a first step towards identification. Hereby, features of perineal pattern of females or head shape of males in *Meloidogyne*; head annulation number in *Pratylenchus* and tail length of *Radopholus* genera were the most helpful features to reduce the number of possible species.

For *Meloidogyne*, the perineal pattern was very useful in our research as a first step to apportion the Colombian population to the six recognized morphogroups. Additionally, a unique pattern as shown for populations P9, P16, P17, P18, P19a, P19b, P21 and P23 for example, allowed identification at species level of *Meloidogyne hapla.* Four main *Meloidogyne* species were easily identified based on head of males using LM of specimens in appropriate position (lateral). The combination of LM and SEM observations provided even more accurate results e.g. based on details of face views. The number of projections protruding from the stylet shaft of males was a helpful tool to separate some Colombian populations at species level. It was the most useful diagnostic character for *M. konaensis* (6-12 large projections) (Eisenback *et al*, 1994) and also for *M. paranaensis* and *M. brasilensis* (1-2 projections) (Carneiro *et. al,* 1996b; Charchar & Eisenback, 2002). Because *M. konaensis* and *M. paranaensis* shared the same phenotype

P1 in protein analysis, the number of projections of male stylet represented the diagnostic character to separate both species.

For *Pratylenchus*, morphometric characters such as number of annulations (two), tail shape (non-crenate tail), spermatheca features (filled with sperm- amphimictic) allowed reduction of the number of putative species to a small group (11 out of about 80 valid species) with similar features as the Colombian population. The combination with SEM observations however, provided even more accurate results, e.g. some specimens of *Pratylenchus* showed two annules plus one incomplete annule at one side of the head (clearly observed using SEM) and all of them showed lip sectors fused with the oral disc and resulting in a smooth face. Therefore, the number of annules (2) and face shape (smooth) were the most useful features used for identification of the Colombian *Pratylenchus* populations and allow to reduce the number of species to three (*P. coffeae*; *P. loosi* and *P. jaehni*). *Pratylenchus* species from Colombia share maximum 30% of morphometric characters with *P. coffeae* populations. Thus, *Pratylenchus* from Colombia can be separated, solely based on morphological and morphometrical grounds from most of the species included in the genus; morphometric characters overlap only with *P. jaehni* and *P. loosi.* Finally, comparison of morphometric data between our populations and the latter two species in combination with molecular studies allowed to separate Colombian *Pratylenchus* population from those *Pratylenchus* species and to consider the Colombian population as belonging to a new species.

In the case of *Radopholus*, morphological and morphometric approaches were adequate for identification at species level. Firstly, tail length and length of hyaline portion placed Colombian populations close to *R. bridgei*, *R. colbrani*, *R. kahikateae*, *R. musicola*, *R. arabocoffeae* and *R. duriophilus.* Further, morphology and morphometric data allowed separation from those species. Some characters showed overlap only with the latter two species. Molecular and phylogenetic approaches were included to reinforce our results and separate Colombian populations of *Radopholus similis* from *R. arabocoffeae* and *R. duriophilus*. Molecular analysis confirmed species identity and was also helpful to analyse phylogenetic relationships between our populations and other *Radopholus similis* populations and *Radopholus* spp. populations around the world.

Protein analyses (method used only for Meloidogyne *populations)*

Protein electrophoresis was the first molecular technique to be applied in plant nematology. Identification of most common and some rare *Meloidogyne* species became routine based on isozymes from the soluble protein extract of a single young egg-laying female (Esbenshade & Triantaphyllou, 1985c, 1990; Karssen *et al*., 1995; Molinari *et al*., 2005), various females (Carneiro *et al*., 1996a, 2000, 2004b) or from galled root pieces harboring a female (Ibrahim & Perry, 1993).

Extensive characterization of isozyme phenotypes has subsequently been carried out for other plant-parasitic nematode genera e.g. *Aphelenchoides* and *Ditylenchus* (Ibrahim *et al*., 1994); *Heterodera* (Esbenshade & Triantaphyllou, 1987, 1988; Ibrahim & Rowe, 1995; Andres *et al*., 2001); *Radopholus* (Fallas *et al*., 1996; Huettel *et al*., 1983); Globodera (Fox & Atkinson, 1988); *Pratylenchus* (Ibrahim *et al*., 1995; Andres *et al*., 2000). For many genera, these studies revealed a wide variation in isozyme phenotypes between populations of the same species with the exception of *Meloidogyne*, in which limited intraspecific variation was detected. In the relatively few studies where protein-based diagnostics have been used, nematologists have obtained a much better insight into the diversity of *Meloidogyne* species present and the frequency of occurrence and abundance of the individual species (De Waele & Elsen, 2007).

Because number of described species have increased and populations with similar esterase patterns showed different malate dehydrogenase (MDH) patterns (more than one band or different Rm), combination of esterase with malate dehydrogenase on a unique gel has become more appropriate to use than esterase alone. This was confirmed in our study in which phenotype number 16 (E1bN1b) differered from specific-species phenotype of *M. hapla* just in faster Rm of malate dehydrogenase band.

Based on number of esterase bands and its rate of migration (Rm) our Colombian phenotype E1aN1a fitted to the phenotype P1 described for *M. paranaensis* and *M. konaensis* (Carneiro *et al.* , 1996a, 1996b, 2000, 2004b). This result combined with morphological analysis, i.e. mainly based on number projections protruding from the

shaft in stylet of males, confirmed the presence of *M. paranaensis* and *M. konaensis* in Colombia.

Species-specific PCR (*method used only for* Meloidogyne *populations*)

Our results confirm previous studies (Williamson *et al.*, 1997; Zijlstra *et al.*, 2000; Wishart *et al*.,2002) which showed that the use of a species-specific PCR is a rapid and useful tool for differentiating mixtures of root-knot nematodes However, three apparently wrong signals were obtained: two false-positive and one false-negative.

False-positive were the populations P1and P15 which produced the expected band sizes for *M. arenaria* and *M. hapla,* respectively. However, P15 did not show the typical autapomorphic features characterizing sequences of *M. hapla*. Both populations were included in clade 2 (unknown species) in phylogenetic trees. Only morphological data of juvenile stages were obtained (no perineal pattern, no isozymes results available) from populations P1 and P15 which clustered with unknown species in CDA (Fig. 4.7), hereby confirming their position in clade 2 i. e. not closely related to *M. hapla* and *M. arenaria*. Additionally, population P15 was associated with *Rubus glaucus,* a host so far never recorded as host of root knot nematodes. For populations P1 and P15, a wrong specific primer signal could be possible, or an alternative reproductive strategy might separate population P15 from other *M. hapla* populations from Colombia. The species-specific primers used in our case were built based on a gene pool from different countries.

The false negative was the population P25 (named 1 in specific primer) which did not produce the expected band of *M. javanica* or control populations used for protein analysis.

Using species-specific PCR, Adam *et al.* (2005) reported inconsistent results with lines of *M. incognita* "when DNA of single females was used and a band of the expected size was produced with DNA extracted from a bulk sample of nematodes suggesting that variation in the primer site(s) or sensitivity to DNA concentration may occur with the Finc and Rinc primers".

Molecular and phylogenetic analyses

Analyses of Colombian *Meloidogyne* populations at different rDNA regions (D2D3, 18S and ITS1-5.8-ITS2) yielded phylogenetic trees with similar topologies: Clade 1 contained *M. hapla* populations; clade 2 grouped six populations (P1, P2, P5a, P15, P22 and P23) not identified at species level and therefore their reproductive strategies are unknown, and clade 3 contained *M. incognita, M. javanica* and *M. arenaria*.

These topologies agreed with morphological and morphometric data, protein analyses and species-specific PCR. An exception was formed by population P15 (see species-specific PCR for explanation). The tree topology and analyses of individual sequences allowed to infer that the species-specific PCR results of populations P1 and P15 were wrong. For the latter population the absence of autapomorphic characters typical of *M. hapla* species was enough to recognize the error. Additionally, GenBank accessions of *Meloidogyne* containing mainly temperate species were consistently placed as a separate clade (clade 0). All of the generated trees contained three identical clades which were strongly supported by high PP values.

In the same way, topologies of trees and analyses of autapomorphic characters of nucleotide sequences were decisive tools to support the Colombian population of *Pratylenchus* as a new species.

Additionally, molecular and phylogenetic data suggested that the evolutionary history of some *Radopholus similis* populations included in previous studies (Elbadri, 2000; Elbadri *et al.*, 2002) is not clear. For example, populations from the same host (banana plants) and from the same country (Sudan) were positioned in different clades in our phylogenetic trees and showed the largest differences in percentage of divergence.

Additional to taxonomical approaches, analyses of sequences and phylogenetic trees allowed to infer important aspects of evolutionary history and biology, *i.e.* the populations reported by Elbadri (2000) from Sudan, Uganda, Cuba and Germany, sharing the same clade with the Colombian populations of *R. similis* in the phylogenetic tree, were found to be the most pathogenic populations for banana among all the populations tested by this author. He stated that those findings agreed with the results obtained by

Pastrick *et al.* (1995), Li *et al.* (1996), and Thiéry *et al.* (1997) who were able to separate populations of nematode species with similar virulence using RAPD primers (Elbadri, 2000).

Diversity of Meloidogyne, Pratylenchus *and* Radopholus *from Colombia: hosts and localities.*

Studies on nematode biodiversity focusing at species level are needed since wide distributions of plant-parasitic nematodes have been recorded in plantain and fruit crops, resulting in significant economic losses (as shown in chapter 2). Thus, this thesis focused mainly on two composition components of biodiversity (species and genes) as a contribution to analyse the biodiversity of the nematofauna in Colombia.

Although only one *Pratylenchus* population was considered in this thesis, a new species was identified. This finding might suggest that, we can expect a high diversity in future research when samples from different hosts and localities will be included. For *Radopholus*, only one species, *R. similis*, was identified from the two populations studied, but small differences were shown for the different regions, *i.e.* the stylet of females was 18.5 μm (15.9-21.5) and 19.5 μm (17.2-20.2) for Urabá and Llanos, respectively; stylet of males was 12.8 μm (9-15.3) and 13.9 μ m (13.5-15.3) for Urabá and Llanos, respectively. Morphometric data together with molecular analyses, confirmed the high intraspecific variability of *R. similis.*

Since a large number of *Meloidogyne* populations (24) were included in this study, the highest diversity was analysed in this genus. Taxonomical studies on *Meloidogyne* in Colombia are based mainly on the perineal pattern. The most recent species descriptions and results of current study have shown that the perineal pattern is a good criterion as a first step to group/separate species but further combined methods (morphological, protein analyses and relationships) are essential for accurate identification. Only combined methods allowed identification of the four most common species worldwide as well as recognizing new records that were probably misidentified before. These were *M. paranaensis* and *M. konaensis* and probably one or more new

species, all included in clade 2 of the phylogenetic analyses and representing phenotypes 16 to 19 of the protein analyses. However, additional specimens are needed for complete analysis of the latter populations.

Concerning the distribution of the species identified in the present study, the results showed that *M. incognita* is the species most widely distributed (present in 11 of 24 localities), followed by *M. javanica* (8); *M. paranaensis* (7), *M. hapla* (6) and *M. arenaria* (4). In relationship with the host, *M. incognita* was also the species associated to the largest number of crops (four out of six crops considered in this study): *S. quitoense, C. betacea, P. ligularis* and plantain), followed by *P. arenaria* and *P. paranaensis* (three of six: *C. betacea* and plantain for both nematode species plus banana and *P. ligularis*, respectively) and *M. javanica* and *M. hapla* (two of six: *S. quitoense* and *C. betacea*). The putative *M. hapla* (only in species-specific PCR test) population P15 was associated with *R. glaucus*, however analysis of the absence of autapomorphic characters typical of *M. hapla* species and the position of the population in clade 2 of phylogenetic tree suggested that this population probably represents a new species. Additionally, no *Meloidogyne* species have been reported from this host.

Previous studies on nematodes associated with fruit crops in Colombia (Figs. 3.4, 3.5) have already demonstrated that *M. incognita, M. javanica* and *M. hapla* are associated with *S. quitoense, C. betacea* and *P. ligularis; M. arenaria* is also associated with the first two crops. Some specimens/populations of *M. arenaria* are associated with fungi resulting in a dangerous pathological complex. Additionally, *M. exigua* was recorded on *C. betacea* in two localities (Gaviria & Navarro, 2003) but this nematode species was not identified during this study. Differences between these former studies and the present study are likely because no identical localities and host were analysed. Atypical phenotypes, E3N1, E3N2, were found in Colombian *Meloidogyne* populations P5a, P19a and P19b. Those phenotypes represent a new species which will be described from Brazil, Chile, Iran and Africa by Karssen and Carneiro (personal communication; December 10, 2007). Similar phenotype (named M3) was observed by Esbenshade & Triantaphyllou (1985c) in three populations from South America (from Argentina, Bolivia and Ecuador) and one population from Turkey (E923). At present, this

phenotype was found in Colombia associated with *S. quitoense* and *C. betacea* in Tolima and Caldas, respectively, but probably its distribution and host range could be more widespread.

On *Musa* plants (plantain and banana) *Meloidogyne* and *Pratylenchus* were already recorded from different localities as shown in previous studies (chapter 3, tables 3.12 to 3.16), however, samples from those localities were not included in this study. Because data of previous studies fit to different agro-ecological regions and a wide spectrum of production conditions (banana fields with high technology for export in Carepa to almost primitive production conditions under natural primary forestry in Guapi) high diversity can be expected.

Further considerations

Correct species identification is crucial for reliable crop management because of differences in host range and virulence between the species. Diversity of nematodes found in the present study matched with high biodiversity expected from Colombia.

As stated in the introduction, studies on biodiversity represent a long-term multidisciplinary task.

The first future task will be to recover and purify those populations included in clade 2 in the phylogenetic trees (P1, P2, P5a, P5b, P15 and P23) and those that fit to phenotypes 16 and 19 (P14a and P3a, P10, P11, respectively) in the protein analyses. They probably represent new species. Descriptions of the new species, their distribution, host associates and potential damage will be considered.

At the same time, although *M. paranaensis* and *M. konaensis* were record in Colombia in this study, more research is needed to define their geographical distribution and host range in Colombia. Carneiro *et al* .(2004) found two phenotypes associated with *M. paranaensis* and *M. konaensis*, P1 and K3 (the first one associated with both species and the latter one specific to *M. konaensis*) but only P1 (recorded in this study as E1aN1a or phenotype number 15) was detected in Colombia. Further information about clear differentiation of these species using isozyme phenotypes is required, moreover because important damage to crops has been recorded from *M. paranaensis* and *M.*

konaensis. Since both species have not yet been recorded from *C. betacea* and *P. ligularis,* evaluating possible damage to these fruit crops is also needed.

Similar studies should be conducted in Colombia for the new species that will be described from Brazil, Chile, Iran and Africa populations by Karssen and Carneiro (personal communication; December 10, 2007).

Analyses of *Radopholus* populations on phylogenetic trees suggested that additional research is needed to bring more insight in the observed high divergence within *R. similis* from the same host (banana plants) and from the same country (Sudan), a diversity even higher than between different species (*R. duriophilus* and *R. araboccofeae*).

To obtain appropriate and reliable identification, access to different methods for taxonomic research (mainly those molecular, protein analysis, analyses of sequences and phylogenetic studies) should be recommended to Institutions and Government. Because "Biodiversity" refers not simply to the number of genes, species, ecosystems, or any other group of things in a defined area", but also to composition, structure, and function determining, and in fact constituting, the biodiversity of an area, multidisciplinary and inter-institutional project should planned to adequate approach and more efficient research results on biodiversity.

Our results suggest that if more host and localities would be included in future studies, the number of new species, not only of *Meloidogyne* but also of other plant parasitic nematodes, will increase in correspondence with the high biodiversity of fauna and flora in Colombia.

Summary

Wide distributions of economically important plant-parasitic nematodes belonging to the genera *Meloidogyne, Pratylenchus* and *Radopholus* have been recorded in plantain and fruit crops in Colombia. Despite of significant economic losses, taxonomic research on these taxa in Colombia is limited. This thesis focuses mainly on two composition components of biodiversity (species and genes) as a contribution to the analyses of biodiversity of economically important plant-parasitic nematodes in Colombia. Samples were taken from 16 different regions of the country.

Identification, characterization, and description of known and new nematode species should be based on a combination of as many data as possible, using different approaches. Morphometrical and morphological studies were based on LM and SEM observations, combined with other taxonomical methods such as analyses of proteins and use of species-specific primers (only for *Meloidogyne* populations), molecular analyses of sequences and phylogenetic analyses. In the current study, morphometric and/or morphological features allowed to separate populations into small species groups as a first step towards identification. Hereby, features of perineal pattern of females or head shape of males in *Meloidogyne*; head annulation number in *Pratylenchus* and tail length of *Radopholus* were the most helpful features at species level and reduced the number of species to be taken into account. From the combination of these analyses it turned out that several species ocurred together in the field. Thus, purification of populations is recommended as a second step in the identification process. In this study, only three *Meloidogyne* "populations" could be successfully purified. However, to overcome the mixed species problem for all other species, *Meloidogyne* identification was based on a single young egg-laying female (protein analysis) and DNA (for species-specific PCR and sequence analyses) was always extracted from a single juvenile.

The highest diversity was analyzed in the genus *Meloidogyne* since a large number of populations (24) were available for this study. Six *Meloidogyne* species: *M. incognita, M. javanica, M. arenaria, M. hapla, M. paranaensis* and *M. konaensis* were found associated with four fruit crops and plantain in Colombia. The last two species

had never been recorded previously in Colombia but since they show morphological features of *M. incognita*, they were probably misidentified in the past. Only the use of combined methods allows accurate identification to species level. Analyses of esterase and malate dehydrogenase resulted in a profile that is typical of both species, P1 fitted both *M. paranaensis* and *M. konaensis*. Though, the morphological feature, number of projections protruding from the stylet shaft of males allowed their separation. Isozyme analysis also recovered phenotypes that could not be connected to known *Meloidogyne* species. **E3N1, E3N2** phenotypes were found in populations P5a, P19a and P19b. These phenotypes represent a new species that is currently under investigation based on populations from Iran, Africa, Chile and Brazil by Karssen, Carneiro and collaborators (personal communication; December 10, 2007). Furthermore, the phenotype **E1bN1b** (population P14a) matched with *M. hapla* but the Mdh band was very slow and morphological features of this population agreed with *M. javanica*. Populations P3a, P10 and P11 yielded two esterase bands (**E2N2**): one slow band, and one fast band with a migration rate largely similar to the I1 band of the *M. incognita* phenotype. **E1bN1b and** phenotypes probably represent a new species. However additional specimens are needed for accurate morphological and morphometrical studies.

Analyses of Colombian *Meloidogyne* populations (based on 65 nematode specimens) in combination with *Meloidogyne* populations from GenBank at different rDNA regions (D2D3, 18S and ITS1-5.8-ITS2) yielded phylogenetic trees with similar topologies. All of the generated trees contained three identical clades, strongly supported by high PP values. *Meloidogyne* populations containing mainly temperate species (as obtained from GenBank) were consistently placed as a separate clade (clade 0). Clade 1 contained *M. hapla* populations; clade 2 grouped six populations (P1, P2, P5a, P15, P22 and P23) not identified at species level and therefore with unknown reproductive strategies, and clade 3 contained *M. incognita*, *M. javanica* and *M. arenaria*. These topologies agreed with morphological and morphometric data, protein analyses and species-specific PCR. However, populations P1 and P15 formed exceptions. The tree topology and analyses of individual sequences showed that species-specific PCR results of populations P1 and P15 were wrong. For the latter populations the absence of autapomorphic characters typical of *M. hapla* species allowed to recognize the error.

Only one *Pratylenchus* population was obtained, but morphological analyses were completed with comprehensive molecular divergence and phylogenetic analyses (based on eight nematode specimens from Colombia and additional Genbank material). Their outcome resulted in the description of a new species, indicated here as *Pratylenchus* sp. n. The new species is closely related to three *Pratylenchus* species but clearly differs from the other species within genus. It shares with *P. coffeae*, *P. loosi* and *P. jaehni* morphology features: amphimictic lesion nematodes with two lip annuli, and lip sectors fused together and also fused with the oral disc to a smooth face. Molecular distinctions between *Pratylenchus* n. sp., *P. coffeae, P. jaehni* and *P. loosi* are more clear-cut than are morphological differences. The sequence comparison of the D2D3 rDNA expansion region clearly separates the new species from *P. jaehni, P. loosi* and *P. coffeae*. *Pratylenchus* n. sp. had a sister relation with *P. jaehni*. *P. loosi* and *P. coffeae* were more distinctly related. Clear autapomorphic characters of the new species were present in all specimens studied, differentiating its D2D3 expansion region of 28S rDNA from the observations of its sister species *P. jaehni*. Our results fulfill the requirements of the evolutionary species concept and phylogenetic species concept *sensu* Adams (1998). Additionally, our new species is a typological morphospecies compared with most species included in the genus; morphometric characters overlap only with *P. jaehni* and *P. loosi*. In general, *Pratylenchus* species are polyphagous, but differences in host preference occur among the species. Type host for *Pratylenchus* n. sp. is *Musa* ABB (plantain, hartón cultivar), a host also reported for *P. coffeae*. So far, *P. jaehni* and *P. loosi* have not yet been recorded on *Musa* plants.

Morphological data of two obtained *Radopholus* populations were supplemented with sequence divergence and phylogenetic analyses (D2D3, 18S and ITS1-5.8-ITS2) based on 23 specimens of nematodes of Colombia and additional Genbank material. All specimens were identified as *R. similis.* With respect to morphological characters and morphometric data, Colombian populations of *R. similis* females only slightly extend the inferior limits of the other *R. similis* populations described from different localities around the world, particularly with respect to body length, diameter at mid body and anus, length of the neck region, hyaline part of tail and length of the ovaries. The topology of ITS1-5.8-ITS tree positioned Colombian populations of *R. similis* together

with populations from different countries and hosts in the same clade. Percentage of divergence yielded similar results except for the Costa Rican population which showed 11.7% divergence with respect to Colombian populations from the same clade. The populations of *R. similis* did not cluster together according to geographical distribution, nor did they for host. For example, accessions from the same host (banana plants) and from the same country (Sudan) were positioned in different clades and showed the largest differences in percentage of divergence. The populations analyzed by Elbadri (2000) from Sudan, Uganda, Cuba and Germany and sharing clade with Colombian populations in phylogenetic tree, were found to be the most pathogenic populations for banana among all the tested populations by this author. The phylogenetic position of *Radopholus* species in the ITS1-5.8-ITS tree, more particularly of *R. arabocoffee*, *P. duriophilus*, *R. similis* from Sudan and Belgium suggests that *"R. similis"* could represent a species complex; more research is needed to unravel these relationships.

In conclusion, all retrieved *Meloidogyne* species were widely distributed and present in most of the fruit crops. *R. similis* and *Pratylenchus* n. sp. were restricted to *Musa* plants, but an incomplete distribution picture related to flawed sampling/transported processing can not be excluded for the Pratylenchidae. Correct species identification is crucial for reliable crop management because of differences in host range and virulence between species. Our results suggest that if more hosts and localities would be included in future studies, the number of new species, not only of *Meloidogyne* but also of other plant parasitic nematodes, will increase in correspondence with the high biodiversity of fauna and flora in Colombia. Additionally, studies on biodiversity represent a long-term multidisciplinary task, including descriptions of the new species, data on distribution, host associations, potential damage and relationships with other ecosystem components.

References

Adam, M.A.M., Phillips, M.S. & Blok, V.C. (2005). Identification of *Meloidogyne* spp. from North East Libya and comparison of their inter- and intra-specific genetic variation using RAPDs. *Nematology* 7, 599-609.

Adam, M.A.M., Phillips, M.S. & Blok, V.C. (2007). Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathology* 56, 190-197.

Adams, B.J. (1998). Species concepts and the evolutionary paradigm in modern nematology. *Journal of Nematology* 30, 1-21.

Adams, B.J. (2001). The species delimitation uncertainty principle. *Journal of Nematology* 33, 153-160.

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

Al-Banna, L., Ploeg, A.T., Williamson, V.M. & Kaloshian, I. (2004). Discrimination of six *Pratylenchus* species using PCR and species-specific primers. *Journal of Nematology* 36, 142-146.

Allen, M.W. (1952). Observations on the genus *Meloidogyne* Goeldi, 1887. *Proceedings of the Helminthological Society of Washington* 19, 44–51.

Anderson, R.V. & Townshend, J.L. (1980). Variation of the first head annule in Canadian populations of *Pratylenchus penetrans* (Nematoda, Pratylenchidae) from three host plants. *Canadian Journal of Zoology-Revue Canadiense de Zoologie* 58, 1336-1340.

Andres, M.F., Pinochet, J., Hernandez-Dorrego, A. & Delibes, A. (2000). Detection and analysis of inter- and intraspecific diversity of *Pratylenchus* spp. using isozyme markers. *Plant Pathology* 49, 640-649.

Andres, M.F., Romero, M.D., Montes, M.J. & Delibes, A. (2001). Genetic relationships and isozyme variability in the *Heterodera avenae* complex determined by isoelectrofocusing. *Plant Pathology* 50, 270-279.

Bajaj, H.K. & Bhatti, D.S. (1984). New and known species of *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae) from Haryana, India, with remarks on interspecific variations. *Journal of Nematology* 16, 360-367.

Barriga, R. & Cubillos, G. (1981). Principales nemátodos fitoparásitos asociados con cultivos de plátano (*Musa* AAB y *Musa* ABB) en cuatro regiones de Colombia. *Fitopatología Colombiana*, 9, 80-92.

Bergé. J.B. & Dalmasso, A. (1975). Caractéristiques biochimiques de quelques populations de *Meloidogyne hapla* et *Meloidogyne* sp. *Cahiers Orstom, série Biologie* 10, 263- 271.

Berkeley, M.J. (1855). “Vibrio forming cysts on the roots of cucumber”. *Gardeners Chronicle*. April 7th, 220.

References

Bert, W., Leliaert, F., Vierstraete, A., Vanfketeren, J.R. & Borgonie, G. (2008). Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nematoda: Rhabditida). *Molecular Phylogenetics and Evololution.* In press.

Blok, V.C., Phillips, M.S. & Fargette, M. (1997a). Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root-knot nematodes. *Journal of Nematology* 29, 16-22.

Blok, V.C., Phillips, M.S., McNicol, J.W. & Fargette, M. (1997b). Genetic variation in tropical *Meloidogyne* spp. as shown by RAPDs. *Fundamental and Applied Nematology* 20, 127-133.

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

Brzeski. M.W. (1998). *Nematodes of Tylenchina in Poland and temperate Europe*. Warszawa, Muzeum i Instytut Zoologii Polska Akademia Nauk, 397p.

Café-Filho, A.C. & Huang, C.S. (1989). Description of *Pratylenchus pseudofallax* n. sp. with a key to species of the genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae). *Revue de Nématologie* 12, 7-15.

Carneiro, R.M.D.G., Almeida, M.R.A. & Carneiro, R.G. (1996a). Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp. *Fundamental and Applied Nematology* 19, 555-560.

Carneiro, R. M. D. G., Carneiro, R. G., Abrantes, I. M. O., Santos, M. S. N. A. & Almeida, M. R. A. (1996b). *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. *Journal of Nematology* 28, 177-189.

Carneiro, R. M. D. G., Castagnone-Sereno, P. & Dickson, D. W. (1998). Variability among four populations of *Meloidogyne javanica* from Brazil. *Fundamental and Applied Nematology* 21, 319-326.

Carneiro, R.M.D.G., Almeida, M.R.A. & Queneherve, P. (2000). Enzyme phenotypes of *Meloidogyne* spp. populations. *Nematology* 2, 645-654.

Carneiro, R.M.D.G., Randig, O., Almeida, M.R.A. & Gomes, A.C. M. M. (2004a). Additional information on *Meloidogyne ethiopica* Whitehead, 1968 (Tylenchida: Meloidogynidae), a root-knot nematode parasitising kiwi fruit and grape-vine from Brazil and Chile. *Nematology* 6, 109-123.

Carneiro, R.M.D.G., Tigano, M.S., Randig, O., Almeida, M.R.A. & Sarah, J.L. (2004b). Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. *Nematology* 6, 287-298.

Carneiro, R.M.D.G., Almeida, M.R.A., Gomes, A.C.M.M. & Hernandez, A. (2005). *Meloidogyne izalcoensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising coffee in El Salvador. *Nematology* 7, 819-832.

Carneiro, R.M.D.G., Almeida, M.R.A., Cofcewicz, E.T., Magunacelaya, J.C. & Aballay, E. (2007). *Meloidogyne ethiopica*, a major root-knot nematode parasitising *Vitis vinifera* and other crops in Chile. *Nematology* 9, 635-641.

Carneiro, R.M.D.G., Mendes, M. D., Almeida, M.R.A., dos Santos, M.F.A., Gomes, A.C.M.M. & Karssen, G. (2008). Additional information on *Meloidogyne inornata* Lordello, 1956 (Tylenchida: Meloidogynidae) and its characterisation as a valid species. *Nematology* 10, 123-136.

Carta, L.K., Skantar, A.M. & Handoo, Z.A. (2001). Molecular, morphological and thermal characters of 19 *Pratylenchus* spp. and relatives using the D3 segment of the nuclear LSU rRNA gene. *Nematropica* 31, 193-207.

Castagnone-Sereno, P. (2006). Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity* 96, 282-289.

Castagnone-Sereno, P., Vanlerberghe-Masutti, F. & Leroy, F. (1994). Genetic polymorphism between and within *Meloidogyne* species detected with RAPD marker. *Genome* 37, 904-909.

Castagnone-Sereno, P., Leroy, F., Bongiovanni, M., Zijlstra, C. & Abad, P. (1999). Specific diagnosis of two root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, with satellite DNA probes. *Phytopathology* 89, 380-384.

Castillo, P., Vovlas, N., Subbotin, S. & Troccoli, A. (2003). A new root-knot nematode, *Meloidogyne baetica* n. sp. (Nematoda: Heteroderidae), parasitizing wild olive in Southern Spain. *Phytopathology* 93, 1093-1102.

Castro, J.M.C.E. (2001). Caracterização de *Meloidogyne* spp. de regiões brasileiras produtoras de soja. Tese de Doutorado, Universidade Federal de Viçosa. 82p.

Castro, J.M.C.E., Lima, R.D & Carneiro, R.M.D.G. (2003). Variabilidade isoenzimática de populações de *Meloidogyne* spp. provenientes de regiões brasileiras produtoras de soja. *Nematologia Brasileira* 27, 1-12.

Charchar, J.M. & Eisenback, J.D. (2002). *Meloidogyne brasilensis* n. sp. (Nematoda : Meloidogynidae), a root-knot nematode parasitising tomato cv. Rossol in Brazil. *Nematology* 4, 629-643.

Charchar, J.M., Eisenback, J.D., Charchar, M.J. & Boiteux, M.E. (2008). *Meloidogyne phaseoli* n. sp. (Nematoda : Meloidogynidae), a root-knot nematode parasitising bean in Brazil. *Nematology* 10, 515-538.

Charchar, J.M., Eisenback, J.D., Charchar, M.J. & Boiteux, M.E. (2008). *Meloidogyne pisi* n. sp. (Nematoda : Meloidogynidae), a root-knot nematode parasitising pea in Brazil. *Nematology* 10, 479-493.

Chen, D.Y., Ni, H.F., Chen, R.S., Yen, J.H. & Tsay, T.T. (2006). Identification of spiral nematode (Nematoda: Rotylenchinae) collected from Taiwan and Kinmen. *Zhi Wu Bing Li Xue Hui Kan* 15, 153-169.

Chitwood, B.G. 1949. Root-knot nematodes, part I. A revision of the genus *Meloidogyne* Goeldi, 1887. *Proceedings of the Helminthological Society of Washington* 16, 90-104.

References

Cobb, N.A. (1893). Nematodes, mostly Australian and Fijian. *Macleay Memorial Volume of the Linnean Society of New South Wales.* pp: 252-308.

Cobb, N.A. (1917). Notes on nemas. *Contribution to a Science of Nematology* 5, 117-128.

Cofcewicz, E.T., Carneiro, R.M.D.G., Castagnone-Sereno, P. & Queneherve, P. (2004). Enzyme phenotypes and genetic diversity of root-knot nematodes parasitising *Musa* in Brazil. *Nematology* 6, 85-95.

Cofcewicz, E.T., Carneiro, R.M.D.G., Randig, O., Chabrier, C. & Queneherve, P. (2005). Diversity of *Meloidogyne* spp. on *Musa* in Martinique, Guadaloupe, and French Guiana. *Journal of Nematology* 37, 313-322.

Colbran, R.C. (1970). Studies of plant and soil nematodes. 15. Eleven new species of *Radopholus* Thorne and a new species of *Radopholoides* de Guiran (Nematoda: Tylenchoidea) from Australia. *Queensland Journal of Agricultural and Animal Sciences* 27, 437-460.

Coomans, A. (1988). How to distinguish between plesiomorphic and apomorphic characters in nematodes. *Journal of Nematology* 20, 632.

Coomans, A. (2000). Nematode systematics: past, present and future. *Nematology* 2, 3-7.

Coomans, A. (2002). Present status and future of nematode systematics. *Nematology* 4, 573-582.

Corrales, S. (1996). Reconocimiento e identificación de nematodos asociados con el cultivo del lulo *Solanum quitoense* Lam. en el departamento de Valle del Cauca. Tesis. Ingeniera Agrónoma. Universidad Nacional de Colombia, Palmira. 30 p.

Corbett, D.C.M. (1969). *Pratylenchus pinguicaudatus* n. sp. (Pratylenchinae, Nematoda) with a key to genus *Pratylenchus*. *Nematologica* 15, 550-556.

Corbett, D.C.M. (1983). Three new species of *Pratylenchus* with a redescription of *Pratylenchus andinus* Lordello, Zamith and Boock, 1961 (Nematoda, Pratylenchidae). *Nematologica* 29, 390-403.

Corbett, D.C.M. & Clark, S.A. (1982). SEM studies of *Pratylenchus* spp. *Nematologica* 28, 142.

Courtright, E.M., Wall, D.H., Virginia, R.A., Frisse, R.A., Vida, J.T. & Thomas, W.K. (2000). Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode *Scottnema lindsayae*. *Journal of Nematology* 32, 143-53.

Dalmasso, A. & Berge, J.B. (1978). Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp. - application to taxonomy of *Meloidogyne*. *Journal of Nematology* 10, 323-332.

Das, V.M. & Sultana, S. (1979). Five new species of the genus *Pratylenchus* from vegetable crops of Hyderabad (Andhra Pradesh). *Indian Journal of Nematology* 11, 172-175.

De Giorgi, C., Veronico, P., De Luca, F., Natilla, A., Lanave, C. & Pesole,G. 2002. Structural and evolutionary analysis of the ribosomal genes of the parasitic nematode *Meloidogyne artiellia* suggests its ancient origin. *Mol. Biochem. Parasitol*. 124:91-94.

De Grisse, A.T. (1969). Redescription ou modification de quelques techniques utilisees dans l'etude des nematodes phytoparasitaires. Mededelingen Rijksfakulteit Landbouwwetenschappen Gent 34, 351-369.

De Guiran, G. & Siddiqi, M.R. (1967). Characters differentiating genera *Zygotylenchus* Siddiqi 1963(syn. *Mesotylus* de Guiran 1964) and *Pratylenchoides* Winslow 1958 (Nematoda: Pratylenchinae). *Nematologica* 13, 235-240.

de la Peña, E., Moens, M., van Aelst, A. & Karssen, G. (2006). Description of *Pratylenchus dunensis* sp. n. (Nematoda: Pratylenchidae), a root-lesion nematode associated with the dune grass *Ammophila arenaria* (L.) Link. *Nematology* 8, 79-88.

de la Peña, E., Karssen, G. & Moens, M. (2007). Distribution and diversity of root-lesion nematodes (*Pratylenchus* spp.) associated with *Ammophila arenaria* in coastal dunes of Western Europe. *Nematology* 9, 881-901.

De Ley, I.T., De Ley, P., Vierstraete, A., Karssen, G., Moens, M. & Vanfleteren, J. (2002). Phylogenetic analyses of *Meloidogyne* small subunit rDNA. *Journal of Nematology* 34, 319-327.

De Ley, P. & Bert, W. (2002). Video capture and editing as a tool for the storage, distribution, and illustration of morphological characters of nematodes. *Journal of Nematology* 34, 296-302.

De Ley, P., Felix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W. & Thomas, W.K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1, 591-612.

De Ley, P., De Ley, I.T., Morris, K., Abebe, E., Mundo-Ocampo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Burr, A.H.J., Baldwin, J.G. & Thomas, W.K. (2005). An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences* 360, 1945-1958.

De Luca, F., Fanelli, E., Di Vito, M., Reyes, A. & De Giorgi, C. (2004). Comparison of the sequences of the D3 expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of *Pratylenchus* from the Mediterranean region. *European Journal of Plant Pathology* 110, 949-957.

De Waele, D. & Elsen, A. (2007). Challenges in tropical plant nematology. *Annual Review of Phytopathology* 45, 457-485.

References

Díaz, C. & Múnera, G.E. (2002). Contribución al conocimiento de los sistemas productivos de tomate de árbol, lulo, granadilla y mora en Colombia. En : *IV Seminario Nacional de Frutales de Clima Frío Moderado*. Memorias. Medellín, Noviembre 20-22. pp: 22-28.

Díaz, C. & Múnera, G.E. (2003). Características de los sistemas de producción de tomate de árbol, granadilla y lulo en colombia y los problemas sanitarios prevalentes. En: *Seminario Internacional Entrega de resultados Proyecto Manejo Integrado de Plagas para el mejoramiento de la producción sostenible de frutas en la Zona Andina*. Medellín, Abril 22-25. pp: 43-49.

Doucet, M., Lax, P., Di Rienzo, J.A., Pinochet, J. & Baujard, P. (2001). Temperature-induced morphometrical variability in an isolate of *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida). *Nematology* 3, 1-8.

Dropkin, V.H. (1953). Studies on the variability of anal plate patterns in pure lines of *Meloidogyne* spp. the root-knot nematode. *Proceedings of the Helminthological Society of Washington* 20, 32-39.

DuCharme, E.P. & Birchfield, W. (1956). Physiologic races of the burrowing nematode. *Phytopathology* 46, 615-616.

Duncan, L.W., Inserra, R.N., Thomas, W.K., Dunn, D., Mustika, I., Frisse, L.M., Mendes, M.L., Morris, K. & Kaplan, D.T. (1999). Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. *Nematropica* 29, 61-80.

Ebsary, B.A. 1991. Catalog of the Order Tylenchidae (Nematoda). Publication 1869/B. Research Program Branch, Agriculture Canada, Ottawa. 196 p.

Egunjobi, O.A. (1968). Three new species of nematodes from New Zealand. *New Zealand Journal of Science* 11, 488-491.

Eisenback, J.D. (1985a). Detailed morphology and anatomy of second-stage juveniles, males and females of the genus *Meloidogyne* (root-knot nematodes). In: Sasser, J.N. & Carter, C.C. (Eds). *An advanced treatise on* Meloidogyne. *Volume I. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics, pp. 47-77.

Eisenback, J.D. (1985b). Diagnostic characters useful in the identification of four most common species of root-knot nematodes (*Meloidogyne* spp.). In: Sasser, J.N. & Carter, C.C. (Eds). *An advanced treatise on* Meloidogyne. *Volume I. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics, pp. 95-112.

Eisenback, J.D. (1993). Morphological comparisons of females, males, and second stage juveniles of cytological race A and race B of *Meloidogyne hapla* Chitwood, 1949. *Fundamental and Applied Nematology* 16, 259-271.

Eisenback, J.D. & Hirschmann, H. (1980). Morphological comparison of *Meloidogyne* males by scanning electron-microscopy. *Journal of Nematology* 12, 23-32.

Eisenback, J.D. & Hirschmann, H. (1981). Identification of *Meloidogyne* species on the basis of head shape and stylet morphology of the male. *Journal of Nematology* 13, 513-521.

Eisenback, J.D., Hirschmann, H. & Triantaphyllou, A. C. (1980). Morphological comparison of *Meloidogyne* female head structures, perineal patterns, and stylets. *Journal of Nematology* 12, 300-313.

Eisenback, J.D., Bernard, E. C. & Schmitt, D.P. (1994). Description of the kona coffee root-knot nematode, *Meloidogyne konaensis* n. sp. *Journal of Nematology* 26, 363-374.

Elbadri, G.A.A. (2000). Diversity of *Radopholus similis* (Cobb, 1893) (Nematoda: Tylenchida). Doctoral Thesis, Gent University, Gent, Belgium, 146p.

Elbadri, G.A.A., Geraert, E. & Moens, M. (1999a). Morphological differences among *Radopholus similis* (Cobb, 1893) Thorne, 1949 populations. *Russian Journal of Nematology* 7, 139-153.

Elbadri, G. A. A., Geraert, E. & Moens, M. (1999b). Morphological differences among *Radopholus* populations (Nematoda: Tylenchida) from bananas in Africa. *Journal of Nematode Morphology and Systematics* 2, 1-16.

Elbadri, G.A.A., De Ley, P., Waeyenberge, L., Vierstraete, A., Moens, M. & Vanfleteren, J. (2002). Intraspecific variation in *Radopholus similis* isolates assessed with restriction fragment length polymorphism and DNA sequencing of the internal transcribed spacer region of the ribosomal RNA cistron. *International Journal for Parasitology* 32, 199-205.

Esbenshade, P.R. & Triantaphyllou, A.C. (1985a). Characterization of *Meloidogyne* esterases by their reaction to specific inhibitors and substrates. *Journal of Nematology* 17, 495.

Esbenshade, P.R. & Triantaphyllou, A.C. (1985b). Identification of major *Meloidogyne* species employing enzyme phenotypes as differentiating characters. In: Sasser, J.N. & Carter, C.C. (Eds). *An advanced treatise on* Meloidogyne. *Volume I. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics. pp:135-140.

Esbenshade, P.R. & Triantaphyllou, A.C. (1985c). Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17, 6-20.

Esbenshade, P.R. & Triantaphyllou, A.C. (1986). Biochemical relationships of *Meloidogyne* species. *Journal of Nematology* 18, 607.

Esbenshade, P.R. & Triantaphyllou, A.C. (1987a). Enzymatic relationships and evolution in the genus *Meloidogyne* (Nematoda, Tylenchida). *Journal of Nematology* 19, 8-18.

Esbenshade, P.R. & Triantaphyllou, A.C. (1987b). Genetic-analysis of esterase polymorphisms in *Heterodera glycines*. *Phytopathology* 77, 1696.

Esbenshade, P.R. & Triantaphyllou, A.C. (1988). Genetic-analysis of esterase polymorphism in the soybean cyst nematode, *Heterodera glycines*. *Journal of Nematology* 20, 486-492.

Esbenshade, P.R. & Triantaphyllou, A.C. (1990). Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* 22, 10-15.

Esser, R.P. & Perry, V.G. (1974). Diagnostic compendium of genus *Meloidogyne* (Nematoda: Heteroderidae). *Journal of Nematology* 6, 139.

Esser, R.P., Perry, V.G. & Taylor, A.L. (1976). Diagnostic Compendium of Genus *Meloidogyne* (Nematoda: Heteroderidae). *Proceedings of the Helminthological Society of Washington* 43, 138-150.

Fallas, G.A., Hahn, M.L., Fargette, M., Burrows, P.R. & Sarah, J.L. (1996). Molecular and biochemical diversity among isolates of *Radopholus* spp. from different areas of the world. *Journal of Nematology* 28, 422-430.

Fargette, M. (1987a). Use of the esterase phenotypes in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype. *Revue de Nématologie* 10, 39-43.

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

Ferris, V.R. (1961). A new species of *Pratylenchus* (Nemata: Tylenchida) from roots of soybeans. *Proceedings of the Helminthological Society of Washington* 28, 109-119.

Filipjev, I.N. (1936). On the classification of the Tylenchinae. *Proceedings of the Helminthological Society of Washington* 3, 80-82.

Filipjev, I.N. & Schuurmans Stekhoven, J.H. Jr. (1941). *A Manual of Agricultural Helminthology*. Leiden, The Netherlands, E.J. Brill, 878p.

Flores-Romero, P. & Navas, A. (2005). Enhancing taxonomic resolution: distribution dependent genetic diversity in populations of *Meloidogyne*. *Nematology* 7, 517-530.

Fox, P.C. & Atkinson, H.J. (1988). Non-specific esterase variation in field populations of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica* 34, 156–63.

Franklin, M.T. (1965). *Meloidogyne*-root-knot eelworms. In: Southey, J.F. (Ed.). *Plant nematology*. London, UK, HMSO. pp:59-88.

Franklin, M.T. (1972). The present position on the systematic of *Meloidogyne*. OEPP/EPPO *bull*. 6, 5-15.

Franklin, M.T. (1979). Taxonomy of the genus *Meloidogyne*. In: Root-knot nematodes (*Meloidogyne* species). Systematics, biology and control. pp. 37-54. Ed. F. Lamberti & C.E. Taylor. London, Academic Press.

Frederick, J.J. & Tarjan, A.C. (1989). A compendium of the genus *Pratylenchus* Filipjev,

1936 (Nemata: Pratylenchidae). *Revue de Nématologie* 12, 243-256.

Gaviria, B. & Navarro, R. (2003). Identificación de especies de *Meloidogyne* asociadas con los cultivos de tomate de árbol, lulo y granadilla en Colombia. En: *Seminario Internacional Entrega de resultados Proyecto Manejo Integrado de Plagas para el mejoramiento de la producción sostenible de frutas en la Zona Andina*. Medellin, Abril 22-25. pp: 144-157.

Georgi, L.L. & Abbott, A.G. 1998. Variation in ribosomal genes in *Meloidogyne arenaria*. *Fundamental Applied Nematology* 21: 685-694.

Geraert, E. (2006). Functional and detailed morphology of the Tylenchida (Nematoda). *Nematology monographs and perspectives* 4. 215p.

Göldi, E. A. (1892). Relatoria sôbre a molestia do cafeeiro na província de Rio de Janeiro. *Archos Mus. nac., Rio de Janeiro* 8, 7-112.

Golden, A.M., Lopez, Ch.R. & Vilchez, R.H. (1992). Description of *Pratylenchus gutierrezi* n. sp. (Nematoda: Pratylenchidae) from coffee in Costa Rica. *Journal of Nematology* 24, 298-304.

Gomez, J. (1980). Determinación de la infestación de fitonemátodos en plantaciones bananeras de Urabá, Colombia. *Fitopatología Colombiana.* 9, 19-32.

Gowen S.R., Quénéhervé, P. & Fogain, R. 2005. Nematodes parasites of bananas and plantains. Pp. 611-643. *In*: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, 2nd edition (M. Luc, R. A. Sikora & J. Bridge, eds.). CAB International, Wallingford, UK.

Grant, V. (1957). The plant species in theory and practice. In: Mayr E (ed). *The Species Problem American*. Association for the advancement of Science, Washington, DC, USA. pp: 67-77.

Guirao, P., Moya, A. & Cenis, J.L. (1995). Optimal use of random amplified polymorphic DNA in estimating the genetic-relationship of four major *Meloidogyn*e spp. *Phytopathology* 85, 547-551.

Guzmán, O. & Castaño, J. (2004). Reconocimiento de nemátodos fitopatógenos en plátanos dominico hartón (*Musa* AAB Simmonds), Africa, FHIA-20 y FHIA-21 en la granja Montelindo, Municipio de Palestina (Caldas), Colombia. *Rev. Acad. Colomb. Cienc.* 27: 295-301.

Hahn, M.L., Sarah, J.L., Boisseau, M., Vines, N.J., Wright, D.J. & Burrows, P.R. (1996). Reproductive fitness and pathogenicity of selected *Radopholus* populations on two banana cultivars. *Plant Pathology* 45, 223-231.

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

Hajieghrari, B.; Torabi-Giglou, M. & Waeyenberge, L. 2007. Comparative D2/D3 LSU-rDNA sequence study of some Iranian *Pratylenchus loosi* populations. *African Journal of Biotechnology* 6, 2458-2466.

Handoo, Z.A. & Golden, A. M. (1989). A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). *Journal of Nematology* 21, 202-218.

Handoo, Z.A., Carta, L.K. & Skantar, A.M. (2001). Morphological and molecular characterisation of *Pratylenchus arlingtoni* n. sp., *P. convallariae* and *P. fallax* (Nematoda: Pratylenchidae). *Nematology* 3, 607-618.

Handoo, Z.A., Nyczepir, A.P., Esmenjaud, D., van der Beek, J.G., Castagnone-Sereno, P., Carta, L.K., Skantar, A. & Higgins, J.A. (2004). Morphological, molecular, and differential-host characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing peach in Florida. *Journal of Nematology* 36, 20-35.

Handoo, Z.A., Skantar, A.M., Carta, L.K. & Erbe, E.F. (2005). Morphological and molecular characterization of a new root-knot nematode, *Meloidogyne thailandica* n. sp. (Nematoda: Meloidogynidae), parasitizing ginger (*Zingiber* sp.). *Journal of Nematology* 37, 343-353.

Harris, T.S., Sandall, L.J. & Powers, T.O. (1990). Identification of single *Meloidogyne* juveniles by polymerase chain reaction amplification of mitochondrial-DNA. *Journal of Nematology* 22, 518-524.

Hartman, K.M. & Sasser, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (Eds). *An advanced treatise on* Meloidogyne*. Vol. II. Methodology*. Raleigh, NC, USA, North Carolina State University, Graphics, pp. 69-77.

Hernandez, A., Fargette, M. & Sarah, J.L. (2004a). Characterisation of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. *Nematology* 6, 193-204.

Hernandez, A., Fargette, M. & Sarah, J.L. (2004b). Pathogenicity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) isolates from Central America and Brazil on four genotypes of *Coffea arabica*. *Nematology* 6, 205-213.

Hewlett, T.E. & Tarjan, A.C. (1983). Synopsis of the genus *Meloidogyne* Goeldi, 1887. *Nematropica* 13, 79-102.

Hirschmann, H. (1985). The genus *Meloidogyne* and morphological characters differentiating its species. In: Sasser, J.N. & Carter, C.C. (Eds). *An advanced treatise on* Meloidogyne. *Volume I. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics. pp: 79-93.

Holdeman, Q.L. (1986). The burrowing nematode *Radopholus similis* sensu lato. Nematology Publication, Sacramento, CA, USA, California Department of Food and Agriculture, Division of Plant Industry, 52p.

Holdridge, L.R. (1964). *Life zone ecology*. Tropical Science Center. San Jose, Costa Rica. 206p.

Huettel, R.N. & Yaegashi, T. (1988). Morphological differences between *Radopholus citrophilus* and *Radopholus similis*. *Journal of Nematology* 20, 150-157.

Huettel, R.N., Dickson, D.W. & Kaplan, D.T. (1983). Biochemical identification of the two races of *Radopholus similis* by polyacrylamide gel electrophoresis. *Journal of Nematology* 15, 345-348.

Huettel, R.N., Dickson, D.W. & Kaplan, D.T. (1984). *Radopholus citrophilus* sp. n. (Nematoda), A sibling species of *Radopholus similis*. *Proceedings of the Helminthological Society of Washington* 51, 32-35.

Hugall, A., Stanton, J. & Moritz, C. (1997). Evolution of the AT-rich mitochondrial DNA of the root knot nematode, *Meloidogyne hapla*. *Molecular Biology and Evolution* 14, 40-48.

Hugall, A., Stanton, J. & Moritz, C. (1999). Reticulate evolution and the origins of ribosomal internal transcribed spacer diversity in apomictic *Meloidogyne*. *Molecular Biology and Evolution* 16, 157-164.

Hunt, D.J., Bridge, J. & Machon, J.E. (1989). On *Achlysiella*, a new genus of obese Pratylenchidae (Nematoda: Tylenchoidea). *Revue de Nématologie* 12, 401-407.

Hussey, R.S. (1985). Biochemistry as a tool in identification and its probable usefulness in understanding the nature of parasitism. In: Sasser, J.N. & Carter, C.C. (Eds). *An advanced treatise on* Meloidogyne. *Volume I. Biology and control*. Raleigh, NC, USA, North Carolina State University Graphics. pp: 127-133.

Hyman, B.C. & Powers, T.O. (1991). Integration of molecular data with systematic of plant parasite nematodes. *Annual Review of Phytopathology* 29, 89-107.

Ibrahim, S.K. & Perry, R.N. (1993). Use of esterase patterns of females and galled roots for the identification of species of *Meloidogyne*. *Fundamental and Applied Nematology* 16, 187-190.

Ibrahim, S.K. & Rowe, J.A. (1995). Use of isoelectric focusing and polyacrylamide-gel electrophoresis of nonspecific esterase phenotypes for the identification of cyst nematodes *Heterodera* species. *Fundamental and Applied Nematology* 18, 189-196.

Ibrahim, S.K., Perry, R.N. & Hooper, D.J. (1994). Use of esterase and protein-patterns to differentiate two new species of *Aphelenchoides* on rice from other species of *Aphelenchoides* and from *Ditylenchus angustus* and *D. myceliophagus*. *Nematologica* 40, 267-275.

Ibrahim, S.K., Perry, R.N. & Webb, R.M. (1995). Use of isoenzyme and protein phenotypes to discriminate between six *Pratylenchus* species from Great Britain. *Annals of Applied Biology* 126, 317-327.

Inserra, R. N., Duncan, L.W., Vovlas, N. & Loof, P.A.A. (1996). *Pratylenchus loosi* from pasture grasses in central Florida. *Nematologica* 42, 159-172.

Inserra, R.N., Duncan, L.W., Dunn, D., Kaplan, D.T. & Porazinska, D. (1998). *Pratylenchus pseudocoffeae* from Florida and its relationship with *P. gutierrezi* and *P. coffeae*. *Nematologica* 44, 683-712.

Inserra, R.N., Duncan, L.W., Troccoli, A., Dunn, D., dos Santos, J.M., Kaplan, D. & Vovlas, N. (2001). *Pratylenchus jaehni* sp. n. from citrus in Brazil and its relationship with *P. coffea*e and *P. loosi* (Nematoda: Pratylenchidae). *Nematology* 3, 653-665.

Inserra, R.N., Troccoli, A., Gozel, U., Bernard, E.C., Dunn, D. & Duncan, L.W. (2007). *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*. *Nematology* 9, 25-42.

Janati, A., Berge, J.B., Triantaphyllou, A.C. & Dalmasso, A. (1982). Nouvelles données sur l'utilisation des isoestérases pour l'identification des *Meloidogyne*. *Revue de Nématologie* 5, 147-154.

Jepson, S.B. (1983). Identification of *Meloidogyne*: a general assessment and a comparison of males morphology using light microscopy, with a key to 24 species. *Revue de Nématologie* 6, 291-306.

Jepson, S. B. (1987). *Identification of root-knot nematodes* (Meloidogyne *species*). Wallingford, UK, CAB International, 265p.

Kaplan, D.T. & Opperman, C.H. (1997). Genome similarity implies that citrus-parasitic burrowing nematodes do not represent a unique species. *Journal of Nematology* 29, 430-440.

Kaplan, D.T. & Opperman, C.H. (2000). Reproductive strategies and karyotype of the burrowing nematode, *Radopholus similis*. *Journal of Nematology* 32, 126-133.

Kaplan, D.T., Vanderspool, M.C., Garrett, C., Chang, S. & Opperman, C.H. (1996). Molecular polymorphisms associated with host range in the highly conserved genomes of burrowing nematodes, *Radopholus* spp. *Molecular Plant-Microbe Interactions* 9, 32-38.

Kaplan, D.T., Vanderspool, M.C. & Opperman, C.H. (1997). Sequence tag site and host range assays demonstrate that *Radopholus similis* and *R. citrophilus* are not reproductively isolated. *Journal of Nematology* 29, 421-429.

Kaplan, D.T., Thomas, W.K., Frisse, L.M., Sarah, J. L., Stanton, J.M., Speijer, P.R., Marin, D.H. & Opperman, C.H. (2000). Phylogenetic analysis of geographically diverse *Radopholus similis* via rDNA sequence reveals a monomorphic motif. *Journal of Nematology* 32, 134-142.

Karssen, G. 2002. The plant-parasitic nematode genus *Meloidogyne* Göldi, 1892 (Tylenchida) in Europe. Leiden: Brill. Leiden, The Netherlands, E. J. Brill, 157p.

Karssen, G. & Moens, M. (2006). Root knot nematodes. In: Perry, R.N. & Moens, M. *Plant Nematology*. CABI, Wallingford. pp: 59-90.

Karssen, G. & Van Aelst, A.C. (2001). Root-knot nematode perineal pattern development: a reconsideration. *Nematology* 3, 95-111.

Karssen, G., Vanhoenselaar, T., Verkerkbakker, B. & Janssen, R. (1995). Species identification of cyst and root-knot nematodes from potato by electrophoresis of individual females. *Electrophoresis* 16, 105-109.

Karssen, G., Waeyenberge, L. & Moens, M. (2000). *Pratylenchus brzeskii* sp. nov. (Nematoda: Pratylenchidae), a root-lesion nematode from European coastal dunes. *Annales Zoologici* 50, 255-261.

Karssen, G., Bolk, R.J., Van Aelst, A.C., Van Den Beld, I., Kox, L.F.F., Korthals, G., Molendijk, L., Zijlstra, C., Van Hoof, R. & Cook, R. (2004). Description of *Meloidogyne minor* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode associated with yellow patch disease in golf courses. *Nematology* 6, 59-72.

Kumar, A.C. (1980). Studies of nematodes in coffee soils of South India. 3. A report on *Radopholus similis* and description of *Radopholus colbrani* n. sp. new taxa. *Journal of Coffee Research* 10, 43-46.

Li, J., Faghihi, J., Ferris, J.M. & Ferris, V.R. (1996). The use of RAPD amplified DNA as markers for virulence characteristic in soybean cyst nematodes. *Fundamental and Applied Nematology* 19, 143-150

Liao, J.L., Yang, W.C., Feng, Z.X. & Karssen, G. (2005). Description of *Meloidogyne panyuensis* sp. n. (Nematoda : Meloidogynidae), parasitic on peanut (*Arachis hypogea* L.) in China. *Russian Journal of Nematology* 13, 107-114.

Loof, P.A.A. (1960). Taxonomic studies on the genus *Pratylenchus* (Nematoda). *LEB Fonds Publication* 39. 66p.

Loof, P.A.A. (1978). The genus *Pratylenchus* Filipjev, 1936 (Nematoda: Pratylenchidae): a review of its anatomy, morphology, distribution, systematic and identification. *Vaxtskyddsrapporter*, 5. 50p.

Loof, P.A.A. (1991). The family Pratylenchidae Thorne, 1949. In: Nickle, W.R. (Ed.). *Manual of agricultural nematology*. New York, NY, USA, Marcel Dekker, pp: 363-421.

Lozada, S., Varon, F. & Gómez, E. (2002). Nematodos asociados al tomate de árbol S*olanum betaceum* en el Valle del Cauca. En: *IV Seminario Nacional de Frutales de Clima Frío Moderado*. Memorias. Medellín, Noviembre 20-22. pp: 126 -134.

Luc, M. (1987). A reappraisal of Tylenchina (Nemata). 7. The family Pratylenchidae Thorne, 1949. *Revue de Nématologie* 10, 203-218.

Luc, M. & Goodey, J.B. (1964). *Hirschmanniella* n. g. differentiated from *Radopholus* Thorne, 1949 (Nematoda: Tylenchoidea). *Nematologica* 7, 197-202.

Machon, J.E. & Bridge, J. (1996). *Radopholus citri* n. sp. (Tylenchida: Pratylenchidae) and its pathogenicity on citrus. *Fundamental and Applied Nematology* 19, 127-133.

Marin, D.H., Sutton, T.B. & Barker, K.R. 1998. Dissemination of bananas in Latin America and the Caribbean and its relationship to the occurrence of *Radopholus similis. Plant Disease* 82:964-974.

References

McKenry, M.V. & Roberts. P.A. 1985. *Phytonematology study guide*. Publication No. 4045. Cooperative Extension, University of California. Division of Agricultural and Natural Resources. Oakland.

Meldal, B.H.M., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., Austen, M.C., Blaxter, M.L., Rogers, A.D. & Lambshead, P.J.D. (2007). An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Molecular Phylogenetics and Evolution* 42, 622-636.

Minagawa, N. (1984). Description of *Radopholoides triversus* n. sp. from Japan with a reference to the classification of the family Pratylenchidae (Nematoda, Tylenchida). *Applied Entomology and Zoology* 19, 21-26.

Mizukubo, T. (1989). Description of *Radopholus samoi* n. sp. from Kyushu, Japan. *Japanese Journal of Nematology* 18, 39- 44.

Mizukubo, T. (1992). *Pratylenchus pseudocoffeae* n.sp. (Nematoda, Pratylenchidae) from Composite plants in Japan. *Applied Entomology and Zoology* 27, 437-444.

Molinari, S., Lamberti, F., Crozzoli, R., Sharma, S.B. & Sanchez-Portales, L. 2005. Isozyme patterns of exotic *Meloidogyne* spp. populations. *Nematologia Mediterranea* 33, 61–65.

Múnera, G.E. (2000). Nematofauna asociada al cultivo de la granadilla (*Passiflora ligularis* Juss.). En: *XXI Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI*. Memorias. Palmira. Agosto 30 - Septiembre 1. p: 17.

Múnera, G.E. (2001). Interacción M*eloidogyne incognita - Fusarium solani* en granadilla (P*assiflora ligularis* Juss.). En: *33ª Reunión Anual de la Organización de Nematólogos de los Trópicos Americanos - ONTA y IV Seminario Científico Internacional de Sanidad Vegetal*. Resumenes. Varadero, Cuba. Junio 11 - 15. p: 31.

Múnera, G.E. (2002a). Nematodos asociados con el cultivo de lulo. En: *IV Seminario Nacional de Frutales de Clima Frío Moderado*. Memorias. Medellín, Noviembre 20-22. pp: 135-141.

Múnera, G.E. (2002b). Nematofauna asociada con el cultivo de tomate de árbol en cuatro regiones de Colombia. En: *XXIII Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI*. Resumenes. Bogotá. Julio3-6. pp: 80-81.

Múnera, G. E. (2002c). Nematofauna asociada con la Colección Colombiana de tomate de árbol. En: *XXIII Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI*. Resumenes. Bogotá. Julio3-6. p: 82.

Múnera, G.E. (2003a). Problemas de nematodos y su manejo en *Solanum quitoense, Cyphomandra betacea* y *Passiflora ligularis* en Colombia. En: *XXXV Annual Meeting ONTA*, Memorias. Guayaquil. Julio 21-25. p: 25.

Múnera, G.E. (2003b). Evaluación de pérdidas de campo ocasionadas por nematodos en lulo en el Oriente Antioqueño. En: *XXIV Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI*. Resumenes. Armenia. Julio 25 -27. p. 33.

Múnera, G. E. (2003c). Respuesta de tres clones de lulo La Selva a la inoculacion con tres especies de *Meloidogyne*. En: *XXIV Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines - ASCOLFI*. Resumenes. Armenia. Julio 25 -2. p: 110.

Múnera, G.E. (2004a). Algunas investigaciones realizadas por Corpoica sobre nematodos asociados con lulo, tomate de arbol y granadilla. Quinquenio 1998-2003. En: *V Seminario Nacional e internacional de frutales, tecnología para la transformación de frutas*. Memorias. Manizales, Caldas, 11-13. pp: 197-207 .

Múnera, G.E. (2004b). Nematodos asociados con musaceas en los municipios de Guapi y Timbiquí, Departamento del Cauca. En: Saldarriaga, A. Validación y ajuste de tecnología apropiada para el cultivo de musaceas en el Pacífico Caucano (Informe Final Proyecto PRONATTA-CORPOICA). Medellín. 25p.

Múnera, G.E. & Navarro, R. (2000a). Nematodos asociados a frutales de clima frío moderado. En: *III Seminario Nacional de Frutales de Clima Frío Moderado*. Memorias. Manizales, Noviembre 15 - 17. pp: 136-140.

Múnera, G.E. & Navarro, R. (2000b). Nematofauna asociada a la mora de castilla (*Rubus glaucus* Benth.) en el Oriente Antioqueño. En: *XXI Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI.* Memorias. Palmira. Agosto 30 - Septiembre 1. p: 20.

Múnera, G.E. & Navarro, R. (2001). Nematodos asociados con la colección Colombiana de Passifloras. En: *XXII Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI*. Resumenes. Medellín. Julio 11 -13. p: 36.

Múnera, G.E. & Navarro, R. (2002). Phytoparasitic nematodes associated with the rhizosphere of lulo, mora, tree tomato and granadilla plants in Colombia. In: *Fourth International Congress of Nematology*, Tenerife, Spain, June 8 -13. p: 420.

Múnera, G.E. & Saldarriaga, A. (2003). Evaluación de la interacción de tres especies de *Meloidogyne* con *Fusarium solani* en granadilla (P*assiflora ligularis* Juss.) bajo condiciones de invernadero. En: *Seminario Internacional Entrega de resultados Proyecto Manejo Integrado de Plagas para el mejoramiento de la producción sostenible de frutas en la Zona Andina*. Medellín, Abril 22-25. p:114.

Muñoz L., Múnera, G.E. & Díaz, C. (2003). Literatura gris y proyectos recientes sobre problemas fitosanitarios, con énfasis en nematodos, en los cultivos de lulo (S*olanum quitoense* Lam), tomate de árbol (C*yphomandra betacea* (Cav.) Sendt.) y Passifloras en Colombia. En: *Seminario Internacional Entrega de resultados Proyecto Manejo Integrado de Plagas para el mejoramiento de la producción sostenible de frutas en la Zona Andina*. Medellín, Abril 22-25. pp: 20-31.

Nadler, S.A. (2002). Species delimitation and nematode biodiversity: phylogenies rule. *Nematology* 4, 615-625.

Nadler, S.A., De Ley, P., Mundo-Ocampo, M., Smythe, A.B., Stock, P., Bumbarger, D., Adams, B. J., De Ley, I. T., Holovachov, O. & Baldwin,J.G. 2006. Phylogeny of

Cephalobina (Nematoda): Molecular evidence for recurrent evolution of probolae and incongruence with traditional classifications. *Mol. Phylogenet. Evol*. 40 :696-711.

Navarro, R. (1986). Algunos problemas patológicos de lulo (*Solanum quitoense*) en Antioquia. *Fitopatología Colombiana. Vol. I. Resúmenes y Conferencias Congresos Ascolfi IV-VIII* p: 111.

Navarro, R. (1990). Enfermedades del tomate de árbol y su control. *Seminario Problemas fitosanitarios y perspectivas económicas de algunos frutales de interés actual.* Universidad Nacional de Colombia-Palmira. Marzo 22. p: 8.

Navarro, R. & Múnera, G.E. (2000). Parasitismo de nematodos asociados a la rizosfera de mora de castilla (*Rubus glaucus* Benth.) en el Oriente Antioqueño. En : *XXI Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines- ASCOLFI.* Memorias. Palmira. Agosto 30 - Septiembre 1. p: 20.

Netscher, C. (1978). Morphological and physiological variability of species of *Meloidogyne* in West Africa and implications for their control. Thesis, Landbouwhogeschool,Wageningen, The Netherlands, 47p.

Nguyen, C.N., Subbotin, S.A., Madani, M., Trinh, P.Q. & Moens, M. (2003). *Radopholus duriophilus* sp. n. (Nematoda: Pratylenchidae) from western highland of Vietnam. *Nematology* 5, 549-558.

Noss, R.F. (1990). Indicators for monitoring biodiversity - A hierarchical approach. *Conservation Biology* 4, 355-364.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004). Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53, 47-67.

O'Bannon, J.H., Radewald, J.D., Tomerlin, A.T. & Inserra, R.N. (1976). Comparative influence of *Radopholus similis* and *Pratylenchus coffeae* on *Citrus*. *Journal of Nematology* 8, 58-63.

Orton Williams, K.J. (1972). *Meloidogyne javanica*. *C.I.H. Descriptions of plant-parasitic nematodes.* Set 1, No 3, St. Albans, UK, Commonwealth Agricultural Bureau.

Orton Williams, K.J. (1973). *Meloidogyne incognita*. *C.I.H. Descriptions of plant-parasitic nematodes.* Set 2, No 18, St. Albans, UK, Commonwealth Agricultural Bureau.

Orton Williams, K. J. (1974). *Meloidogyne hapla*. *C.I.H. Descriptions of plant-parasitic nematodes.* Set 3, No 31, St. Albans, UK, Commonwealth Agricultural Bureau.

Orton Williams, K.J. (1975). *Meloidogyne arenaria C.I.H. Descriptions of plant-parasitic nematodes.* Set 5, No 62, St. Albans, UK, Commonwealth Agricultural Bureau.

Orton Williams, K.J. & Siddiqi, M. R. (1973). *Radopholus similis*. *C.I.H. Descriptions of plant-parasitic nematodes.* Set 2, No 27, St. Albans, UK, Commonwealth Agricultural Bureau.

Orui, Y. (1996). Discrimination of the main *Pratylenchus* species (Nematoda: Pratylenchidae) in Japan by PCR-RFLP analysis. *Applied Entomology and Zoology* 31, 505-514.

Pastrick, K.H., Rumpenhorst, H.J. & Burgermeister, W. (1995). Random amplified polymorphic DNA analysis of *Globodera pallida* populations selected for virulence. *Fundamental and Applied Nematology* 18, 109-114.

Peterson D.J. & Vrain, T.C. (1996) Rapid identification of *Meloidogyne chitwoodi*, *M. hapla* and *M. fallax* using PCR primers to amplify their ribosomal intergenic spacer. *Fundamental and Applied Nematology* 19, 601–605.

Peterson, D.J., Zijlstra, C., Wishart J, Blok V & Vrain, T. (1997) Specific probes efficiently distinguish root-knot nematode species using signature in the ribosomal intergenic spacer. *Fundamental and Applied Nematology* 20, 619–626.

Pourjam, E., Kheiri, A. & Geraert, E. (1997a) .The genus *Pratylenchus* Filipjev, 1936 (Tylenchida: Pratylenchidae) from North of Iran. Med. Fac. Landbouww. Univ. Gent 62/3a: 741-756.

Pourjame, E., Waeyenberge, L., Moens, M. & Geraert, E. (1997b). Morphological, morphometrical and molecular studies of *Pratylenchus coffeae* and *P. loosi* (Nematoda: Pratylenchidae). *Med. Fac. Landbouww*. Univ;Gent 64/3a.

Powers, T.O. & Harris, T.S. (1993). A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* 25, 1-6.
Powers, T.O. & Sandall, L. J. (1988). Estimation of genetic divergence in *Meloidogyne* mitochondrial-DNA. *Journal of Nematology* 20, 505-511.

Powers, T.O., Platzer, E. G. & Hyman, B.C. (1986a). *Meloidogyne* Mitochondrial-DNA. *Journal of Nematology* 18, 627.

Powers, T.O., Platzer, E.G. & Hyman, B.C. (1986b). Nematode Mitochondrial-DNA for Molecular Diagnostics. *Phytopathology* 76, 1139.

Powers, T.O., Todd, T. C., Burnell, A. M., Murray, P.C.B., Fleming, C.C., Szalanski, A.L., Adams, B.A. & Harris, T.S. (1997). The rDNA internal transcribed spacer region as a taxonomic marker for nematodes. *Journal of Nematology* 29, 441-450.

Powers, T.O., Mullin, P.G., Harris, T.S., Sutton, L.A. & Higgins, R.S. (2005). Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey. *Journal of Nematology* 37, 226-235.

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

Randig, O., Bongiovanni, M., Carneiro, R.M.D.G. & Castagnone-Sereno, P. (2002). Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. *Genome* 45, 862-870.

Rashid, A. & Khan, A.M. (1976). Morphometric studies on *Pratylenchus coffeae* with description of *Pratylenchus typicus* Rashid, 1974. *Indian Journal of Nematology* 6, 63-72.

Roman, J. & Hirschmann, H. (1969). Morphology and morphometrics of six species of *Pratylenchus. Journal of Nematology* 1, 363-386.

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.

Ryss, A.Y. (1988). Parasitic root nematodes of the family Pratylenchidae (Tylenchida) of the world fauna. *Zool.Inst.Acad.Sci.USSR*, No. 154. 367 p.

Ryss, A.Y. (2002). Genus *Pratylenchus* Filipjev (Nematoda: Pratylenchidae): multientry and monoentry keys and diagnostic relationships. *Zoosystematica Rossica* 10, 11-25.

Ryss, A.Y. (2003). Taxonomy, evolution and phylogeny of the genus *Radopholus* (didelphic species) according to morphological data, with a key to species (Nematoda: Tylenchida). *Zoosystematica Rossica* 11, 243-256.

Ryss, A.Y. & Wouts, W.M. (1997). The genus *Radopholus* (Nematoda: Pratylenchidae) from native vegetation in New Zealand, with descriptions of two new species. *International Journal of Nematology* 7, 1-17.

Saeki, Y., Kawano, E., Yamashita, C., Akao, S. & Nagatomo, Y. (2003). Detection of plant parasitic nematodes, *Meloidogyne incognita* and *Pratylenchus coffeae* by multiplex PCR using specific primers. *Soil Science and Plant Nutrition* 49, 291-295.

Sarah, J.L., Sabatini, C. & Boisseau, M. (1993). Differences in pathogenicity to banana (*Musa* sp cv. *poyo*) among isolates of *Radopholus similis* from different production areas of the world. *Nematropica* 23, 75-79.

Sasser, J.N. (1954). Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). Agricultural Experimental Station, University of Maryland, MD, USA, Bulletin No A-77 (Technical), 30p.

Sasser, J.N. & Freckman, D.W. (1987). A World perspective on Nematology: the role of the society. In: Veech, J.A. & Dickson, D.W. (Eds.). *Vistas on Nematology*. Hyattsville, Society of Nematologists. pp: 7-14.

Sauer, M.R. (1958). *Hoplolaimus gracilidens, Radopholus inequalis* and *Radopholus neosimilis*, three new tylenchs native of Victoria, Australia. *Nematologica* 3, 97-107.

Seinhorst, J.W. (1959). A rapid method for transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* 4, 67-69.

Seinhorst. J.W. (1966). Killing nematodes for taxonomic study with hot Fa 4-1. *Nematologica* 12, 178-188.

Shahina, F. & Maqbool, M.A. (1996). Two new species of the genus *Radopholus* Thorne, 1949 (Nematoda: Pratylenchidae) from Pakistan. *Fundamental and Applied Nematology* 19, 289-292.

Sher, S.A. (1968a). Revision of the genus *Hirschmanniella* Luc & Goodey, 1962 (Nematoda: Tylenchoidea). *Nematologica* 14, 243-257.

Sher, S.A. (1968b). Revision of Genus *Radopholus* Thorne 1949 (Nematoda - Tylenchoidea). *Proceedings of the Helminthological Society of Washington* 35, 219-237.

Sher, S.A. & Allen, M.W. (1953). Revision of the genus *Pratylenchus* (Nematoda: Tylenchidae). *University of California Publications in Zoology* 57, 441-470.

Siddiqi, M.R. (1963). Occurrence of *Radopholus similis* (Cobb, 1893) Thorne, 1949, in Abbottabad, West Pakistan. *Current Science* 32, 462-463.

Siddiqi, M.R. (1964). *Radopholus williansi* n. sp. (Nematoda: Pratylenchidae) a parasite of sugar cane roots at L'Etoile, Mauritius. Farnham Royal, Slough, UK, Commonwealth Agricultural Bureaux, 645 pp. *Indian Journal of Entomology* 26, 207-208.

Siddiqi, M.R. (1986). *Tylenchida parasites of plants and insects*. Farnham Royal, Slough, UK, Commonwealth Agricultural Bureaux, 645p.

Siddiqi, M.R. (1991). *Tanzanius coffeae* gen. n., sp. n. and *Zygradus rector* gen. n., sp. n. (Nematoda: Tylenchina) from Africa. *Afro-Asian Journal of Nematology* 1, 101-107.

Siddiqi, M.R. (1994). Nematodes of tropical rainforests. 3. Three new genera and five new species of tylenchs. *Afro-Asian Journal of Nematology* 4, 22-31.

Siddiqi, M.R. (2000). *Tylenchida parasites of plants and insects*. 2nd edition. Wallingford, UK, CABI Publishing, 833p.

Siddiqi, M.R. & Hahn, M.L. (1995). *Radopholus bridgei* sp. n. (Tylenchida: Pratylenchidae) from Indonesia and its differentiation by morphological and molecular characters. *Afro-Asian Journal of Nematology* 5, 38-43. *Afro-Asian Journal of Nematology* 5, 38-43.

Siddiqi, M.R. & Lenne, J.M. (1990). *Xiphinema llanosum* and *Trophuru vultus*, two new plant nematode species from pasture soils in Colombia. *Journal of Nematology* 22, 262-267.

Stanton, J., Mundo-Ocampo, M., Baldwin, J.G. & Kaplan, D.T. (2001). *Radopholus musicola* n. sp., a new pathogenic species from Australia (Nematoda: Pratylenchidae). *Nematology* 3, 689-698.

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. & Baldwin, J.G. (2006). Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455-474.

Subbotin, S.A., Sturhan, D., Vovlas, N., Castillo, P., Tambe, J.T., Moens, M. & Baldwin, J.G. (2007a). Application of the secondary structure model of rRNA for phylogeny: D2-

D3 expansion segments of the LSU gene of plant-parasitic nematodes from the family Hoplolaimidae Filipjev, 1934. *Molecular Phylogenetics and Evolution* 43, 881-890.

Subbotin, S.A., Ragsdale, E.J., Mullens, T., Roberts, P. & Baldwin, J.G. (2007b). Molecular diagnostics and phylogenetic relationships of some species of root-lesion nematodes of the genus *Pratylenchus*. *Journal of Nematology* 39, 80.

Swofford, D.L., Waddell, P.J., Huelsenbeck, J.P., Foster, P.G., Lewis, P.O. & Rogers, J.S. (2001). Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. *Systematic Biology* 50, 525-539.

Tandingan De Ley, I., De Ley, P., Vierstraete, A., Karssen, G., Moens, M. & Vanfleteren, J. (2002). Phylogenetic analysis of *Meloidogyne* SSU rDNA. *Journal of Nematology* 34, 319-327.

Tanha Maafi, Z., Subbotin, S.A. & Moens, M. (2003). Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences *Nematology* 5, 99-111.

Tarjan, A.C. & Frederick, J.J. (1978). Intraspecific morphological variation among populations of *Pratylenchus brachyurus* and *Pratylenchus coffeae*. *Journal of Nematology* 10, 152-160.

Tarte, R. & Mai, W.F. (1976a). Morphological Variation in *Pratylenchus penetrans*. *Journal of Nematology* 8, 185-195.

Tarte, R. & Mai, W. F. (1976b). Sex expression and tail morphology of female progenies of smooth-tail and crenate tail females of *Pratylenchus penetrans*. *Journal of Nematology* 8, 196-200.

Taylor, D.P. & Jenkins, W.R. (1957). Variation within the nematode genus *Pratylenchus*, with the descriptions of *P. hexincisus*, n. sp. and *P. subpenetrans*, n. sp. *Nematologica* 2, 150-174.

Taylor, A. L. & Sasser, J. N.. 1978. *Biology, identification, and control of root-knot nematodes* (Meloidogyne *species*). Raleigh, NC: North Carolina State University Graphics. 111p.

Tenente, G.C.M.V., De Ley, P., De Ley, I.T., Karssen, G. & Vanfleteren, J.R. (2004). Sequence analysis of the D2/D3 region of the large subunit RDNA from different *Meloidogyne* isolates. *Nematropica* 34, 1-12.

Thiéry, M., Fouville, D. & Mugniéry, D. (1997). Intra- and interspecific variability in *Globodera*, parasites of *Solanaceous* plants, revealed by random amplified polymorphic DNA (RAPD) and correlation with biological features. *Fundamental and Applied Nematology* 20, 495-504.

Thompson J.D., Higgins D.G. & Gibson T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22, 4673-4680.

Thorne, G. (1949). On the classification of the Tylenchida, new order (Nematoda, Phasmidia). *Proceedings of the Helminthological Society of Washington* 16, 37-73.

Tigano, M.S., Carneiro, R.M.D.G., Jeyaprakash, A., Dickson, D.W. & Adams, B.J. (2005). Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. *Nematology* 7, 851-862.

Tomaszewski, E.K., Khalil, M.A.M., Eldeeb, A.A., Powers, T.O. & Starr, J.L. (1994). *Meloidogyne javanica* Parasitic on Peanut. *Journal of Nematology* 26, 436-441.

Triantaphyllou, A.C. (1963). Polyploidy and parthenogenesis in root-knot nematode *Meloidogyne arenaria*. *Journal of Morphology* 113, 489-495.

Triantaphyllou, A.C. (1966). Polyploidy and reproductive patterns in root-knot nematode *Meloidogyne hapla*. *Journal of Morphology* 118, 403-413.

Triantaphyllou, A.C. (1985). Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: Barker, K.R., Carter, C.C., Sasser, J.N. & Raleigh, N.C. (Eds.) *An Advanced treatise on Meloidogyne*. North Carolina State University, Department of Plant Pathology. pp: 113-126.

Triantaphyllou, A.C. & Hirschmann, H. (1980). Cytogenetics and morphology in relation to evolution and speciation of plant parasitic nematodes. *Annual Review of Phytopathology* 18, 333-359.

Triantaphyllou, A.C. & Sasser, J.N. (1960). Variation in perineal patterns and host specificity of *Meloidogyne incognita*. *Phytopathology* 50, 724-735.

Trinh, P. Q., Nguyen, C.N., Waeyenberge, L., Subbotin, S.A., Karssen, G. & Moens, M. (2004). *Radopholus arabocoffeae* sp. n. (Nematoda: Pratylenchidae), a nematode pathogenic to *Coffea arabica* in Vietnam, and additional data on *R. duriophilus*. *Nematology* 6, 681-693.

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

Valette, C., Mounport, D., Nicole, M., Sarah, J.L. & Baujard, P. (1998). Scanning electron microscope study of two African populations of *Radopholus similis* (Nematoda: Pratylenchidae) and proposal of *R. citrophilus* as a junior synonym of *R. similis*. *Fundamental and Applied Nematology* 21, 139-146.

Van den Bergh, E., (1971). *The root lesion nematodes of South Africa* (genus *Pratylenchus*, familia Hoplolaimidae). Department of Agricultural Technical Services. Technical Communication No.99. Plant Protection Research Institute, Pretoria. 13p.

Van der Beek, J.G. & Karssen, G. (1997). Interspecific hybridization of meiotic parthenogenetic *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology* 87, 1061-1066.

References

Van der Beek, J. G., Folkertsma, R., Poleij, L. M., vanKoert, P. H. G. & Bakker, J. (1997). Molecular evidence that *Meloidogyne hapla, M. chitwoodi*, and *M. fallax* are distinct biological entities. *Fundamental and Applied Nematology* 20, 513-520.

Van der Beek, J.G., Loss, J.A. & Pijnacker, L.P. (1998). Cytology of parthenogenesis of five *Meloidogyne* species. *Fundamental and Applied Nematology* 21, 393-399.

Vanegas, D. (2006). Identificación morfológica y molecular de las species de *Meloidogyne* en la zona tabacalera de Los Santos-Santander y evaluación de su parasitismo en tres variedades comerciales de tabaco Burley y Negro (*Nicotiana tabacum* L.). Tesis. Maestría en Biotecnología. Universidad Nacional de Colombia. Medellín. 100p.

Van Oppen, M.J., McDonald, B.J., Willis, B. & Miller, D.J. (2001) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence?. *Molecular Biology and Evolution* 18, 1315–1329.

Van Weerdt, I.G. (1958). Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne, 1949. *Nematologica* 3: 184-196.

Vargas, Z., Varon, F. & Gómez, E. (2002). Nematodos asociados a la granadilla (*Passiflora ligularis* Juss.). En : *IV Seminario Nacional de Frutales de Clima Frío Moderado*. Memorias. Medellín, Noviembre 20-22. pp: 142-152.

Varón, F. (1994). Manejo integrado de nematodos en frutales. XV *Congreso de la Asociación Colombiana de Fitopatología y Ciencias Afines - ASCOLFI*. Memorias. Agosto 31 a Septiembre 2. Bogotá. pp: 93-98.

Volcy, Ch. (1998). *Nematodos. Tomo 2. Diversidad y parasitismo en plantas*. Universidad Nacional de Colombia. Sede Medellín. 182p.

Volcy, Ch. (2003). El nematodo *Hoplolaimus seinhorsti* Luc: Registro nuevo para la región bananera de Urabá. *Revista Augura*. pp: 50-55.

Vovlas, N., Mifsud, D., Landa, B.B. & Castillo, P. Host-parasite relationships of *Meloidogyne javanica* on potato. 2005. *Plant Pathology* 54:657-664.

Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J. & Vrain, T. C. (2000). Molecular characterisation of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. *Nematology* 2, 135-142.

Whitehead, A.G. (1968). Taxonomy of *Meloidogyne* (Nematoda: Heteroderidae) with description of four new species. *Transactions of the Zoological Society of London* 31, 263- 401.

Williamson, V.M., Caswell-Chen, E.P., Westerdahl, B. B.,Wu, F.F. & Caryl, G. (1997). A PCR assay to identify and distinguish single juveniles of *Meloidogyne hapla* and *M. chitwoodi*. *Journal of Nematology* 29, 9-15.

Wishart, J., Phillips, M.S. & Blok, V.C. (2002). Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. *Phytopathology* 92, 884-892.

Xihua, Li. (2006). Morphological Characterization of various *Pratylenchus* populations (Nematoda: Tylenchina). Master Thesis, Gent University, Gent, Belgium, 87p.

Zijlstra, C. (2000). Identification of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology* 106, 283-290.

Zijlstra, C., Lever, A.E.M., Uenk, B.J. & van Silfhout, C.H. (1995). Differences Between ITS Regions of Isolates of Root-Knot Nematodes *Meloidogyne hapla* and *M. chitwoodi*. *Phytopathology* 85, 1231-1237.

Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2, 847-853.