Molecular characterisation of *Pratylenchus coffeae* populations from Vietnam

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Summary. Random amplified polymorphic DNA (RAPD) analysis of the complete genome and sequencing of the D2-D3 expansion segments of the 28S rDNA gene were carried out to compare the intraspecific genomic variability of ten *Pratylenchus coffeae* populations collected from different agricultural crops in different agro-ecological regions in Vietnam. In addition, the RAPD bands were compared with these of *P. speijeri*, a species recently described from Ghana, while the D2-D3 sequences were compared with these of *P. coffeae* and closely related *Pratylenchus* species available in the GenBank database. As determined by RAPD bands analysis of the complete genome, genomic similarity did not correspond either with geographic or original host plant origin of the Vietnamese *P. coffeae* populations. As determined by sequence analysis of the D2-D3 28S rDNA expansion fragments, all ten *P. coffeae* populations from Vietnam examined were closely related to each other and with the *P. coffeae* populations of which the D2-D3 28S rDNA expansion fragments sequences were available from GenBank. Both the RAPD bands analysis of the complete genome and the sequence analysis of the D2-D3 28S rDNA expansion fragments indicate genetic divergence among the ten *P. coffeae* populations from Vietnam examined on the one hand and *P. speijeri* from Ghana on the other hand, confirming the validity of the latter species.

Key words: D2-D3 expansion segments, genomic variability, molecular taxonomy, *Pratylenchus speijeri*, RAPD analysis, root-lesion nematodes, sequencing, 28S rDNA gene.

It is very difficult to separate the species of the genus *Pratylenchus* based on their morphology and morphometrics alone (Castillo & Vovlas, 2007). During the past two decades, several molecular tool boxes have been developed for the identification and comparison of nematode genera, species and populations, including the polymerase chain reaction (PCR) to amplify, detect and compare DNA fragments, PCR-restriction fragment length polymorphism (PCR-RFLP) to reveal variation in sequences in PCR products obtained by restriction endonuclease digestion, random amplified polymorphic DNA (RAPD) and sequencing (Subbotin & Moens, 2013). For the taxonomy of *Pratylenchus* species, DNA-based techniques have been used since the early 1990s (Castillo & Vovlas, 2007).

Random amplified polymorphic DNA PCR analysis has shown its potential usefulness in distinguishing seven populations of *Pratylenchus vulnus* from one population of *P. neglectus*, the patterns of the amplified DNA bands of the *P. neglectus* population being clearly different from those of the *P. vulnus* populations (Pinochet *et al.*,

1994). However, a high level of polymorphism was observed among the *P. vulnus* populations. To estimate the phylogenetic relationship among 15 populations of *P. coffeae*, *P. pseudocoffeae*, *P. guttierezi* and *P. loosi*, Duncan *et al.* (1999) constructed a dendrogram based on 227 RAPD bands obtained with 18 primers. The authors observed a low level of similarity among the populations suggesting that the *Pratylenchus* genome is highly variable and, therefore, that phylogenetic relationships among *Pratylenchus* species and populations cannot be based solely on RAPD data.

For the taxonomy of plant-parasitic nematode taxa, including *Pratylenchus* (see for instance Handoo *et al.*, 2001; Subbotin *et al.*, 2006, 2008; de la Peña *et al.*, 2007; De Luca *et al*., 2012), the sequence of the D2-D3 expansion segments of the 28S rDNA gene has been examined especially and has been shown to be an important molecular tool. Duncan *et al.* (1999) sequenced the DNA of this segment of 19 populations of *P. coffeae* and closely related species (*P. pseudocoffeae*, *P. guttierezi* and *P. loosi*) collected worldwide. The DNA sequences indicated a close relationship among the *P. coffeae* populations isolated from coffee in Indonesia and from citrus, yam, banana and miscellaneous plants isolated from the USA, Costa Rica, Honduras, Brazil, Martinique, Ghana, Oman, Malaysia and China, although the sequence similarity was not absolute: the sequences of some populations varied by 1-5 nucleotides. The same study also showed that *P. coffeae* and *P. loosi* could be distinguished based on these DNA sequences. The morphology and morphometrics of these two nematode species are almost identical, the only difference being the shape of the spermatheca (Pourjame *et al.*, 1997). Recently, De Luca *et al*. (2012) sequenced the D2-D3 expansion segments of the 28S rRNA gene, the ITS rRNA gene and a portion of the *hsp*90 gene of a *Pratylenchus* population originally isolated from banana in Ghana and for a long time considered to be *P. coffeae*, and *P. coffeae* species complex populations from different sources, including the ten *P. zeae* populations from Vietnam included in our study. Based on the (phylo)genetic data, De Luca *et al*. (2012) described the *Pratylenchus* population from Ghana as a new species (*P. speijeri*), although an extensive comparison of the morphological characters and morphometrics of *P. speijeri* did not result in an unambiguous separation from *P. coffeae*, the closest related species. With the exception of a *Pratylenchus* population isolated from taro in Japan, the other *Pratylenchus* populations, including those from Vietnam, were identified as *P. coffeae* (De Luca *et al*., 2012).

The objectives of our study were to compare the intraspecific genomic variability of ten *P. coffeae* populations collected from different agricultural crops in different agro-ecological regions in Vietnam (Tuyet *et al*., 2012) based: a) on RAPD analysis of the complete genome; b) on sequences of the D2-D3 expansion segments of the 28S rDNA gene; and c) on a comparison of these sequences with the sequences of *P. coffeae* and closely related *Pratylenchus* species available in the GenBank database).

MATERIALS AND METHODS

Nematode populations. Ten *P. coffeae* populations collected from different agricultural crops in different agro-ecological regions in Vietnam and one *P. speijeri* population originally isolated from banana in Ghana (Table 1) were included in our study. The specimens studied were obtained from *in vitro* carrot disc cultures (Moody *et al*., 1973). Carrot disc cultures with vigorous developing nematode populations (many active nematodes in the Petri dish) were selected. The nematodes were collected in a test tube by rinsing the Petri dishes with sterile water. The nematodes were immediately used for DNA extraction.

Table 1. Origin and population codes of the ten *Pratylenchus coffeae* populations from Vietnam used in the present work.

No.	Host plant	Province	Agro-ecological region	Population code
1	Banana	Dien Bien	Northwest	NW
2	Coffee	Yen Bai	Northeast	NE1
3	Banana	Yen Bai	Northeast	NE2
4	Banana	Phu Tho	Northeast	NE3
5	Banana	Bac Kan	Northeast	NE4
6	Banana	Ha Tay	Red River Delta	RRD1
7	Ornamental tree	Hung Yen	Red River Delta	RRD2
8	Banana	Thanh Hoa	North Central Coast	NCC1
9	Coffee	Nghe An	North Central Coast	NCC2
10	Coffee	Dak Lak	Central Highlands	CH

Table 2. Oligonucleotides used for the RAPD-PCR study.

No.	Primer sequence (5' to 3')	Code
1	GGC ACT GAGG	OPG2
2	GAG CCC TCCA	OPG3
3	GTG CCT AACC	OPG6
4	AGG GCC GTCT	OPG10
5	CAG CTC ACGA	OPG11
6	TGC CCG TCGT	OPG12
7	CTC TCC GCCA	OPG13
8	AGC GTC CTCC	OPG16
9	GTC AGG GCAA	OPG19
10	CCG AAG CCCT	SC10-30

Random amplified polymorphism DNA (RAPD) study. Approximately 1,000 nematodes of each *P. coffeae* population and of the *P. speijeri* population obtained from the *in vitro* carrot disc cultures were transferred into 1.5 ml Eppendorf tubes and centrifuged at 13,000 rpm for 20 min (minutes). After centrifugation, the supernatants were discarded and the tubes stored at –80°C for at least 30 min. Then the pellets were crushed with a sterile plastic stick. Genomic DNA was isolated according to the manufacturer's instructions (Purification of Genomic DNA from Tissue Culture Cell Lysates Using a Microcentrifuge, Wizard SV Genomic DNA Purification System, Promega Benelux, Leiden, The Netherlands). The purified DNA was stored at –20°C.

The extracted DNA was used for PCR. Ten random selected primers, ten oligonucleotides long, were used (Table 2). RAPD-PCR was performed as follows: 5 µl extracted DNA suspension were added to the PCR reaction mixture containing 1 × PCR Rxn buffer, 2 mM $MgCl_2$, 250 µM of each dNTP, 0.005 µM of a primer, 2 U *Taq* DNA polymerase recombinant (Invitrogen, Merelbeke, Belgium) and double distilled water (ddH_2O) to a final volume of 35 µl. The PCR reaction mixture was placed in a thermocycler preheated at 95°C. The DNA amplification profile consisted of 7 min at 95°C, 44 cycles of 1 min at 92°C, 1 min at 36°C and 3 min at 72°C. A final step of 10 min at 72°C completed the DNA amplification. Following DNA amplification, 5 µl of each PCR product was loaded on a 1% agarose gel. A 100 bp and 1 Kb DNA mass ladder were included as size markers. After electrophoresis for 45 min at 100 V, 100 mA, 10 W, the DNA bands were stained with 0.003% ethidium bromide (0.02 µg ml^{-1}) for 10 min. The gel was viewed on a UV-transilluminator and photographed. Ten independent reactions were performed three times for each nematode population/primer combination.

The DNA bands which were retained after the process described above (repeated three times) were scored visually as present (1) or absent (0). The data were gathered in a matrix file and analysed using PAUP version 4.0b10 (Swofford, 1998) to construct a Neighbour-Joining (NJ) tree. The genetic distances among the *P. coffeae* populations and the *P. speijeri* population were calculated by means of the pair group method using arithmetic averages (UPGMA) according to Dice's coefficient. In addition, a bootstrap analysis generating 1,000 random data sets from the original data was carried out to check the support for the groupings within the NJ tree.

D2-D3 of 28S rRNA gene sequencing and phylogenetic analysis. DNA extraction was done as described by Waeyenberge *et al*. (2000). The crude DNA extract was stored at –20°C.

The extracted DNA was used for PCR. The forward primer D2A 5'-ACAAGTACCGTGAGGG AAAGTTG-3' and the reverse primer D3B 5'-TCG GAAGGAACCAGCTACTA-3' (De Ley *et al.*, 1999) were used for amplifying the D2-D3 expansion segment of the 28S rDNA gene. Five µl of the extracted DNA were added to the PCR reaction mixture containing 1 × DNA *Taq* polymerase, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 2 U *Taq* polymerase (Qiagen, West Sussex, UK), and ddH_2O to a final volume of 45 µl. The PCR reaction mixture was incubated in a thermocycler preheated for 5 min at 92°C. The PCR amplification temperature and time conditions were 40 cycles of 30 s at 94°C, 45 s at 55°C and 1 min at 72°C. A 7 min polymerisation period at 72°C followed the last cycle. Following DNA amplification, 5 µl of each PCR product were loaded on a 1% agarose gel. A 100 bp and 1 Kb DNA mass ladder were included as size markers. After electrophoresis for 45 min at 100 V, 100 mA, 10 W, the DNA bands were stained with 0.003% ethidium bromide (0.02 µg ml^{-1}) for 10 min. The gel was viewed on a UV-transilluminator and photographed.

The PCR reaction mixture was loaded on a 2% agarose gel in 1 × TAE buffer. Electrophoresis was done until the DNA band of interest was isolated from adjacent contaminating fragments, primer-dimers and left-overs. The bands were identified by staining the gel with 0.003% ethidium bromide (0.02 µg ml^{-1}) for 10 min. The DNA bands of interest were cut from the gel by using an ethanol-cleaned scalpel on a UV light box. The excised agarose gel slices were placed in 1.5 ml

microcentrifuge tubes. Gel mass was determined by first pre-weighting the tubes and then re-weighting the tubes with the excised gel slice. The instructions as described in the MSB Spin PCRapase Kit (Invitek, Berlin, Germany) were followed to purify the PCR products from the gel.

Five µl purified PCR product was loaded on a 1% agarose gel in 1 × TAE buffer. A low DNA mass ladder was also loaded on the gel to estimate the mass of the purified DNA. After electrophoresis for 45 min at 100 V, the gel was stained with 0.003% ethidium bromide (0.02 µg ml^{-1}) for 10 min. The DNA bands were visualised on a UV-transilluminator and photographed. The concentration of purified PCR products was estimated visually by comparing the brightness of their bands with the DNA bands of the low DNA mass ladder.

The purified PCR products were cloned using pGEM-T vectors and JM109 competent cells as described in the manufacturer's protocols (pGEM-T Vector System II, Promega Benelux, Leiden, The Netherlands). From each sample, ten individual white colonies were re-cultured in tubes containing liquid LB/ampicilin. These tubes were incubated at 37°C while shaken (150 rpm) overnight. PCR was done for each selected colony to control whether the correct product was ligated into the vector and transformation was successful. Two PCR vector-primers were used: M13 forward (5'-TGTAAAACGACGGCCAGT-3') and pGEM-T reverse (5'-CAGGAAACAGCTATGAC-3'). The PCR products were checked on an agarose gel after electrophoresis as described before. Only the colonies which produced a PCR band of approximately 1 Kb (PCR product of approximately 800 bp + part of the vector approximately 200 bp) were used for sequencing. The plasmid DNA was purified from the corresponding cultures following the instructions included in a Wizard Plus SV Minipreps kit (Promega Benelux, Leiden, The Netherlands).The microcentrifuge tube containing the eluted DNA was stored at –20°C.

Sequencing was done according to the manufacturer's instructions (ABI Prism® BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit, AB Applied Biosystems, Lennik, Belgium 2002). The sequences were created using an ABI Prism 310 Genetic Analyser. ABI Prism DNA Sequencing Analysis Software version 3.7 was used to analyse the results (Applied Biosystems, Foster City, CA, USA). Due to cycle sequencing and electrophoresis limitations, only about 700 bp could be sequenced. Therefore, from all the samples, both DNA-strands were sequenced by using one of the vector-primers to be able to construct the complete sequence of the cloned PCR products which are longer than 1 Kb. Software-package Chromas version 1.45 (Technelysium, Helensvale, QLD, Australia) was used to visualise the sequences on the computer. Both sequences from the same sample (forward sequence and inverted complementary of the reverse sequence) were exported together in one text file in order to find the overlapping sequence. After removing this overlapping part in one of the sequences and the sequence part belonging to the plasmid in both sequences, one complete sequence was generated for each population and saved as a text file. ClustalX 2.0 (Thomas *et al.*, 1997) was used to make an alignment of all complete sequences together with ten *P. coffeae* sequences from GenBank (NCBI, Table 3). The D2-D3 28S rDNA expansion segment sequences were obtained with BLAST (basic local alignment search tool) from GenBank (http://www.ncbi.nlm.nih.gov).

Table 3. Origin, GenBank accession numbers, codes and number of base pairs of *Pratylenchus* species accessions available in GenBank (deposited by Duncan *et al.*, 1999) used for comparison with the *Pratylenchus coffeae* populations from Vietnam.

Number	*Pratylenchus* species	Origin	Accession number	Code	Number of base pairs (D2-D3 sequence size)
1	*P. coffeae*	Florida, USA	AF170428	C4	745
2	*P. coffeae*	Dhofar, Oman	AF170429	C6	735
3	*P. coffeae*	Martinique	AF170430	Y1	730
4	*P. coffeae*	Pernambuco, Brazil	AF170431	Y2	741
5	*P. coffeae*	Puerto Rico, USA	AF170432	Y3	726
6	*P. speijeri*	Ghana	AF170433	B1	754
7	*P. coffeae*	Honduras	AF170434	B2	731
8	*P. coffeae*	Sao Paulo, Brazil	AF170435	M1	731
9	*P. coffeae*	Florida, USA	AF170436	M3	750
10	*P. coffeae*	Kaliwining, Indonesia	AF170443	K6	735
11	*P. jaehni*	Sao Paulo, Brazil	AF170426	C1	750
12	*P. jaehni*	Sao Paulo, Brazil	AF170427	C2	756
13	*P. loosi*	USA	AF170437	N1	738

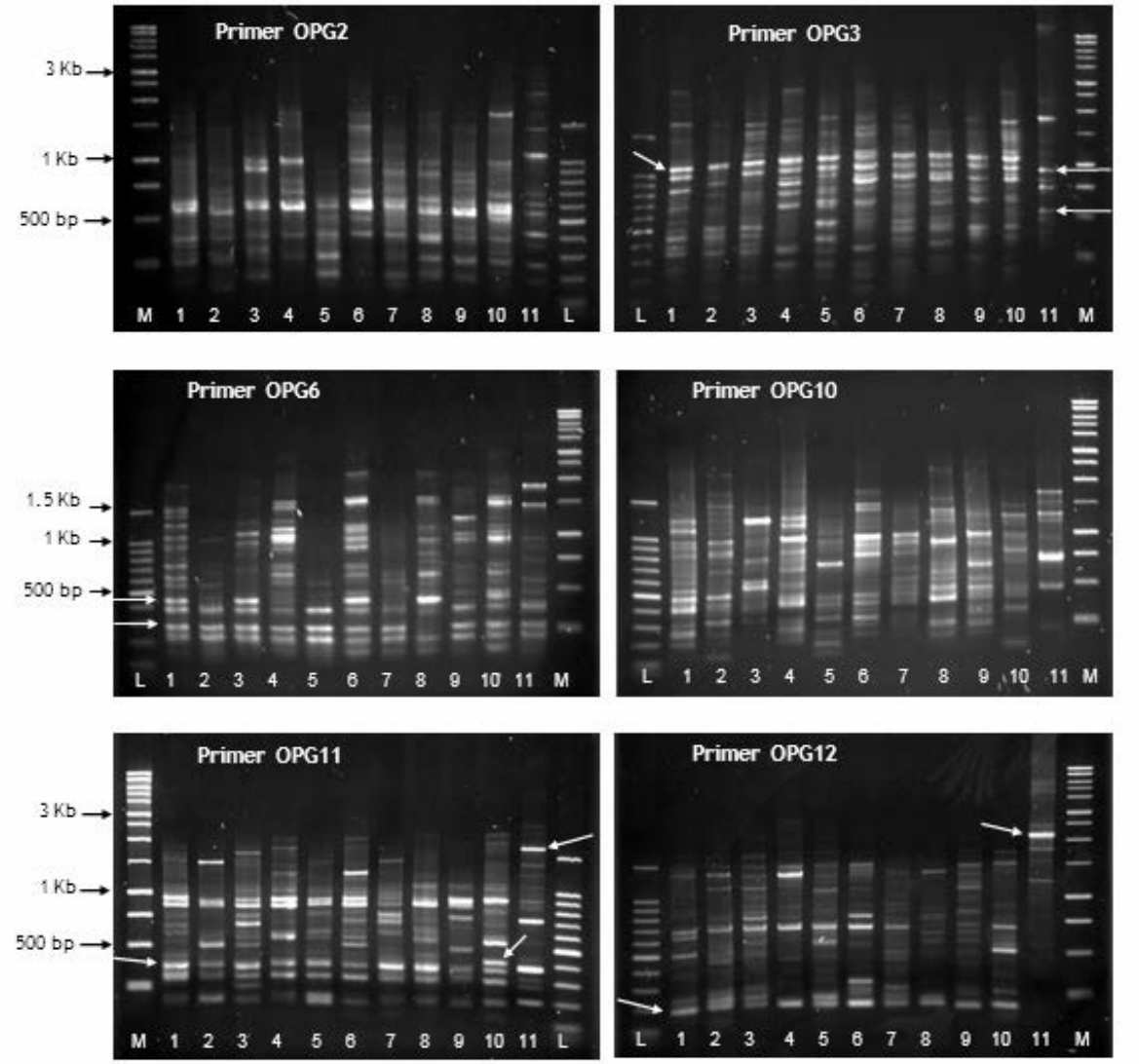

Fig. 1. RAPD bands generated by ten 10-oligonucleotide-long primers for ten *Pratylenchus coffeae* populations from Vietnam and a *Pratylenchus speijeri* population from Ghana. L: 100 bp DNA ladder; M: 1 Kb DNA ladder; 1: Northwest; 2: Northeast 1; 3: Northeast 2; 4: Northeast 3; 5: Northeast 4; 6: Red River Delta 1; 7: Red River Delta 2; 8: North Central Coast 1; 9: North Central Coast 2; 10: Central Highlands population; 11: Ghana population. Arrows show the fragments of approximately 600, 950 and 1,200 bp generated by primer OPG3; 280 and 450 bp generated by primer OPG6; 350, 410 and 1,700 bp generated by primer OPG11; 190 and 2,200 bp generated by primer OPG12.

RESULTS

Random amplified polymorphism DNA (RAPD) study. The ten 10-oligonucleotide-long primers used generated a total of 152 polymorphic DNA bands, ranging from 0.2 to 2.2 Kb in size (Fig. 1). All primers produced complex patterns but at least one band of the same size was observed in all *P. coffeae* populations. Primer OPG3 amplified a fragment of approximately 1,200 bp for all *P. coffeae* populations from Vietnam but not for the *P. speijeri* population. By contrast, a fragment of approximately 600 bp was amplified only in the *P. speijeri* population. A similar observation was made for the primers OPG11, OPG12 and SC10.30. These primers amplified fragments of approximately 350, 190 and 750 bp, respectively, for all *P. coffeae* populations from Vietnam examined but not for the *P. speijeri* population. By contrast, fragments of approximately 1700, 2200 and 1400 bp were amplified in the *P. speijeri* population but not in the *P. coffeae* populations from Vietnam.

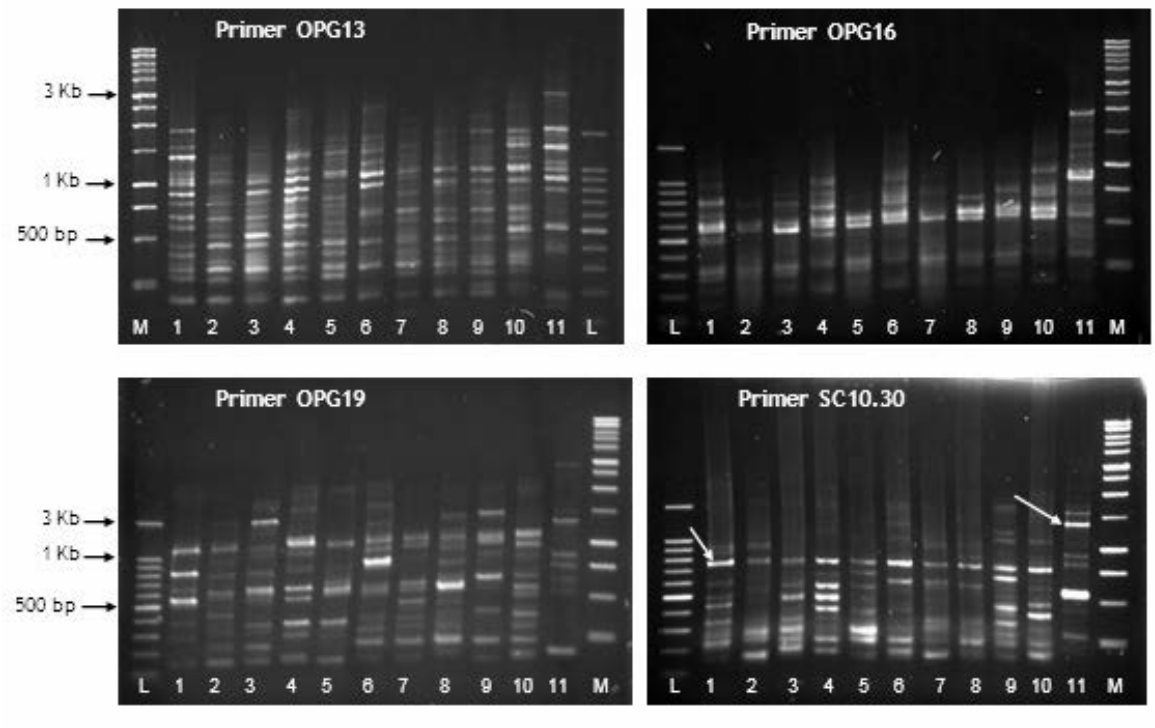

Fig. 1 (continued). Arrows show the fragments of approximately 750 and 1,400 bp generated by primer SC10.30.

The primer OPG3 amplified a band of approximately 950 bp in all *P. coffeae* populations from Vietnam, except in the Northeast 1 population. Primer OPG6 amplified one band of approximately 280 bp in all *P. coffeae* populations from Vietnam, except in the North Central Coast 1 population. And one band of approximately 450 bp was observed only in the Northwest, Northeast 2, Red River Delta 1, North Central Coast 1 and Central Highlands populations while a band of approximately 410 bp was only generated in the Central Highlands population by primer OPG11.

All the results obtained with the ten 10-oligonucleotide-long primers are summarised in a NJ tree that shows the genetic distance among the ten *P. coffeae* populations from Vietnam and the *P. speijeri* population based on 152 DNA bands (Table 4, Fig. 2). Table 4 shows that the pairwise distance between the *P. coffeae* populations from Vietnam and the *P. speijeri* population was the highest, ranging from 0.41 to 0.57. The pairwise distance among the *P. coffeae* populations from Vienam ranged from 0.23 to 0.42. In this NJ tree, the *P. coffeae* populations from Vietnam were separated into two groups with less than 50 bootstrap value support. The Northeast 1 and 4 populations (originally isolated from coffee and banana roots, respectively) and the Red River Delta 2 populations (originally isolated from the roots of an ornamental tree) were clustered together. This group is separated from another cluster including all the other *P. coffeae* populations from Vietnam, which were originally isolated either from banana or coffee. Within the latter group, the Red River Delta 1 and Northeast 3 populations, both originally isolated from banana, clustered into a subgroup with 81 bootstrap value support.

D2-D3 of 28S rRNA gene sequence and phylogenetic analysis. A total of 759 bp was analysed (data not shown). Among the ten *P. coffeae* populations from Vietnam examined, the D2-D3 28S rDNA expansion segment sequences of the Red River Delta 2 and Northeast 4 populations were identical while among the North Central Coast 1 population and the Red River Delta 1 and Northeast 1 populations these sequences varied at ten sites. The D2-D3 28S rDNA expansion segments sequences of the *P. coffeae* populations from Vietnam differed at least at 1 to 23 sites compared with those of the ten *P. coffeae* populations available in GenBank, with the exception of the North Central Coast 2 population where the sequences were identical with those of the *P. coffeae* populations from Honduras

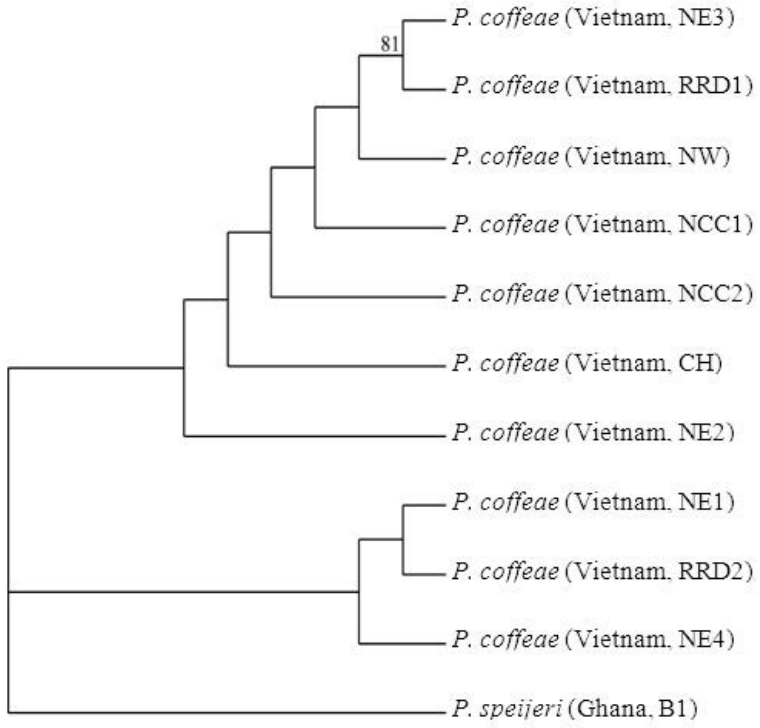

Fig. 2. Neighbour-Joining tree of ten *Pratylenchus coffeae* populations from Vietnam and a *P. speijeri* population from Ghana (Gha) based on RAPD bands generated by ten 10-ologonucleotide-long primers. A bootstrap value > 50% is given in the appropriate clade. For the list with the abbreviations of the Vietnamese nematode population codes see Table 1.

Table 4. Pairwise distances between ten *Pratylenchus coffeae* populations from Vietnam and a *P. speijeri* population from Ghana (Gha) based on RAPD bands generated by ten 10-oligonucleotide-long primers.

	NW	NE1	NE2	NE3	NE4	RRD1	RRD2	NCC1	NCC2	CH	Gha
NW	-	0.336	0.342	0.283	0.349	0.342	0.322	0.269	0.316	0.362	0.526
NE1	51	-	0.296	0.408	0.263	0.362	0.237	0.303	0.362	0.355	0.414
NE2	52	45	-	0.388	0.296	0.355	0.269	0.309	0.329	0.362	0.460
NE3	43	62	59	-	0.382	0.257	0.395	0.329	0.388	0.368	0.572
NE4	53	40	45	58	-	0.388	0.250	0.342	0.401	0.368	0.427
RRD1	52	55	54	39	59	-	0.296	0.309	0.421	0.335	0.579
RRD2	49	36	41	60	38	45	-	0.263	0.335	0.355	0.441
NCC1	41	46	47	50	52	47	40	-	0.335	0.382	0.454
NCC2	48	55	50	59	61	64	51	51	-	0.388	0.487
CH	55	54	55	56	56	51	54	58	59	-	0.493
Gha	80	63	70	87	65	88	67	69	74	75	-

Below diagonal: total character differences.

Above diagonal: mean character differences (adjusted for missing data).

For the list with the abbreviations of the Vietnamese nematode population codes see Table 1.

(B2-AF170434), Brazil (M1-AF170435), USA (M3-AF170436) and Martinique (Y1-AF170430). The D2-D3 28S rDNA expansion segments sequences of the *P. speijeri* population (B1-AF170533) differed from those of all other *P. coffeae* populations at 18 to 23 sites.

The sequence divergence of the D2-D3 28S rDNA expansion segments among the *P. coffeae* populations was low. Among the *P. coffeae* populations from Vietnam, the North Central Coast 1 population showed the highest difference in pairwise distance (> 0.006; data not shown). All sequences of the D2-D3 28S rDNA expansion segments of the ten *P. coffeae* populations from Vietnam matched the sequences available in GenBank with a similarity higher than 99% except

for those of the *P. speijeri* population and the two *P. jaehni* populations (C1-AF170426 and C2-AF170427). The pairwise distance between the ten *P. coffeae* populations from Vietnam and the *P. speijeri* population was higher than 0.024.

The maximum parsimony tree constructed on the basis of the D2-D3 28S rDNA expansion segment sequences is shown in Figure 3. Eight out of ten *P. coffeae* populations from Vietnam are placed in two exclusive groups. The first group contains the Northwest, Red River Delta 2, Northeast 4 and Central Coast 1 populations with a bootstrap value of 63%. The second group contains the Northeast 1, 2 and 3 populations and the Red River Delta 1 population with a bootstrap value of 65%. These two groups are in turn part of a larger group with a bootstrap value of 99% that includes the other two *P. coffeae* populations from Vietnam (North Central Coast 2 and Central Highlands) and the other *P. coffeae* populations available in GenBank. The three other *Pratylenchus* species (*P. speijeri*, *P. jaehni* and *P. loosi*) included in the analysis are clearly separated from all the *P. coffeae* populations.

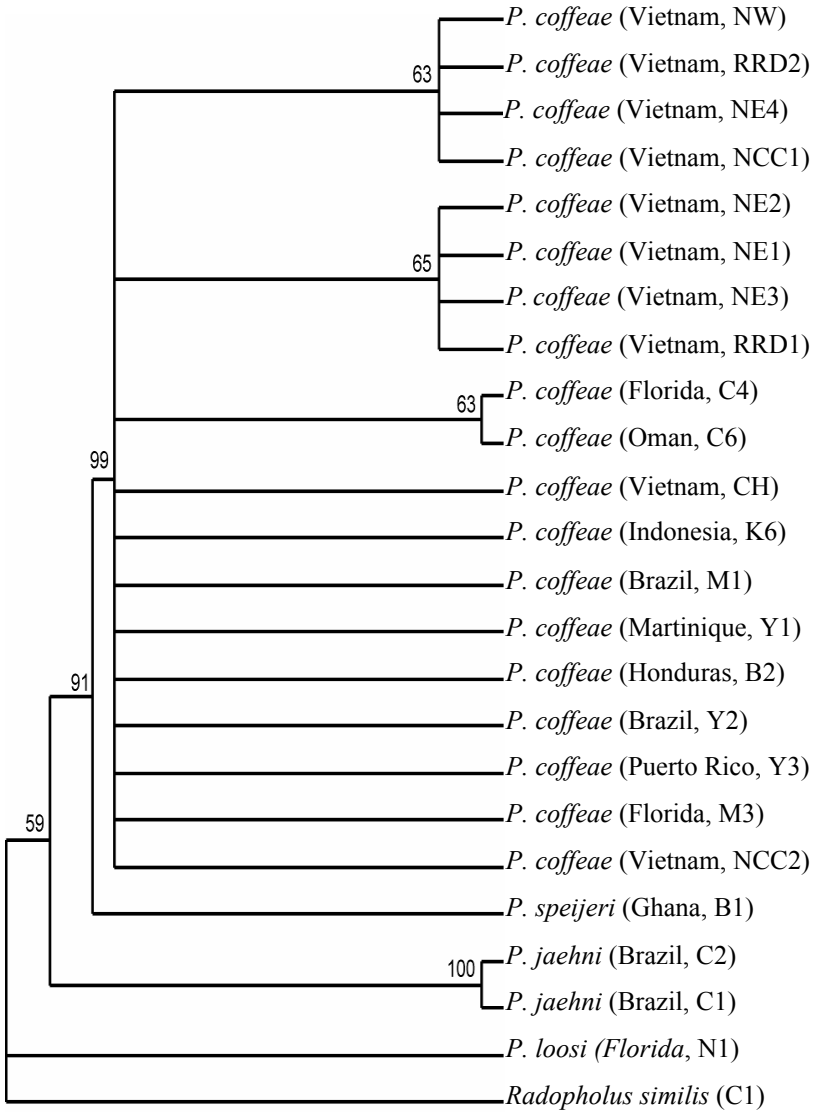

Fig. 3. Single maximum parsimony tree of ten *Pratylenchus coffeae* populations from Vietnam, nine other *P. coffeae*, two *Pratylenchus jaehni*, one *Pratylenchus loosi*, one *P. speijeri* population from Ghana and one *Radopholus similis* population available in GenBank based on the sequence alignment of the D2-D3 28S rDNA expansion segments. Bootstrap values > 50% are given in the appropriate clades. For the list with the abbreviations of the Vietnamese nematode population codes see Table 1; for the other nematode populations see Table 3.

DISCUSSION

RAPD bands analysis of the complete genome clustered the ten *P. coffeae* populations from Vietnam examined in two groups. The largest group consisted of seven populations. This group included populations collected in all the different agro-ecological regions of Vietnam: the only population collected in the Central Highlands clustered together with both populations collected in the North Central Coast and four populations collected in the north. The smallest group consisted of the remaining three populations; all collected in the two most northeastern situated agro-ecological regions (Red River Delta and Northeast). These results indicate that genomic similarity as determined by RAPD bands analysis of the complete genome did not correspond with geographic proximity. Similar results were obtained when Pinochet *et al*. (1994) compared the PCR-RAPD bands of seven *P. vulnus* populations from different geographical areas (USA, Argentina, France, Spain and Italy). By contrast, Mizukubo *et al*. (2003) examined the genomic similarity of 20 *P. coffeae* populations isolated from ten different agricultural crops in Japan using PCR-RFLP analysis. Based on this analysis, they suggested the existence of at least three groups of *P. coffeae* in Japan which they designated as A, B and C according to the digestion pattern using *Hin*f I, *Alu* I, *Dde* I and *Hha* I restriction endonucleases.

Although in our study the largest group included five out of the six *P. coffeae* populations originally isolated from banana, our results indicate that genomic similarity as determined by RAPD bands analysis of the complete genome also did not correspond with host plant origin. In the largest group two *P. coffeae* populations originally isolated from coffee were also included, while in the smallest group populations originally isolated from banana, coffee and the only population originally isolated from the roots of an ornamental tree was included. In contrast, Siciliarno-Wilcken *et al*. (2002) reported that, based on PCR-RAPD bands analysis, seven *P. coffeae* populations isolated from different host plants and regions of Brazil could be grouped in four clusters according to the host plants.

Our results show that the genetic differences among the *P. coffeae* populations from Vietnam examined are low, which indicates a low divergence. Moreover, the sequences of the D2-D3 28S rDNA expansion fragments of all the *P. coffeae* populations from Vietnam examined were closely related with the sequences of the *P. coffeae* populations available in GenBank.

Partial or complete sequence analysis of D2-D3 28S rDNA expansion segments have been used to separate different *Pratylenchus* species including *P. coffeae* (Duncan *et al*., 1999; Inserra *et al*., 2007; Subbotin *et al*., 2008). Our sequence analysis clearly supports the separation of the ten *P. coffeae* populations from Vietnam examined from *P. speijeri*, *P. jaehni* and *P. loosi*.

Interestingly, two out of three *P. coffeae* populations originally isolated from coffee in Vietnam (one collected in the North Central Coast, the other one collected in the Central Highlands) appears to be more related to the *P. coffeae* populations available in GenBank, which were all originally isolated from coffee from a wide range of countries from the Americas (USA, Honduras, Brazil), the Caribean (Puerto Rico, Martinique), Africa (Ghana) and Asia (Oman, Indonesia).

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Nguyen Thi Tuyet, L. Waeyenberge, A. Elsen, Ho Huu Nhi and D. De Waele. Молекулярно-таксономическая характеристика популяций *Pratylenchus coffeae* из Вьетнама.

Резюме. Анализ полного генома с помощью RAPD и данные по нуклеотидным последовательностям D2-D3 сегмента 28S rDNA были использованы для сравнения внутривидовой изменчивости десяти популяций *Pratylenchus coffeae*, собранных в различных агро-экологических регионах Вьетнама. Полученные при RAPD-анализе спектры сравнили с таковыми у описанного недавно из Ганы *P. speijeri*, тогда как D2-D3 последовательности сравнивали с таковыми у *P. coffeae* и близких видов рода *Pratylenchus*, депонированными в ГенБанке. Как показал RAPD-анализ, генетическое сходство не коррелирует ни с географической близостью мест выделения, ни с общим растением-хозяином. Аанализ нуклеотидных последовательностей D2-D3 28S rDNA показал, что все 10 популяций *P. coffeae* из Вьетнама сходны друг с другом и последовательностями *P. coffeae*, депонированными в Генбанке. Оба вида анализа показали, что уровень генетических различий между популяциями *P. coffeae* из Вьетнама и *P. speijeri* из Ганы подтверждают видовую самостоятельность последнего вида.