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A novel species-specific satellite DNA family in the invasive root-knot nematode *Meloidogyne mayaguensis* and its potential use for diagnostics

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Abstract The root-knot nematode (RKN) *Meloidogyne mayaguensis* is considered as one of the most damaging RKN species because of its extremely wide host range. Recent surveys have shown the rapid spread of this parasite in agro-ecosytems, often making crop cultivation not viable in the heavily infested areas. Here, we report the identification, molecular cloning, genomic organisation and sequence analysis of a new satellite DNA (satDNA) family from *M. mayaguensis* (named pMmPet). It is comprised of two groups of A+T rich, tandemly repeated units of 174 and 180 bp, respectively. Using these sequences as targets, hybridisation and PCR experiments performed on a wide collection of 44 populations belonging to 15 RKN species showed that the pMmPet family could only be detected in the 16 *M. mayaguensis* populations tested. In addition, because of their repetitive nature, positive detection of pMmPet sequences was achieved in single individual nematodes. Therefore, the repeated sequence described here possesses features that make it an excellent candidate for use as a specific and extremely sensitive tool for the accurate detection and identification of this invasive pest on a routine basis. Clearly, monitoring the occurrence and spread of *M. mayaguensis* at the domestic and international levels are needed to avoid wholesale loss of agricultural resources in the infested regions.

Keywords Bioinvasion · Diagnostics · Genetic marker · Root-knot nematode · Satellite DNA

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Introduction

The root-knot nematode (RKN) *Meloidogyne mayaguensis* was originally described in 1988 from specimens obtained from galled roots of eggplant from Puerto Rico (Rammah and Hirschmann 1988). Because of its wide host range, including crops carrying genes that confer resistance to the main *Meloidogyne* species (Brito et al. 2007), this species is considered as one of the most damaging RKNs. Early reports highlighted the presence and economic importance of this parasite in West Africa (Fargette et al. 1996). In 2000, the geographic distribution of *M. mayaguensis* was restricted to Africa, Central and South America, and the Caribbean (CAB International 2000). More recently, it was reported in Brazil (Carneiro et al. 2001) and in

the continental USA in Florida (Brito et al. 2004). So far, it has not been conclusively detected in Europe, although its occurrence was suspected in a greenhouse in France (Blok et al. 2002), but its specific status there was not clearly established. From all these observations, it is realistic to consider *M. mayaguensis* as an invasive species, i.e. a species occurring, as a result of human activities, beyond its accepted normal distribution and which has a quantitative and statistically significant direct or indirect adverse biological impact that threatens valued environmental, agricultural or other social resources by the damage it causes (Davis and Thompson 2000; Parker et al. 1999). The current situation in Brazil illustrates further the risks of dispersal of this nematode. It was originally detected in guava orchards in 2001 in the Pernambuco and Bahia states (Carneiro et al. 2001). Since then, the nematode has been spreading rapidly in the country, making the cultivation of guava not viable in the heavily infested areas (Carneiro et al. 2007). In addition, because of its morphological resemblance to *M. incognita* (Brito et al. 2004; Carneiro et al. 2001, 2007), *M. mayaguensis* has possibly not been detected or has been misidentified in a number of surveys, which strengthened the need for molecular diagnostic methods to accurately and reliably detect and identify this invasive species (Brito et al. 2004). Previous work has shown that *M. mayaguensis* could be differentiated from other RKN species on the basis of PCR amplification of selected mitochondrial (Blok et al. 2002; Brito et al. 2004) or ribosomal DNA regions (Adam et al. 2007), but these studies have been performed on a limited number of species/populations, and thus their practical output for routine diagnostic purposes still require validation on a large set of representative nematode populations.

Repetitive DNA sequences are a major component of the eukaryotic genome. Among them, a large fraction is constituted of non-coding, tandemly repeated sequences, organised as long arrays located in the constitutive heterochromatin, commonly known as satellite DNA (satDNA) (Plohl et al. 2008). SatDNA repeats evolve in a concerted manner, being continuously homogenised within a genome and fixed within a group of reproductively linked organisms (Dover 2002). The outcome is lower sequence variability within than between taxa. As a consequence, these repetitive sequences have proven to be powerful tools as species-specific markers in nematodes of agronomical interest (Grenier et al. 1997), including RKNs (Castagnone-Sereno et al. 1995; Piotte et al. 1995; Randig et al. 2002). Here, we report the identification, molecular cloning, genomic organisation and sequence analysis of a new satDNA family from the RKN *M. mayaguensis*. Moreover, we investigated the distribution of this repetitive family in a wide RKN collection (44 populations belonging to 15 *Meloidogyne* species, including 16 *M. mayaguensis* populations), in order to evaluate the potential use of this novel satDNA family as a genetic marker for the successful diagnostic of this nematode. Clearly, failure to implement pest management programmes aimed at identifying *M. mayaguensis* populations and taking effective steps to prevent their dispersal and establishment could effectively result in wholesale loss of agricultural resources in infested areas.

Materials and methods

Nematode populations and genomic DNA extraction

Fourty-four *Meloidogyne* spp. populations, belonging to 15 species, were used in this study, including 16 *M. mayaguensis* populations. Their sampling geographic location and the original host plant of samples are given in Table 1. They were multiplied under greenhouse conditions in the living nematode collections of either EMBRAPA, Recursos Genéticos e Biotecnologia (CENARGEN, Brazilia, Brazil) or INRA (Sophia Antipolis, France) on tomato plants (*Solanum lycopersicum*), cvs Santa Clara or Saint Pierre, respectively. Prior to multiplication, each population was identified specifically according to its isoesterase electrophoretic pattern according to the procedure of Carneiro et al. (2000). Nematode eggs were prepared according to McClure et al. (1973) and stored at −80°C until use. For each nematode population, genomic DNA was purified from aliquots of 200–300 µl eggs by a phenol-chloroform method (Sambrook et al. 1989).

Identification, cloning and sequencing of satellite DNA

Nematode genomic DNA was digested with a set of restriction endonucleases (*Alu*I, *Bgl*II, *Dra*I, *Eco*RI,

Table 1 *Meloidogyne* spp. populations used in this study

No.	Species	Geographic origin	Original host plant
1	*M. mayaguensis*	Petrolina, PE, Brazil	Guava
2	*M. mayaguensis*	Limoeiro do Norte, CE, Brazil	Guava
3	*M. mayaguensis*	Jan May Pic, Puerto Rico	Eggplant
4	*M. mayaguensis*	Maloua, Congo	Potato
5	*M. mayaguensis*	Godet, Guadeloupe, FWI	Tomato
6	*M. mayaguensis*	Martinique, FWI	Guava
7	*M. mayaguensis*	Qezaltenaco, Guatemala	Coffee
8	*M. mayaguensis*	Guanacaste, Costa Rica	Coffee
9	*M. mayaguensis*	Sao Joao da Barra, RJ, Brazil	Guava
10	*M. mayaguensis*	Pedro Canario, ES, Brazil	Guava
11	*M. mayaguensis*	Reginopolis, SP, Brazil	Pepper
12	*M. mayaguensis*	Tupa, SP, Brazil	Guava
13	*M. mayaguensis*	Campos Novos Paulista, SP, Brazil	Pepper
14	*M. mayaguensis*	Piraju, SP, Brazil	Pepper
15	*M. mayaguensis*	Munic de Santa Mariana, Pr, Brazil	Guava
16	*M. mayaguensis*	Novo Horizonte do Sul, MS, Brazil	Guava
17	*M. arabicida*	Juan Vinas, Cartago, Costa Rica	Coffee
18	*M. arenaria*	San Julian, Guadeloupe, FWI	Banana
19	*M. arenaria*	Caspesterre-Belle-Eau, Guadeloupe, FWI	Banana
20	*M. arenaria*	Goyave, Guadeloupe, FWI	Banana
21	*M. arenaria*	Marmande, France	Tomato
22	*M. chitwoodi*	The Netherlands	Potato
23	*M. cruciani*	Macouria, Guiana	Banana
24	*M. ethiopica*	Farropilha, RS, Brazil	Kiwi
25	*M. ethiopica*	Itapetinga, SP, Brazil	Soybean
26	*M. ethiopica*	Kenya	Tomato
27	*M. ethiopica*	Curico, Chile	Grapevine
28	*M. exigua*	San José, Costa Rica	Coffee
29	*M. exigua*	Lavras, MG, Brazil	Coffee
30	*M. exigua*	Sao Sebastian do Paraiso, MG, Brazil	Coffee
31	*M. fallax*	The Netherlands	Potato
32	*M. floridensis*	Florida, USA	Peachs
33	*M. hapla*	Botucatu, SP, Brazil	Strawberry
34	*M. hispanica*	Sevilla, Spain	Peach
35	*M. hispanica*	Pernambuco, PE, Brazil	Sugar cane
36	*M. incognita*	Londrina, PR, Brazil	Coffee
37	*M. incognita*	Apucarana, PR, Brazil	Pumpkin
38	*M. incognita*	Avilandia, SP, Brazil	Coffee
39	*M. incognita*	Jacuipe, AL, Brazil	Pumpkin/okra
40	*M. inornata*	Capa Bonito, SP, Brazil	Yakon (*polymia sonchifolia*)
41	*M. javanica*	Macouria, Guiana	Banana
42	*M. javanica*	Petrolina, PE, Brazil	Tomato
43	*M. paranaensis*	Apucarana, PR, Brazil	Coffee
44	*M. paranaensis*	Panorama San Marcos, Guatemala	Coffee

*Hae*III, *Pst*I, *Rsa*I, *Sal*3A, *Sty*I, *Taq*I) according to manufacturer's instructions and the resulting fragments separated on 1% agarose gels according to standard procedures (Sambrook et al. 1989). DNA bands corresponding to putative satellite DNA monomers were purified from the gel, ligated into the plasmid vector pBluescript SK+ and used to transform competent *Escherichia coli* cells according to standard procedures (Sambrook et al. 1989). The transformants were selected on ampicillin (100 mg ml^{-1}) agar plates containing X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside; 80 mg ml^{-1}) and IPTG (isopropyl-beta-D-thiogalactopyranoside; 120 mg ml^{-1}), and screened by PCR using the universal vector-localised T7 and T3 primers. Positive clones were sequenced using an automatic sequencing system Applied Biosystems 3700 at the DNA sequencing facility of EMBRAPA CENARGEN, Brazilia, Brazil. The sequenced satDNA repeat units were deposited in EMBL databank under Accession Numbers AM980481-AM980501.

Southern and dot-blot analysis

For Southern analysis, genomic DNA from *M. mayaguensis* population #1 (1–2 µg) was digested with restriction enzyme(s) for 4 h at 37°C, electrophoresed and transferred to nylon membranes according to standard procedures (Sambrook et al. 1989). Hybridisation probes were labelled with ^{32}P-dCTP in random-priming reaction, and hybridisations were performed overnight at 65°C. Conditions for washing consisted of 65°C in a 1 X SSPE, 0.1% SDS final solution. For time-course analysis, 10 U μg^{-1} of appropriate restriction endonuclease were used to digest genomic DNA and samples containing 1 µg of DNA were taken from the reaction mixture after time intervals ranging from 1 min to 24 h. The reaction was stopped by adding Na_2EDTA pH 8.0 to 20 mM concentration and chilling on ice. For dot-blot analysis, one µg genomic DNA of each nematode population tested was spotted on the membranes, and the hybridisation and washing conditions described above were used.

Sequence analysis of monomers

Multiple sequence alignments were performed using ClustalW2 (Larkin et al. 2007) at the European Bioinformatics Institute (http://www.ebi.ac.uk), and the corresponding consensus sequences were determined by the majority principle. Phylogenetic analyses were performed using the distance criterion in PAUP*, version 4b10 (Swofford 1998), and a Neighbour-Joining tree of monomers was constructed. Bootstrap values were calculated on 1,000 replicates.

PCR analysis

The nucleotide sequence of the two primers used in these experiments, MayaF.1 (5′-TCATGTATTCGAG GAGAAACACC-3′) and MayaR.1 (5′-TCATTC GAAACTACATTAAAAACAGTC-3′), was deduced from the consensus sequence of the monomeric unit of the pMmPet_174 satDNA sub-family (Fig. 2). They were purchased from Eurogentec. PCR amplifications were carried out in a final volume of 25 µl containing DNA template; 80 pM of each primer; dNTPs (Boehringer) each at 200 mM final concentration; 1x Taq incubation buffer and 1 U Taq polymerase (MP Biomedicals). DNA template consisted either of 10 ng of genomic DNA purified as indicated above or the individual nematode material prepared according to a previously described simplified procedure, using proteinase K in combination with the alternation of low and high temperatures (Castagnone et al. 2005). Amplifications were performed on a Biometra TRIO-Thermoblock thermal cycler. Thermal cycling conditions consisted of 3 min at 95°C followed by 15 cycles of 1 min at 94°C, 1 min at 61°C, 2 min at 72°C, and a final extension of 5 min at 72°C.

Results

Detection of a satellite DNA family in *Meloidogyne mayaguensis*

Total genomic DNA of *M. mayaguensis* (population #1) was digested to completion with a set of 10 restriction enzymes (*Alu*I, *Bgl*II, *Dra*I, *Eco*RI, *Hae*III, *Pst*I, *Rsa*I, *Sal*3A, *Sty*I, *Taq*I), electrophoresed and hybridised with total genomic DNA used as a probe in order to detect highly repetitive sequences. The available genomic DNA of nematodes of the genus *Meloidogyne* is usually rather limited due to the small size of animals and laborious process of their

cultivation. Consequently, only 1–2 µg of *M. mayaguensis* genomic DNA was available for digestion with each restriction enzyme. With three enzymes (*Alu*I, *Dra*I and *Taq*I), the presence of a band of < 200 bp was observed after autoradiography (Fig. 1a). The ~180 bp band detected in the *Alu*I digestion, which was assumed to correspond to the monomeric unit of a satDNA family, was purified from the gel, ^{32}P-labelled and used as a probe in the Southern blot analysis. The autoradiograph confirmed the presence of a prominent ~180 bp hybridisation signal in the *Alu*I and *Taq*I digests, while a faint discrete band was detected in the *Dra*I digest (Fig. 1b). Also, the *Rsa*I digestion revealed a ladder pattern of ~180 bp putative multimers (Fig. 1b). To further examine the arrangement of these sequences, DNA fragments produced after a time-course digestion of *M. mayaguensis* DNA with *Alu*I were electrophoresed and hybridised with the probe described above. Autoradiography showed a typical ladder pattern, with an increasing amount of monomers released during digestion (Fig. 1c). This result confirmed the tandem arrangement of ~180-bp long monomer repetitive sequences in the genome of the nematode. After complete digestion (24 h), dimers were still detected, which indicated the loss of *Alu*I restriction sites in some monomers.

Monomer sequence analysis

The ~180-bp *Alu*I restriction fragments from *M. mayaguensis* genomic DNA were isolated from the gel and subcloned. Twenty-one positive clones, named pMmPet(n) (n = clone number), were selected at random and sequenced. Sequencing revealed two groups of repeated units of 174 and 180 bp, respectively. Such lengths closely agreed with the estimate based on the electrophoretic mobility of the restriction fragments. Alignment of the 21 sequences indicated that they belonged to two subfamilies, named pMmPet_174 and pMmPet_180, respectively (Fig. 2). As shown in Fig. 3a, Neighbour-Joining analysis confirmed the clear-cut separation between both subfamilies. A consensus sequence for each subfamily was derived from the data set, and alignment showed a low level of sequence conservation between them (54.6%; Fig. 3b). On the contrary, analysis of the primary structure of the

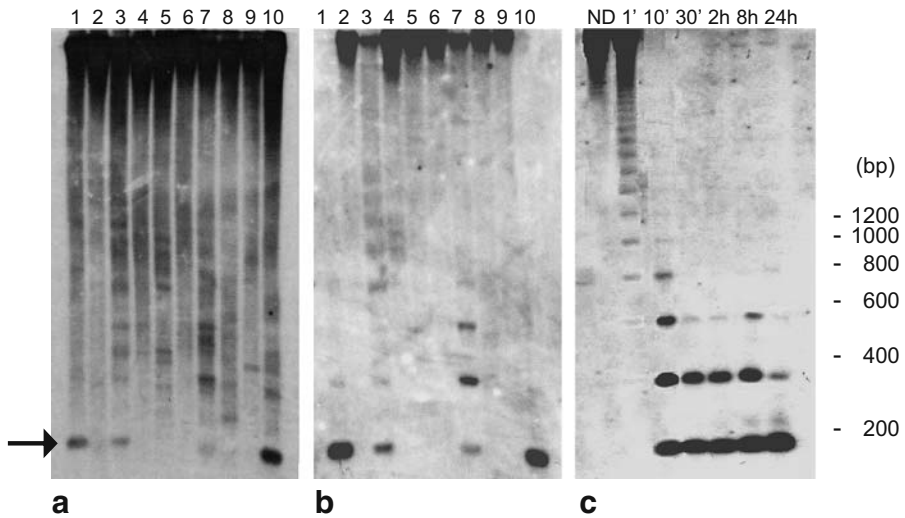

Fig. 1 Southern Blot analysis of *Meloidogyne mayaguensis* genomic DNA. **a** Genomic DNA was digested by a set of restriction endonucleases and hybridised with a ^{32}P-labelled probe consisting of whole genomic DNA. 1 = *Alu*I; 2 = *Bgl*II; 3 = *Dra*I; 4 = *Eco*RI; 5 = *Hae*III; 6 = *Pst*I; 7 = *Rsa*I; 8 = *Sau*3A; 9 = *Sty*I; 10 = *Taq*I. Among others, a repetitive fragment of ~200 bp was identified (arrow) in the *Alu*I digest. **b** Genomic DNA was digested by a set of restriction endonucleases and hybridised with a ^{32}P-labelled probe consisting of ~180 bp-DNA fragments released by the *Alu*I digestion of genomic DNA (arrow in panel A). Restriction endonuclease codes are as above. **c** Digestion kinetics of *M. mayaguensis* genomic DNA. Genomic DNA was digested by *Alu*I for increasing time (1 min to 24 h) and hybridised with a ^{32}P-labelled probe consisting of ~180 bp-DNA fragments released by the *Alu*I digestion of genomic DNA (arrow in panel A). ND = non-digested DNA. Molecular weights are given in base pairs

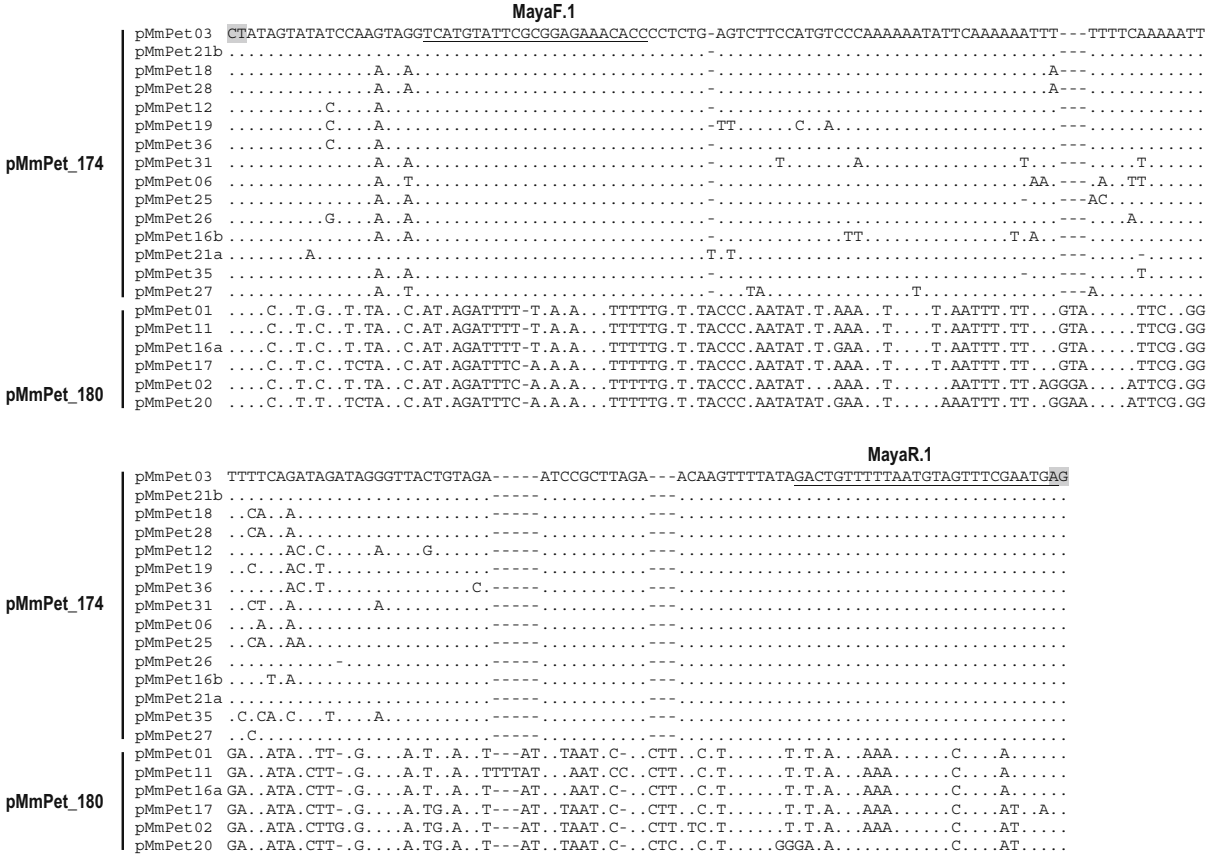

Fig. 2 Sequence alignment of the 21 cloned monomers of the pMmPet satellite DNA family from *Meloidogyne mayaguensis* (accession numbers AM980481-AM980501). Dots and dashes represent conserved and deleted nucleotides, respectively. The grey boxes indicate the *Alu*I restriction site (AG/CT) used for detection and cloning of the pMmPet family. Underlined are the primers designed for PCR amplification

monomers in relation to their consensus sequence revealed a high homogeneity within each subfamily: the individual identity of the cloned monomers compared to their consensus ranged from 94.8 to 98.3% and from 91.7 to 98.9% for pMmPet_174 and pMmPet_180, respectively. In both subfamilies, most of the mutations to the consensus sequence were nucleotide substitutions, except for a few short indels of 1 to 3 bp (Fig. 2). The A + T content of each subfamily was high (67.2 and 76.7% for pMmPet_174 and pMmPet_180, respectively), with frequent stretches composed exclusively of As and (or) Ts. Analysis of the restriction sites in the consensus sequences showed a good correlation with the patterns observed on the Southern blots (Fig. 1a and b), with the detection of one *Alu*I (in both consensus), one *Dra*I (in pMmPet_180 only) and two *Taq*I restriction sites (in both consensus), respectively (Fig. 3b). A search in the EMBL and GENBANK nucleic acid databases revealed no significant similarity with any recorded sequence, thus suggesting that the pMmPet family represents a novel satDNA family in RKNs.

Distribution of the pMmPet satellite DNA family in *Meloidogyne* species

In order to evaluate the distribution of the *Alu*I satDNA in RKNs, the genomic DNA of a representative subset of populations (including four *M. mayaguensis* populations, and 14 populations belonging to nine additional species) was dotted on to a nylon membrane and successively hybridised with a probe consisting of a cloned monomer of each subfamily, respectively. The autoradiographies shown in Fig. 4 illustrate such an experiment, and clearly indicate, whatever the probe

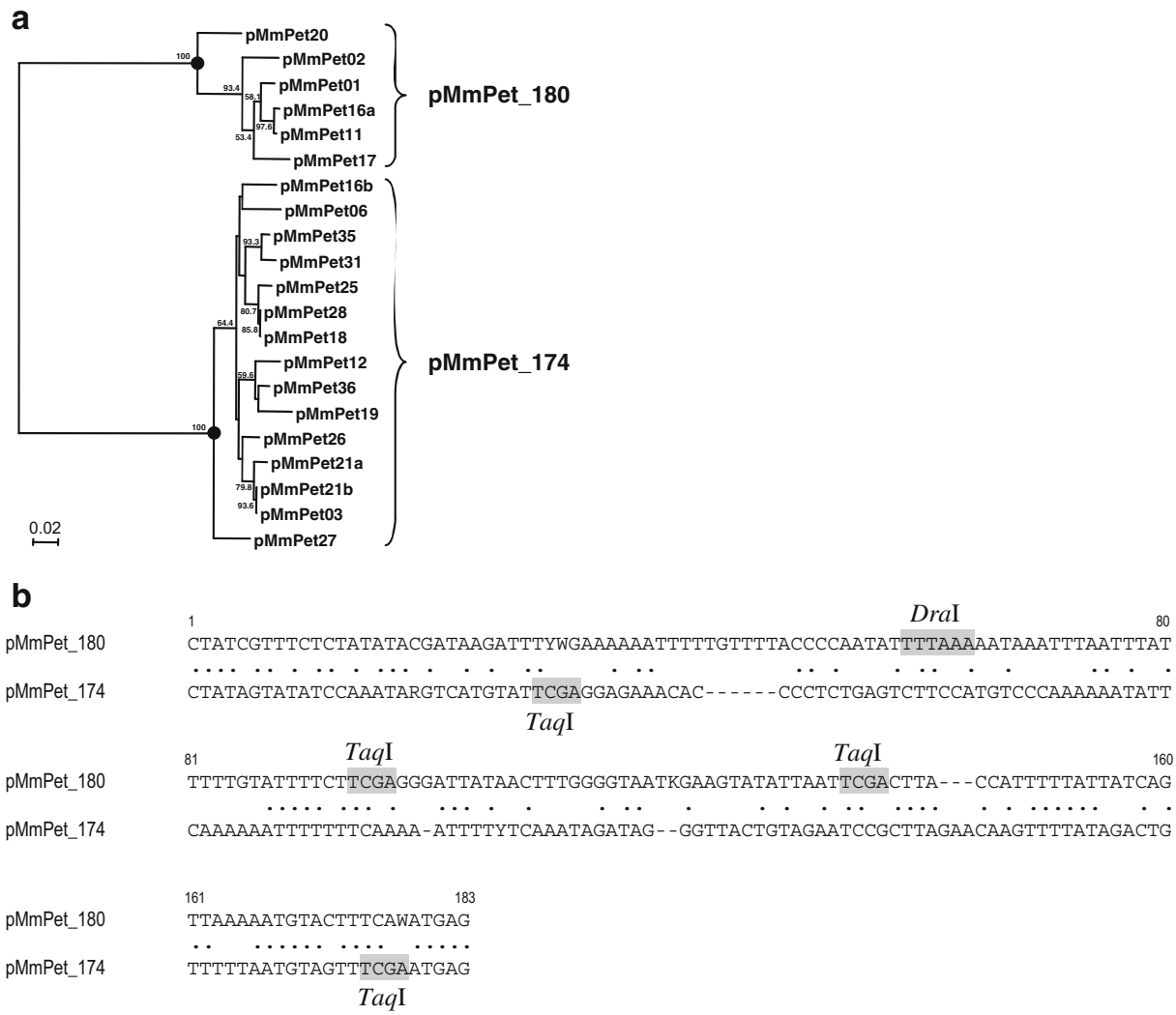

Fig. 3 Sequence analysis of the pMmPet satellite DNA in *Meloidogyne mayaguensis*. **a** Neighbour-joining tree of the 21 cloned *Alu*I satellite DNA monomers. Numbers at nodes indicate bootstrap support in % (1,000 replicates). The black dots identify the two pMmPet subfamilies (100% bootstrap support). **b** Alignment of the consensus sequences defined from the two pMmPet subfamilies shown in panel A. Dots and dashes represent conserved and deleted nucleotides, respectively. Grey boxes illustrate the conserved restriction sites (see text for details)

used, that hybridisation occurred only with the *M. mayaguensis* populations tested.

PCR experiments were further set up on a larger number of RKN populations to investigate the potential use of this sequence for diagnostic purposes. Since the pMmPet_174 sequences were very conserved in both their 5' and 3' ends (Fig. 2), these regions were used to design primers that could readily anneal to the borders of the monomeric unit. Indeed, two sequences were thus selected, named MayaF.1 and MayaR.1 (Fig. 2). Because of their location, these primers should theoretically result in amplification of a monomeric element shorter than the consensus one (151 bp instead of 174 bp). But since the repeat family has previously been shown to be organised in tandem arrays (Fig. 1c), and apart from the monomeric unit, the amplification of a ladder of multimers of the complete 174-bp monomer was expected from the use of these oligonucleotides as primers in PCR experiments. To select optimal PCR conditions, the effects of DNA template concentration and the number of amplification cycles were tested, alone or

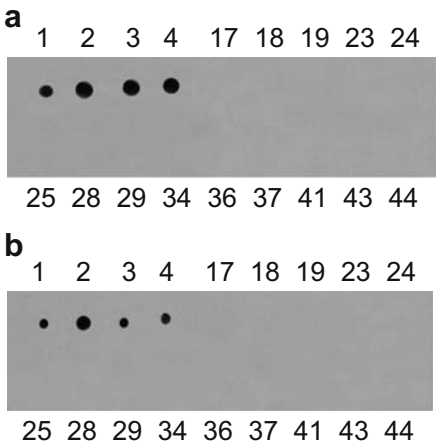

Fig. 4 Distribution of the pMmPet satellite DNA family in the genus *Meloidogyne* as inferred from dot-blot analysis, using a monomer from the pMmPet_174 (**a**) or the pMmPet_180 (**b**) subfamily as hybridisation probe, respectively. For population codes, see Table 1

in combination. The best reliable results were obtained with 5–10 ng of genomic DNA per reaction, and as low as 15 cycles of amplification (data not shown). Indeed, ladder patterns of monomers and multimers (up to the pentamer) were amplified from genomic DNA of all the *M. mayaguensis* populations tested, while no amplification was detected with any of the other *Meloidogyne* species tested (Fig. 5).

To test the sensitivity of the detection, amplification of DNA from single nematodes (either second-stage juveniles or females) was considered. As shown in Fig. 6, individual amplification patterns were always very similar to the one obtained from *M. mayaguensis* purified genomic DNA. As expected, no amplification occurred in samples belonging to other *Meloidogyne* species prepared in the same manner (data not shown).

Discussion

In the present study, we isolated and characterised a novel *Alu*I satDNA family in the genome of the RKN *M. mayaguensis*, constituted of tandemly arranged 173 to 184 bp-long monomeric units. Twenty-one such monomers were cloned and sequenced. Because of their random selection, it is expected that the range of nucleotide diversity they displayed is representative of the pMmPet family. One notable result is the clear identification of two subfamilies, named pMmPet_174 and pMmPet_180, exhibiting as little as 54.6% average identity at the nucleotide level. Based on the numbers of monomers of each subfamily randomly characterised, and the very similar hybridisation signals detected in the dot-blot analyses, it can reasonably be hypothesised that both subfamilies are present in the nematode genome at a comparable rate. Although such a feature is not the general rule, the concomitant occurrence of satDNA subfamilies, detected at a comparable rate in one genome, has already been documented in RKNs, e.g. in *M. chitwoodi* (Castagnone-Sereno et al. 1998) and *M. paranaensis* (Mestrovic et al. 2005, 2006a). Such genomic distribution appears rather different from the library model recently described in RKNs (Mestrovic et al. 2006b), where one satDNA family is predominant whereas others remain present as very low-copy number sequences.

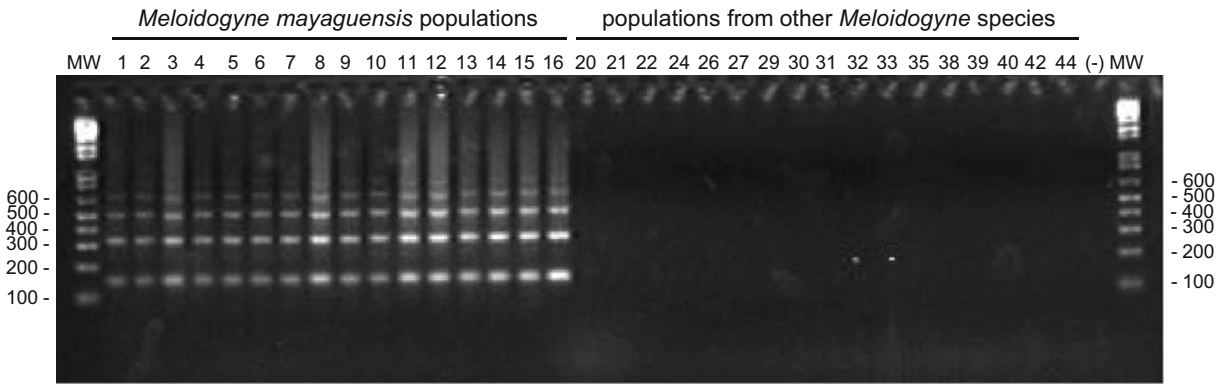

Fig. 5 Specific PCR amplification of the pMmPet satellite DNA in *Meloidogyne mayaguensis* populations. For population codes, see Table 1. (−) represents negative control (water). Molecular weights are given in base pairs

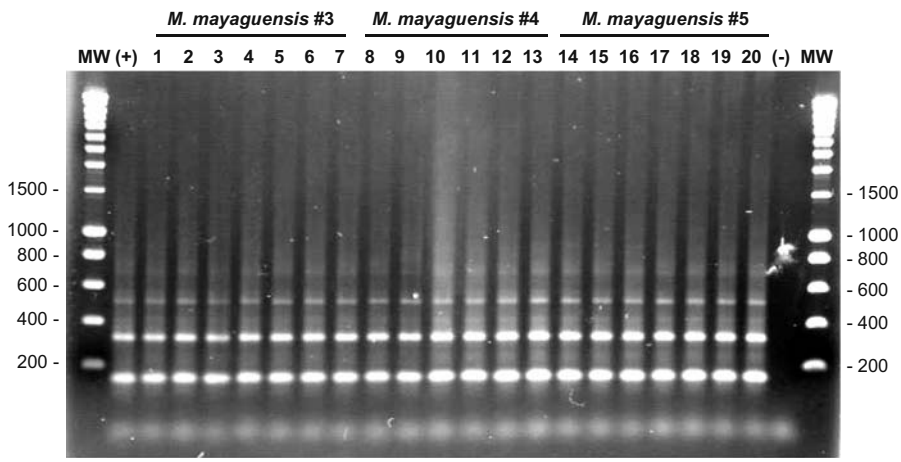

Fig. 6 PCR amplification of the pMmPet satellite DNA from *Meloidogyne mayaguensis* single individuals. (+), (−) represent positive (genomic DNA) and negative (water) controls, respectively. For population codes, see Table 1. Molecular weights are given in base pairs

The 21 monomers of the pMmPet satDNA family sequenced in this study have in common a high A + T content and share the presence of short stretches of As and/or Ts, a feature commonly observed in satDNAs (Plohl et al. 2008). In a number of cases, it has been suggested that during the evolution of satDNAs, C-to-T and G-to-A transitions are more frequent than the reverse transitions, which results in A + T enrichment of these sequences (Ugarkovic et al. 1989; Rojas-Rousse et al. 1993). In this hypothesis, the high A + T content observed here within pMmPet suggests it is of ancient origin. However, a significant difference was nevertheless noticed between pMmPet_174 and pMmPet_180 (67.2 and 76.7% average A + T content, respectively), suggesting that both subfamilies arose in the genome as the result of independent amplification bursts. Since the pMmPet satDNA family could not be detected, under our experimental conditions, in the genome of the 14 other RKN species tested, we further hypothesise that these bursts probably occurred after the speciation event that individualised *M. mayaguensis* from the other taxa of the genus. Within each subfamily, another remarkable trait of the pMmPet satDNA family is the very high degree of sequence similarity displayed among monomeric units. Considering that this satDNA could not have arisen through some recent amplification burst, an alternative view to explain such lack of variation would be that some highly effective homogenisation mechanism is acting upon the monomers, as a consequence of molecular drive (Dover 2002). Recently, such a model of evolution was supported by conclusive data from various kinds of repetitive elements, including ribosomal DNA (Ganley and Kobayashi 2007), protein coding genes (Thomas 2006) and mitochondrial DNA (Tatarenkov and Avise 2007).

Both dot-blot and PCR experiments detected pMmPet sequences in *M. mayaguensis* populations only, while no hybridisation/amplification was observed with populations belonging to the 14 other species tested. This result strongly suggests that this satDNA family is indeed specific for this taxon. In addition, although we did not precisely evaluate the genomic content of the pMmPet satDNA family in *M. mayaguensis*, it was shown to be abundant enough to allow the unambiguous identification of single nematodes. Conversely to other molecular markers investigated for the specific characterisation of *M. mayaguensis* (Adam et al. 2007, Blok et al. 2002, Brito et al. 2004), the satDNA-based procedure developed here was unambiguously validated on a large collection of RKN populations, which were almost exclusively sampled in tropical regions from where *M. mayaguensis* probably originates. Therefore, the repeated sequence described here possesses features that make it an excellent candidate for use as a specific and extremely sensitive tool for the accurate detection and identification of this invasive pest on a routine basis. For that purpose, further adaptation of the technique may include the development of a squash-blot procedure and cold-labelling of the probe, as previously described for *M. chitwoodi* and *M. fallax* (Castagnone-Sereno et al. 1999), thus avoiding time-consuming DNA extraction, PCR and gel staining procedures, and the use of radioactive labelling compounds. Since *M. mayaguensis* shares the same wide host range as *M. incognita*, and the females

harbour perineal patterns (an important taxonomic character for RKNs) morphologically similar to *M. incognita* (Carneiro et al. 2001; Brito et al. 2004), the possibility that the nematode has been misidentified in different regions of the world is not to be excluded. In addition, it has been recently suggested that *M. enterolobii* could be a senior synonym of *M. mayaguensis* (Xu et al. 2004), and it is worthwhile noting that *M. enterolobii* has just been added to the EPPO Alert List due the risk of introduction of this pest into the EPPO region (EPPO 2008). Although *M. mayaguensis* (or *M. enterolobii* if synonymisation is validated) has not yet been recognised as a quarantine organism, monitoring its occurrence and spread at the domestic and international levels appears to be an urgent need, an action that should be facilitated by the specific genetic marker developed in this study.

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