Molecular characterization of genetic diversity within the Africa/Middle East/Asia Minor and Sub-Saharan African groups of the Bemisia tabaci species complex

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Molecular characterization of genetic diversity within the Africa/Middle East/Asia Minor and Sub-Saharan African groups of the *Bemisia tabaci* species complex

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Bemisia tabaci Gennadius is a major plant pest in many agricultural systems worldwide. It is a complex of cryptic species that differ in many ecological respects, including damage-causing potential. Monitoring the genetic composition of *B. tabaci* populations in the field is, therefore, essential for achieving effective control. Sequencing is costly and time consuming; thus, efficient diagnostic tools must be developed to perform epidemiological studies involving hundreds of individuals. Here, we describe a polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) method for identifying all the putative species comprising the Africa/Middle East/Asia Minor group, including those that are the most devastating crop pests. Moreover, intra-specific diversity associated with specific symbiotic bacteria known to manipulate insect host phenotypes can also be detected. In addition, this method discriminates between the Africa/Middle East/Asia Minor and the Sub-Saharan African groups, which are sympatric in some areas. This simple, reliable and cost-effective diagnostic tool is ideal for the rapid analysis of a high number of individuals and, thus, has potential applications in field contexts, where it could provide valuable baseline information for pest management programs.

Keywords: PCR-RFLP; *Bemisia tabaci*; *mtCOI* gene; molecular diagnostic; Sub-Saharan African group; MEAM1 species; Mediterranean species; pest management program

1. Introduction

The whitefly *Bemisia tabaci* Gennadius is one of the most important pests worldwide, causing significant damage to food and ornamental crops directly by feeding on sap and, to an even greater extent, indirectly by transmitting many plant-pathogenic viruses (Jones 2003). *B. tabaci* displays considerable genetic and biological variability despite its morphological homogeneity, which has led to the conclusion that it is in fact a complex of morphologically indistinguishable "cryptic" species (Perring 2001; De Barro et al. 2011). The genetic complexity of *B. tabaci* has mainly been investigated using mitochondrial cytochrome oxidase gene (*mtCOI*) sequence data (Frohlich et al. 1999; Boykin et al. 2007). This has led to the identification of several genetic groups known as biotypes, but the use of the term "biotype" is now subjected to debate. The taxonomy and systematics of *B. tabaci* have not yet been completely elucidated, but recently it was proposed that a value of 3.5% *mtCOI* sequence divergence should be used to distinguish 28 putative cryptic species within the *B. tabaci* complex (Dinsdale et al. 2010; Hu et al. 2011; see De Barro et al. 2011 and Liu et al. 2012 for review). Those species fall into 11 major groups (>11% divergence), which include those previously described as biotypes. In this study, we have adopted the nomenclature proposed by Dinsdale et al. (2010), but we have also included the biotype designation so as to make it easier to relate our study to the previously published literature.

Among the 28 putative species, two stand out as being the most predominant and devastating worldwide, the Middle East-Asia Minor species 1 (commonly known as biotype B; referred to hereafter as MEAM1) and the Mediterranean MED species (which includes the Q, J, L and ASL biotypes). These two species belong to the same Africa/Middle East/Asia Minor major group of *B. tabaci* identified by Dinsdale et al. (2010), which also includes MEAM2 and Indian Ocean IO species (formerly known as the Ms biotype). The MED and MEAM1 species have colonized large areas of most continents as a result of introduction/invasion processes (Bedford et al. 1994; Liu et al. 2007; De Barro and Ahmed 2011). Their invasive ability is mainly attributable to their having a broad spectrum of host plants, better reproductive and competitive potential and greater insecticide resistance. In the Mediterranean basin, the MED species predominates. In some areas, it coexists locally with MEAM1. The MED species is common in North Africa and has spread, probably recently, into West Central Africa and South Africa, where it coexists with the Sub-Saharan African indigenous species (also known as the AnSL biotypes; referred to hereafter as SSAF species) (Gueguen et al. 2010; Esterhuizen et al. 2013).

The MED species appears to be genetically diverse not only because it includes the Q, J, L and Sub-Saharan African Silverleaf ASL biotypes (De Barro et al. 2011), but also because high genetic variability has been observed within the Q group, leading to the recognition of

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several cytotypes on the basis of *mtCOI* gene sequencing and symbiotic bacterial infection (Gueguen et al. 2010; Chu et al. 2012). Chu et al. (2012) recognized five sub-clades, designated as Q1–Q5, four of which correspond to the previously known Q1, Q2, Q3 and ASL genetic groups (Gueguen et al. 2010). These genetic groups display some particular features. First, they have different geographical and host plant ranges. For example, Q1 and Q2 have been sampled at a large geographical scale on a wide spectrum of host plants, whereas Q3 has only been observed in Western Africa on *Lantana camara* L. and tobacco (Gnankiné et al. 2012). The Q subclades also differ with regard to the composition of the bacterial symbiotic communities they harbor (Gueguen et al. 2010; Gnankiné et al. 2012). Some of these bacteria are known to induce drastic phenotypic change in *B. tabaci*, conferring features such as a fitness benefit (Kontseladov et al. 2008; Himler et al. 2011). Finally, they also differ as regards the frequencies of alleles resistant to pyrethroids and organophosphates (Mouton et al. unpublished data).

The spread of the MED and MEAM1 species worldwide has had a considerable impact on agriculture (for review, see Oetting and Buntin 1996), and many studies have focused on identifying the mechanisms underlying their invasiveness and the best methods of control. This requires monitoring *B. tabaci* populations to track the evolution of the species composition in the invaded areas. Gene sequencing is costly and time consuming; therefore, other effective diagnostic tools need to be developed in order to perform epidemiological studies involving hundreds of individuals. One of the various polymerase chain reaction (PCR) based methods, PCR-RFLP, consists of digestion with restriction enzymes after a PCR, and it provides a simple, fast and effective way to detect some of the variations in DNA sequences without sequencing. This technique has already been developed for use in *B. tabaci*, mainly to distinguish MEAM1, MED and non-MEAM1, non-MED species (Bosco et al. 2006; Tsagkarakou et al. 2007; Vassiliou et al. 2008; Ma et al. 2009; Shatters et al. 2009). Moreover, other PCR-RFLP protocols have been proposed to detect genetic variability in the Mediterranean basin, but these do not identify all the genetic diversity known to exist in the Africa/Middle East/Asia Minor group (Sartor et al. 2008; Chu et al. 2012). In this study, we describe a PCR-RFLP method that can be used to identify all the putative species that belong to the major groups (MED, MEAM1, MEAM2 and IO species) and also to recognize within-species variations. We also considered the Sub-Saharan African group, which is composed of several Sub-Saharan African species (SSAF1–5 species), which are sympatric with the MED species in some localities (Berry et al. 2004; Gueguen et al. 2010; Gnankiné et al. 2012; Esterhuizen et al. 2013).

2. Materials and methods

2.1. *Phylogenetic analyses*

We performed a phylogenetic analysis to determine the genetic diversity within *B. tabaci* populations in the two major groups under consideration in this study, i.e. the Africa/Middle East/Asia Minor and the Sub-Saharan African clades. All the *mtCOI* sequences of *B. tabaci* available in Genbank for these two groups were analyzed, and only the sequences of around 657 bp with no undefined nucleotides, and no gaps or indels, were used. This gave us 143 sequences originating from samples collected worldwide (Table 1). Multiple sequence alignment was carried out using MUSCLE software (Edgar 2004) implemented in CLC DNA Workbench (CLC bio). Only non-redundant sequences were retained to construct the tree; this gave us 25 haplotypes (for each haplotype, the sequence used for the analysis is shown in bold type in Table 1). Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inferences. The appropriate model of evolution selected with jModelTest v0.1.1 (Posada 2008) was GTR + I + G for both methods. ML analyses were performed with PhyML v3.0 (100 bootstrap replicates) (Guindon et al. 2010). Bayesian analyses were done using MrBayes v3.1.2 with 500,000 generations (Ronquist and Huelsenbeck 2003). Results were plotted using FigTree v1.3.1.

2.2. In silico *selection of restriction enzymes*

Restriction enzymes were selected by *in silico* analyses of the 25 haplotypes using the CLC DNA Workbench 6.0 (CLC Bio) program. CLC sequence viewer was used to predict restriction sites on a 657-bp fragment of the *mtCOI* gene sequence with 50 restriction enzymes.

2.3. *PCR-RFLP on field samples*

The PCR-RFLP tool developed in this study was tested on almost 1100 *B. tabaci* individuals originating from France, Spain, Israel, Greece, Togo, Benin and Burkina Faso, and collected from various host plants (Table 2). DNA was extracted using the procedure of Gueguen et al. (2010). Briefly, individual insects were ground in 25 μL of extraction buffer containing 50 mM KCl, 10 mM Tris-base pH 8.0, 0.45% Nonidet P-40, 0.45% Tween 20 and 500 mg/mL proteinase K. Samples were incubated at 65 °C for 3 h and then at 100 °C for 15 min. A 35-μL aliquot of pure water was added to this extract, which was then stored at −20 °C until use. An 819-bp fragment of *mtCOI* was amplified with the primers C1-J-2195 and L2-N-3014 (Simon et al. 1994). PCR amplifications were performed in a final volume of 25 μL containing 200 μM dNTPs, 200 nM of each primer, 1.5 mM of $MgCl_2$, 0.5 U of *Taq* polymerase (EueblueTaq, Eurobio, France) and 2 μL of DNA template. The cycling profile consisted of an initial denaturing phase at 95 °C for 2 min, followed by 35 cycles consisting of: 94 °C for 1 min (denaturing), 50 °C for 1 min (annealing) and 72 °C for 1 min (extension) followed by a final extension phase at 72 °C for 10 min. PCR products (10 μL) were digested with *XapI*, and another 10 μL was digested with *BfmI* (10 UI) at 37 °C for 3 h. The products obtained were then separated by electrophoresis on a 2% agarose gel at 100 V for 1 h and visualized

Table 1. Geographic origin and types of restriction profiles of *Bemisia tabaci* strains retrieved from Genbank for the *in silico* analysis. Numbers in bold indicate the sequences used for the phylogenetic analysis.

B. tabaci major group	B. tabaci species	Biotype	Geographic origin	Type of restriction profile			Genbank accession number
				Xap1	*BfmI*	Profile number	
Africa/Middle East/Asia Minor	Middle East-Asia Minor1	B	India	2	2	Profile 1	AF321927, AJ748368
			Morocco				AM176570, AJ517768
			Israel				AY766369, AY766373, DQ174536
			USA				EF080824, AY057123
			Australia				DQ174535
			Guadeloupe				AM180064
			France				AJ550170
			Argentina				AF340215
			South Africa				AY057140
			Japan				AB204578, AB204580, AB204581
			Pakistan				AJ510071, AJ510074, AJ510075, AJ510076, AJ510079, AJ510081
			Indian Ocean				AJ550173, AJ550174, AJ877260
			China				AY686062, AY686063, AY688073
			United Arab Emirates				DQ133382
			Italy				GU086341
			Taiwan				GU086342
			Kuwait				GU086346
			Jordan				GU086347
			Spain				GU086348
			Dominican Republic				GU086349
			Kuwait	10	2	Profile 2	GU086354, GU086355
			Iran				GU086350, GU086352, GU086353
			Saudi Arabia				GU086357, GU086358, GU086344, GU086345
			Yemen				GU086343, GU086359, GU086360
			South Korea	2	9	Profile 3	DQ174538
	Middle East-Asia Minor2		Reunion	1	5	Profile 4	AJ550177
	Mediterranean	Q1	Algeria	1	1	Profile 5	AM176575
			Morocco				EU760753, AY0571387
			France				EU760737
			Burkina Faso				FJ766425
			Cameroon				EU760722
			China				HM802268
			Japan				AB204586, AB204587, AB204588
			USA				EF080823
		Q2	Turkey	8	1	Profile 6	AF342776
			Israel				EU760756
			USA				FJ188686, EF080821
		Q3	Burkina Faso	8	2	Profile 7	FJ766384, FJ766429
		J	Cameroon	5	4	Profile 8	AF344258

(continued)

Table 1. (*Continued*)

B. tabaci major group	B. tabaci species	Biotype	Geographic origin	Type of restriction profile			Genbank accession number
				Xap1	BfmI	Profile number	
			Zimbabwe				AF344285
			Ivory Coast				AY057136
			Ghana				AY827579, AY827580, AY827587
			Ghana	8	4	Profile 9	AY827581
			Ghana	5	6	Profile 10	AY827588, AY827590
		L	Sudan	1	1	Profile 5	AY827612, AY827614, AY827615
			Sudan	1	2	Profile 11	AY827613
		ASL	Zimbabwe	5	4	Profile 8	AF344286
			Cameroon				EU760758
			Burkina Faso				FJ766410, FJ766435
			Uganda				AY903571
			Côte d'Ivoire				FJ766383
			Ghana				AY827589
			Croatia	1	1	Profile 5	GU086334, GU086335
	Indian Ocean	Ms	Reunion	9	8	Profile 12	EU760759
			Uganda				AY903539
Sub-Saharan Africa	Sub-Saharan Africa 1	AnSL1	South Africa	6	3	Profile 13	AF344267, AF344268, AF344264
			Ghana				AF418668, AY827591
			Zambia				AF344281
			Swaziland				AF344276, AF344270
			Tanzania				AF418667
			Uganda				AY057162
			Uganda	4	3	Profile 14	AY057168, AY057149, AY057151, AY057178, AY057179, AY057180, AY057182, AY057183, AY057185, AY057210,AY057181
			Uganda	7	3	Profile 15	AY057169
	Sub-Saharan Africa 2	AnSL2	Uganda	4	3	Profile 14	AY057141, AY057208, AY827611, AF418669, AY057143, AY057146, AY057173, AY057194, AY827605
			Mali	4	7	Profile 16	AY827604
			Nigeria				AY827607
	Sub-Saharan Africa 3	AnSL3	Cameroon	4	3	Profile 14	AF344257
	Sub-Saharan Africa 4	AnSL4	Cameroon	3	3	Profile 17	AF344245, AF344246, AF344249, AF344251, AF344252, AF344254, AF344255, AF344247
	Sub-Saharan Africa 5	AnSL5	South Africa	3	3	Profile 17	JN104711, JN104712, JN104713, JN104714, JN104715, JN104716, JN104717, JN104718, JN104719, JN104720

Table 2.   Samples of *Bemisia tabaci* used in this study.

B. tabaci major group	*B. tabaci* species	Biotype	Geographic origin	Host plant	PCR-RFLP	Sequencing
Sub-Saharan Africa	Sub-Saharan Africa 1	AnSL1	Togo	Cassava	11	2
			Benin	Cassava	15	1
	Sub-Saharan Africa 2	AnSL2	Togo	Cassava	7	1
			Benin	Cassava	1	3
Africa/Middle East/ Asia Minor	Mediterranean	ASL	Burkina Faso	Cotton	6	0
				Tomato	74	0
				Eggplant	2	0
				Marrow	8	11
				Tobacco	119	0
			Benin	Cotton	10	6
				Tomato	26	6
		Q1	Burkina Faso	Cotton	175	21
				Tomato	91	8
				Eggplant	9	4
				Marrow	26	3
				Tobacco	2	1
				Cucumber	29	0
			Togo	Cotton	29	2
			Benin	Cotton	2	2
			Spain	Melon	5	1
				Tomato	28	1
				Cucumber	38	1
			France	Hibiscus	15	1
				Eggplant	15	1
			Greece	Cucumber	20	1
			Greece	Eggplant	10	1
		Q2	Spain	Cucumber	7	1
				Melon	26	1
				Sweet pepper	38	1
				Tomato	42	1
			France	Hibiscus	47	1
				Eggplant	15	1
			Israel	Cotton	20	1
		Q3	Burkina Faso	Tomato	4	0
				Tobacco	16	9
				Lantana camara	83	21
	Middle East-Asia Minor1	B	Spain	Cotton	7	4
			Israel	Cotton	1	1

Note: Numbers indicated in the "PCR-RFLP" and "sequencing" columns correspond to the number of individuals tested by PCR-RFLP and the number of samples sequenced, respectively.

by ethidium bromide staining. In parallel, PCR products were directly sequenced from 120 individuals.

3.   Results

3.1.   *Phylogenetic structure*

The phylogenetic tree obtained with the 25 selected haplotypes is consistent with the most recent *B. tabaci* phylogenies available in the literature (Dinsdale et al. 2010; De Barro et al. 2011). Since the ML and the Bayesian analyses led to substantially the same topology, we present only the ML analysis (Figure 1). Genetic variability was observed within the major groups and species recognized by De Barro et al. (2011). Fifteen haplotypes were identified in the Africa/Middle East/Asia Minor group, and seven in the Sub-Saharan African group. Genetic diversity was also found within species, with up to nine haplotypes in the MED species and four in MEAM1. Polymorphism was lower in the other species analyzed (five haplotypes

for SSAF5, three for SSAF1, two for SSAF2 and one for MEAM2, IO, SSAF3 and SSAF4), but this may have been due to the limited number of sequences available in Genbank for these species.

3.2.   **In silico** *PCR-RFLP*

Two restriction enzymes were selected after *in silico* analysis on the basis of their capacity to discriminate between the Africa/Middle East/Asia Minor and Sub-Saharan African major groups, and their ability to discriminate within these groups between the putative species described by Dinsdale et al. (2010) and De Barro et al. (2011). Ten and nine profile types were found for *XapI* and *BfmI*, respectively (Figure 2). Both enzymes were reliably able to distinguish between the two major groups. For *XapI,* we found profile types 1, 2, 5, 8, 9 and 10 for the Africa/Middle East/Asia Minor group, and profile types 3, 4, 6 and 7 for the Sub-Saharan African major group. For *BfmI,*
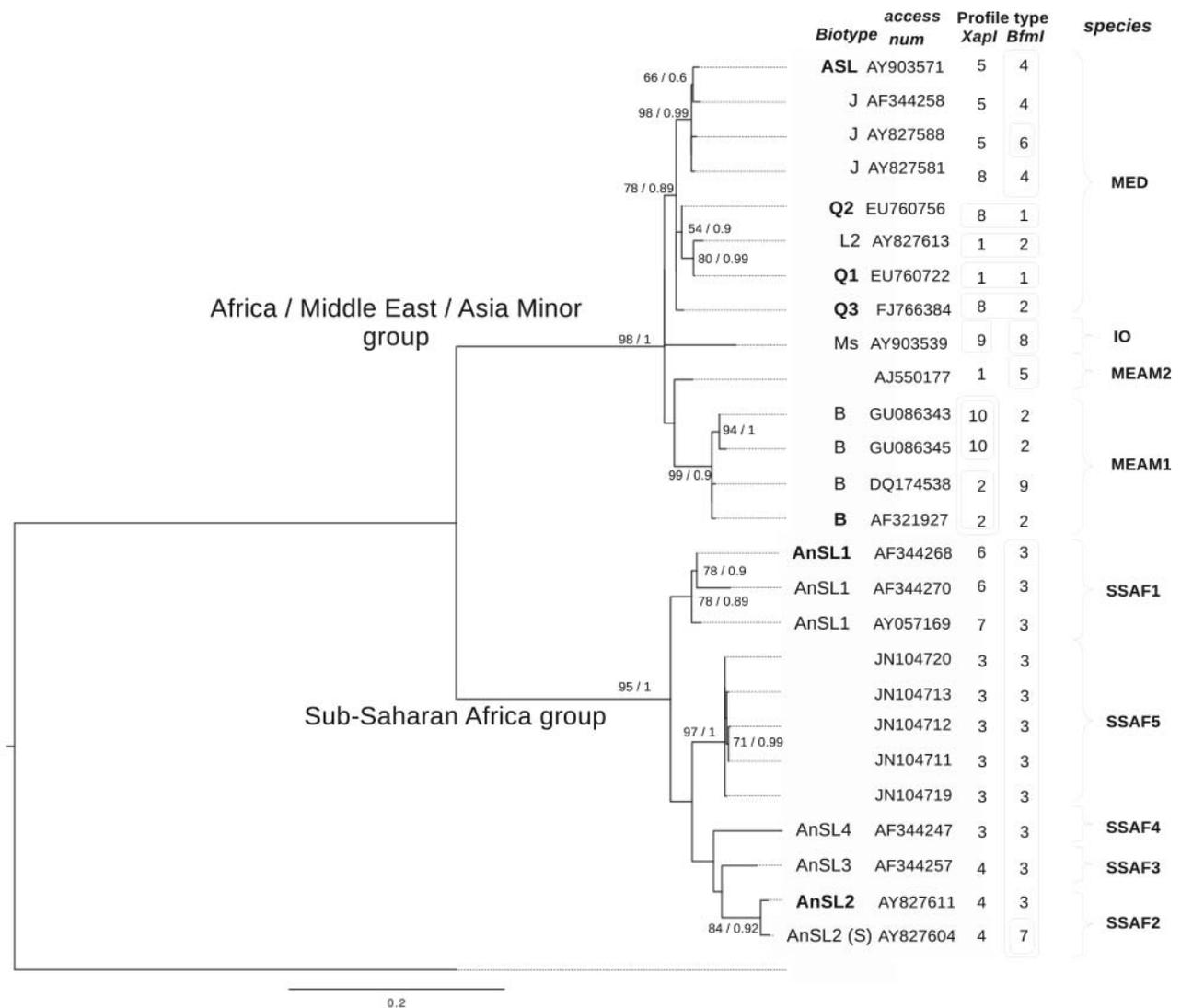
Figure 1.   *mtCOI* gene-based phylogeny of the Africa/Middle East/Asia Minor and Sub-Saharan African major groups of *B. tabaci* using maximum likelihood (ML) analyses. Bootstrap values are shown at the nodes for ML analysis, followed by the corresponding posterior probabilities obtained by Bayesian inferences. Sequences were retrieved from Genbank. *Bemisia afer* was used as the outgroup. The analysis was based on a 657-bp sequence. Biotypes and access numbers are indicated at each branch, as is the type of profile obtained with each enzyme (*XapI*/*BfmI*). Frames indicate the specific profiles that can be used to distinguish between the sequences (for example, profiles 10 and 2 obtained with *XapI* are specific to the MEAM1 species, whereas restriction with both enzymes is required to identify Q1, Q2, Q3 and L2).

profiles 1, 2, 4, 5, 6, 8, 9 correspond to the Africa/Middle East/Asia Minor group, while profiles 3 and 7 characterized the Sub-Saharan African major group (Figure 1 and Table 1). Seventeen profiles were obtained when simultaneous DNA digestion by both enzymes was performed: 12 for the Africa/Middle East/Asia Minor group and 7 for the Sub-Saharan African group (Table 1). Within the Sub-Saharan African group, we were unable to distinguish all the SSAF species. In contrast, within the Africa/Middle East/Asia Minor group, PCR-RFLP profiles distinguished all the putative species: profiles 1–3 were particular to the MEAM1 species; profiles 5–11 were specific to the MED species and profiles 4 and 12 characterized the MEAM2 and IO species, respectively. In summary, *in silico* analysis suggests that *XapI* and *BfmI* acting simultaneously offer good potential identification of all four putative

species in the Africa/Middle East/Asia Minor group, including the ability to detect within-species diversity, since 12 profiles were obtained for 15 known haplotypes.

3.3.   *PCR-RFLP on field samples*

The *in silico* predictions were checked on field samples belonging to the Africa/Middle East/Asia Minor (MEAM1 and MED species) and the Sub-Saharan African (SSAF1 and SSAF2 species) groups. We performed both PCR-RFLP and *mtCOI* gene sequencing on 120 *B. tabaci* individuals collected worldwide from several plants (Table 2). For another 959 individuals, the identification was done using the PCR-RFLP method only, in order to check the reliability of the method. Digestion with *XapI* and *BfmI* generated six and four distinct restriction
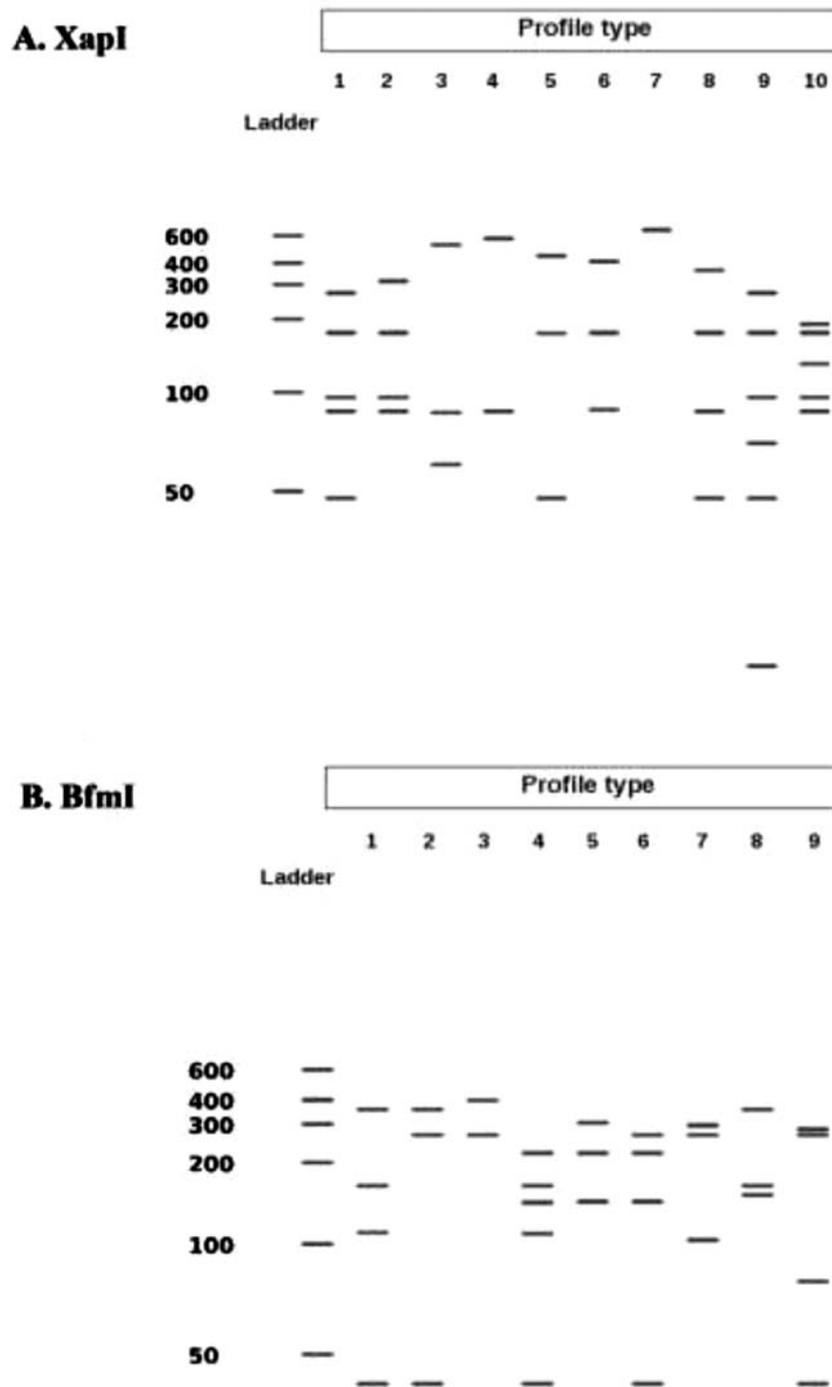
Figure 2. *In silico* restriction profiles obtained with *XapI* **(**A) and *BfmI* (B). The sizes of the bands corresponding to the ladder are indicated (bp).

patterns in our samples, respectively, differing by the number and/or size of the fragments, making it possible to identify the MEAM1, MED and SSAF species (Figure 3) as predicted by the *in silico* analysis (Table 1). Moreover, restriction profiles differentiated haplotypes within the MED species, making it possible to recognize the commonly known ASL, Q1, Q2 and Q3 genetic groups previously described as different biotypes or cytotypes, which harbor different bacterial endosymbionts. Fragments of less than 50 bp were not detected, because they were too small to be visualized by electrophoresis in a routine agarose gel assay. Moreover, the size of the bands observed for some restriction profiles deviated slightly from predictions; this was because the *in silico* analysis was performed on 657 bp, which is only a part of the 867-bp amplicon obtained with the primers used to amplify the *mtCOI* gene in *B. tabaci* (C1-J-2195 and L2-N-3014; Simon et al. 1994).

For all 120 individuals in which biotype identification was carried out using both standard *mtCOI* gene
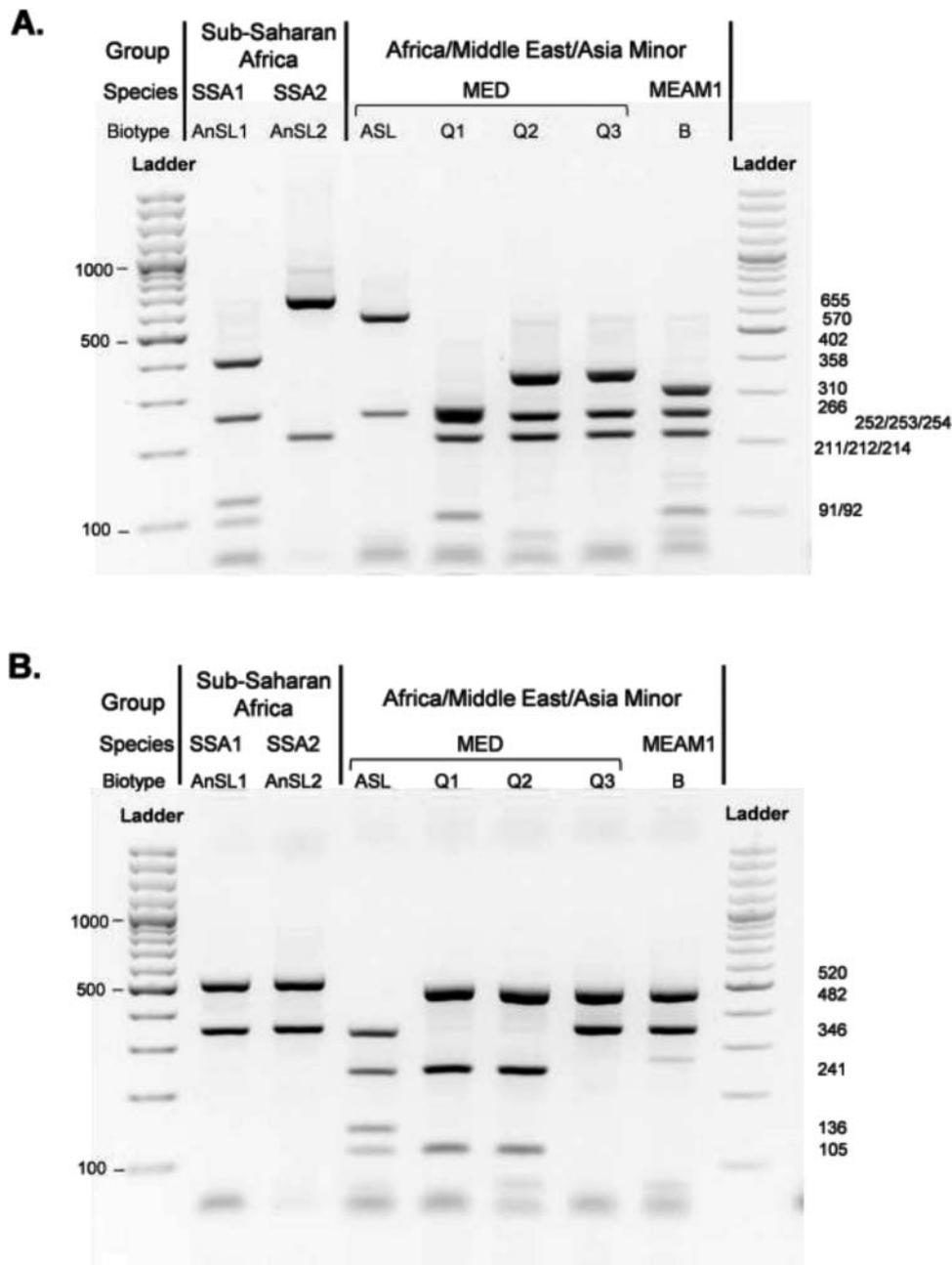
Figure 3. PCR-RFLP assays with *XapI* (A) and *BfmI* (B). The sizes of the different bands obtained and the sizes (bp) of the bands corresponding to the ladder are indicated on the left and the right of the figure, respectively.

sequencing and PCR-RFLP assay, the same results were obtained by both methods (Table 2). Moreover, we did not detect any unexpected restriction profiles for the 959 other individuals screened by PCR-RFLP only. This implies there was no other sequence variation at the *XapI* and *BfmI* restriction sites than those detected by the *in silico* analysis.

4. Discussion

In this paper, a simple diagnostic tool based on a PCR-RFLP method on the *mtCOI* gene was used:

(1) to reliably distinguish between the two major groups of *B. tabaci* that coexist in sympatry in

some places in the Mediterranean basin and Africa (Gueguen et al. 2010; Gnankiné et al. 2012; Esterhuizen et al. 2013): the Africa/Middle East/Asia Minor group, which includes the two most invasive members (B and Q biotypes), and the Sub-Saharan African group, which is regarded as the basal clade of *B. tabaci;*

(2) to detect all the putative species defined in the Africa/Middle East/Asia Minor group by De Barro et al. (2011) and Dinsdale et al. (2010). These species include MEAM1 (formerly referred to as biotype B) and MED (biotype Q), which are widespread and considered to be the ones that cause the most damage (Oetting and Buntin

1996). In many places, displacement of MEAM1 by MED has been observed (Horowitz et al. 2005; Crowder et al. 2010), and the mechanism(s) underlying this phenomenon have been investigated for many years; this requires monitoring *B. tabaci* populations to track the evolution of the species composition in these areas. One explanation for this displacement could be the fact that these two species differ in their susceptibility towards various insecticides. Indeed, it has been demonstrated that the use of neonicotinoids or pyriproxyfen positively selects for the MED species, which exhibits greater resistance to these insecticides than the MEAM1 species (Horowitz et al. 2005).

Genetic diversity at the mitochondrial level has also been detected at a finer scale, i.e., within species. This PCR-RFLP protocol makes it possible to identify entities previously recognized as the Q1, Q2, Q3 and ASL genetic groups on the basis of *mtCOI* gene sequencing. While it is still not completely clear whether these groups are of any biological significance, some arguments suggest that this distinction should be taken into account. First, these haplotypes harbor particular symbiont communities that can potentially modify a number of biological traits in their host (Chiel et al. 2007; Gueguen et al. 2010; Gnankiné et al. 2012). Second, all the genetic groups of MED species are structured geographically (Gueguen et al. 2010; Gnankiné et al. 2012), so that tracking them provides a way of identifying the invasion routes of *B. tabaci*. Finally, they are associated with biological features, such as host plant use (Gnankiné et al. 2012) and the frequency of insecticide-resistant alleles (Mouton et al. unpublished data). This further suggests that the mitochondrial diversity could also be associated with nuclear variation and possible genetic isolation. This last point is important because species definition by Dinsdale et al. (2010) is based on *mtCOI* divergence, which means that some so far unidentified, genetically isolated entities may exist at a finer phylogenetic scale. In a recent study, Chu et al. (2012) found five haplotypes within the MED species by *mtCOI* sequencing. However, the PCR-RFLP method they developed for the *mtCOI* gene using the restriction enzyme *VspI* only distinguished one haplotype, which corresponds to the commonly known Q1 biotype, but the method was unable to detect the other four. The tool we developed here can be used to identify four out of the five *mtCOI* haplotypes described in the MED species. It can thus detect diversity at a very fine scale, which may make it very useful in population studies and may have major implications for pest management programs.

Levels of resistance and resistance mutation frequencies to insecticides differ between *B. tabaci* species (Horowitz et al. 2005; Alon et al. 2006, 2008; Wang et al. 2010) but also within species as has been observed within the MED species (Mouton pers.com) Therefore, to be effective, strategies developed to control this pest must differ according to the local *B. tabaci* composition and the chemical classes of insecticides used. Monitoring the evolution of genetic diversity is, thus, clearly required for effective control strategies of this pest. The tool developed here has already been successfully used for describing the diversity of *B. tabaci* genetic groups in western Africa (Gnankiné et al. 2012; Gnankiné et al. 2013; Mouton, pers.com) and could be useful to monitor the dynamics of *B. tabaci* populations in relation to insecticide resistance in countries, like west African countries, where insecticides are widely used and lead to high levels of resistance (Houndété et al. 2010).

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