Evolutionary biology of *Fusarium oxysporum* f.sp. *cubense*

by

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I can do everything through Him who gives me strength.

PHILIPPIANS 4:13

Declaration

I, the undersigned, declare that the work in this thesis is the result of my own investigation and that it has not previously in its entirety or partially been submitted for a degree at any other University.

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PREFACE

The genus *Fusarium* is one of the most adaptive and versatile genera in the fungal kingdom. One of the best known species within the genus, *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen, consists of more than 150 *formae speciales* that are defined by their pathogenicity to different hosts, and a diverse range of non-pathogenic members. *Fusarium oxysporum* f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*), the causal agent of Fusarium wilt of banana (Panama disease), is regarded as one of the economically most important and destructive *forma specialis* of *F. oxysporum*. Apart from regions bordering the Mediterranean, Melanesia, Somalia and some islands in the South Pacific, Fusarium wilt of banana has been reported in all banana-growing regions of the world.

Foc is particularly diverse for an asexually reproducing fungus, and consists of three races and 24 vegetative compatibility groups (VCG). In **Chapter 1**, the current knowledge on the taxonomy, biology and evolution of *F. oxysporum* and *Foc* is summarized. It explains why evolution is taking place, the forces that bring about change in a pathogen, and how diversity and evolution can be measured. Phenotypic markers for assessing diversity in *Foc* include virulence to a set of differential host cultivars and the placement of isolates into VCGs. Several DNA-based markers have been used to study genotypic diversity in *Foc*, such as random amplification of polymorphic DNA, DNA amplified fingerprinting analysis, amplified fragment length polymorphisms and gene sequencing. Such markers consistently divided *Foc* into two major clades and between five and nine clonal lineages that more or less reflect the grouping of *Foc* according to VCG.

Despite extensive research on the phenotypic and genotypic diversity in *Foc*, the relationship between the two clades and the lineages representing the banana pathogen has not been fully elucidated. For this reason, the relatedness among and within VCGs of *Foc*, as well as their relationships with other *formae speciales* and non-pathogenic members of *F. oxysporum*, were investigated. In **Chapter 2**, a worldwide collection of *Foc* was used to investigate the morphological, pathogenic, genetic and phylogenetic relationships among isolates. To determine the potential of *Foc* to reproduce sexually, *MAT*-1 and *MAT*-2 idiomorphs were identified using mating type-specific makers, and sexual crosses were conducted among isolates of opposite mating-types. A diagnostic PCR-restriction fragment length polymorphism procedure was developed to rapidly and accurately differentiate the phylogenetic lineages of *Foc*.

Control of Fusarium wilt is not always possible once the disease becomes established in banana plantations. Resistant cultivars can be grown, but replacement varieties for Cavendish bananas are often not acceptable to markets. Control of *Foc*, therefore, relies heavily on early detection of the pathogen and the isolation of *Foc*-infested fields. In **Chapter 3**, a molecular marker for the quick and accurate detection of *Foc* 'subtropical' race 4 was developed. This marker will be particularly valuable to the South African banana industry as VCG 0120 is the only variant of the pathogen present in the country. Gene regions were screened for polymorphisms, and PCR primer pairs developed and tested to distinguish *Foc* 'subtropical' race 4 from other *Foc* VCGs, non-pathogenic *F. oxysporum* and other *formae speciales* of *F. oxysporum*.

The phylogenetic relationships between individuals belonging to the same fungal species are often difficult to determine because of a lack of variation within gene sequences of closely related species. Microsatellite regions, however, have high mutation rates and could differentiate closely related species, or subpopulations within species. In **Chapter 4,** nine microsatellite markers were applied to a global collection of *Foc* to measure gene and genotypic diversity, their reproductive mode, population differentiation, gene flow and population structure.

CHAPTER 1

Biology and evolution of *Fusarium oxysporum*, with specific reference to the banana wilt pathogen: A review

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INTRODUCTION

The genus *Fusarium* is regarded as one of the most adaptive, versatile and pioneering genera in the fungal kingdom. One of its economically more important members is *Fusarium oxysporum* Schlechtendahl emend. Snyder and Hansen that consists of both pathogenic and non-pathogenic strains (Gordon & Martyn, 1997). The pathogenic strains are divided into special forms or *formae speciales* according to the crop species that they cause disease to, and into races according to crop cultivar specificity. To date more than 150 different *formae speciales* have been described (Hawksworth *et al.*, 1995; O'Donnell & Cigelnik, 1999; Baayen *et al.*, 2000). Although non-pathogenic strains of *F. oxysporum* are widespread and genetically more diverse than their pathogenic counterparts (Gordon & Okamoto, 1992), they are not as well studied (Edel *et al.*, 2001). Alabouvette (1990) indicated that non-pathogenic *Fusarium* strains are among the biological factors that influence the effectiveness of Fusarium wilt suppressive soils (Alabouvette *et al.*, 1990). It was only after this discovery that native non-pathogenic isolates were considered of any value (Elias *et al.*, 1991; Katan *et al.*, 1994).

The great diversity in *F. oxysporum* raises questions about its evolutionary biology, and whether the fungus represents a single species or a species complex (Kistler, 1997; O'Donnell & Cigelnik, 1997), and various techniques have been utilized to gain more insight into this question. Earlier techniques used in *F. oxysporum* to assess variation include assigning isolates to vegetative compatibility groups (Buxton, 1962; Puhalla, 1985), determining virulence to crops (Waite & Stover, 1960; Su *et al.*, 1986; Brake *et al.*, 1990; Pegg *et al.*, 1993; Pegg *et al.*, 1994; Ploetz, 1994), applying isozyme markers as indicators of genetic relationships (Ploetz, 1990), grouping of isolates according to volatile compound production (Moore *et al.*, 1991), and measuring chromosome number and genome size differences (Boehm *et al.*, 1994). More recently, DNA-based techniques such as, random amplified polymorphic DNA (RAPD) profiles (Bentley *et al.*, 1994), amplified fragment length polymorphisms (AFLPs) (Groenewald *et al.*, 2006b), short sequence repeats (SSR) or microsatellite markers (Brave *et al.*, 2001; Bogale *et al.*, 2006), and DNA sequence data (O'Donnell, 1998) were used to determine diversity within *F. oxysporum*. The purpose of this review is to summarize current knowledge of the evolution and biology of *F. oxysporum*, with specific reference to strains that attack bananas.

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THE FUSARIUM WILT PATHOGEN, *FUSARIUM OXYSPORUM*

Taxonomic classification

The genus *Fusarium* was first introduced by Link in 1809 (Leslie & Summerell, 2006), with its primary character being the presence of canoe- or banana-shaped conidia. The genus, however, was only properly classified when Wollenweber and Reinking (1935) divided approximately 1000 described *Fusarium* spp. into 16 sections and reduced it to 65 spp. Since 1935, various attempts have been made to improve the Wollenweber and Reinking classification system. These efforts often led to *Fusarium* taxonomists being labelled as either "splitters" or "lumpers". Where the splitters' approach was complex with the description of too many species, many of which were described considering a single isolate, the lumpers' approach was over-simplified and was based on a few general characteristics only. Snyder and Hansen (1940) were regarded as the ultimate lumpers, as they compressed Wollenweber and Reinking's 16 sections into nine species. The species contained in the section Elegans were merged into a single species, called *F. oxysporum. Fusarium oxysporum* and *Fusarium solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen, are the only *Fusarium* spp. described by Snyder and Hansen (1940) that remained unchanged, although it is clear that both represent more than one species (Baayen *et al.*, 2000; Leslie & Summerell, 2006). Snyder and Hansen's classification system was eventually replaced by a system proposed by Nelson *et al.* (1983) that presented a more complicated taxonomy with more recognised species. Recently, Leslie and Summerell (2006) produced a laboratory manual in which 70 species of *Fusarium* are described. This manual represents the first *Fusarium* classification system where morphological characters were considered together with genetic and phylogenetic information for the description of new species.

The morphological taxonomy of species in the genus *Fusarium* is based primarily on the form and abundance of their asexual reproductive structures and on their cultural characteristics. Morphological characteristics include the shape and size of the macroconidia and their basal foot cells, the presence and abundance of microconidia and chlamydospores, and the nature of the phialides from which conidia are produced (Booth, 1971; Nelson *et al.*, 1983; Nelson, 1991; Gordon & Martyn, 1997; Edel *et al.*, 2000; Lorens *et al.*, 2006). Cultural characteristics include colony texture, colour and sometimes aroma (Lorens *et al.*, 2006). The subdivision of isolates into different morphological *Fusarium* spp., however, is complex and difficult. Individuals of the same species sometimes vary considerably (Snyder & Hansen, 1940; Gaudet *et al.*, 1989), as some characters are not stable and can easily be altered by varying environmental conditions (Nelson, 1991). There are also not enough morphological characters to easily define new species.

Fusarium oxysporum is one of the oldest and morphologically most diverse *Fusarium* spp. It is, therefore, not surprising that many researchers regard *F. oxysporum* as a species complex rather than a single species (Kistler, 1997; O'Donnell & Cigelnik, 1997; Baayen *et al.*, 2000). The phylogenetic species concept which overcomes some limitations with regards to the morphological species concept by providing a wealth of variable characters, have both contributed to and complicated designation of new species (Leslie *et al.*, 2007). Based on a multi-gene phylogeny of *F. oxysporum* using the translation elongation factor 1α (TEF) and mitochondrial small subunit ribosomal RNA (MtSSU), O'Donnell *et al.* (1998) suggested that *F. oxysporum* consists of at least five phylogenetic lineages. Various other authors have confirmed the phylogenetically diverse nature of *F. oxysporum* and its *formae speciales* by means of AFLP and multi-gene sequence analyses (Baayen *et al.*, 2000), SSR, AFLP and TEF sequence analyses (Bogale *et al.*, 2006), and AFLPs (Groenewald *et al.*, 2006b).

The biological species concept where sexual reproduction can be induced to occur under controlled laboratory conditions has proven invaluable for many *Fusarium* spp. This concept, however, is not taxonomically useful for species such as *F. oxysporum*. Sexual reproduction in ascomycetes is determined by the genes encoded at the mating type (*MAT*) loci (Menzenburg & Glass, 1990; Kronstad & Staben, 1997). In order for mating to occur, both *MAT* loci, namely *MAT*-1 and *MAT*-2, need to be present. In homothallic fungi these loci are present in a single individual, thus conferring self-fertility. In heterothallic fungi, however, two individuals of opposite mating type are required for sexual reproduction. In other words, isolates can only be sexually compatible if one individual harbours the *MAT*-1 locus while the other harbours the *MAT*-2 locus. Based on sexual crosses between *Fusarium* isolates, a number of mating populations or biological species have been described. For example, the *Gibberella fujikuroi* (Sawada) Ito it & K. Kimara. species complex currently contains ten heterothallic mating populations (Leslie *et al.*, 2007) while the *F. solani* complex harbours various heterothallic and homothallic mating populations (Leslie & Summerell, 2006). Although no sexual structures are known for *F. oxysporum*, the *MAT* loci of its members

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appear to be similar in structure and function to those of sexually reproducing *Fusarium* spp. (Arie *et al.*, 2000; Yun *et al.*, 2000). It is, therefore, speculated that the absence of sexual reproduction may be due to mutations in the genes encoded at these loci (Arie *et al.*, 2000; Yun *et al.*, 2000).

Formae speciales:

Pathogenic members of *F. oxysporum* cause wilt diseases to a number of agronomically important crops. Isolates that attack the same crop are considered to belong to the same *forma specialis*. Isolates of *F. oxysporum* that are pathogenic to bananas and plantains are called *F. oxysporum* f.sp. *cubense* (E.F. Smith) Snyder and Hansen (*Foc*) (Waite & Stover, 1960). Most *formae speciales* are pathogenic to a single crop, such as *F. oxysporum* f.sp. *dianthi* to carnations and *F. oxysporum* f.sp. *vasinfectum* to cotton, but in some instances a *forma specialis* can attack more than one crop. Cafri *et al.* (2005), for instance, found cross-pathogenicity of *F. oxysporum* f.sp. *cucumerimum*, a pathogen of cucumber, towards melon. This pathogenicity, however, was not as aggressive as to the original plant species and not all the isolates included in their study caused disease to melon. Furthermore, no disease was observed when cucumber plants were challenged with *F. oxysporum* f.sp. *melonis*, the pathogen of melon (Cafri *et al.*, 2005).

The underlying genetic basis of host specificity in *F. oxysporum* is unclear (Baayen *et al.*, 2000). It would, therefore, be premature to assume that all individuals in a specific *forma specialis* are closely related or derived from a common ancestor (O'Donnell *et al.*, 1998). Koenig *et al.* (1997) showed that some of the individuals of the banana wilt pathogen were more closely related to isolates of *F. oxysporum* f.sp. *niveum*, a pathogen of watermelon, than to each other. Baayen *et al.* (2000) also reported a similar close association between *F. oxysporum* f.sp. *tulipae* and *F. oxysporum* f.sp. *asparagi*, as well as between *F. oxysporum* f.sp. *gladioli* and *F. oxysporum* f.sp. *dianthi.* These findings are not completely unexpected as a *forma specialis* describes a group of isolates sharing a physiological capability which may sometimes be associated with genetically diverse isolates (Correl, 1991). A *forma specialis* designation, therefore, does not necessarily form part of a taxonomic hierarchy (Gordon & Martyn, 1997).

Not all isolates of *F. oxysporum* can be classified as pathogenic. Non-pathogenic strains of *F. oxysporum* are cosmopolitan and can be found in native soils, water and plant residues. They

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have also been isolated from various plant species with no external or internal wilting symptoms (Armstrong & Armstrong, 1948; Wilson, 1995). Non-pathogenic *F. oxysporum* isolates can be found inside plant roots and in the root rhizosphere (Elias *et al.*, 1991; Olivain & Alabouvette, 1999). Individuals that live asymptomatically inside roots and other plant tissue are known as endophytes (Agrios, 2005). These isolates are non-pathogenic, either because they lack the ability to enter the vascular tissue, or because plants in their native environment are able to rapidly counteract infection (Gao *et al.*, 1995). Non-pathogenic strains of *F. oxysporum* could possibly serve as a source for new pathogenic strains (Gordon & Okamoto, 1992). Apple and Gordon (1994) reported a close phylogenetic relationship between some pathogenic *F. oxysporum* f.sp. *melonis* and non-pathogenic *F. oxysporum* soil isolates.

Race:

Races in *F. oxysporum* are recognized by their pathogenicity to a differential set of cultivars. Although most *formae speciales* of *F. oxysporum* are grouped into different races, some exceptions occur where different races have not yet been reported for example within *F. oxysporum* f.sp. *radicis-lycopersici* (Di Primo *et al.*, 2001). The subdivision of pathogens into races could be relatively simple, with a single cultivar defining a single race, or it could be more complex, such as when several cultivars are hosts to the same race. An example of a pathogen with a simple race structure is *F. oxysporum* f.sp. *lycopersici* where the tomato cultivar and pathogen have a gene-for-gene interaction. In this instance, race-specific virulence genes *I*, *I2* and *I3* of the pathogen relates to the avirulence genes of the cultivar that defines races 1, 2 and 3, respectively (Mes *et al.*, 1999; Kawabe *et al.*, 2005). Most other *formae speciales*, however, have a much more complex race structure or system.

The classification of individuals into races according to host cultivars can sometimes be problematic. This is because pathogenicity tests are often influenced by variables such as temperature, host age and method of inoculation (Correl, 1991). Pathogenicity tests conducted in different laboratories around the world, therefore, could easily generate different results (Davis *et al.*, 1996). Field testing is also time consuming and expensive and appropriate testing sites are often not readily available. In the case of *F. oxysporum* f.sp. *dianthi*, a pathogen of carnation, only six of the eight races can still be used to type new pathogens, as cultivars of lines 3 and 7 are no longer available (Migheli *et al.*, 1995).

Foc is subdivided into four races (Waite & Stover, 1960; Su *et al.*, 1986; Moore *et al.*, 1993). Race 1 individuals attack Gros Michel, Silk, Apple, Lady Finger and Latundan cultivars; race 2 attacks Bluggoe and other plantains; and race 3 attacks *Heliconia* spp. (Su *et al.*, 1977; Ploetz, 1988). Race 3 does not cause disease to banana and is therefore not considered part of the *Foc* race structure any more (Ploetz, 2005b). *Foc* race 4 is pathogenic to Cavendish bananas and all cultivars susceptible to races 1 and 2. Individual isolates belonging to race 4 are further subdivided into 'tropical' and 'subtropical' race 4 isolates, based on the ability to cause disease to Cavendish bananas under tropical and subtropical environmental conditions, respectively (Ploetz, 1990; Viljoen, 2002).

Vegetative compatibility:

Vegetative compatibility has been exploited by many researchers to explore diversity in and among the *formae speciales* of *F. oxysporum*, and is based on the formation of a stable heterokaryon between compatible mutants. Buxton (1962) was first to demonstrate heterokaryon formation between two mutants of *Fusarium*. The technique required ultra-violet (UV) irradiation to produce mutants, and the process was considered very laborious (Katan, 1999). Puhalla (1985) then demonstrated heterokaryon formation by the complementation of nitrate-reducing (*nit*) mutants on minimal medium (Puhalla, 1985). Normal growth of *nit*-mutants on minimal medium is thin but once two compatible individuals come together, dense mycelial growth reveals formation of a heterokaryon. The mutants generated using Puhalla's (1985) approach are readily recovered and stable, therefore making it the preferred method for vegetative compatible group (VCG) determination (Katan, 1999). One major advantage of the use of vegetative compatibility instead of race classification is that VCGs involve only the pathogen, and not the host's genes.

Vegetative compatibility is controlled by at least seven vegetative or heterokaryon incompatibility loci, designated as either *vic* or *het*, in *F. oxysporum* (Ploetz, 1999). Such high numbers of *vic* loci have also been reported for *F. circinatum* (Nirenburg & O'Donnell emend. Britz, Coutinho, Wingfield & Marasas) that has at least six (Gordon *et al.*, 2006) and *F. moniliforme* (syn. *F. verticillioides* (Saccardo) Nirenberg) that has at least ten (Leslie, 1991). For two individuals to be vegetatively compatible and to form a stable heterokaryon, they need to share a common allele at each of those loci (Correll, 1991). One could, therefore, assume that the rest of the genomes of the interacting individuals of asexual species that have not undergone recent sexual reproduction is also very similar (Leslie, 1990; Leslie, 1993).

Although VCGs are good phenotypic characters for assessing diversity within a population, genetic relationships between VCGs must be assessed by other means. The latter is especially important as a single mutation at the individual *vic*/*het* loci would for example allow the placing of closely related individuals in different VCGs (Bentley & Dale, 1995; Bentley *et al.*, 1998).

In most cases, the relationship between VCG and race is complex, with multiple VCGs comprising a single race. For example, Katan *et al.* (1993) found that VCG 0138 of *F. oxysporum* f.sp. *melonis* in Israel was associated with races 0, 1 and 1-2, whereas VCG 0135 contained isolates of race 0 and 2. Similar findings have also been reported for *F. oxysporum* f.sp. *lycopersici* (Elias *et al.*, 1991; Marlatt *et al.*, 1996; Mes e*t al.*, 1999; Cai *et al.*, 2003), *F. oxysporum* f.sp. *dainthi* (Aloi & Baayen, 1993; Kalc Wright *et al.*, 1996; Migheli *et al.*, 1998) and *F. oxysporum* f.sp. *melonis* (Jacobson & Gordon, 1990; Jacobson & Gordon, 1991; Katan *et al.*, 1993; Elena & Pappas, 2006). A notable exception is *F. oxysporum* f.sp. *vasinfectum* that has a one-on-one relationship between VCG and race (Katan & Katan, 1988; Assigbetse *et al.*, 1994).

Twenty-four VCGs have been described for *Foc* (Ploetz and Correll, 1988; Moore *et al.*, 1993; Bentley & Dale, 1995; Katan, 1999; Katan & Di Primo, 1999). A few of these have a cosmopolitan distribution and are found in Asia, the Americas and in Africa, while others are limited to geographical regions such as Asia (VCG 0121, 0122, 0124, 0126, 01217, 01218, 01219 and 01221, 01223, 01224), Australia (VCG 01211, 01220) and Africa (VCG 01212, 01214, 01217 and 01222). It was suggested that VCG 01214, isolated from Enset plants in Malawi, might have evolved in Africa (Koenig *et al.*, 1997). Some VCGs have also shown to be bridging or cross-compatible and are, therefore, referred to as VCG complexes. These include VCGs 0120 and 01215, VCG 0124 and 0125, as well as VCG 0129 and 01211 (Brake *et al.*, 1990). VCGs 0120, 0129, 01211, 0122 and 01215 cause disease to Cavendish bananas in the subtropics and are referred to as *Foc* 'subtopical' race 4 isolates (Ploetz & Correll, 1988; Brake *et al.*, 1990; Moore *et al.*, 1993; Bentley *et al.*, 1998). In the tropics, isolates from these VCGs do not attack Cavendish bananas and are, therefore, referred to as *Foc* race 1. VCGs that cause disease to Cavendish bananas in the tropics include VCG 01213 and VCG 01216, and are referred to as *Foc* 'tropical' race 4 (Bentley *et al.*, 1998). *Foc* 'tropical' and 'subtropical' race 4 isolates are related, but genetically distinct from each other (Ploetz, 1990).

Growth, survival and spread

The optimal temperature for growth of *F. oxysporum* is between 25 and 28ºC, and no growth will occur above 33ºC and below 17ºC (Cook & Baker, 1983). According to Wilson (1946), acid soil (pH 4.2) supports *Fusarium* growth. A recent study by Groenewald *et al.* (2006a), however, showed that *Foc* grows slowly at pH 4, and more rapidly at pH 6 or 7 than pH 4 in culture (Groenewald *et al.*, 2006a). Only some isolates grew at pH 8, thus, the authors argued that ecological factors in the soil, rather than the ability of *F. oxysporum* to grow better at a low pH might be responsible for the increase of Fusarium wilt at lower pH (Groenewald *et al.*, 2006a). Reduction of disease incidence because of high soil pH could be due to the domination of disease-suppressive bacteria in the root rhizosphere or because of unavailability of essential micronutrients (Jones *et al.*, 1989). Some of the most essential nutrients for fungal growth, sporulation and virulence are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, magnesium, sulphur, iron, manganese, molybdenum and zinc (Steinberg, 1950). Depletion of these nutrients will result in a less aggressive pathogen that is unable to grow and sporulate.

Fusarium oxysporum survives in soil and crop residues for several decades during its saprophytic phase (Beckman & Roberts, 1995). Smith *et al.* (2001) were able to isolate *F. oxysporum* f.sp. *vasinfectum* isolates in soil ten years after the last planting of cotton. Although mycelia and asexual spores have been found in soil, the species survives by the colonization on roots of non-host crops or formation of chlamydospores (Beckman & Roberts, 1995). These survival spores are usually one- or two-celled and thick-walled, and form on older mycelia just before plant death. Macroconidia that are produced in the host plant may also be converted or structurally modified into chlamydospores once the plant dies, and the chlamydospores are released back into the soil or preserved in crop residues (Griffin, 1981). Chlamydospores will germinate only after it comes into contact with plant roots or when stimulated by nutrients such as carbon, nitrogen and amino acids (Stover, 1962; Griffin, 1981; Beckman & Roberts, 1995). This usually happens when healthy plants are planted in infested soils.

Humans play an important role in the development or prevention of Fusarium wilt diseases. The most important contributing factor to new outbreaks of the disease is the spread of the pathogen to unaffected fields in or on planting material, soil and farm equipment, or in irrigation water (Green, 1981). *Fusarium oxysporum* can also be disseminated for short distances by wind, rain splash and root contact. Its movement from farm to farm, country to country and across continents is, therefore, primarily because of human involvement.

Pathogenesis

Invasion of plants by *F. oxysporum* is complex and follows three main processes, namely adhesion, penetration, and colonization. Adhesion to the root surface is not a specific process, as both pathogenic and non-pathogenic *F. oxysporum* isolates can adhere to host and non-host plants (Bishop & Cooper, 1983a). Penetration can take place directly, but *F. oxysporum* enters plant tissue mostly through wounds. The most common site for direct penetration is the tip or near the tip of both tap and lateral roots (Griffin, 1981). There is no apparent difference between penetration by pathogenic and non-pathogenic isolates, but non-pathogens are unable to invade the cortex, or their presence in the cortex and xylem is limited (MacHardy & Beckman, 1981). The formation of physical barriers and the production of phenolic compounds appear to be the deciding factors in stopping non-pathogenic isolates to further penetrate the host (MacHardy & Beckman, 1981).

The mycelium of pathogenic *F. oxysporum* isolates will advance inter- and intracellularly through the root cortex and penetrate the primary and secondary xylem through the pits (Bishop & Cooper, 1983b). Mycelia may also move laterally into adjacent vessels through the pits. Once in the xylem vessels, spores, predominantly microconidia are produced and the pathogen will spread upwards until they encounter an end wall or perforation plate (Griffin, 1981; Bishop & Cooper, 1983b). The spores that are unable to move further will germinate and the newly formed mycelia will penetrate the barriers (Beckman *et al.*, 1961; Beckman *et al.*, 1962). After the barriers are penetrated, more microconidia are produced and these spores proceed upwards until another end wall or perforation plate is encountered and the process of germination, penetration and spore production is repeated.

Invasion of the vascular system of the plant by *F. oxysporum*, together with host defence responses, lead to symptom development (Beckman *et al.*, 1961; Beckman *et al.*, 1962; Stover, 1962). To localize infection, the host plant produces vascular gels. The production of cell wall degrading enzymes by the pathogen, such as pectin methylesterase, polygalacturonase and cellulase, in turn, leads to the weakening of vascular gels. It has been proposed that the degraded vascular gels might contribute to wilt symptoms by blockage of the vascular vessels.

Fusarium wilt symptoms are characterised by the rapid wilting and browning of the older leaves, followed by the younger leaves and shoots (Beckman *et al.*, 1961; Stover, 1962). Dying of the leaves eventually leads to plant death. A brown ring will form internally in the vicinity of the vascular bundles (MacHardy & Beckman, 1981). After plant death, the mycelia move towards the surface of the plant where they form chlamydospores that are, once again, released into the soil.

Control

Restriction of the introduction of the pathogen by quarantine and sanitation methods, and the early detection and isolation of the disease, are the only means whereby Fusarium wilt disease spread can be effectively prevented. Once the disease is established, control by means of chemical, biological and cultural practices becomes extremely difficult, if not impossible. Using disease resistant planting material then remains the only effective way of controlling Fusarium wilt diseases (Moore *et al.*, 1999).

THE EVOLUTION OF *FUSARIUM OXYSPORUM* AND *FOC*

Why fungi evolve and diversify

Fungi, like any living organism, have the ability to change in response to a changing or ‘new’ environment. This is due to selection pressure that the ‘new’ environment enforces on the organism (McDonald, 1997), and only individuals that adapt will survive. ‘New’ environments can arise due to natural causes or human intervention. Examples of human intervention include the introduction of a pathogen into a new area, the introduction of a new host into an area where the pathogen is already present, and the planting of large-scale monocultures (McDonald, 1997).

The rate at which a pathogen adapts to its environment directly reflects the diversity that exists within the population (McDonald & McDermott, 1993). Genetic variation, therefore, may be used to predict how long an effective control measure will last (McDonald & McDermott, 1993; McDonald & Linde, 2002). The more diverse the pathogen population, the greater the gene pool it can sample from to find the appropriate genotype/phenotype that will survive in the new or changing environment. Once this new genotype/phenotype, that could be a new race or VCG, is selected, a phase of clonal reproduction usually follows. This will ultimately lead to inoculum levels that, without intervention, can possibly lead to an epidemic.

How fungi evolve

Change in pathogens may be brought about by five evolutionary forces: Natural mutation, natural selection, genetic drift, gene flow and mating system (McDonald & Linde, 2002). Natural mutations are changes that take place in the DNA base sequence of the genome. These are rare and change the genetic constitution of a population slowly (McDonald & Linde, 2002). Natural selection and random genetic drift are processes by which changes in genotype and gene frequency occur over time. Natural selection favours genotypes with a reproductive advantage, while random genetic drift is a change in gene frequency within a population that takes place due to chance alone. These processes will ultimately decrease genetic diversity and favoured genotypes might become fixed. Gene flow refers to the movement of gametes, individuals or populations from one area to the next, thereby preventing fixation in the pathogen population. Gene flow causes a decrease in genetic differentiation between populations and an increase in genetic diversity within populations (McDonald & Linde, 2002). It may, therefore, limit or promote a population’s ability to persist in local conditions, and could change the reproductive mode of the organism (Taylor *et al.*, 1999). The final evolutionary force to bring about change in pathogens is their mating system, whether sexual or asexual. The consequence of sexual reproduction and meiotic recombination is that the genes of parental strains are rearranged into new combinations in the progeny. Asexual organisms, such as *F. oxysporum*, in turn evolve by means of neutral mutations, but also through the processes of parasexuality and heterokaryosis (Buxton, 1964; Kuhn *et al.*, 1995). Parasexuality is a non-sexual mode of genetic exchange without meiosis or the development of sexual structures, and is unique to some fungi (Buxton, 1964; Kuhn *et al.*, 1995). Heterokaryosis is a form of genetic exchange initiated by fusion of vegetative hyphae (anastomosis) between individuals with very similar genomes (Kuhn *et al.*, 1995).

Four evolutionary models have been described for the relationship between VCG and *formae speciales* in *F. oxysporum* (Kistler & Momol, 1990). The first is host specialization followed by infrequent genetic isolation by way of vegetative incompatibility. In other words, the pathogen and host co-evolve (Burdon, 1992). The second is host specialization with more frequent genetic isolation. This model will be supported with more sequence variation within *formae speciales* and less within VCG. Then, infrequent genetic isolation followed by specialization. This model could describe the loss of a sexual stage, and the model would be supported if members of different *formae speciales* and VCGs were genetically equally distant, regardless of host relationships. The last model is frequent genetic isolation followed by speciation. All of the above-mentioned models fit the diverse relationships between the various *formae speciales* and their VCGs of *F. oxysporum*.

How evolution and diversity is measured

Various phenotypic techniques have been used to measure diversity within populations of *F. oxysporum* (Ploetz, 1999). These include characterizing vegetative compatibility groups within the same *forma specialis*, pathogenecity testing, isozyme markers to group different amino acid patterns, grouping isolates according to volatile compound production and, lastly, measuring chromosome number and genome size differences. However, a major disadvantage when using these techniques is that they utilize characters with limited variation or characters under strong selection pressure, for example virulence and resistance to host plants. This will lead to an underestimation of diversity and to biased results (McDonald & McDermott, 1993). The development of DNA markers to examine population dynamics of plant pathogens enabled us to use techniques that measure and quantify diversity much more efficiently by providing levels of precision not previously available (McDermott & McDonald, 1993). Most of these techniques measure variety in both coding and non-coding regions and are, therefore, more variable (McDonald & McDermott, 1993). The markers include DNA sequence information, RAPD profiles, restriction fragment length polymorphisms (RFLP), AFLP and SSRs or microsatellite markers.

DNA sequence analysis:

DNA sequence analysis makes it possible to assess relationships between individuals at the nucleotide per nucleotide level. Sequence data are relatively easy to generate, robust and inexpensive. Depending on the genomic region targeted, these data can potentially be used

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for studies at all taxonomic levels, especially the species and subspecies levels. For this purpose, sequence data are usually explored for indels (insertions or deletions) or single nucleotide polymorphisms (SNPs) to be used as possible species- or group-specific markers for diagnostics. Alternatively, sequence information can be used directly using similarity-based DNA comparisons and phylogenetic analyses. For DNA analysis of a specific genomic region, closely related individuals are normally expected to have more similar sequences, although this is not often a true reflection of their phylogenetic histories (Goldstein *et al.*, 1995; Goldstein *et al.*, 1999). Therefore, to obtain a reliable classification or a realistic idea of the diversity among a group of isolates, phylogenetic trees are inferred. These trees are usually robust and well-resolved only when combined information for a number of genes or regions are included in the analysis. Gene genealogies of the different regions will usually be congruent for clonal populations and incongruent for recombining populations (Taylor *et al.*, 1999). It could also be used to identify phylogenetic species, which seems to be a good alternative to the biological species concept (Taylor *et al.*, 1999).

The phylogenetic species concept has effectively been used to help resolve several species of *Fusarium* (Nirenberg & O'Donnell, 1997; Nirenberg & O'Donnell, 1998; Zeller *et al.*, 2003; O'Donnell *et al.*, 2004b) and to resolve groups within the *F. oxysporum* complex. Bogale *et al.* (2006) sequenced the TEF and MtSSU region of 32 *F. oxysporum* isolates from Ethiopia and separated these isolates into three groups. These groups corresponded with the three clades of *F. oxysporum* previously defined by O'Donnell *et al.* (1998). Skovgaard *et al.* (2002) divided a global population of *F. oxysporum* f.sp. *vasinfectum* into four lineages with the use of TEF, MtSSU, nitrate reductase and phosphate permase gene sequencing. They could confirm that this *forma specialis* is not monophyletic, having at least two independent or evolutionary origins. Isolates in their Lineage I that comprised of isolates representing race 3 and 5 evolved independently from the other races of *F. oxysporum* f.sp. *vasinfectum*. Kawabe *et al.* (2005) confirmed the polyphyletic nature of *F. oxysporum* f.sp. *lycopersici* with the use of IGS, *MAT* and endopolygalacturonase sequence analyses. The isolates used in their study, which represents a worldwide collection of *F. oxysporum* f.sp. *lycopersici*, formed three distinct evolutionary lineages, each containing single or closely related VCGs. With respect to *Foc*, O'Donnell *et al.* (1998) used a multi-gene phylogenetic approach to show that there might be as many as five possible phylogenetic species.

RAPDs:

RAPDs is a PCR-based technique that relies on the amplification of various regions of the genome. Short oligonucleotide sequences are used as primers to amplify multiple bands of different lengths by binding to complementary sequences in the genome by chance (Welsh & McClelland, 1990; Williams *et al.*, 1990). These bands are then separated using polyacrylamide or agarose electrophoresis to obtain fingerprints that can be compared among individual isolates. Advantages of this technique include that no prior knowledge of sequence data is needed, that amplification sites are distributed throughout the genome, and that low quantities of DNA template are required. Disadvantages involve poor reproducibility between laboratories, and the amplification of DNA from any source that the short primers can identify (McDonald, 1997). It is, therefore, of great importance that extra precautions are taken to avoid contamination. RAPDs are dominant markers and band profiles can, therefore, not be interpreted as alleles and loci (McDonald, 1997). It only confirms clonal or genetically isolated populations.

RAPDs have been applied to study populations of various *formae speciales* of *F. oxysporum*. Bentley *et al.* (1994), for instance, found identical or near identical haplotypes for each VCG of *Foc* from an Australian population. In 1998, Bentley *et al.* (1998) also used DNA amplified fingerprinting (DAF) analysis, a technique closely related to RAPDs, to identify 33 genotypes among 241 *Foc* isolates, and separated these into nine clonal lineages. VCGs of *F. oxysporum* f.sp. *gladioli* were separated into three groups using RAPDs (Mes *et al.*, 1999), while Manualis *et al.* (1994) was able to separate *F. oxysporum* f.sp. *dainthi* from non-pathogenic *F. oxysporum* isolates. Assigbetse *et al.* (1994) also grouped a global collection of *F. oxysporum* f.sp. *vasinfectum* isolates according to race and geographic origin using RAPDs.

AFLPs:

For AFLP analysis, two restriction enzymes, usually a frequent and a rare cutter, are used to digest genomic DNA. Target-specific adapters are subsequently ligated to the digested genomic DNA, followed by two individual PCR amplification reactions, called pre-amplification and selective amplification. The adapter-ligated fragments are then size-determined on a polyacrylamide gel (Vos *et al.*, 1995). AFLPs can be used for population diversity studies by comparing the fingerprint of individual isolates. The technique is highly reproducible, amenable to automation, and no sequence data for primer construction is required. It has the advantage over RAPDs that more loci are screened and longer primers are used, making AFLPs more reproducible (McDonald, 1997). Some disadvantages, however, are that purified, high molecular weight DNA is required, and band profiles cannot be interpreted in terms of loci and alleles, therefore similar sized fragments may not be homologous.

Baayen *et al.* (2000) utilized AFLP and DNA sequencing to test the monophyletic origin of eight *formae speciales* of *F. oxysporum*. All isolates included in their study grouped into three clades that correspond to the three clades earlier described by O'Donnell *et al.* (1998). The monophyly of two *formae speciales*, namely *F. oxysporum* f.sp. *lilli* and *F. oxysporum* f.sp. *tupipae*, as well as multiple evolutionary origins for *F. oxysporum* f.sp. *asparagi*, *F. oxysporum* f.sp. *dainthi*, *F. oxysporum* f.sp. *gladioli*, *F. oxysporum* f.sp. *lini* and *F. oxysporum* f.sp. *opuntiarum,* were confirmed. O'Donnell *et al.* (2004a) used AFLP and DNA sequencing to do comparative and genetic diversity analysis among clinically important *F. oxysporum* isolates from San Antonio and Houston Texas, Maryland and Washington. The authors concluded that the majority of the isolates were associated with a single clonal lineage that was recently dispersed. Groenewald *et al.* (2006b) identified seven genotypic groups from a global collection of *Foc* with the use of AFLPs and confirmed the pathogens polyphyletic nature.

Microsatellite markers or SSRs:

SSR markers are used to study the evolution and diversification of pathogen populations that are closely related. The technique involves comparison of highly polymorphic di-, tri- or tetra-nucleotide repeats that are scattered throughout the genome of most organisms (Queller *et al.*, 1993). The up- and downstream regions of these polymorphic repeats are usually highly conserved, thus allowing for primer development. PCR products are subsequently separated and analysed using a DNA sequencer. A major disadvantage of SSRs is that markers/primer pairs are expensive and time consuming to develop. Advantages of this technique are that SSRs are usually present in large numbers, evenly spaced throughout the genome, provide high levels of polymorphisms, and are easily reproducible. With the calculation of index of association, one can measure recombination and test if alleles are identical by convergence and not by decent, and *vice versa*. Indirect gene flow in a population can also be measured with the use of Wright's F_{st} (Weir, 1996; Agapow & Burt, 2001). Direct methods of gene flow are usually inferred from the direct observation of the movement of individuals. Such observations, however, are difficult in areas where the pathogen is endemic and rare periodic episodes might be overlooked.

Brave *et al.* (2001) used a modified microsatellite technique to study *F. oxysporum* f.sp. *ciceri* isolates from India and were able to differentiate between the four races of the pathogen. The authors showed that race 1 and 4 were closely related, whereas races 2 and 3 were distantly related to the other races. Bogale *et al.* (2006) used DNA sequence, SSR repeats and AFLPs to examine the diversity of *F. oxysporum* from Ethiopia and found lineages that corresponded to the three major groups defined by multi-gene sequencing of *F. oxysporum.* The authors also found a low genetic diversity and stated that it correctly reflects the nature of Ethiopian agriculture. The above mentioned studies are of the few studies within *F. oxysporum* that utilized SSR data to detect genetic variation and currently no published data are available for *Foc*.

DISTRIBUTION AND EVOLUTION OF *FOC*

Foc, the pathogen responsible for Fusarium wilt of banana (Panama disease), is believed to have co-evolved with its host in Southeast Asia (Stover, 1962; Ploetz, 1990). From there it was introduced into new banana-producing countries by the movement of infected planting material (Pegg *et al.*, 1996). Today, Fusarium wilt can be found in all the banana-growing countries of the world except those bordering the Mediterranean, Melanesia, Somalia and some islands in the South Pacific (Stover & Simmonds, 1987; Ploetz, 1994).

Fusarium wilt was first reported in Australia in 1876, and shortly thereafter in Central America where the disease caused substantial losses in countries such as Panama and Costa Rica (Stover, 1962). In response to the devastation that Fusarium wilt caused to banana export plantations, the highly susceptible Gros Michel cultivar was replaced by the Fusarium wilt-resistant Cavendish dessert banana in the 1960's. While many believed that resistance to Fusarium wilt was found, Cavendish banana cultivars succumbed to the disease both in the subtropics and later in the tropics (Ploetz, 1990; Pegg *et al.*, 1993; Pegg *et al.*, 1994). Fusarium wilt now, once again, is threatening banana production in many Cavendish-producing countries of the world (Ploetz, 2005a; Ploetz, 2005b)

Most members of a VCG in *Foc* are closely related and have a common clonal origin despite their broad geographical distribution. This becomes evident when one considers RAPD, RFLP, AFLPs, mitochondrial DNA and electropheric karyotype data that have produced similar fingerprinting patterns, mitochondrial haplotypes and electrophoretic karyotype profiles for individuals of the same VCG (Bentley *et al.* 1994; Boehm *et al.*, 1994; Pegg *et al.*, 1995; Koenig *et al.*, 1997; Groenewald *et al.*, 2006b). Despite the observed asexuality, *Foc* has proved to be a highly diverse fungus, consisting of multiple lineages. This may be due to genetic exchange through parasexuality and heterokaryosis (Buxton, 1962; Kuhn, 1995), although Ploetz (1993) argued that variation in *Foc* is rather due to mutation.

A complex relationship exists between VCG and race in *Foc* (Ploetz, 1990; Correll, 1991; Bentley *et al.*, 1995; Pegg *et al.*, 1995) and a similar complex relationship exists between race and *formae specialis*. Several phylogenetic studies have now shown that some individuals of *Foc* race 4 have a closer relationship to individuals of other *formae speciales* of *F. oxysporum* than to other *Foc* race 1 or 2 isolates (Baayen *et al.*, 2000; Groenewald *et al.*, 2006b).

In a study to analyze a global population of *Foc*, Boehm *et al.* (1994) separated *Foc* into two major groups by means of electrophoretic karyotyping, with one group generally associated with banana cultivars with partial B genomes, and the other associated with banana cultivars with pure A genomes. This grouping of a global collection of *Foc* was confirmed by means of RFLP data (Koenig *et al.*, 1997), RAPD and DAF analysis (Bentley and Dale, 1995; Bentley and Bassam, 1996; Bentley *et al.*, 1998), sequence analysis of the TEF and MtSSU genes (O'Donnell *et al.*, 1998), and AFLP analysis (Groenewald *et al.*, 2006b). Ploetz and Pegg (2000) suggested that the two groups represent evidence of two separate incidents that led to the evolution of *Foc* as a pathogen of bananas. In his analysis, Ploetz (2005a; 2005b) argued that the groups defined by Koenig *et al.* (1997) might represent separate species. Ploetz and Pegg (2000) reported that Wallace's line appears to be the eastern boundary for the natural distribution of both groups. One VCG of *Foc* (01214), however, appeared to originate outside the Indo-Malayan region (Ploetz and Pegg, 1997). This VCG comes from Enset plants in Malawi, and has been implicated as a third putative species in *Foc* (Ploetz, 2005a; 2005b).

More recently, Taylor *et al.* (1999) reanalyzed a reduced data set of Koenig *et al.* (1997) and showed that recombination within some of the clonal lineages may exist. They suggested that the lack of association between DNA fingerprint and VCG within DNA fingerprinting group (DFG) IV defined by Bentley *et al.* (1998) is further evidence of recombination in addition to strictly clonal reproduction. Taylor *et al.* (1999) utilized the index of association and the parsimony tree length permutation test, and with neither method could they reject the null hypothesis that individuals within DFG IV are recombining in nature. They do, however, suggest that this question should be reinvestigated with other approaches, such as congruity of gene genealogies. If recombination was present in the early history of *Foc*, it was probably followed by lineage divergence and subsequent clonal propagation of a few highly fit genotypes.

CONCLUSION

Studies on the phylogenetics and population biology of *F. oxysporum* over the past decade have made it possible to better understand the evolutionary history, genetic structure and diversity of the Fusarium wilt pathogen. Many of the population genetic studies performed to date, however, are biased, as researchers focused primarily on the interaction between pathogenic individuals that attack a single agricultural crop. While this approach might have led to an overestimation of clonality, the information gained is still important and should be interpreted and incorporated accordingly to implement effective control strategies (McDonald, 1997). Understanding the composition of the pathogenic population not only contributes to the selection of resistant cultivars, but could possibly also predict the duration of resistance of a new plant variety. The early detection of new and different phylogenetic groups in a geographical area could help set up quarantine measures to prevent spread in the absence of alternative control measures.

Good progress has been made with investigating the phylogenetic and evolutionary history of *Foc* by using molecular techniques. Consistency in the research results of Koenig *et al.* (1997), Bentley *et al.* (1998), O'Donnell *et al.* (1998b) and Groenewald *et al.* (2006b) confirmed that *Foc* has a polyphyletic origin, is divided into two major clades, and that certain *Foc* isolates are more closely related to other *formae speciales* of *F. oxysporum* than to other *Foc* isolates. Taylor *et al.* (1999) also showed that recombination might have contributed to the evolution of *Foc*. The presence of *MAT* genes could possibly help to better understand the evolutionary origin of a species or specific lineage. If both mating types are present in a group that have close affinity it implies a sexual origin (Abo *et al.*, 2005). There

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are still uncertainties about the true relationship between the VCGs, races and DNA lineages of *Foc*, and whether the pathogen might consists of two or even more phylogenetically distinct species. With the use of a more comprehensive sequencing study, including *MAT* genotyping, mating type studies as well as SSR analysis on a worldwide population of *Foc*, one might gain more insight into the evolutionary history of *Foc*.

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CHAPTER 2

Evolutionary relationships among the vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense*

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ABSTRACT

Fusarium oxysporum is a highly diverse soil-borne fungus. The pathogenic members are responsible for Fusarium wilt diseases of many agricultural crops. The species is subdivided into *formae speciales*, of which *F. oxysporum* f.sp. *cubense* (*Foc*), the causal agent of Fusarium wilt of banana (*Musa* spp.), is one of the most destructive. Isolates of this pathogen are routinely characterized using vegetative compatibility, and 24 so-called vegetative compatibility groups (VCGs) are known for *Foc*. To study the genetic relatedness among and within the VCGs of *Foc* two nuclear and two mitochondrial DNA regions were sequenced. A total of 70 isolates were included in this study that represents 20 of 24 different VCGs, other *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* isolates. Phylogenetic analysis separated the isolates into two main clades. Both *Foc* clades include *formae speciales* of *F. oxysporum* other than *Foc* and three lineages according to their VCG status, while the non-pathogenic isolates of *F. oxysporum* grouped together in Clade B only. No cultural, morphological or pathogenic characteristics among isolates were consistently associated with a single clade or lineage. The mating types of all isolates were determined using PCR-based assays for detection of conserved regions in the *MAT*-1 and *MAT*-2 mating type idomorphs. Isolates of opposite mating type were then crossed in an attempt to induce a sexual stage. All of the examined isolates harboured either one of the mating-type idiomorphs, and never both. This is consistent with the idea that the mating system of this fungus is heterothallic, should sexual reproduction occur. Although, no sexual structures were observed, some lineages of *Foc* harboured *MAT*-1 isolates and *MAT*-2 isolates, suggesting a potential that these lineages have a sexual origin. Overall these findings therefore show that *Foc* is a polyphyletic taxon consisting of multiple lineages that might have more recent sexual origins than initially anticipated.

INTRODUCTION

Fusarium oxysporum Schlechtendahl emend. Snyder and Hansen is a cosmopolitan species (Booth, 1971) comprising of both pathogenic and non-pathogenic members (Gordon & Martyn, 1997). The pathogenic members of *F. oxysporum* cause Fusarium wilt of several agricultural crops, and are subdivided into *formae speciales* according to the crop they causes disease to (Hawksworth *et al.*, 1995; O'Donnell & Cigelnik, 1999; Baayen *et al.*, 2000). One of the economically more important and destructive *forma specialis* is *F. oxysporum* f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*), the causal agent of Fusarium wilt (Panama disease) of banana (*Musa s*pp.). This disease has been reported in all production regions of the world except those bordering the Mediterranean, Melanesia, Somalia and some islands in the South Pacific (Stover & Simmonds, 1987; Ploetz, 1994).

Based on virulence to specific banana cultivars, *Foc* is subdivided into three races (Ploetz, 1990; Ploetz, 1994). *Foc* race 1 causes disease to Gros Michel, Silk, Apple, Lady Finger and Latundan cultivars; race 2 affects Bluggoe bananas (Waite & Stover, 1960; Su *et al.*, 1977; Su *et al.*, 1986; Moore *et al.*, 1993); while race 4 causes disease to Cavendish cultivars, as well as all the cultivars susceptible to *Foc* races 1 and 2 (Su *et al.*, 1977). *Foc* race 4 is further subdivided into 'tropical' and 'subtropical' isolates, based primarily on their ability to cause disease to Cavendish bananas in the tropics and subtropics, respectively (Ploetz, 1990; Viljoen, 2002). Race 3 is not considered a race of *Foc* anymore, as these isolates do not cause disease to banana but to *Heliconia* spp. (Ploetz, 2005a; Ploetz, 2005b). Race classification of *Foc* isolates, however, is not always reliable. For instance, genetically identical isolates of *Foc*, are classified as race 4 isolates in the subtropics, and as race 1 isolates in the tropics, because they cause disease to Cavendish bananas under sub-tropical conditions only (Ploetz, 1990; Viljoen, 2002). Race designations currently are based on field evaluations of a small set of differential cultivars. This raises important concerns to both plant pathologists studying *Foc* and banana plant breeders interested in improving the crop for disease resistance to the pathogen.

Vegetative compatibility groups (VCGs) and a variety of DNA-based tools are now used for a more accurate characterization of *Foc* isolates. Isolates belong to the same VCG when their hyphae can anastomose to form a stable heterokaryon, because of identical alleles at all of their vegetative incompatibility (*vic*) loci. When isolates have different alleles at one or more of their *vic* loci, stable heterokaryons are not formed (Leslie, 1993). Based on this system, *Foc* is currently divided into 24 VCGs (Ploetz, 1988; Moore *et al.*, 1993; Bentley & Dale, 1995; Katan, 1999). DNA-based techniques separate *Foc* into a number of clonal lineages that more or less correspond to the grouping based on VCGs. These DNA-based techniques include genomic fingerprinting methods such as random amplification of polymorphic DNA (RAPD) (Bentley & Dale, 1995; Bentley & Bassam, 1996), DNA amplified fingerprinting (DAF) analysis (Bentley *et al.*, 1998) and amplified fragment length polymorphisms (AFLPs) (Groenewald *et al.*, 2006). The nucleotide sequence information for genes encoding the translation elongation factor-1α (TEF) and the mitochondrial small subunit (MtSSU) ribosomal RNA (rRNA) have also been used for *Foc* classification (O'Donnell *et al.*, 1998b).

For and evolutionary point of view, it has been demonstrated that *Foc* has multiple independent origins. Previous DNA-based studies (Bentley & Dale, 1995; Bentley & Bassam, 1996; Bentley *et al.*, 1998; O'Donnell *et al.*, 1998a; Groenewald *et al.*, 2006) separated *Foc* into two clades. Within each clade, some *Foc* isolates are more closely related to isolates from other *formae speciales* of *F. oxysporum* than to *Foc* isolates in the other clade (O'Donnell *et al.*, 1998a; Baayen *et al.*, 2000), indicating that isolates of a specific *forma specialis* or race are not necessarily derived from a recent common ancestor. New pathogenic forms of *F. oxysporum* may be derived from other pathogenic and non-pathogenic members (Gordon & Okamoto, 1992). Overall, these evolutionary patterns is likely to be the results of co-evolution with the plant host, as suggested by some authors (Ploetz & Pegg, 1997; Ploetz, 2005a; Ploetz, 2005b), and other factors such as horizontal gene transfer (HGT) via parasexuality, heterokaryosis or sexual recombination. Although parasexuality and heterokaryosis are known to occur in *F. oxysporum* (Buxton, 1962; Kuhn *et al.*, 1995), evidence for recombination is less concrete, with only indirect evidence suggesting potential sexual recombination (Taylor *et al.*, 1999). While sexual fruiting structures have never been observed in *F. oxysporum*, the organization of its *MAT*-loci, that determines sexual reproduction (Menzenburg & Glass, 1990; Kronstad & Staben, 1997), is similar to those found in the closely related *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura complex and other heterothallic pyrenomycetes (Arie *et al.*, 2000; Yun *et al.*, 2000).

Development of appropriate disease management strategies for *Fusarium* wilt of banana and the selection of *Foc*-resistant banana cultivars require knowledge of the diversity and evolutionary history of the pathogen. Although most previous DNA-based studies (Koenig *et al.*, 1997; Bentley *et al.*, 1998; O'Donnell *et al.*, 1998a) provided knowledge regarding the diversity of *Foc*, they were not always consistent in defining the genetic relationships among lineages (Groenewald *et al.*, 2006). Multi-gene phylogenies have effectively been used to resolve several species of *Fusarium* (Nirenberg & O'Donnell, 1997; Nirenberg & O'Donnell, 1998; Zeller *et al.*, 2003; O'Donnell *et al.*, 2004b) and to differentiate groups within *F. oxysporum* (Skovgaard *et al.*, 2002; Kawabe *et al.*, 2005; Bogale *et al.*, 2006). The objectives of this study were, therefore, to determine the relationships among and diversity within the VCGs of *Foc*, as well as their relationships with other *formae speciales* and non-pathogenic members of *F. oxysporum*. For this purpose the DNA sequence information for TEF and MtSSU, as well as the rRNA intergenic spacer (IGS) region and a previously characterised repeated region encoded on the mitochondrion (MtR) (Tom Gordon, unpublished data) was investigated. To allow rapid differentiation of the main lineages of *Foc*, we also aimed to develop a diagnostic PCR-RFLP (restriction fragment length polymorphism) procedure. To evaluate the potential of *Foc* to reproduce sexually, sexual crosses among isolates of opposite mating types were performed after PCR-based detection of the *MAT*-1 and *MAT*-2 idiomorphs (Kerenyi *et al.*, 1999).

MATERIALS AND METHODS

Fungal isolates**:** A total of 70 *F. oxysporum* isolates were selected for this study (Table 1). These included a global collection of 51 *Foc* isolates representing 20 of the 24 VCGs. Isolates representing the little known VCGs 01222, 01223 and 01224 occurring in Malaysia and VCG 01221 occurring in Malawi were not available. Nine isolates of *F. oxysporum* representing *formae speciales* other than *Foc*, an isolate of unknown VCG, two *F. oxysporum* isolates from heliconia (*Heliconia* sp.) and eight non-pathogenic *F. oxysporum* isolates from the rhizosphere of banana plants in South Africa (Nel *et al.*, 2006a) were also included. All cultures are maintained in the culture collection (CAV) of the Forestry Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. DNA for cultures that

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were not available was kindly supplied by Dr Suzy Bentley from the Queensland Department of Primary Industries (QDPI) in Brisbane, Australia.

***Pathogenicity tests*:** Twenty-seven isolates were selected for pathogenicity tests on banana plants. These included isolates of *Foc* representing 17 known VCGs, the unknown VCG from Vietnam, and isolates obtained from heliconia (Table 1). VCGs 0121, 01211, and 01212 were not included because of their limited geographic distribution and cultivar specificity (Ploetz, 2005a; Ploetz, 2005b). All cultures were grown on 20 g/l potato dextrose agar (PDA) (Biolab Diagnostics, Wadeville, South Africa) for 10 days. A spore suspension was prepared by using sterile distilled water to wash spores from the mycelia, followed by filtration through cheese cloth, and adjusting the spore concentration to 1 x 10^6 spores/ml with a haemacytometer. Pathogenicity tests were performed with all isolates on either Gros Michel tissue culture banana plantlets or Gros Michel and Bluggoe tissue culture banana plantlets for the unknown VCG from Vietnam and the *F. oxysporum* isolates from heliconia. The tests were conducted in a hydroponics system as described by Nel *et al.* (2006b), and disease severity was measured after six weeks using a disease rating scale developed by Carlier *et al.* (2002).

***Morphological characterization*:** Cultural and morphological characteristics of *Foc* isolates were studied using the techniques described by Nelson *et al.* (1983) and Leslie and Summerell (2006). All *Foc* isolates were cultured on PDA (40 g/l) and carnation leaf agar (CLA) (Leslie & Summerell, 2006) and incubated at 25°C under white and near-ultraviolet fluorescent light for 12 days. Colony colour and colony diameter were recorded after 3, 7 and 10 days' growth on PDA, and the presence of sclerotia and sporodochia documented after 12 days. Morphological features such as the presence and abundance of micro- and macroconidia, chlamydospores and coiled hyphae, and the size and shape of the macroconidia produced on CLA were examined using light microscopy.

***DNA isolation, PCR amplification and Sequencing*:** Isolates of *Foc* and *F. oxysporum* were grown on 20 g/l PDA medium for 7 days. Fungal tissue was harvested by scraping it off the surface of the growth media and homogenizing it in 300 µl DNA extraction buffer (200 mM Tris-HCl, pH 8; 150 mM NaCl; 25 mM ethylenediaminetetra-acteic acid di-sodium salt (EDTA), pH 8; 0.5% sodium dodecyl sulphate (SDS)) (Reader & Broda, 1985). Extraction samples were frozen in liquid nitrogen, followed by incubation at 60°C for 5 min, after which DNA was purified with phenol-chloroform extractions (Sambrook *et al.*, 1989). DNA was precipitated with 0.1 volumes 3 M sodium acetate (pH 5.5) and 2 volumes of ethanol at 5ºC and centrifuged for 10 min, washed with 70% ethanol and resuspended with sterile distilled water. To remove excess RNA, *RNase A* (0.2 U/µl; Sigma-Aldrich, Steinheim, Germany) was added to DNA samples and incubated at 37°C for 3 hr. Extracted DNA was quantified using a Nanodrop spectrophotometer (NanoDrop, Wilmington, USA) and stored at -20°C until use.

Approximately 650-base pair (bp), 700-bp and 1500-bp regions of TEF, MtSSU, and IGS, respectively, were amplified with an Eppendorf Mastercycler Gradient PCR machine (Eppendorf Scientific, Hamburg, Germany) using primer sets EF1 and EF2 (O'Donnell *et al.*, 1998b), MS1 and MS2 (White *et al.*, 1990), and PNFo and PnF22 (Edel *et al.*, 1995), respectively. Primers R117 (GTC AAC CAG GAG CAG ACT G) and U9 (GTA ACC TCT GAC TCA CCG) (Tom Gordon, unpublished data) that target the MtR region on the *Foc* mitochondrion were also included. Each amplification reaction contained ~5 ng/µl DNA, 0.3 µM of each primer, 250 µM dNTPs (Fermantas, Nunningen, Switzerland), 0.04 U/µl *Taq* DNA polymerase (Roche Molecular Biochemicals, Manheim, Germany) and PCR buffer with $MgCl_2$ (Roche). PCR cycling conditions consisted of 35 cycles at 94°C for 45 sec, 60°C (TEF), 53°C (MtSSU), 50°C (IGS), or 59°C (MtR) for 45 sec, and 72°C for 90 sec. Each PCR was preceded by an initial denaturation step at 94°C for 2 min and concluded by a final extension step at 72°C for 5 min.

PCR products were purified using the High Pure PCR Product purification Kit (Roche Applied Biochemicals) and sequenced in both directions with the original PCR primers and in the case of IGS, with the internal reverse primer IGS2 (5' GCC GGA TTT GCT CCC TTC T 3'), developed in this study using BioEdit version 6.0.7 (Hall, 1999). The Big Dye^{TM} Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) and an ABI 377 automated sequencer (Applied Biosystems) were used. Raw sequences were visualised and corrected, where necessary with Chromas Lite version 2.01 (Technelysium).

***Phylogenetic Analysis*:** Multiple sequence alignments were constructed using MAFFT version 5.85 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) with the L-INS-i option effective (Kazutaka *et al.*, 2002; Katoh *et al.*, 2005). Four datasets for the sequenced gene regions were constructed, three of which each comprised the MtR, IGS and TEF+MtSSU sequences of *Foc*. For outgroup purposes these datasets included the relevant sequence data for *F. circinatum*, *F. oxysporum* (NRRL 28687) or *Fusarium* spp. (NRRL 22903 and NRRL 25184), respectively. The fourth dataset was also composed of combined TEF+MtSSU data, but included sequences for other *formae speciales* of *F. oxysporum*, non-pathogenic *F. oxysporum* from South Africa, *F. oxysporum* isolates from heliconia and sequences for *F. oxysporum* isolates that were obtained from GenBank. For outgroup purposes, the TEF and MtSSU sequences for the two *Fusarium* isolates, NRRL 22903 and NRRL 25184, were used in the extended TEF+MtSSU dataset. All ambiguously aligned sites were excluded from further analyses.

To test for the combinability of datasets, the partition-homogeneity test (Farris *et al.*, 1994) implemented in PAUP* version 10b (Swofford, 1998) was used on parsimony informative sites only (Dolphin *et al.*, 2000; Lee, 2001; Darlu & Lecointre, 2002). These tests were based on 1 000 repartitions and heuristic searches using 100 rounds of random sequence additions with tree bisection reconnection branch swapping. Phylogenies based on Bayesian inference (BI) and maximum likelihood (ML) methods were inferred for the different datasets using MrBayes version 3.b.4 (Heulsenbeck *et al.*, 2001) and PhyML version 2.4.3 (Guidon & Gascuel, 2003), respectively. For these analyses, the best-fit models of evolution, as indicated by MrModeltest 2.2 (Nylander, 2004) for BI and Modeltest 3.7 (Posada & Crandall, 1998) for ML, were used. The TEF+MtSSU dataset utilized the Hasegawa, Kishino and Yano (1985) model (HKY) plus proportion invariable sites (I) for BI and the transitional model with equal base frequencies (TIMef) (Tavare, 1986) for the ML analysis. The IGS dataset used the General Time Reversible (GTR) model (Tavare, 1986) plus proportion invariable sites (I) and a gamma correction for among site variation (G) for BI and the Kimura’s (1981) 3-parameter model for the ML analysis. For the MtR dataset, the BI analysis used Felsenstein’s (1981) evolutionary model, while ML used Tumara and Nei’s (1993) model with equal base frequencies (TNef). BI and ML analysis of the extended TEF+MtSSU dataset utilized Tumara and Nei’s (1993) model with gamma distribution to correct for among site variation. BI trees were constructed using the Metropolis-coupled Monte Carolo Markov Chain and 2 000 000 generations using one cold and three heated chains. After discarding a burn-in corresponding to about 50 000 generations post-stationarity, Bayesian posterior probabilities were calculated. For the TEF+MtSSU dataset, the BI analysis used separate parameters for each gene (Heulsenbeck *et al.*, 2001). ML bootstrap confidence values were based on 1000 replications.

DNA-based diagnosis of the Foc lineages: All sequences were screened using BioEdit for VCG- or lineage-specific polymorphisms. As the IGS region contained polymorphisms for the different *Foc* lineages, four restriction enzymes were used for diagnostic PCR-RFLP purposes. These enzymes included *AvaI* (New England BioLabs, Hitchin, England), *BbvI* (New England BioLabs), *BceAI* (New England BioLabs) and *Csp6I* (Fermentas). All enzymes were used separately in PCR-RFLP digestion reactions and consisted of 5 µl IGS PCR product, 2 U of the restriction enzyme and 2 µl of the supplied restriction buffer. After incubation at 37°C for 3 hr, the restricted fragments were separated using agarose (3%, w/v) gel electrophoresis (Sambrook *et al.*, 1989).

Mating type diagnoses and mating studies: Mating types of the various *Foc* isolates were determined by PCR using the primer set Falpha 1 and Falpha 2 for *MAT*-1 (Arie *et al.*, 2000), and the primers GFmat2c (Steenkamp *et al.*, 2000) and FF1 (Visser, 2003) for *MAT*-2. PCR conditions were similar to those described earlier, apart from the use of annealing temperatures of 55°C for *MAT*-1 and 54°C for *MAT*-2. Selected *MAT*-1 and *MAT*-2 products were also sequenced in both directions with the original PCR primers, as described above. These sequences were then compared to those in GenBank (http://www.ncbi.nlm.nih.gov/) using *blastn*.

Once the mating type of the isolates was known, two *Foc* isolates in each VCG were crossed in all possible combinations with isolates of the opposite mating type in other VCGs on carrot agar (Leslie & Summerell, 2006). For the *Foc* lineages containing both mating types, all isolates of opposite mating types were crossed with each other. For all the crosses, isolates were treated both as males and females. *Foc* isolates were also crossed with the mating type tester of *F. circinatum* (MRC 6213 or MRC 7488) (Britz *et al.*, 1999). Crosses between these tester isolates were included as positive controls.

RESULTS

Pathogenicity tests: All isolates designated *Foc* in this study caused disease symptoms typical of *Fusarium* wilt on Gros Michel and/or Bluggoe plantlets. After symptom development, the inoculated pathogens were re-isolated from randomly selected plants to confirm Koch's postulates. The two *F. oxysporum* isolates obtained from heliconia did not cause any symptoms on the respective banana hosts.

***Morphological characterisation*:** Isolates of *Foc* developed cultural and morphological characteristics typical of those described for *F. oxysporum* (Nelson *et al.*, 1983; Leslie & Summerell, 2006). No significant differences were found in growth rate between isolates representing different VCGs, lineages or clades of *Foc* (data not presented). Isolates representing *Foc* VCGs 0126, 01210 and 01219, however, grew unevenly at colony margins and produced a dense aerial mycelial growth, while the remaining isolates produced even colony margins with intermediate to dense mycelial development. No sclerotia-like structures or sporodochia were produced by any of the isolates after 12 days. Colony colours varied from cream to peach (*Foc* VCGs 0121, 01216, 01213, 01213/16, 0120, 01215, 0120/15, 0129, 0122, 01211, 0129/11), cream to dark purple (*Foc* VCGs 0126, 01210, 01219, 0123, 01217, 01218), cream to peach-purple (*Foc* VCG 0124, 0125, 0128, 01220, 01212) and cream to purple-red (*F. oxysporum* from heliconia).

Microconidia were produced in false heads on short monophialides and were mostly single-celled and kidney-shaped. All the VCGs produced microconidia in abundance, with the exception of *Foc* VCGs 0126, 01210 and 01219, that produced a few microconidia only. Thin, sickle-shaped macroconidia were sparse or absent in most isolates, except for one isolate each representing *Foc* VCGs 01210, 0126 and 0123. These isolates produced an intermediate number of macroconidia. After 12 days, chlamydospores were produced by *F. oxysporum* isolates from heliconia only. The chlamydospores were formed singly and sometimes in pairs with a coarse protective wall. Chlamydospores, with characteristics similar to those described earlier, were produced in the other isolates after 4 weeks, and in some cases only after 6 weeks of incubation.

Sequence and phylogenetic analysis**:** The partition-homogeneity test supported combination of the TEF and MtSSU datasets (p = 0.9), but rejected combination of the other regions (p = 0.02 or p = 0.01). The MtR and IGS datasets were therefore analysed separately, while the TEF and MtSSU datasets were combined for analysis. The multiple sequence alignments for the *Foc* MtR, IGS and TEF+MtSSU datasets and the extended TEF+MtSSU were 1224, 1577, 1328 and 853 characters in length, respectively (Appendices A, B and C). The *Foc* MtR region harboured mostly 1-base insertions or deletions (indels) and a few 9-17-base indels, as well as a long 148- or 156-base indel. For phylogenetic analyses, this large indel was excluded. The *Foc* IGS and TEF+MtSSU datasets mostly harboured short 1-4-base indels as well as a couple of 9-12-base indels.

Phylogenetic analyses of the *Foc* TEF+MtSSU dataset separated the 48 isolates into two distinct clades (Clades A and B)(Fig 1). Clade A consisted of three lineages (I, II and III). Lineage I contained representatives for *Foc* VCGs 0126, 01210, 01219, Lineage II contained isolates representing *Foc* VCGs 0121, 01216, 01213 and 01213/16 and Lineage III consisted of isolates associated with *Foc* VCGs 0120, 01215, 0120/15, 0129, 0122, 01211 and 0129/11. Isolates in Lineage II are commonly referred to as *Foc* 'tropical' race 4 and all isolates belonging to *Foc* 'subtropical' race 4 are located in Lineage III. Clade B included three lineages (IV, V and VI), with Lineage IV consisting of *Foc* VCG 0123, 01217, and 01218 isolates, Lineage V consisting of *Foc* VCG 0124, 0125, 0128, 01220 and 01212 isolates and Lineage IV of *Foc* VCG 01214.

The IGS and MtR (Figs. 2 and 3) phylogenies data did not support the two main clades revealed by the TEF+MtSSU data. The MtR data did, however, cluster the 48 *Foc* isolates into five groups that match the TEF+MtSSU-based lineages (Fig. 3). The IGS sequences also allowed separation of the isolates into groups that broadly match those based on the TEF+MtSSU and MtR datasets. The only exceptions were the divergent placement of *Foc* VCGs 0121, 0122, 01210 and 01214 in the IGS phylogeny. *Foc* VCG 0121 formed part of Lineage II based on the TEF+MtSSU dataset, whereas it is associated with an exceptionally long branch in the IGS tree that are unrelated to other Lineage II members (*Foc* VCGs 01216, 01213 and 01213/16). In the TEF+MtSSU tree, *Foc* VCGs 0122 and 01210 formed part of Lineages III and I, respectively, but grouped together in the IGS tree separate from isolates representing TEF+MtSSU-based Lineages I and III. In the IGS and MtR trees, the Lineage VI taxon, *Foc* VCG 01214, were nested in a clade of Lineage IV isolates, but displayed a sister-group relationship with this lineage based on the TEF+MtSSU data.

The presence of the 148- or 156-bp indel (Fig. 4) divided the *Foc* isolates into three groups. Isolates (Group A) representing *Foc* VCGs 0120, 0120/15, 01215, 0126, 01210, 01219, 0121, 01213, 01213/16, 01216 and 01218 harboured a 156-bp fragment deletion in this position, while isolates (Group B) harbouring a 156-bp insertion position were represented by *Foc* VCGs 01211, 0122, 0129 and 0129/11. Isolates (Group C) that harboured a 148-bp insertion at this position were those representing *Foc* VCGs 0124, 0125, 0128, 01220, 01212, 0123, 01214 and 0129/11. The composition of Groups A and C corresponds roughly with that of Clade A (Fig. 1). The same is also true for Group B and Clade B. The only exception is the Clade B taxon, *Foc* VCG 01218 that harboured the 156-bp deletion and VCGs *Foc* 0122, 01211, 0129/11 and 0129 that formed part of Group B, but reside in Clade A.

The extended TEF+MtSSU dataset that included the *Foc* sequences, as well as those for other *F. oxysporum* isolates determined in this study and those obtained from GenBank, separated the isolates into four well-supported clades (Fig. 5). Two of these clades (A and B) correspond with those identified using the smaller TEF+MtSSU dataset (Fig. 1) and included a representative set of all the *Foc* isolates examined. Clade C included human pathogens (O'Donnell *et al.*, 2004a) as well as *formae speciales* of *F. oxysporum* other than *Foc*. Clade D included the *F. oxysporum* isolates from heliconia and a single *F. oxysporum* isolate from human tissue. Although Clade A consisted predominantly of *Foc* isolates, it also included isolates of *F. oxysporum* f.sp. *canariensis* and *F. oxysporum* f.sp. *perniciosum*. Clade B included a number of non-pathogenic *F. oxysporum* isolates, two *F. oxysporum* isolates from human tissue, *F. inflexum*, and several *formae speciales* of *F. oxysporum.* Within Clade B, an *Foc* isolated typed as VCG 01214 appeared to be more closely related to non-pathogenic *F. oxysporum* isolates, other *formae speciales* of *F. oxysporum* such as *raphanai*, *vasinfectum*, *melonis* and *dianthi* and *F. inflexum* than to *Foc*. CAV 1020, an unknown *Foc* VCG from Vietnam, was included in Clade B, but was also more closely related to non-pathogenic isolates than to known *Foc* VCGs.

IGS PCR-RFLP**:** Of the four regions sequenced, IGS was most useful for identifying the different TEF+MtSSU-based lineages of *Foc*. As the *Foc* IGS region contains at least 102 polymorphic nucleotide positions that allowed separation of the individual lineages and/or VCGs, it was possible to select four restriction enzymes to apply for diagnostic purposes (Figs. 6 and 7). Enzyme *AvaI* allowed separation of Clades A and B (Figs. 6A and 7A). Among the Clade A lineages, *BceAI* separates Lineage II from I and III (Figs. 6B and 7B), while *Csp6I* separates Lineages I and Lineage III (Figs. 6C and 7C). Within Clade B, *BbvI* separates Lineages V and from Lineages IV and VI (Figs. 6D and 7D). No restriction enzyme was able to separate isolates of Lineages IV and VI from one another. However, isolates from Lineage VI (VCG 01214) harbour a 94-bp deletion within the MtR gene region, and can therefore be separated from Lineage IV by means of conventional agarose gel electrophoresis.

Mating type diagnoses and mating studies**:** The mating types of *Foc* isolates were identified as *MAT*-1 and *MAT*-2 based on the presence of 370- and 700-bp fragments, respectively. Only one *MAT* amplicon was present per *Foc* isolate. *MAT*-1 was present in *Foc* VCGs 0122, 01210, 01219, 01213, 01213/16, 01216, 01214 and 01218, and *MAT*-2 was present in *Foc* VCGs 0120, 01215, 0120/15, 0126, 0121, 0124, 0124/5, 0125, 0128, 01220, 0123, 0129, 01211, 01212, 01217 and 0129/11 (Table 1). Both *MAT* amplicons were present within Clades A and B, as well as within Lineages I, II and III of Clade A and Lineage IV of Clade B. Lineage V included isolates of *MAT*-2 only.

No sexual fruiting structures were produced in any of the crosses between the *Foc* isolates 8 weeks after incubation. Protoperithecia-like structures were, however, formed in some crossing combinations. These protoperithecial structures were dark purple to black and superficially resembled the perithecia that were produced by crosses between the *F. circinatum* tester strains. Structures that were too small to be protoperithecia were also observed. Protopertihecia were abundantly produced when individuals from Clade A were crossed with one another, particularly when *MAT*-2 isolates (*Foc* VCGs 0120, 01215, 0120/15) from Lineage III were crossed with *MAT*-1 isolates from Lineage III, and to a lesser extent with those from Lineages I and II. Abundant protoperithecia also formed when *MAT*-1 isolates (*Foc* VCGs 01213, 01216, 01213/16) from Lineage II were crossed with *MAT*-2 isolates from Lineage II and to a lesser extent those from Lineages I and III. Only a few protoperithecia-like structures were observed when Lineage I isolates were crossed with isolates of opposite mating type from Lineages II and III, as well as Lineage I itself. No protoperithecia-like structures were produced when individuals from Clade A were mated with those in Clade B and *vice versa,* or when individuals within Lineage IV of Clade B, were crossed. One or two protoperithecial structures were observed when *Foc* isolates were crossed with the two *F. circinatum* tester strains. The tester strains, when crossed with each other, produced fertile perithecia.

DISCUSSION

In this study, the evolution of *Foc*, the causal agent of Fusarium wilt of banana, was investigated. Based on the DNA sequence information for two nuclear (TEF and IGS) and two mitochondrial (MtSSU and MtR) regions, *Foc* is separated into a number of distinct lineages that broadly correspond to VCGs. To facilitate rapid identification, a PCR-RFLP procedure for discriminating between these lineages was developed. The phylogenies constructed here also separated the pathogen into two distinct clades. Within these clades some lineages of *Foc* were more closely associated with isolates representing *formae speciales* than to other *Foc* isolates, thus emphasizing that *Foc*'s ability to cause disease on banana emerged multiple times, independently. Despite the fact that a sexual stage for *F. oxysporum* is not known, the *Foc* lineages included isolates of opposite mating type that produced structures resembling protoperithecia in abundance when they were crossed with each other. Sexual recombination, therefore, might have played an important role in shaping the evolutionary history of this polyphyletic causal agent of Fusarium wilt of banana.

Various concepts have been described to demarcate new species of filamentous fungi (Sites & Marshall, 2004). These concepts are roughly subdivided into two broad categories namely tree-based concepts, such as the phylogenetic species concept, and non-tree based methods such as the biological and morphological species concepts (Sites & Marshall, 2004). Although tree-based and non-tree based methods are generally used for accurate identification of *Fusarium* spp. (Leslie *et al.*, 2001), the application of non-tree based methods for classifying the different lineages of *F. oxysporum* is complicated. This is mainly

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due to the overall lack of morphological variation for application of a morphological species concept, as well as the absence of sex for application of a biological species concept. As expected, all 51 of the *Foc* isolates examined in this study displayed similar morphological characters and no fertile sexual crosses were observed in crosses between isolates of opposite mating type. However, according to the phylogenetic species concept, the *Foc* isolates were separated into at least six distinct and mostly unrelated lineages. These appeared to be scattered among other non-*Foc* lineages within two of the four *F. oxysporum* clades (Kistler, 1997; O'Donnell & Cigelnik, 1999; O'Donnell *et al.*, 2000; O'Donnell *et al.*, 2004a; Bogale *et al.*, 2006) supported by the extended TEF+MtSSU dataset.

The separation of *Foc* VCGs into distinct phylogenetic lineages consisting of clusters of related VCGs correlates well with earlier studies using DNA fingerprinting techniques such as RFLPs (Koenig *et al.*, 1997), RAPDs and DAFs (Bentley & Dale, 1995; Bentley & Bassam, 1996; Bentley *et al.*, 1998), and AFLPs (Groenewald *et al.*, 2006). This is also true for previous DNA-based phylogenetic studies (O'Donnell *et al.*, 1998b). For all three of the *Foc* datasets (TEF+MtSSU, IGS and MtR) used in the current study, isolates associated with the same VCG generally also had identical sequences and clustered together irrespective of their geographic origin. All three of these datasets also consistently clustered the same sets of VCGs into each of the *Foc* lineages, with the notable exceptions of *Foc* VCGs 0121, 0122 and 01210. In the TEF+MtSSU and MtR phylogenies, VCG 0121 forms part of Lineage II, *Foc* VCG 0122 forms part of Lineage III and *Foc* VCG 01210 forms part of Lineage I. However, in the IGS tree (Fig. 3), *Foc* VCGs 01210 and *Foc* VCG 0122 group together, while isolates representing *Foc* VCG 0121 form a group to the exclusion of all other *Foc* isolates. These discrepancies may potentially be associated with the specific nature of the IGS region as a relatively fast evolving region and thus could complicate the inference of the true phylogenetic history (Appel & Gordon, 1996). In some *Fusarium* spp. the inference of the true phylogenetic relationships using nuclear encoded rRNA regions may also be complicated by the presence of multiple non-orthologous copies (O'Donnell & Cigelnik, 1997). Nevertheless, despite these potential limitations, the IGS region has been proven to be an excellent marker for *Fusarium* spp. diagnoses (Schweigkofler *et al.*, 2004; Kawabe *et al.*, 2005; Kawabe *et al.*, 2007).

Race designation in *Foc* and other *F. oxysporum formae speciales* are based on field evaluation, and is generally known not to produce stable classifications (Correll, 1991; Migheli *et al.*, 1995; Davis *et al.*, 1996). In this study, isolates representing *Foc* races 1 and 2 are scattered among Lineages I, III, IV and V in Clades A and B, and isolates representing *Foc* race 4 are mostly restricted to Clade A (Lineages II and III). The only exception is VCG 01220 (Clade B, Lineage IV) from Australia that caused disease in stressed Cavendish bananas (Pegg *et al.*, 1995). *Foc* race 4 is subdivided into 'tropical' and 'subtropical' race 4 isolates that form part of Lineages II and III, respectively. However, isolates belonging to VCG 0120 in Lineage III is separated into *Foc* race 1 and 'subtropical' race 4, demonstrating the effect of the environment on the current race system. This inconsistency is a consequence of the predisposition of Cavendish bananas to infection by VCG 0120 in the cooler winter temperatures of the subtropics, but not in the tropics (Viljoen, 2002). The classification of *Foc* into races in the greenhouse is even more difficult, as virulence is influenced by variables such as temperature, host age and method of inoculation (Correll, 1991), and different pathogenicity tests used in different laboratories around the world could easily generate different results (Davis *et al.*, 1996). Once universally acceptable greenhouse inoculation techniques have been developed and new potentially differential banana cultivars have been selected for race designation in *Foc*, the lineages in this study could serve as candidates for developing a new race structure.

The IGS PCR-RFLP fingerprinting method developed in this study presents a quick, easy and accurate method to identify the lineages of *Foc*. These fingerprints also allow separation of *Foc* from non-pathogenic isolates of *F. oxysporum*. They could, therefore, be used for the early detection and characterisation of *Foc* in infected planting material, whether symptomatic or not, in water, and in the soil. In laboratories without sequencing facilities and where VCG testers of *Foc* cannot be used because of national quarantine regulations, these fingerprints could be of great value in the characterisation of the Fusarium wilt pathogen of banana. It is, specifically, its ability to rapidly and accurately detect *Foc* 'tropical' race 4 isolates (Lineage II) in new regions where this pathogen is introduced, that could be invaluable in the isolation and management of this most devastating form of *Foc*.

The occurrence of both mating types in *Foc* is reported for the first time in this study. The results presented here therefore confirm that *Foc* would be heterothallic should sexual reproduction take place, as either *MAT*-1 or *MAT*-2 sequences (never both) were detected in each of the isolates examined. The fact that in some cases, both *MAT*-1 and *MAT*-2 individuals were detected in a single group implies that the different lineages of the Fusarium wilt pathogen have sexual origins that could be more recent than initially anticipated. These results, therefore, support the hypothesis that all fungi were originally sexual (Lobuglio *et al.*, 1993; Taylor *et al.*, 1999; Lobuglio & Taylor, 2002), and that sexual recombination is usually followed by phases of clonal propagation of opportunistic varieties (Maynard Smith *et al.*, 1993). These findings again raise questions regarding the true asexuality of *Foc*, although it was not possible to obtain any perithecia with viable ascospores with the attempted mating studies.

The results of all previous phylogenetic studies (O'Donnell *et al.*, 1998b; Groenewald *et al.*, 2006) clearly demonstrate multiple origins for the evolution of *Foc* as a pathogen of bananas. However, the results presented here, suggest that co-evolution with the plant host in its centre of origin in Wallace's Indo-Malayan region in southeast Asia (Ploetz & Pegg, 1997) has played an important role during this process. For example, the majority of *Foc* isolates in Clade B originate from banana cultivars that represent *M. balbisiana* X *M. acuminata* hybrids with at least one chromosome derived from *M. balbisiana* (e.g. Lady finger and Bluggoe), while those in Clade A mostly originate from banana cultivars with pure "A" genomes (*i.e.* all chromosomes derived from *M. acuminata*; e.g. Cavendish and Gros Michel)(Boehm *et al.*, 1994). It is therefore possible that *Foc* Lineages I-III derived their ability to cause disease on banana, specifically on *M. acuminata*, from the ancestor of Clade A. The ancestor of Clade B, on the other hand has appeared to have potentially imparted to its descendents the ability to cause disease to banana cultivars of pure and hybrid background, as well as to plants in the related genus *Ensete*. This is because one of Clade B Lineage VI VCG (VCG 01214) has the capacity to cause disease not only to enset, but also banana cultivars with pure A and mixed A-B genomes. The fact that *Foc* VCG 01214, which originated in Malawi, was proven to be pathogenic to Gros Michel and Bluggoe in this study, also indicates that it should be regarded a true banana pathogen in contrast to previous suggestions (Ploetz, 2005b; Ploetz, 2005a).

In addition to co-evolution with the banana host in its centre of origin, the evolution of *Foc* might also be determined by factors such as HGT. Although *F. oxysporum* is considered to be strictly mitotic (Gordon & Martyn, 1997; Taylor *et al.*, 1999), previous research has suggested that genetic exchange among and within individual lineages might occur more frequently than initially thought. This possibility is further emphasized by the results of the current study showing that one of the mitochondrial and nuclear regions examined (MtR and IGS, respectively) supported phylogenies that were highly incongruent with the *Foc* TEF+MtSSU tree. For example, the fact that *Foc* VCGs 0122 and 01210 cluster together in the IGS tree and separate in the TEF+MtSSU tree potentially reflects ancient recombination or genetic exchange beween *Foc* Lineages III and I. Such genetic exchange or recombination could be due to parasexuality, a non-sexual mode of genetic exchange, or heterokaryosis, a process that is initiated by fusion of vegetative hyphae (anastomosis) between individuals with very similar genomes, and have been shown to occur in *F. oxysporum* (Buxton, 1962; Kuhn *et al.*, 1995). Taylor *et al.* (1999) also demonstrated the possibility of recombination within some of the *Foc* clonal lineages. In their study, they re-analysed previous RFLP data (Koenig *et al.*, 1997) and showed that recombination within some of the clonal lineages may exist. They further concluded that the lack of association between DAF-based DNA fingerprint groups (Bentley *et al.*, 1998) and VCGs is evidence for recombination. The findings presented in the current study also support the potential for sexual recombination in certain lineages. Both mating types were detected in some *Foc* lineages, and crosses between many pairs of isolates of opposite mating type resulted in the production of structures resembling immature perithecia.

Inclusion of isolates representing other *formae speciales*, non-pathogenic *F. oxysporum* isolates and *F. oxysporum* isolates from human tissue in phylogenetic analyses illustrates the great diversity that exists within the *F. oxysporum* complex. In this study, a single isolate from Vietnam (CAV 1020) belonging to an unknown *Foc* VCG (Fig. 5) (Bentley *et al.*, 1998) grouped separate from all other isolates in the *Foc* tree, suggesting that many more distinct lineages of *Foc* remain to be discovered. By studying *Foc* using phylogenetic analyses of the entire *F. oxysporum* complex, it was also possible to detect possible instances where taxon designations have been erroneously applied. A good example of this is the divergent positions of the two *F. oxysporum* isolates from heliconia in the extended *F. oxysporum* tree that has

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been proven here to be non-pathogenic to banana, thus confirming that *Foc* race 3 needs to be placed in new *formae speciales* of *F. oxysporum* (Bentley *et al.*, 1998). It also demonstrates that focusing on a single agricultural crop may lead to an overestimation of clonality, although important information is gained (McDonald, 1997).

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Table 1. Origin, pathogenicity, vegetative compatibility grouping and mating type of isolates of *Fusarium oxysporum* f.sp. *cubense* (*Foc*), *F. oxysporum* and *F. circinatum* selected for a multi-gene phylogenetic comparison.

Isolate number	Other number[1]	Species and *forma specialis*	VCG	Host/Cultivar	Origin	Collector	Pathogenicity [2]	Mating type [3]	Lineage[2]
CAV 009		*Foc*	0120	Cavendish	South Africa	A. Viljoen	Not tested	MAT 2	III
CAV 045		*Foc*	0120	Cavendish	South Africa	A. Viljoen	Not tested	MAT 2	III
CAV 105		*Foc*	0120	Cavendish	South Africa	A. Viljoen	Not tested	MAT 2	III
CAV 293	IC-1	*Foc*	0120	Dwarf Cavendish	Canary Islands	Unknown	Pathogen	MAT 2	III
CAV 294	34661	*Foc*	0120	Highgate	Honduras	R. Ploetz	Pathogen	MAT 2	III
CAV 296	STH1	*Foc*	0120	Highgate	Honduras	R. Ploetz	Not tested	MAT 2	III
CAV 298	BR18	*Foc*	0120/15	Banana	Brazil	Unknown	Not tested	MAT 2	III
CAV 299	PD14-1	*Foc*	0120/15	Gros Michel	Nigeria	Unknown	Not tested	MAT 2	III
CAV 612	RPCR1-1	*Foc*	01215	Gros Michel	Costa Rica	Unknown	Pathogen	MAT 2	III
CAV 607	RP 13	*Foc*	0122	Cavendish	Philippines	Unknown	Pathogen	MAT 1	III
CAV 605	RP 14	*Foc*	0122	Cavendish	Philippines	Unknown	Pathogen	MAT 1	III
CAV 613	Phil 7	*Foc*	0126	Latundan	Philippines	Unknown	Pathogen	MAT 2	I
CAV 793	Indo 33	*Foc*	0126	Pisang Rubus	Indonesia	R. Shivas	Pathogen	MAT 2	I
CAV 794	Indo 38	*Foc*	0126	Pisang Rubus	Indonesia	Unknown	Not tested	MAT 2	I
CAV 1051	RP 52	*Foc*	01210	Apple	USA	R. Ploetz	Pathogen	MAT 1	I
CAV 632	RP26	*Foc*	01210	Highgate	Honduras	R. Ploetz	Pathogen	MAT 1	I
CAV 847	Indo 35	*Foc*	01219	Pisang Raja Sereh	Indonesia	H. Stover	Pathogen	MAT 1	I
CAV 195	INDO 25	*Foc*	01219	Pisang Ambon	Indonesia	N. Moore	Not tested	MAT 1	I
	RP7[1]	*Foc*	0121	Cavendish	Taiwan	Unknown	Pathogen	MAT 2	II
	RP8[1]	*Foc*	0121	Cavendish	Taiwan	Unknown	Not tested	MAT 2	II

CAV	Other	Species and *forma specialis*	VCG	Host/Cultivar	Origin	Collector	Pathogenicity	Mating type	Lineage[2]
	RP9[1]	*Foc*	0121	Cavendish	Taiwan	Unknown	Not tested	MAT 2	II
CAV 810	Indo 34	*Foc*	01213	Pisang Berangan	Indonesia	I. Buddenhagen, J.C. Barlett	Pathogen	MAT 1	II
CAV 811	Indo 30	*Foc*	01213	Pisang Susu	Indonesia	H. Stover	Pathogen	MAT 1	II
CAV 300	CV-1	*Foc*	01213	Valery	Indonesia	Unknown	Not tested	MAT 1	II
CAV 312	RPML 25	*Foc*	01213/16	Pisang udang	Malaysia	R. Ploetz	Pathogen	MAT 1	II
CAV 313	RPML 47	*Foc*	01213/16	Pisang awak legor	Malaysia	R. Ploetz	Not tested	MAT 1	II
CAV 814	Indo 47	*Foc*	01216	Cavendish	Indonesia	I. Buddenhagen, R. Shivas	Pathogen	MAT 1	II
CAV 815	Indo 56	*Foc*	01216	Cavendish	Indonesia	I. Buddenhagen, G.P. Salingay	Pathogen	MAT 1	II
CAV 604	Indo 50	*Foc*	01216	Cavendish	Indonesia	Unknown	Not tested	MAT 1	II
CAV 602	23534	*Foc*	0124	Lady finger	Australia	Unknown	Pathogen	MAT 2	V
CAV 609	23538	*Foc*	0124	Lady finger	Australia	Unknown	Pathogen	MAT 2	V
CAV 786	23734	*Foc*	0124	Lady finger	Australia	K. Pegg	Not tested	MAT 2	V
	8611[1]	*Foc*	0125	Lady finger	Australia	Unknown	Pathogen	MAT 2	V
	23480[1]	*Foc*	0125	Lady finger	Australia	Unknown	Not tested	MAT 2	V
	23487[1]	*Foc*	0125	Lady finger	Australia	Unknown	Not tested	MAT 2	V
CAV 1097	22993	*Foc*	0128	Blue Java	Australia	Unknown	Pathogen	MAT 2	V
CAV 1096	22994	*Foc*	0128	Bluggoe	Australia	Unknown	Pathogen	MAT 2	V
	24211[1]	*Foc*	01220	Cavendish	Australia	Unknown	Not tested	MAT 2	V
	24219[1]	*Foc*	01220	Cavendish	Australia	Unknown	Not tested	MAT 2	V
CAV 957	Thai 37	*Foc*	0123	Kluai Namwa	Thailand	S. Kooariyakul	Not tested	MAT 2	IV

CAV	Other	Species and *forma specialis*	VCG	Host/Cultivar	Origin	Collector	Pathogenicity	Mating type	Lineage[2]
CAV 929	PHIL 13	*Foc*	0123	Latundan	Philippines	L. Magnaye	Pathogen	MAT 2	IV
CAV 933	Thai 2-1	*Foc*	0123	Kluai Namwa	Thailand	N. Singburaudom	Pathogen	MAT 2	IV
	23510[1]	*Foc*	0129	Lady finger	Australia	Unknown	Not tested	MAT 2	III
CAV 1100	23518	*Foc*	0129	Lady finger	Australia	K. Pegg	Pathogen	MAT 2	III
	23631[1]	*Foc*	01211	SH3142	Australia	Unknown	Not tested	MAT 2	III
	RP58[1]	*Foc*	01212	Ney poovan	Tanzania	Unknown	Not tested	MAT 2	V
CAV 189	RPMW 40	*Foc*	01214	Harare	Malawi	R. Ploetz	Pathogen	MAT 1	VI
CAV 871	MAL 7	*Foc*	01217	Pisang Rastali	Malaysia	Unknown	Pathogen	MAT 2	IV
CAV 791	Indo 5	*Foc*	01218	Pisang Siem	Indonesia	N. Moore	Pathogen	MAT 1	IV
CAV 1107	Viet 6	*Foc*	0129/11	Chuoi xiem	Vietnam	I. Buddenhagen, N. Moore, S. Bentley	Not tested	MAT 2	III
CAV 1020	Viet 19	*Foc*	unknown	Chuoi xiem	Vietnam	I. Buddenhagen, N. Moore, S. Bentley	Pathogen		
CAV 1788		*F. oxysporum*		*Heliconia* sp.	South Africa	S. Tween	Non-pathogen		
CAV 1787		*F. oxysporum*		*Heliconia* sp.	South Africa	S. Tween	Non-pathogen		
		F. circinatum							
CAV 211		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 273		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 202		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 261		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 246		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 275		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			
CAV 231		*F. oxysporum* non-pathogen		soil	South Africa	B. Nel			

CAV	**Other**	**Species and *forma specialis***	**VCG**	**Host/Cultivar**	**Origin**	**Collector**	**Pathogenicity**	**Mating type**	**Lineage**[2]
CAV 274		*F. oxysporum* non pathogen		soil	South Africa	B. Nel			
CAV 330	CBS 413.90	*F. oxysporum* f.sp. *lycopersici*		tomato	Israel	R. Cohn			
CAV 337	CBS 411.90	*F. oxysporum* f.*sp. vasinfectum*		cotton	Israel	J. Katan			
CAV 342	CBS 101.97	*F. oxysporum* f.sp. *lupine*		lupinus	Netherlands	M. Guranowska			
CAV 343	CBS 424.90	*F. oxysporum* f.sp. *melonis*		melon	Israel	J. Katan			
CAV 336	CBS 488.76	*F. oxysporum* f.sp. *raphanai*		radish	Germany	W. Gerlach			
CAV 341	CBS 794.70	*F. oxysporum* f.sp. *perniciosum*		silktree	Iran	W. Gerlach			
CAV 329	CBS 259.51	*F. oxysporum* f.sp. *lini*		flax	Canada	J.W. Groves			
CAV 328	CBS 137.97	*F. oxysporum* f.sp. *gladioli*		freesia	Netherlands	E.J.A. Roebroeck, L.B.O. Lisse			
CAV 335	CBS 101587	*F. oxysporum* f.sp. *radicis-lycopersici*		tomato		G.V. Bloemberg			

[1] CAV = Culture Collection at FABI, University of Pretoria, South Africa; NRRL = Regional Research Laboratory, NCAUR, Peoria IL, USA; digits only = Culture Collection of the Queensland Department of Primary Industries, Brisbane, Australia; RP = Culture Collection of Randy Ploetz at the University of Florida, Homestead FL, USA; DNA supplied by Dr Suzy Bentley from the Queensland department of Primary Industries (QDPI), Brisbane Australia (DNA).

[2] Pathogenicity test conducted in a hydroponics system (Nel *et al.*, 2006b), and disease severity measured using a previously developed rating scale (Carlier *et al.*, 2002).

[3] Mating-types were determined by PCR using the primer set Falpha 1 and Falpha 2 for *MAT*-1 (Arie *et al.*, 2000), and the primers GFmat2c (Steenkamp *et al.*, 2000) and FF1 (Visser, 2003) for *MAT*-2.

[4] *Foc* lineages (I-VI) identified in based on phylogenetic analyses of the combined TEF+MtSSU sequence data (Figure 1).

Figure 1. A maximum likelihood phylogenetic tree of *Fusarium oxysporum* f.sp. *cubense* inferred from combined translation elongation factor-1α and the mitochondrial small subunit ribosomal RNA sequence data. A tree with a similar topology was generated using Bayesian inference. The two major clades are indicated at their respective branches with A and B, while the various *Foc* lineages (I-VI) are indicated to the right of the tree. For each taxon, vegetative compatibility group (VCG) and race designation, geographic origin and mating type are indicated. Bootstrap values (>50%) for the distance, maximum likelihood analyses and Bayesian posterior probabilities (>0.7) are indicated at the internodes. The tree is rooted with *Fusarium* sp. NRRL 22903.

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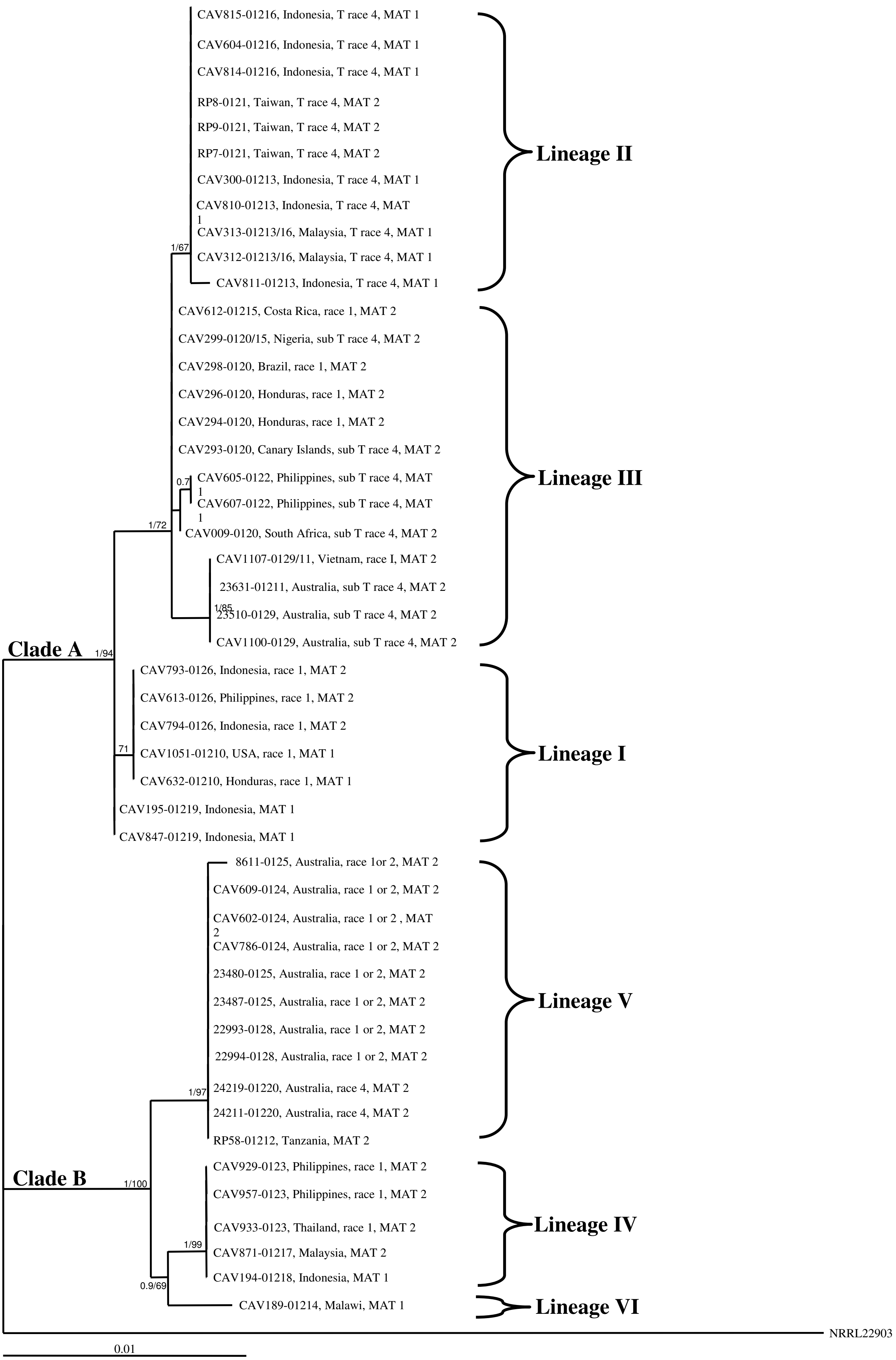

Figure 2. A maximum likelihood phylogenetic tree of *Fusarium oxysporum* f.sp. *cubense* inferred from intergenic spacer (IGS) region of the rRNA operon data. A tree with a similar topology was generated using Bayesian inference. The TEF+MtSSU-based *Foc* lineages (I-VI) identified in Figure 1 are indicated to the right of the tree. For each taxon, vegetative compatibility group (VCG) and race designation, geographic origin and mating type are indicated. Bootstrap values (>50%) for the distance, maximum likelihood analyses and Bayesian posterior probabilities (>0.7) are indicated at the internodes. The tree is rooted with *Fusarium* sp. NRRL28687.

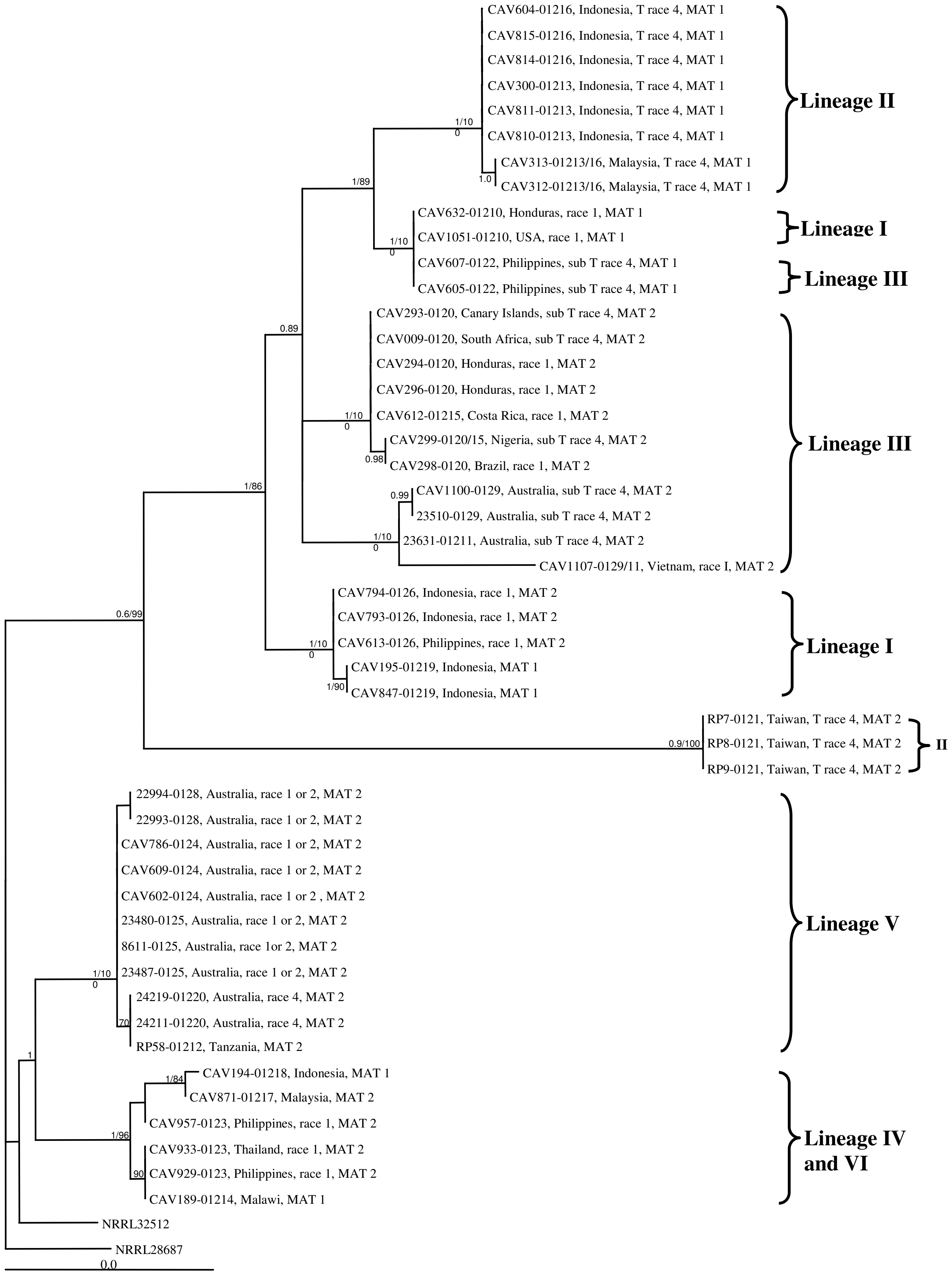
CAV604-01216, Indonesia, T race 4, MAT 1
CAV815-01216, Indonesia, T race 4, MAT 1
CAV814-01216, Indonesia, T race 4, MAT 1
CAV300-01213, Indonesia, T race 4, MAT 1
CAV811-01213, Indonesia, T race 4, MAT 1
CAV810-01213, Indonesia, T race 4, MAT 1
CAV313-01213/16, Malaysia, T race 4, MAT 1
CAV312-01213/16, Malaysia, T race 4, MAT 1
1/100
1.0
1/89
Lineage II
CAV632-01210, Honduras, race 1, MAT 1
CAV1051-01210, USA, race 1, MAT 1
1/100
Lineage I
CAV607-0122, Philippines, sub T race 4, MAT 1
CAV605-0122, Philippines, sub T race 4, MAT 1
Lineage III
0.89
CAV293-0120, Canary Islands, sub T race 4, MAT 2
CAV009-0120, South Africa, sub T race 4, MAT 2
CAV294-0120, Honduras, race 1, MAT 2
CAV296-0120, Honduras, race 1, MAT 2
CAV612-01215, Costa Rica, race 1, MAT 2
1/100
CAV299-0120/15, Nigeria, sub T race 4, MAT 2
0.98
CAV298-0120, Brazil, race 1, MAT 2
0.99
CAV1100-0129, Australia, sub T race 4, MAT 2
23510-0129, Australia, sub T race 4, MAT 2
1/100
23631-01211, Australia, sub T race 4, MAT 2
CAV1107-0129/11, Vietnam, race I, MAT 2
Lineage III
1/86
CAV794-0126, Indonesia, race 1, MAT 2
CAV793-0126, Indonesia, race 1, MAT 2
1/100
CAV613-0126, Philippines, race 1, MAT 2
CAV195-01219, Indonesia, MAT 1
1/90
CAV847-01219, Indonesia, MAT 1
Lineage I
0.6/99
RP7-0121, Taiwan, T race 4, MAT 2
0.9/100
RP8-0121, Taiwan, T race 4, MAT 2
RP9-0121, Taiwan, T race 4, MAT 2
II
22994-0128, Australia, race 1 or 2, MAT 2
22993-0128, Australia, race 1 or 2, MAT 2
CAV786-0124, Australia, race 1 or 2, MAT 2
CAV609-0124, Australia, race 1 or 2, MAT 2
CAV602-0124, Australia, race 1 or 2 , MAT 2
23480-0125, Australia, race 1 or 2, MAT 2
8611-0125, Australia, race 1or 2, MAT 2
1/100
23487-0125, Australia, race 1 or 2, MAT 2
24219-01220, Australia, race 4, MAT 2
70
24211-01220, Australia, race 4, MAT 2
RP58-01212, Tanzania, MAT 2
Lineage V
1
1/84
CAV194-01218, Indonesia, MAT 1
CAV871-01217, Malaysia, MAT 2
CAV957-0123, Philippines, race 1, MAT 2
1/96
CAV933-0123, Thailand, race 1, MAT 2
90
CAV929-0123, Philippines, race 1, MAT 2
CAV189-01214, Malawi, MAT 1
Lineage IV and VI
NRRL32512
NRRL28687
0.0

Figure 3. A maximum likelihood phylogenetic tree of *Fusarium oxysporum* f.sp. *cubense* inferred from mitochondrial direct repeat (MtR). A tree with a similar topology was generated using Bayesian inference. The TEF+MtSSU-based *Foc* lineages (I-VI) identified in Figure 1 are indicated to the right of the tree. For each taxon, vegetative compatibility group (VCG) and race designation, geographic origin and mating type are indicated. Bootstrap values (>50%) for the distance, maximum likelihood analyses and Bayesian posterior probabilities (>0.7) are indicated at the internodes. The tree is rooted with *F. circinatum*.

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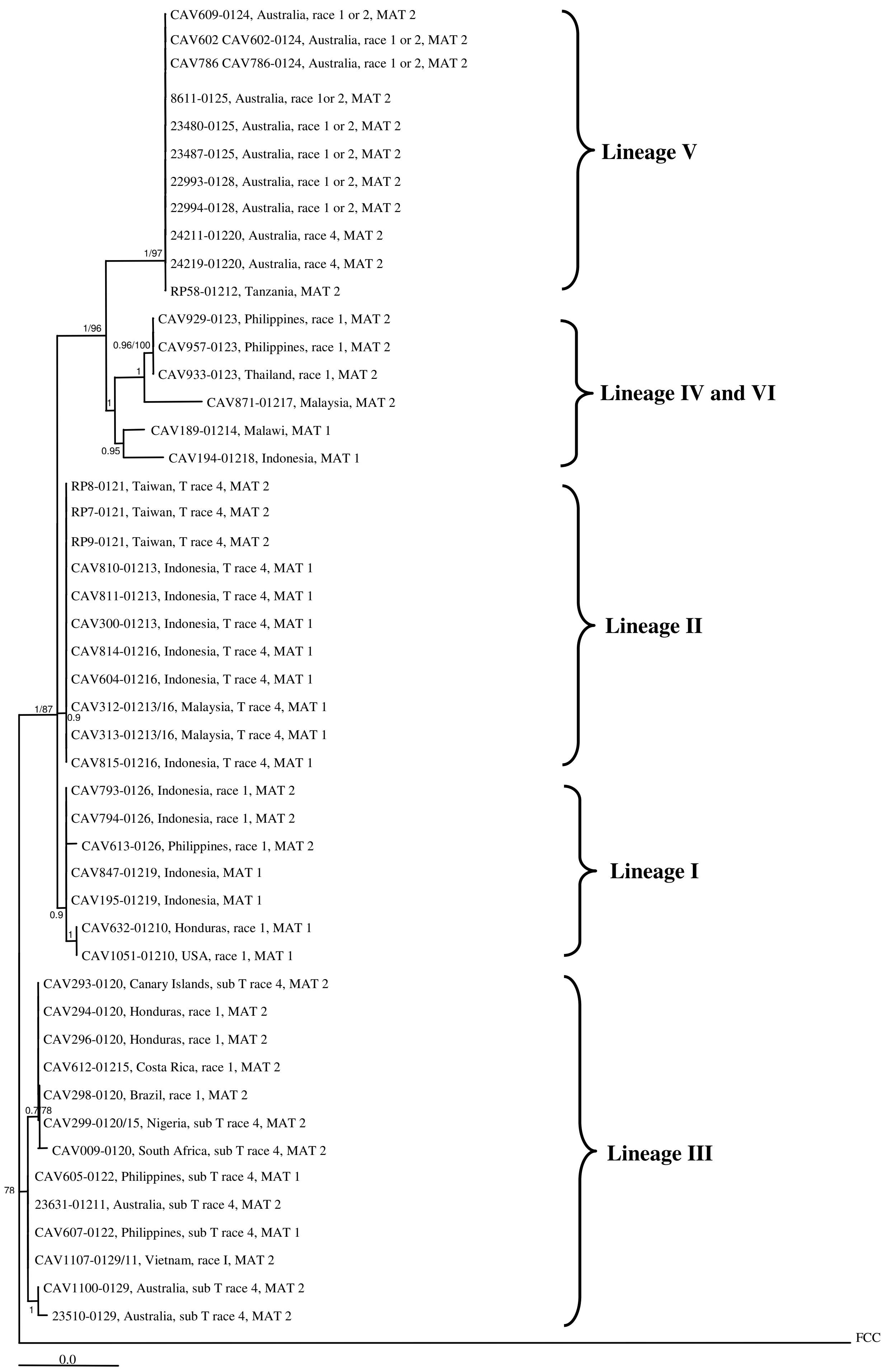

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CAV293-0120      ataaagggatcaatagttttcacagaagcctcttcctcctttgaaggcgcaagctcttct
CAV607-0122      ............................................................
CAV929-0123      .............................................a---------ta...
                         910       920       930       940       950       960
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV293-0120      t-----------------------------------------------------------
CAV607-0122      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV929-0123      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
                         970       980       990      1000      1010      1020
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV293-0120      ------------------------------------------------------------
CAV607-0122      ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV929-0123      ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
                        1030      1040      1050      1060      1070      1080
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV293-0120      -------------------------------------ggttaaatatttcttgctctaat
CAV607-0122      gaagcctcttcctccattgaaggcgcaagctcttctc.......................
CAV929-0123      gaagcctcttcctcctttgaaatatctc---------......................c

CAV293-0120      ataaagggatcaatagttttcacagaagcctcttcctcctttgaaggcgcaagctcttct
CAV607-0122      ............................................................
```

Figure 4. An indel of 156 or 148 base pairs in the mitochondrial region 7 (MtR) of *Fusarium oxysporum* f.sp. *cubense* distinguished isolates in Clade A from those in Clade B. This indel is situated at base 901 with respect to the forward primer R117. Deletions are indicated with -'s.

Figure 5. A maximum likelihood phylogenetic tree of *Fusarium oxysporum* f.sp. *cubense* and other isolates in the *F. oxysporum* complex inferred from combined translation elongation factor-1α and the mitochondrial small subunit ribosomal RNA sequence data. A tree with a similar topology was generated using Bayesian inference. The three main clades are indicated with A, B and C to the right of the tree. For each *Foc* taxon, vegetative compatibility group (VCG) and race designation, geographic origin and mating type are indicated. Taxa representing other *F. oxysporum* isolates are indicated as 'human pathogen', 'non-pathogen' or with the specific *forma specialis*. Bootstrap values (>50%), based on 1000 replications are indicated at the internodes. The tree is rooted with *Fusarium* sp. NRRL 22903 and NRRL 25184.

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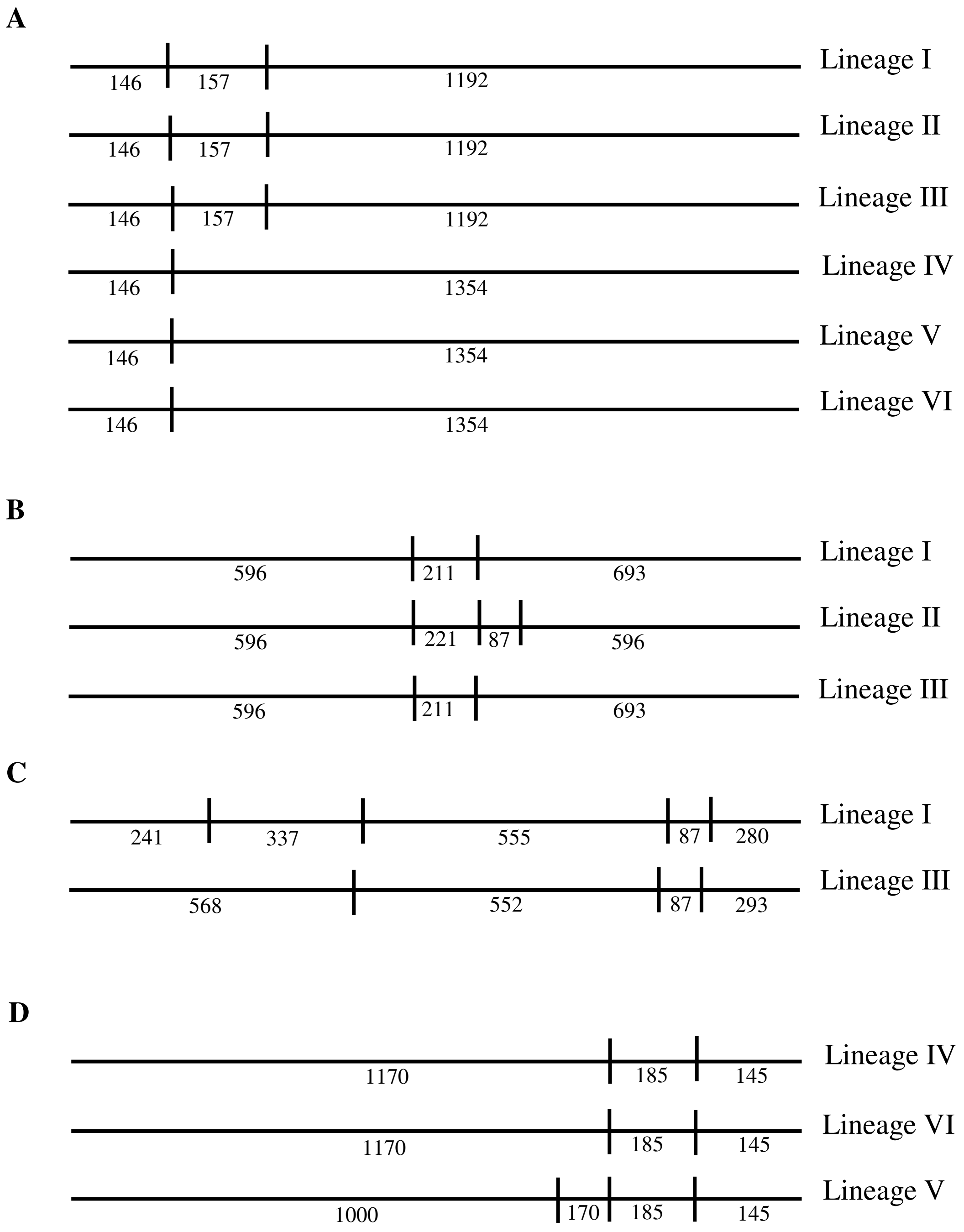

Figure 6. Restriction fragment length polymorphism map of the rRNA intergenic spacer PCR amplicons of the six *Fusarium oxysporum* f.sp. *cubense* lineages digested with restriction enzymes *AvaI* (**A**), *BceAI* (**B**), *Csp6I* (**C**), *BbvI* (**D**).

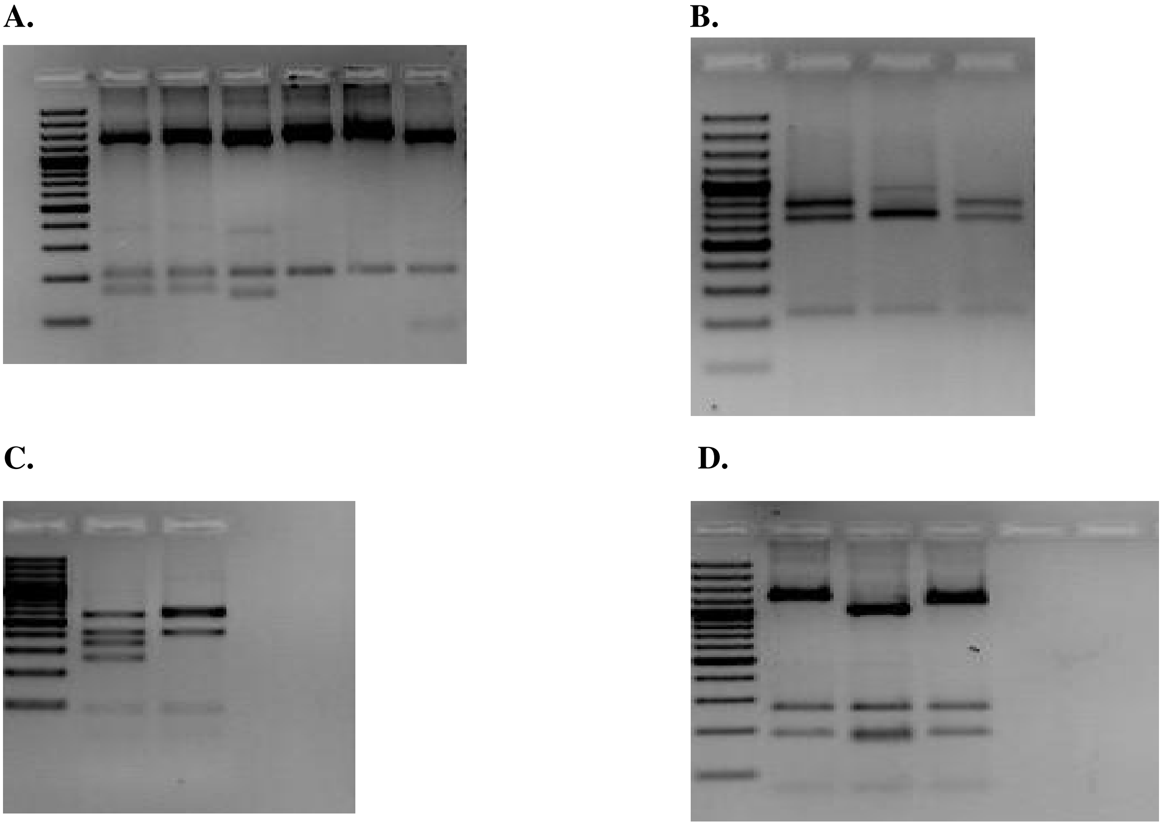

Figure 7. Restriction fragment length polymorphism fingerprints of the rRNA intergenic spacer PCR amplicons of the six *Fusarium oxysporum* f.sp. *cubense* lineages digested with restriction enzymes *AvaI* (A), *BceAI* (B), *Csp6I* (C), *BbvI* (D). **A.** Lane 1: 100-bp marker, lane 2: CAV 794 (lineage I), lane 3: CAV 815 (lineage II), lane 4: CAV 294 (Lineage III), lane 5: CAV 933 (Lineage IV), lane 6: CAV 1098 (Lineage V), lane 7: CAV 189 (Lineage VI). **B.** Lane 1: 100-bp marker, lane 2: CAV 794 (lineage I), lane 3: CAV 815 (lineage II), lane 4: CAV 294 (Lineage III). **C.** Lane 1: 100-bp marker, lane 2: CAV 794 (lineage I), lane 3: CAV 294 (Lineage III) **D.** Lane 1: 100-bp marker, lane 2: CAV 933 (Lineage IV), lane 3: CAV 1098 (Lineage V), lane 4: CAV 189 (Lineage VI).

CHAPTER 3

Rapid PCR-based identification of *Fusarium oxysporum* f.sp. *cubense* ‘subtropical’ race 4

ABSTRACT

Fusarium oxysporum f.sp. *cubense* (*Foc*) 'subtropical' race 4 is responsible for Fusarium wilt, the most important disease of bananas in South Africa. Conventional control practices for Fusarium wilt are ineffective, and disease management relies heavily on the use of clean planting material and the early detection of the disease in order to restrict the spread of the pathogen to unaffected areas. The aim of this study was to develop a PCR-based method for the rapid and accurate identification of *Foc* 'subtropical' race 4. Thirty-five isolates that included representatives of 20 vegetative compatibility groups of *Foc*, *formae speciales* other than *Foc*, and non-pathogenic *F. oxysporum*, were used in this study. Following DNA isolation, PCR and sequencing of a repeated region on the mithochondrion, analysis of the sequence data revealed an 8-base pair (bp) insertion that was subsequently targeted for the design of a *Foc* 'subtropical' race 4-specific PCR primer. Isolates were positively identified as *Foc* 'subtropical' race 4 with the amplification of an 800-bp fragment. The development of the *Foc* 'subtropical' race 4 PCR primer will aid in rapid and accurate detection of the Fusarium wilt pathogen of banana from planting material, water and soil, and can also be used to support quarantine services.

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INTRODUCTION

Fusarium oxysporum f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*) is the causal agent of Fusarium wilt, a disease affecting bananas (*Musa* spp.) in most regions where the crop is cultivated (Stover & Simmonds, 1987; Ploetz, 1994). Of the three races known for *Foc*, race 4 is regarded the most damaging and attacks Cavendish bananas in addition to those cultivars that are susceptible to *Foc* races 1 and 2 (Ploetz, 1990; Ploetz, 1994). *Foc* race 4 is divided into 'tropical' and 'subtropical' isolates based on their ability to cause disease to Cavendish bananas in the tropics and subtropics, respectively (Su *et al.*, 1977; Ploetz, 2005a; Ploetz, 2005b). In South Africa, Cavendish cultivars are the only bananas produced commercially and banana production areas are continuously decreasing due to Fusarium wilt devastation (Viljoen, 2002). Control of *Foc* is therefore crucially important not only in South Africa, but in all countries where *Foc* 'subtropical' race 4 threatens banana production (Ploetz, 2005a; Ploetz, 2005b).

Conventional control practices for Fusarium wilt of banana are ineffective, primarily due to *Foc*'s ability to survive in soil for long periods through the formation of chlamydospores (Beckman & Roberts, 1995). The only sustainable control option is the planting of disease-resistant cultivars (Moore *et al.*, 1999), but markets and producers are not eager to replace the popular Cavendish banana with resistant varieties. In order to restrict the spread of the pathogen to disease-free areas, disease management practices relies heavily on the use of clean planting material and the rapid and accurate detection of the pathogen. Conventional identification of *Foc* involves morphological identification, pathogenicity testing and the determination of vegetative compatibility groups (VCGs) (Waite & Stover, 1960; Su *et al.*, 1986; Moore *et al.*, 1993). *Foc* is separated into 24 known VCGs (Ploetz, 1988; Moore *et al.*, 1993; Bentley & Dale, 1995; Katan, 1999), of which six VCGs (0120, 01215, 0120/15, 0129, 0122 and 01211) represent *Foc* 'subtropical' race 4. Race and VCG identification of *Foc* is expensive, laborious and time consuming. The determination of races is based on field evaluation of a limited set of differential cultivars, while VCG analyses involve the generation and complementation of mutants (Puhalla, 1985).

DNA-based identification techniques provide a feasible alternative for the phenotypic diagnostics of *Foc*, but the fungus' polyphyletic nature complicates their development. Analysis of several DNA regions separated *Foc* into six distinct and mostly unrelated

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phylogenetic lineages (Chapter 2) with complex relationships between races and VCGs, which makes it difficult for a single DNA-based technique to distinguish all forms of the pathogen. This previous study (Chapter 2) has, however, shown that most race 4 isolates of *Foc* share a common ancestor that subsequently diverged to give rise to the lineages (Lineages II and III) that respectively harbour 'tropical' or 'subtropical' race 4 isolates. The aim of the current study was to use one of the regions included in this previous study (Chapter 2), that encodes a repeated region on the mithochondrion (MtR), for developing a PCR-based method for the rapid and accurate identification of isolates that potentially represent *Foc* 'subtropical' race 4.

MATERIALS AND METHODS

Fungal isolates and DNA: *Twenty-three isolates of* Foc*, ten* F. oxysporum *isolates representing* formae speciales *other than* Foc*, one* F. oxysporum *isolate from a* Heliconia *sp. and two non-pathogenic* F. oxysporum *isolates from the rhizosphere of banana plants in South Africa (Nel* et al.*, 2006) were included in this study (Table 1). DNA was extracted from these isolates using*

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sodium dodecyl sulphate (SDS) and phenol-chloroform extractions as described before (Chapter 2).

Development of Foc 'subtropical' race 4 specific PCR primers: The MtR region of the 35 *F. oxysporum* isolates was amplified with primer pair R117 and U9 (Tom Gordon, unpublished data) as described before (Chapter 2). The PCR fragments were purified using a High Pure PCR Product purification Kit (Roche Molecular Biochemicals, Manheim, Germany) and sequenced in both directions using the MtR region PCR primers and the Big Dye™ version 3.1 kit (Applied Biosystems, Foster City, California) and an ABI 377 automated sequencer (Applied Biosystems). The resulting sequences were analyzed using Chromas Lite version 2.01 (Technelysium) and the primers STR4r (5' AAG AAG AGC TTG CGC C 3') specific to *Foc* 'subtropical' race 4 and STR4f (5' CGC CGC TAT TTG AAG C 3') were designed using BioEdit version 6.0.7 (Hall, 1999). Amplification reaction mixtures and cycling conditions were similar to those used for amplification of the MtR region, with the exception of an STR4f and STR4r annealing temperature of 53°C, and the replacement of *Taq* DNA polymerase with FastStart *Taq* DNA polymerase (Roche Molecular Biochemicals) and an extension of the initial denaturation step to 5 min. To confirm that primers STR4f and STR4r amplified the targeted MtR region, selected PCR products were excised from the agarose gels (Roche) following electrophoresis (Sambrook *et al.*, 1989) and extracted with a QIAquick® Gel Extracion Kit (Qiagen, Madison, USA). The products were then sequenced with STR4f and STR4r as described earlier and compared to the MtR dataset.

Verification of Foc 'subtropical' race 4 diagnostic PCR: To test for the specificity and efficacy of the primer set developed for *Foc* 'subtropical' race 4, primers STR4f and STR4r were tested on 35 of the *F. oxysporum* isolates included in this study (Table 1), as well as the genomic DNA of another 204 isolates representing a worldwide collection of *Foc* (Chapter 4).

RESULTS

Development of Foc 'subtropical' race 4 specific PCR primers: Comparison of the MtR sequences for 35 isolates of *F. oxysporum* revealed the presence of an 8-base pair (bp) insertion in all Lineage III 'subtropical' race 4 isolates (Fig 1). Other polymorphisms within this region included a 156-bp indel, a 94-bp deletion within *Foc* VCG 01214 and an 18-bp deletion within *Foc* VCG 0124, 0125, 0124/5, 0128, 01220 and 01212 (Appendix C). The 8-bp insertion was, therefore, the only polymorphism specific for *Foc* 'subtropical' race 4 and was subsequently targeted for designing the reverse primer STR4r that was used in combination with the forward primer STR4f to amplify an 800-bp fragment from all 'subtropical' race 4 *Foc* isolates (Fig. 2).

PCR with primers STR4r and STR4f amplified an 800-bp fragment in *Foc* 'subtropical' race 4 isolates, but not in the other *F. oxysporum* isolates used in this study (Fig. 2). The primers further amplified four more fragments (1100-, 1300-, 2500- and 3000-bp) for *Foc* 'subtropical' race 4 VCGs 0120, 01215 and 0120/15 and two fragments (1100- and 2500-bp) for *Foc* 'subtropical' race 4 VCGs 0122, 0129 and 01211 (Fig 2). *Foc* isolates other than 'subtropical' race 4 produced an 1100-bp fragment and sometimes an additional 3000-bp fragment. Isolates representing *F. oxysporum* from other *formae speciales* and the non-pathogenic *F. oxysporum* isolates either produced the 1100-bp fragment or no product was amplified (Fig 2). The Australian isolate CAV 623 (VCG 01220), previously diagnosed as *Foc* 'subtropical' race 4 (Table 1), did not produce the expected 800-bp fragment, but only the 1100-bp fragment (Fig. 2).

Sequence analyses of the excised 800-bp PCR product showed that it corresponded to the MtR sequences from which the STR4f and STR4r primers were designed (Fig 1). Sequence analyses of the 1100-bp fragment also aligned within this region with respect to the forward primer, but continued beyond the diagnostic STR4r-binding site into the rest of the MtR's upstream region (Fig 1). Apart from insertions or deletions, sequence alignment of the MtR region revealed high sequence similarity between all isolates. The 8-bp insertion region from which the reverse primer was designed is, therefore, the only 'subtropical' race 4 MtR diagnostic sequence motif. The forward primer will therefore bind to all *F. oxysporum* isolates and since the MtR region is a region consisting of various repeating units, fragments other than the 800-bp region were amplified in most of the isolates tested.

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Verification of Foc 'subtropical' race 4 diagnostic PCR: Primers STR4f and STR4r allowed amplification of an 800-bp fragment from the six Lineage III 'subtropical' race 4 isolates included in this study (Table 1), as well as from 92 of the DNA samples isolated from a worldwide population of *Foc* (Chapter 4). The race designations for the majority of the 92 *Foc* isolates are unknown, but all were shown to represent Lineage III and the VCGs associated with *Foc* subtropical' race 4 (i.e. *Foc* VCG 0120, 01215, 0120/15, 0129, 0122 and 01211).

DISCUSSION

A PCR-based molecular marker for the detection of *Foc* 'subtropical' race 4 was successfully developed in this study. This marker, which consistently amplified an 800-bp fragment, distinguished *Foc* 'subtropical' race 4 from a global population of *Foc*, various *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* isolates. The only exception was an isolate from Australia (CAV 623, VCG 01220) previously considered as representing 'subtropical' race 4 (Pegg *et al.*, 1995). This isolate, however, cannot be considered as typical of *Foc* 'subtropical' race 4 as it caused disease to Cavendish bananas only after the plants were severely stressed by waterlogging and drought (Pegg *et al.*, 1995). This discrepancy highlights the fact that field evaluation of bananas to determine *Foc* races is inaccurate, since the results are influenced by environmental factors and variables such as temperature, host age and drought (Correll, 1991; Davis *et al.*, 1996). In contrast, the application of the diagnostic primers developed in this study allows for the consistent identification of *Foc* isolates regarded as *Foc* 'subtropical' race 4.

In this study, the specific detection of a race in *F. oxysporum* is reported for the first time. Most DNA-based diagnostic methods developed to date focussed on distinguishing *F. oxysporum* from other *Fusarium* spp. or to distinguish a *forma specialis* of *F. oxysporum* from other pathogens. For example, Mishra *et al.* (2003) developed a PCR-based assay to differentiate *F. oxysporum* from other toxigenic and pathogenic *Fusarium* spp., and Lievens *et al.* (2003) designed and developed a DNA array for the rapid detection and identification of the tomato vascular wilt pathogen *F. oxysporum* f.sp. *lycopersici* and Verticillium Wilt pathogens *Verticillium albo-atrum* or *Verticillium dahlia*. While the molecular marker developed in this study allows for the unequivocal diagnoses of *F. oxysporum* isolates representing *Foc* 'subtropical' race 4, our results should preferentially be supported by

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pathogenicity tests to confirm race designation, as the targeted mitochondrial region is not linked to specific virulence genes. While reliable pathogenicity tests do not exist, the 8-bp insertion in the MtR gene region appears to accurately reflect the identification of *Foc* isolates that cause disease to Cavendish bananas under subtropical environmental conditions.

The application of a PCR-based diagnostic method for *Foc* 'subtropical' race 4 will dramatically reduce the labour, time and costs associated with the identification of this pathogen by means of VCG analyses and field pathogenicity testing. The *Foc* 'subtropical' race 4 diagnostic method can be applied directly to isolates obtained from infected banana tissue, planting material, as well as water and soil samples. After the PCR-assay, only those isolates containing the 800-bp fragment need further analyses in pathogenicity studies. This presents a great advantage over existing methodologies, as *Foc* 'subtropical' race 4 isolates found in plant material, soil and water is morphologically indistinguishable from *F. oxysporum* isolates that do not cause disease to banana. Endophytic *F. oxysporum* isolates are commonly associated with banana plants and are frequently isolated from the roots, rhizomes and pseudostem bases of plants (Pereira *et al.*, 1999; Dubois *et al.*, 2004). Implementation of the PCR-based diagnostic method for the rapid and accurate detection of *Foc* 'subtropical' race 4 is of great importance and relevance to the growers in subtropical banana production areas, and should be used in routine sample surveys in order to set up and maintain quarantine regulations.

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Table 1. Origin and vegetative compatibility group (VCG) of *Fusarium* isolates used in this study.

CAV	Lineage[1]	Other number	Species or *formae speciales*	VCG	Race	Host/Cultivar	Origin	Collector
CAV 009	III		*Foc*	0120	ST race 4	Cavendish	South Africa	A. Viljoen
CAV 298	III	BR18	*Foc*	0120/15	ST race 4	Banana	Brazil	Unknown
CAV 612	III	RPCR1-1	*Foc*	01215	ST race 4	Gros Michel	Costa Rica	Unknown
CAV 607	III	RP 13	*Foc*	0122	ST race 4	Cavendish	Philippines	Unknown
CAV 613	I	Phil 7	*Foc*	0126	Race 1	Latundan	Philippines	Unknown
CAV 1051	I	RP 52	*Foc*	01210	Race 1	Apple	USA	R. Ploetz
CAV 847	I	Indo 35	*Foc*	01219	Unknown	Pisang Raja Sereh	Indonesia	H. Stover
CAV 180	II	Taiwan 14	*Foc*	0121	T race 4	Cavendish	Taiwan	Unknown
CAV 300	II	CV-1	*Foc*	01213	T race 4	Valery	Indonesia	Unknown
CAV 312	II	RPML 25	*Foc*	01213/16	T race 4	Pisang udang	Malaysia	R. Ploetz
CAV 604	II	Indo 50	*Foc*	01216	T race 4	Cavendish	Indonesia	Unknown
CAV 602	V	23534	*Foc*	0124	Race 1or 2	Lady finger	Australia	Unknown
CAV 184	V	23906	*Foc*	0125	Race 1 or 2	Lady finger	Australia	Unknown
CAV 1097	V	22993	*Foc*	0128	Race 1 or 2	Blue Java	Australia	Unknown
CAV 623	V	24218	*Foc*	01220	ST race 4	Williams	Australia	Unknown
CAV 957	IV	Thai 37	*Foc*	0123	Race 1	Kluai Namwa	Thailand	S. Kooariyakul
CAV 186	III	24234	*Foc*	0129	ST race 4	Cavendish	Australia	Unknown
CAV 617	III	23707	*Foc*	01211	ST race 4	Lady Finger	Australia	Unknown

CAV	**Lineage**[1]	**Other**	**Species or *formae speciales***	**VCG**		**Host/Cultivar**	**Origin**	**Collector**
CAV 188	V	STNPZ	*Foc*	01212	Unknown	unknown	Tanzania	R. Ploetz
CAV 189	VI	RPMW 40	*Foc*	01214	Unknown	Harare	Malawi	R. Ploetz
CAV 871	IV	MAL 7	*Foc*	01217	Unknown	Pisang Rastali	Malaysia	Unknown
CAV 194	IV	Indo 5	*Foc*	01218	Unknown	Pisang Siem	Indonesia	N. Moore
CAV 1020		Viet 19	*Foc*	unknown		Chuoi xiem	Vietnam	I. Buddenhagen, N. Moore, S. Bentley
CAV 1788			*F. oxysporum*			*Heliconia* sp.	South Africa	S. Tween
CAV 211			*F. oxysporum* non pathogen			Soil	South Africa	B. Nel
CAV 273			*F. oxysporum* non pathogen			Soil	South Africa	B. Nel
CAV 330		CBS 413.90	*F. oxysporum* f.sp. *lycopersici*			Tomato	Israel	R. Cohn
CAV 337		CBS 411.90	*F. oxysporum* f.*sp. vasinfectum*			Cotton	Israel	J. Katan
CAV 342		CBS 101.97	*F. oxysporum* f.sp. *lupine*			Lupinus	Netherlands	M. Guranowska
CAV 343		CBS 424.90	*F. oxysporum* f.sp. *melonis*			Melon	Israel	J. Katan
CAV 336		CBS 488.76	*F. oxysporum* f.sp. *raphanai*			Radish	Germany	W. Gerlach
CAV 341		CBS 794.70	*F. oxysporum* f.sp. *perniciosum*			Silktree	Iran	W. Gerlach
CAV 329		CBS 259.51	*F. oxysporum* f.sp. *lini*			Flax	Canada	J.W. Groves
CAV 328		CBS 137.97	*F. oxysporum* f.sp. *gladioli*			Freesia	Netherlands	E.J.A. Roebroeck, L.B.O. Lisse
CAV 335		CBS 101587	*F. oxysporum* f.sp. *radicis-lycopersici*			Tomato		G.V. Bloemberg

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[1] *Foc* lineages as defined by a multi-gene phylogeny (Chapter 2).

```
                  10        20        30        40        50        60
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 gaatatgaagagtgtacggcttcgccgctatttgaagcttcagacatattaacttctata
800 bp   ----------------------nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
1100bp   ----------------------nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn

                  70        80        90       100       110       120
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 gcttgggaggctgaagaagagatccggcttactctttctaccgaattagatgggggtccc
800 bp   ............................................................
1100bp   ............................................................

                 130       140       150       160       170       180
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 tcattattataattattgagagaaatattcgggctatttactaactctttatcatctatt
800 bp   ............................................................
1100bp   ............................................................

                 190       200       210       220       230       240
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 ggttctgaaaatgagaaagaggaccgttctatattatttgaccgttctatgttatttgac
800 bp   ............................................................
1100bp   ............................................................

                 250       260       270       280       290       300
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 ccttctaaattattcgggtgaatataatcttctaaaaacaccaattcaaagaaattaaaa
800 bp   ............................................................
1100bp   .......G....................................................

                 310       320       330       340       350       360
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 taatacacaggattttctggatccccttctggcaaattctctataccatctggatatcct
800 bp   ............................................................
1100bp   ............................................................

                 370       380       390       400       410       420
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 tgggcagcatcggatagatccacactatccacacccggaggtagataaagagtaacatca
800 bp   ............................................................
1100bp   ............................................................

                 430       440       450       460       470       480
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 tgatgattaacatgcaaatccggcacttctggaatatctcaggggaaaactcggtaatta
800 bp   ............................................................
1100bp   ............................................................

                 490       500       510       520       530       540
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 gtatcaatagtcatctcgacataataacacctattattttctacacctagcaaacgcggc
800 bp   ............................................................
1100bp   ............................................................

                 550       560       570       580       590       600
         ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120 tctattccacccctaaccggggtcataactgctaacattctatcaatagctacagtcaag
800 bp   ............................................................
1100bp   ............................................................
```

```
                 610       620       630       640       650       660
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  tacttatatcccctttcacggtgaatagcttcggctacatatcgtaaattatcgtcatac
800 bp    ............................................................
1100bp    ............................................................

                 670       680       690       700       710       720
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  gcctccggagaaggagcctcactagaactatcatctgatgatataataatagcctcagga
800 bp    .......A....................................................
1100bp    ............................................................

                 730       740       750       760       770       780
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  tctcccacttctactggtgcaggaggagaag------------aagtatcctcactagaa
800 bp    ...............................------------.................
1100bp    ...............................------------.................

                 790       800       810       820       830       840
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  ctatcatctgatgatataataataacctcaggatctctcacttctactgctgccagagga
800 bp    ............................................................
1100bp    ............................................................

                 850       860       870       880       890       900
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  ataaagggatcaatagttttcacagaagcctcttcctcctttgaaggcgcaagctcttct
800 bp    .........nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn--------------
1100bp    .............................................a---------ta...

                 910       920       930       940       950       960
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  t-----------------------------------------------------------
800 bp    ------------------------------------------------------------
1100bp    .-----------------------------------------------------------

                 970       980       990       1000      1010      1020
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  ------------------------------------------------------------
800 bp    ------------------------------------------------------------
1100bp    ------------------------------------------------------------

                 1030      1040      1050      1060      1070      1080
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  -------------------------------------ggttaaatatttcttgctctaat
800 bp    ------------------------------------------------------------
1100bp    -------------------------------------.......................

                 1090      1100      1110      1120      1130      1140
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  ttagacagttcttcatctcctatactggagagtggactatcttcagcttctactggctcc
800 bp    ------------------------------------------------------------
1100bp    ............................................................

                 1150      1160      1170      1180      1190      1200
          ....|....|....|....|....|....|....|....|....|....|....|....|
VCG 0120  gg-attacttggtaggatagggagaagtaggataggatctatactttc-tactactgttt
800 bp    ------------------------------------------------------------
1100bp    ..-............................c...........g....-...........
```

```
                   1210      1220      1230      1240
           ....|....|....|....|....|....|....|....|....|.
VCG 0120   ggtta-cctaaacccgcattcatc----------------------
800 bp     ----------------------------------------------
1100bp     .....-......t...........ataannnnnnnnnnnnnnnnnn
```

Figure 1. Sequences for the repeated region encoded on the mitochondion of *Fusarium oxysporum* f.sp. *cubense*. The alignment includes the 800- and 1100-bp fragments sequenced for a VCG 0120 isolate. The grey arrows indicate the target fragment of the forward primer as well as the 8-bp insertion targeted for the design of the reverse primer. An unknown nucleotide is indicated with "n", a deletion in the sequence is indicated with "-" and a "." indicates a sequence position similar to that of the VCG 0120 isolate.

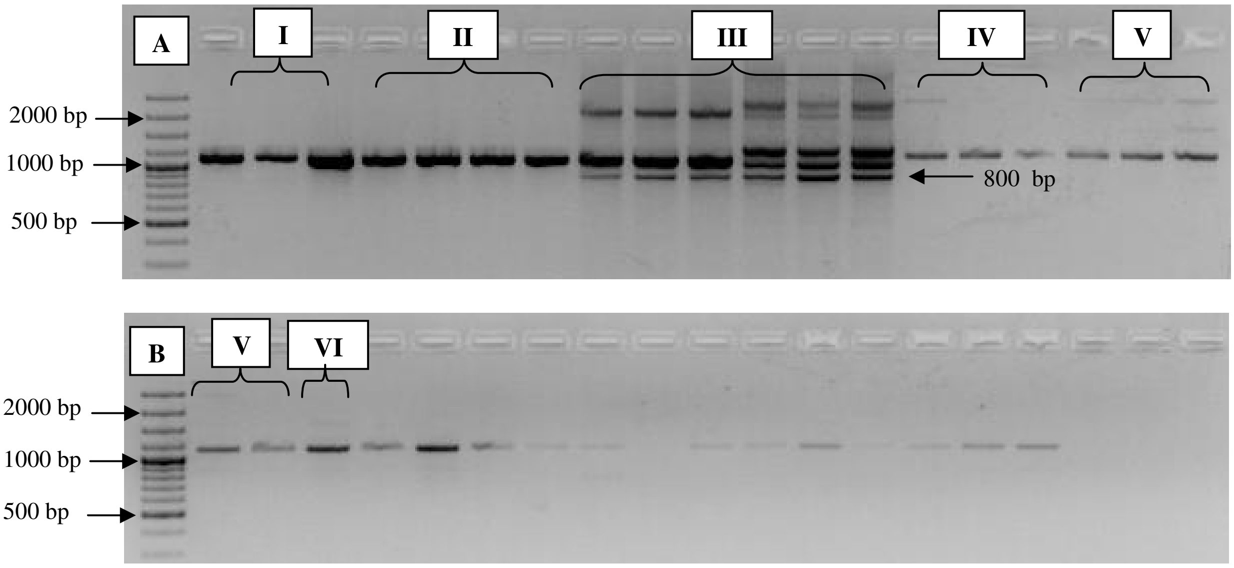

Figure 2. PCR amplification products of the 'subtropical' race 4-specific marker of *Fusarium oxysporum* f.sp. *cubense*. **A:** Lane 1: 100-bp molecular weight marker, lane 2: CAV 613 (VCG 0126), lane 3: CAV 1051 (VCG 01210), lane 4: CAV 847 (VCG 01219), lane 5: CAV 180 (VCG 0121), lane 6: CAV 300 (VCG 01213), lane 7: CAV 312 (VCG 01213/16), lane 8: CAV 604 (VCG 01216), lane 9: CAV 009 (VCG 0120), lane 10: CAV 298 (VCG 0120/15), lane 11: CAV 612 (VCG 01215), lane 12: CAV 607 (VCG 0122), lane 13: CAV 186 (VCG 0129), lane 14: CAV 617 (VCG 01211), lane 15: CAV 957 (VCG 0123), lane 16: CAV 871 (VCG 01217), lane 17: CAV 194 (VCG 01218), lane 18: CAV 602 (VCG 0124), lane 19: CAV 184 (VCG 0125), lane 20: CAV 1097 (VCG 0128). **B:** Lane 1: 100-bp molecular weight marker, lane 2: CAV 623 (VCG 01220), lane 3: CAV 188 (VCG 01212), lane 4: CAV 189 (VCG 01214), lane 5: CAV 1020, lane 6: CAV 1788, lane 7: CAV 211, lane 8: CAV 273, lane 9: CAV 330, lane 10: CAV 337, lane 11: CAV 342, lane 12: CAV 343, lane 13: CAV 336, lane 14: CAV 341, lane 15: CAV 329, lane 16: CAV 328, lane 17: CAV 325.

CHAPTER 4

The use of microsatellite markers to detect variation in a worldwide population of *Fusarium oxysporum* f.sp. *cubense*

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ABSTRACT

The fungal genus *Fusarium* includes species that behave as saprophytes, parasites and pathogens in plants, animals and humans. *Fusarium oxysporum*, a diverse species in this genus, consists of both non-pathogenic and pathogenic strains, and the latter includes more than 150 *formae speciales*. One of the most destructive *formae speciales* is *F. oxysporum* f.sp. *cubense* (*Foc*), causal agent of Fusarium wilt (Panama disease) of banana (*Musa s*pp.). Phenotypic markers have divided *Foc* into three pathogenic races and 24 vegetative compatibility groups (VCGs). Genotypic markers showed that *F. oxysporum* is a polyphyletic taxon that consists of multiple clonal lineages. In this study, a Bayesian approach was used to characterize the population structure of a worldwide collection of *Foc*. Nine microsatellite markers (SSRs) developed for *F. oxysporum*, were used to measure gene and genotypic diversity, the reproductive mode, population differentiation and gene flow among populations. From a geographic point of view, the Southeast Asian isolates showed the highest level of genotypic diversity, supporting the hypothesis that Asia is the centre of origin for *Foc*. The Bayesian structure analyses separated the *Foc* isolates into subpopulations that mostly correspond to previously defined multi-gene phylogenetic lineages. Within the subpopulation representing isolates from Lineage V the hypothesis of sexual recombination could not be rejected. A low gene and genotypic diversity was observed within all defined subpopulations and inferred gene flow between most of these populations appears to be limited. The results of this study therefore indicate that *Foc* consists of multiple unrelated groups or lineages that are reproductively isolated. The data also confirmed that VCG assays together with phylogenetic analyses remain a powerful tool for characterizing isolates of the causal agent of Fusarium wilt of banana.

INTRODUCTION

Fusarium is regarded as one of the most heterogeneous fungal genera (Gaudet *et al.*, 1989), and includes species that have been identified as saprophytes, parasites and pathogens in plants, animals and humans (Booth, 1971; Armstrong & Armstrong, 1981; Booth, 1984). One of the economically more important species in the genus is *Fusarium oxysporum* Schlechtendahl. emend. Snyder & Hansen. This highly diverse species comprises of non-pathogenic and pathogenic members, and the latter includes more than 150 *formae speciales*, recognised by their ability to cause disease to different crops (Hawksworth *et al.*, 1995; O'Donnell & Cigelnik, 1999). Pathogenic members of *F. oxysporum* infect plants mostly through roots and, once inside the xylem, rapidly spread in the vascular vessels by means of conidia. Invasion of conidia within the plant's vascular system, together with host defence responses, eventually block the plant's vascular system, (Beckman *et al.*, 1961; Beckman *et al.*, 1962; Stover, 1962; Beckman & Roberts, 1995) causing it to wilt and die.

Fusarium wilt (Panama disease) of banana (*Musa s*pp.) is a destructive vascular wilt disease caused by *F. oxysporum* f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*). This disease has been reported from all production regions of the world except those bordering the Mediterranean, Melanesia, Somalia and some islands in the South Pacific (Stover & Simmonds, 1987; Ploetz, 1994). Various markers have been used to study genetic diversity in *Foc.* The most widely applied phenotypic markers are pathogenicity and vegetative compatibility, and which grouped *Foc* into three pathogenic races (Ploetz, 1990; Ploetz, 1994) and 24 vegetative compatibility groups (VCGs) (Ploetz, 1988; Moore *et al.*, 1993; Bentley & Dale, 1995; Katan, 1999), respectively. DNA-based markers, such as random fragment length polymorphisms (RFLP) (Koenig *et al.*, 1997), multi-gene sequences (O'Donnell *et al.*, 1998; Chapter 2), random amplification of polymorphic DNA (RAPD) (Bentley & Dale, 1995; Bentley & Bassam, 1996), DNA amplified fingerprinting (DAF) (Bentley *et al.*, 1998) and amplified fragment length polymorphisms (AFLPs) (Groenewald *et al.*, 2006) have also been used to study diversity in *Foc*. These studies all concluded that *Foc* is a polyphyletic taxon that consists of multiple clonal lineages.

Pathogen detection, disease diagnosis and disease prevention are dependent on accurate identification of species, races and VCGs of pathogenic fungi, as well as on information regarding pathogen diversity and population structure (Bickford *et al.*, 2006; Pérez-Losada *et*

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al., 2006). In the absence of sufficient morphological and biological evidence of variation between lineages of *Foc*, DNA-based markers have been more successfully employed to detect variation in the banana pathogen (Koenig *et al.*, 1997; Bentley *et al.*, 1998; O'Donnell *et al.*, 1998). Even these markers, however, have limitations in separating closely related individuals of clonal organisms such as *F. oxysporum.* Studies of phylogenetic relationships based on gene sequencing, for instance, are often hampered by a lack of variation in gene regions among closely related species (Goldstein *et al.*, 1995; 1999). Fingerprinting techniques such as RAPDs have poor reproducibility between laboratories (McDonald, 1997) and interpretation of AFLP band profiles in terms of loci and alleles is compromised by the fact that similar sized fragments may not be homologous (Carbone *et al.*, 1999).

The existence of repetitive elements (microsatellites) or short simple sequence repeats (SSRs) in eukaryotic genomes has been documented since the 1960s (Bruford & Wayne, 1993). These SSRs have high mutation rates that could provide information on relationships among closely related species or subpopulations within species (Goldstein *et al.*, 1995; 1999). Brave *et al.* (2001), for instance, used a modified microsatellite technique in a study of *F. oxysporum* f.sp. *ciceri* isolates from India and were able to differentiate between the four races of the pathogen, which was not possible using other fingerprinting techniques. Bogale *et al.* (2006) used SSRs together with other DNA-based data to examine the diversity of *F. oxysporum* from Ethiopia and found a low genetic diversity among the isolates concluding that it reflects the nature of Ethiopian agriculture. In the current study, the population structure of a worldwide collection of *Foc* was characterized using DNA-based SSR markers targeting nine nuclear loci. For this purpose, a Bayesian approach was used to assign individuals to different populations, after which gene and genotypic diversity were determined, population differentiation was characterized and reproductive mode and gene flow inferred. The mating type for individual isolates was also determined in order to investigate the potential involvement of sexual recombination in *Foc*.

MATERIALS AND METHODS

Fungal isolates: A worldwide collection of 239 isolates representing 19 VCGs and five VCG complexes were used in this study (Table 1). These isolates represent five of the six *Foc* lineages defined from phylogenetic analysis using nucleotide sequence information for genes encoding the translation elongation factor-1α and the mitochondrial small subunit ribosomal

RNA (Chapter 2; Table 1). Thirty isolates were sampled from Africa, mostly South Africa, but also from Nigeria, Malawi, Tanzania, Canary Islands, Uganda and Zaire; 32 from the Americas, including Brazil, Costa Rica, Honduras, Nicaragua, Jamaica and the USA; 91 from Asian countries such as Indonesia, Malaysia, Philippines, Taiwan, Thailand and India; and 86 isolates from Australia. Isolates representing the five remaining VCGs were not included in the study because no isolates of VCG 01221, 01222, 01223 and 01224, and only one isolate of VCG 01214, were available. All the isolates were obtained from Dr Suzy Bentley of the Queensland Department of Primary Industries, Brisbane, Australia, except those from South Africa. All cultures are maintained in the culture collection of the Forestry Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA isolation: The *Foc* isolates were grown at 25°C on 50% potato dextrose agar (PDA) (Biolab Diagnostics, Wadeville, South Africa) (20 g/L) for 7 days. Mycelia were harvested and homogenized in DNA extraction buffer (Reader & Broda, 1985), after which DNA was purified using standard phenol-chloroform extractions (Sambrook *et al.*, 1989) and precipitated using 0.1 volumes of 3 M sodium acetate (pH 5.5) and 2 volumes of ethanol at 5ºC and centrifugation (20817 x *g*) for 10 min at -20°C. The precipitated DNA was then washed with 70% ethanol, air dried, resuspended in sterile distilled water and quantified using a Nanodrop spectrophotometer (NanoDrop, Wilmington, USA).

SSR analysis: Nine loci containing SSR sequences were amplified with six primer pairs developed by Visser (2003) and three by Bogale *et al.* (2005) (Table 2). One primer in each set was fluorescently labelled with either NED, PET, 6-FAM or VIC. PCR cocktails contained ~5 ng/µl DNA, 0.3 µM of each primer, 250 µM dNTPs (Fermantas, Nunningen, Switzerland), 0.04 U/µl *Taq* DNA polymerase (Roche Molecular Biochemicals, Manheim, Germany) and PCR buffer with $MgCl_2$ (Roche). PCR reaction conditions comprised of 35 cycles of 30 sec at 94°C, 45 sec at the specific annealing temperature for the primer pair

(Table 2), and 90 sec at 72°C. An initial step of 2 min at 94°C was included prior to the 35 cycles, and the PCR was completed with a final extension step of 5 min at 72°C.

After visualization of amplicons using agarose gel (Roche) electrophoresis (Sambrook *et al.*, 1989), PCR products were diluted, either 1:25 or 1:50 depending on concentration, and 1 µl of the diluted product was mixed with a LIZ^{TM} 500 internal size standard:formamide mix (1:14) (Applied Biosystems, Foster City, CA, USA). As the primer labels were selected in order to prevent overlapping of similar sized product amplified by different primer sets, all the amplified products for a specific isolate could be multiplexed. PCR products were separated with an ABI PRISM 3100 sequencer (Applied Biosystems), and the DNA fragment sizes were determined using Genescan 2.1 and GENEMAPPER software (Applied Biosystems). In these analyses a unique letter was allocated to each allele of a locus, while a unique number was allocated to each multi-locus haplotype (MLH) (genotype) (Table 1). A binary data matrix was also constructed where each allele was treated as a unique character, and the presence or absence of characters was scored as 1 and 0. This matrix was subsequently used to infer the parsimony-based relationships among MLHs, with the heuristic search option in PAUP* version 10b (Swofford, 1998).

Population structure: To detect the most likely number of populations (*K*) among our *Foc* isolates based on allele frequencies per locus, a Bayesian approach was used by employing the programme STRUCTURE 2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). Four independent runs of *K*=2 to *K*=5 were performed with 1 000 000 Markov chain Monte Carlo (MCMC) iterations following a burn-in period of 10 000 iterations. For these analyses, the admixture ancestry model (Pritchard *et al.*, 2000) and a value of one for the parameter (Lambda) of distribution of allelic frequencies (Pritchard *et al.*, 2000), were used. *K* was identified by plotting the Ln P(D) (posterior probability of the data for a given *K*) values calculated by STRUCTURE over the *K*-values tested. For the identified *K*-value, individuals were assigned to populations based on ≥80% shared ancestry using STRUCTURE's *Q*-matrix (*Q* denotes the admixture proportions for each individual).

Gene and genotypic diversity: To determine gene and genotypic diversity, the programme POPGENE (Yeh *et al.*, 1999) was used. Allele frequency per locus was calculated for each population and Nei's (1973) gene diversity (H) was determined with the formula H = 1 - ∑

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x^2_k, where x_k is the frequency of the k^{th} allele. Stoddard and Taylor's (1988) genotypic diversity (G_{ST}) was calculated from the observed number of genotypes across all loci using $G_{ST} = \sum_{x=0}^{1/N} [f_x(X/N^2)]$, where N is the sample size and f_x is the observed frequency of the x^{th} allele. To compare the percentage of diversity between different populations, the maximum percentage of genotypic diversity ($\hat{G}$) (Chen *et al.*, 1994) was estimated from $\hat{G} = G/N \times 100$, where G is the observed genotypic diversity and N is the population sample size. Gene diversity, genotypic diversity and the maximum percentage of genotypic diversity were calculated for populations defined in terms of geography (*i.e.* Africa, Asia, Australia and the Americas), as well as in terms of their multi-gene phylogenetic lineage associations (*i.e.* lineages I, II, III, IV and V).

***Population differentiation and gene flow**:* To study population differentiation and gene flow, the programme Multilocus (Agapow & Burt, 2001) was used. Population differentiation was calculated from clone-corrected populations using Weir's theta (θ) under 1 000 randomised datasets (Weir, 1996). This value is Weir's formulation of Wrights' F_{ST} and is calculated from $Q = \sum Q_2 - \sum Q_3 / \sum (1-Q_3)$, where Q_2 is the probability that two alleles from the same population are the same, and Q_3 is the probability that two alleles from different populations are the same. From θ, gene flow was calculated as $M = [(1/\theta) - 1]/2$ (Cockerham & Weir, 1993). Population differentiation was estimated for populations defined in terms of geography and multi-gene phylogenetic lineage associations (Chapter 2).

The mode of reproduction: The index of association (I_A), that determines multi-locus linkage disequilibrium, as well as the parsimony tree length permutation test (PTLPT) (Taylor *et al.*, 1999b), was used to infer the mode of reproduction. The observed value of I_A, calculated in Multilocus, was compared to 1 000 randomisations under the null hypothesis of random mating. The PTLPT in PAUP (Swofford, 1998) makes use of a phylogenetic method that was used to test for phylogenetic signal and was amended to test for the reproductive mode (Lobuglio & Taylor, 2002). The null hypothesis for a recombining population requires that the observed tree length not be significantly different from the expected tree length of 1 000 randomised data sets. For both I_A and PTLPT, clone-corrected and uncorrected populations were used where a clone-corrected population represents only one isolate per genotype present in that population and an uncorrected population represents the total population. The mode of reproduction was calculated within populations as defined by the five lineages in *Foc* (Lineage I, II, III, IV and V) (Chapter 2).

Mating type assay: Primer pairs Falpha 1 and Falpha 2 for *MAT*-1 (Arie *et al.*, 2000) and GFmat2c (Steenkamp *et al.*, 2000) and *Foc* FF1 (Visser, 2003) for *MAT*-2 were used to determine the mating types of the various *Foc* isolates. Except for the primers, these amplification reaction mixtures consisted of the same components as those for the SSR PCRs described earlier. PCR cycling conditions comprised of 35 cycles of 30 sec at 94°C, 45 sec at 55°C for *MAT*-1 and 54°C for *MAT*-2, and 90 sec at 72°C. An initial step of 2 min at 94°C was included prior to the 35 cycles, and the PCR was completed with a final extension step of 5 min at 72°C. The presence of the *MAT*-1 and *MAT*-2 idiomorphs was detected with agarose gel electrophoresis (Sambrook *et al.*, 1989).

RESULTS

SSR analysis: The nine SSR primer sets used in this study allowed the identification of 27 different alleles in the 239 isolates examined (Table 2). However, three primer sets (G20/G21, MB11F/MB11R and MB14F/MB14R) did not amplify more than one size of fragment. As this suggested that the examined *Foc* isolates are monomorphic at these loci, they were excluded from further analysis. G42/G43 was the only locus used in this study that displayed different alleles as a direct consequence of the repeat unit of the SSR (Table 2). In the other loci, insertions and/or deletions other than those associated with the repeating unit of the SSR were also involved in the observed polymorphism. For locus G1/MV15, for example, two of the alleles differed by 1 base pair (bp) only, although the repeating unit of the targeted SSR is a di-nucleotide (Table 2).

Irrespective of the nature of the polymorphic marker, some alleles were associated with specific VCGs. For locus G1/MV15, for example, alleles E and F were always associated only with isolates from *Foc* VCG 01210 and 01219, respectively. Similarly, some alleles were associated with specific lineages defined by multi-gene phylogenetic lineage associations (Chapter 2) (e.g. only the Lineage IV isolates the d allele present at locus G1/3). However, each of the 31 genotypes or MLHs (Table 1) was always associated with only one lineage (Table 3). In contrast, MLHs were either unique to a specific VCG, or were shared by closely related VCGs. For example, MLHs 3, 17 and 31 were unique to VCGs 01210, 0122 and 01222, respectively, but MLH 6 was shared by the closely related VCGs 0121, 01213 and 01216 (Table 3). In some cases, a single VCG was associated with one to four different MLHs. VCG 01213, for instance, was associated with MLHs 6, 8, 10 and 11 (Table 3).

Parsimony analysis separated the *Foc* MLHs into five groups (Fig. 1) that correspond to the multi-gene phylogenetic Lineages I-V previously identified (Chapter 2). The Lineage I MLHs included MLH 1-5 and clustered together. The same was also true for the Lineage II MLHs (MLH 6-12), the Lineage III MLHs (MLH 13, MLH 15-16), the Lineage IV MLHs (MLH 18-19) and the Lineage V MLHs (MLH 20-31). The only exception was MLH 14, represented by a single VCG 0120 isolate (CAV289) from Brazil, which clustered with Lineage I MLHs in stead of the VCG 0120 MLH 13 in Lineage III (Fig. 1). Removal of MLH 14 did not change the outcome of the parsimony analysis as a similar unrooted network was generated (results not shown).

Population structure: The results of the STRUCTURE analysis indicated that a *K*-value of 4 was associated with the highest Ln P(D)-value (Fig 2). Overall the global collection of *Foc* isolates used in this study is, therefore, likely to represent four separate populations. Assignment of individuals to the four populations using an ≥80% shared ancestry cut-off (Evanno *et al.*, 2005) resulted in groups of isolates that resemble the multi-gene phylogenetic lineages identified previously for *Foc* (Chapter 2) as well as the parsimony-based clusters identified in this study. Three of the STRUCTURE-based clusters consisted of isolates shown to represent *Foc* Lineages II, IV and V, respectively. The third STRUCTURE-based cluster consisted of isolates from both Lineages I and III of *Foc*. The removal of MLH 14 representing the VCG 0120 isolate from Brazil, also resulted in a *K*-value of four and the same STRUCTURE-based clusters. However, previous phylogenetic analyses based on both nuclear and mitochondrial gene regions showed that Lineages II and III are more closely related to one another than either is to Lineage I (Chapter 2). Therefore, populations were mainly defined in terms of the isolates' specific multi-gene phylogenetic lineage-associations for further analysis. To evaluate the origin and distribution of *Foc*, some populations were also defined in terms of geography.

Gene and genotypic diversity: From a geographic point of view, most alleles (25) were found among the Asian *Foc* isolates, followed by Australia with 21, the Americas with 19 and finally Africa with 17 (Tables 4 and 5). Isolates from the Americas were also monomorphic at locus MB18F/MB18R with all harbouring the a allele (Table 4). Unique alleles were found in the Asian population, and were associated with loci G1/MV15 (alleles b and f) and G2/G3 (alleles b and d) as well as within the American population at locus G1/MV15 (allele f) (Table 4). Fifty-one % of the alleles were shared by all the populations. Most MLHs (18) were also identified among the Asian isolates, and 13 of these were unique (Fig. 1). Among the Australian isolates, 12 MLHs were identified of which five were unique. Among the African isolates there were seven MLHs of which four were unique, and six MLHs of which three were unique among the American isolates (Fig. 3). Only 6.7% of the observed MLHs were shared across all the populations. Since unique MLHs were detected only once or twice, the populations generally consisted of one over-represented genotype. Gene diversity (*H*) across loci and genotypic diversity (G_{ST}) of geographically-defined populations were moderate to low, with Asia having the highest gene and genotypic diversity of 0.4 and 5.35, respectively (Table 5).

Definition of populations in terms of the multi-gene phylogenetic lineage associations of the *Foc* isolates (Chapter 2), revealed 18 alleles among the lineage V isolates, 13 alleles among each of lineages I, II and III, and 11 alleles among the lineage IV isolates (Tables 6 and 7). However, Lineages I and IV were monomorphic at seven loci, Lineage II at six, Lineage III at five, and Lineage V at four loci (Table 6). None of the alleles were unique to a specific lineage, and 11.1% were shared by all the lineages. Of the 31 MLHs, Lineages I and III included five MLHs, Lineage II seven, Lineage IV two and Lineage V 12 MLHs (Fig. 4). All the MLHs were unique to the respective Lineages, although each consisted mostly of one or two over-represented MLHs. MLH 16 and 31 were defined by null alleles at locus MB18F/MB18R. The gene and genotypic diversity was lower in the populations defined by multi-gene phylogeny than by geographic origin, with Lineage V yielding the highest gene diversity and Lineage I the highest genotypic diversity of 0.13 and 2.41, respectively (Table 7).

Population differentiation and gene flow: Values for θ, the population differentiation estimate, that approaches 1 indicate a low number of alleles shared between populations (*i.e.* limited gene flow), while θ-values nearing 0 indicate a significant number of alleles shared by two populations (*i.e.* unlimited gene flow) (Lobuglio & Taylor, 2002). In this study, θ was estimated within Australia and Asia because population differentiation should be determined between populations within a close geographical area where fungi have the opportunity to interbreed, whether or not they can actually do so (Taylor *et al.*, 1999a). For the Australian isolates, populations were defined according to the multi-gene phylogenetic associations (Chapter 2) and represents Lineages II, III and V as these are the only *Foc* lineages presents in Australia. For the Asian isolates, six populations were defined according to geographic origin (i.e. India, Indonesia, Malaysia, Philippines, Taiwan and Thailand) but also according to the multi-gene phylogenetic lineages of *Foc* from Asia (Chapter 2), irrespective of their geographic location.

The Australian *Foc* lineages yielded θ-values above 0.67 for all combinations, suggesting that few alleles were shared between the three populations defined by lineages II, III and V (Table 8). These θ-values, however, were not supported with significant P-values, making it impossible to reject the hypothesis of no population differentiation. The estimated gene flow (M) between the Australian populations was below 0.24 in all combinations (Table 8), which suggests great separation between the three *Foc* lineages in Australia. High M-values are good indicators of gene flow between populations, while values near 0 indicate a very small chance of gene flow (Cockerham & Weir, 1993).

For the geographically-defined Asian isolates, significant θ values were obtained for alleles shared between populations from India and Indonesia, India and Malaysia, India and Taiwan, and Indonesia and Malaysia ($P<0.05$) (Table 9). The highest θ-value of 0.8 between the Indian and Taiwanese populations could be linked to the low number of alleles/MLHs that were shared between these two populations, while the low θ-value of 0.2 between the Malaysian and Indonesian populations indicates sharing of several alleles/ MLHs. Population differentiation values of 0.4 and 0.6 between the Indian and Indonesian, and between the Indian and Malaysian populations, respectively, indicate that the number of alleles shared between these populations and those that were unique were more or less equal. Population differentiation values between several other countries were 0.2 and below (e.g. between the Philippines and India, Indonesia or Malaysia; between Taiwan and Indonesia, Malaysia, or the Philippines; and between Thailand and Indonesia, Malaysia, or the Philippines), suggesting that these populations share alleles/MLHs. These interactions, however, were not supported with significant P-values. M-values ranging from 0.1 to 12 (Table 9), indicated an almost complete lack of gene flow among some populations, and a more abundant gene flow between others.

The θ-values for the Asian *Foc* lineages indicated that most populations share few alleles and the M-values indicated that gene flow was limited (Table 10). The exception with regards to θ-values, however, was between Lineages I and III, with a value of 0.3. This suggests that some alleles are shared between the populations although not with significant P-values. The relative low M value of 1.1, however, indicated that gene flow between lineage I and III are limited or occur only occasionally (Table 10).

Mode of reproduction: Although the I_A and PTLPT for determining mode of reproduction were calculated for all *Foc* lineages defined from their multi-gene phylogenetic associations, simulations of randomisations under the null hypothesis of random mating could only be compiled within Lineage V. The failure to construct these simulations of random mating within lineages I to IV was due to the lack of diversity indicating a clonal reproductive strategy within these *Foc* lineages. The observed value for I_A for isolates in lineage V fell within the range of the randomised dataset for the clone-corrected population (Fig. 5A), suggesting random allele association and, therefore, random mating (Agapow & Burt, 2001). In the case of the uncorrected population (Fig. 5B), the observed I_A-value fell beyond the range of the randomized dataset, suggesting clonal reproduction. However, neither of the two populations' observed I_A-values were statistically significant ($P < 0.05$). In terms of the PTLPT, the observed tree length for lineage V also fell within the range of the randomized dataset for the clone-corrected population, and beyond the range of the randomized dataset for the uncorrected population. These results, however, were supported with significant P-values ($P > 0.05$) (Figs. 5C & D). The conflicting results between clone-corrected and uncorrected populations suggest that *Foc* predominantly follows a clonal reproductive strategy, but based on the PTLPT test, the occurrence of sexual recombination could not be excluded in all cases (Taylor *et al.*, 1999b).

Mating type assay: The isolates included in this study were identified either as *MAT*-1 or as *MAT*-2, based on the presence of a 370-bp amplicon representing a portion of the *MAT*-1 idiomorph (Arie *et al.*, 2000) or a 700-bp amplicon representing a portion of the *MAT*-2 idiomorph (Visser, 2003). With the exception of one Lineage I isolate from Indonesia (CAV 795, VCG 0126) representing *MAT*-1 that differed from the other VCG 0126 isolates (MAT-2 amplicons), only one mating type amplicon was present per VCG. Similarly, MLHs contained only one mating type with the exception of MLH 2 and MLH 6 that included both. *MAT*-1 was present in *Foc* MLH 2, MLH 3, MLH 4, MLH 5, MLH 6, MLH 8, MLH 9, MLH

10, MLH 11, MLH 12, MLH 17, and *MAT*-2 was present in *Foc* MLH 1, MLH 2, MLH 6, MLH 7, MLH 13, MLH 14, MLH 15, MLH 16, and MLH 18 to MLH 31 (Table 1).

DISCUSSION

A fundamental prerequisite to the correct utilization of SSR data for addressing population genetic questions is appropriate definition of populations or subpopulations. Populations are usually defined according to geographic origin, although the correct genetic structure of populations is not always reflected in geography (Evanno *et al.*, 2005). Individuals from different locations are not always genetically differentiated and, similarly, individuals within locations can be genetically structured due to unidentified barriers that would limit gene flow (Evanno *et al.*, 2005). For example, in a population study of Canadian polar bears Paetkau *et al.* (1995) concluded that, although measures of genetic distance reflect the geographic distribution of populations, it also suggested patterns of gene flow which are not obvious from geography. Similarly, Petit *et al.* (2000) sampled migratory bats from the same breeding population thousands of kilometres apart. A Bayesian clustering approach was, therefore, employed to identify subpopulations among the 239 *Foc* isolates. The results of this cluster analysis clearly separated the isolates into four populations, which broadly reflected their multi-gene phylogenetic lineages (Chapter 2). The only exception was that individuals from Lineages I and III were clustered together into a single subpopulation. This association is however, unlikely, as previous multi-gene phylogenies based on nuclear and mitochondrial regions clearly showed that Lineage III shares a more recent common ancestor with Lineage II isolates, than with Lineage I isolates (Chapter 2). Furthermore, Pritchard *et al.* (2000) pointed out that the Bayesian clustering approach merely provides an indication of the subpopulations, and that it should be employed as an *ad hoc* guide for the population analysis (Pritchard *et al.*, 2000). In the current study, populations were therefore defined mostly in terms of the multi-gene phylogenetic lineage associations of the isolates. Whether the variation within these lineages and groups detected by the Bayesian cluster analysis represent interspecific (species-based) or intraspecific (populations-based) genetic variation (Leslie *et al.*, 2007) remains to be clarified.

From a geographic perspective, Asia has long been considered the centre of origin of both the banana plant and its pathogens (Ploetz & Pegg, 1997; Ploetz, 2005a; Ploetz, 2005b). Among the populations of *Foc* investigated, Asia yielded the highest number of alleles, the highest gene and genotypic diversity, and was one of the only populations that harboured unique alleles. Burdon and Silk (1997) suggested that a higher gene diversity could be the result of mutations accumulating over time, and the population with the highest diversity should, therefore, be regarded as the oldest of the populations in question. Therefore, using SSR markers, this study, unequivocally demonstrated that the gene and genotypic diversity of the Asian population are most representative of an ancestral population when compared to populations from other continents.

If Asia is the centre of diversity for *Foc*, one would expect to find all 31 MLHs or genotypes in the Asian population. Unique genotypes, however, were found in all geographically-defined *Foc* populations. One could argue that VCGs represented by these genotypes unique evolved outside the centre of origin, but this is highly unlikely because the genotypes absent from Asia are associated with *Foc* VCGs that have previously been reported from various countries in Asia (Ploetz, 2005a; Ploetz, 2005b). The absence of certain genotypes from the Asian population could, therefore, be due to insufficient and selective sampling. It is also possible that these results reflect the effects of clonal selection (Leslie *et al.*, 2001) in combination with adaptation to the forces of geographic isolation and environmental changes over time (McDonald, 1997; Kohn, 2005).

Despite the fact that unique genotypes were present in all geographically defined populations, the worldwide collection of *Foc* consisted predominantly of six over represented genotypes or MLHs. Of these over represented genotypes, at least two (MLHs 13 and 20), were represented by individuals from all four continents. Similarly, a DNA fingerprinting study by Bentley *et al.* (1998) reported the global distribution of some of their DNA fingerprinting groups. These groups were also associated with similar VCGs than those associated with MLH 13 and 20. Such an over representation of MLHs, together with the overall low genetic diversity within all the defined subpopulations, provides support for the idea that *Foc* mainly reproduces mitotically (see below) and has a clonal genetic structure (Taylor *et al.*, 1999b; Lobuglio & Taylor, 2002). In addition, the occurrence of these genotypes on all continents illustrates that they are probably associated with well-adapted isolates of *Foc*. These genotypes also appear to be easily moved around the globe, most likely via infected planting material (Stover, 1962; Ploetz, 1990).

When one considers diversity among genotypes or MLHs, a specific MLH was mostly associated with only one or two *Foc* VCGs. The VCGs that shared similar MLHs, however, always resided within the same lineage as defined by multi-gene phylogenetic analyses (Chapter 2). In some instances, however, the same VCG was shared by several MLHs, suggesting that these VCGs are more diverse than others. Such diversity depicted in some VCGs is, however, not unusual considering the nature of vegetative compatibility assays. These assays rely on anastomosis of hyphae to form a stable heterokaryon between individuals that have identical alleles at all of their vegetative incompatibility (*vic*) loci. Isolates of the same VCG may, therefore, differ at regions outside the *vic* loci (Leslie, 1993), making diversity within VCGs possible. Despite the diversity within VCGs, these results confirm the close genetic relationship of *Foc* VCGs within specific multi-gene lineages, similar to the findings of others (Bentley & Dale, 1995; Bentley & Bassam, 1996; Koenig *et al.*, 1997; Bentley *et al.*, 1998; Groenewald *et al.*, 2006). The results presented here, therefore, indicate that vegetative compatibility determination, in combination with phylogenetic analyses, remains a powerful tool for characterizing isolates causing Fusarium wilt of banana.

The Australian populations tested for population differentiation and gene flow were represented by *Foc* ‘tropical’ race 4 (Lineage II), ‘subtropical’ race 4 (Lineage III) and isolates representing races 1 and 2 (Lineage V). These populations proved to be genetically isolated with respect to one another, with a limited amount of gene flow between them. The populations, therefore, have most likely been introduced independently into Australia from different countries or locations in Southeast Asia and have remained genetically isolated. Isolates from Lineage II represent *Foc* ‘tropical’ race 4 that has only been associated with diseased Cavendish bananas in geographically restricted areas (Bentley *et al.*, 1998). Lineage III and V, however, co-exist in Queensland (Table 1). Therefore, the limited gene flow depicted between Lineages III and V was not a consequence of geographic separation.

Considering the Asian populations, low population differentiation was detected between countries such as Indonesia, Malaysia, the Philippines and Thailand, all countries within close proximity to one another, and also between Thailand and India. The little population differentiation detected between these countries could, therefore, indicate the possible free movement of the pathogen or infected planting material between the countries within close proximity. It could, however, also indicate separate introductions of similar genotypes into these countries. The population differentiation estimates, therefore, were likely to be a direct consequence of the specific *Foc* lineage present within the countries in question, irrespective of geography. This idea was also supported by the high population differentiation and low gene flow estimates of the *Foc* lineages within Asia. The relatively low θ-value between Lineages I and III that suggest some amount of gene flow between these lineages, together with the population structure analysis reported here that indicated that these lineages should be combined into a single population, could be a direct result of the step-wise mutation nature of SSR regions. In other words, it may be due to the constraint of the range of allele sizes and the high mutation rate of the SSRs markers (Orti *et al.*, 1997; Fisher *et al.*, 2000). Interestingly, though, the structure analysis, un-rooted parsiomony-based network, and the population differentiation estimate, supported some of the same phylogenetic groupings inferred from the intergenic spacer region of the rRNA operon (IGS) (Chapter 2). Apple and Gordon (1996) reported that the IGS region of *F. oxysporum* as a relatively fast evolving and that it could, therefore, contribute to the similarity in associations inferred from the SSR data and the IGS region.

The presence of a small number of genotypes within Lineages I – IV of *Foc* is a strong indication that the pathogen reproduces asexually (Lobuglio & Taylor, 2002). It was, however, not possible to reject the hypothesis of a recombining population for Lineage V, which indicates the potential occurrence of sexual recombination. These results are in agreement with those of Taylor *et al.* (1999b) who reanalysed RFLP data of Koenig *et al.* (1997) with the use of PTLPT, and reported that recombination within some of the lineages might exist. Taylor *et al.* (1999b) then compared their results to a study by Bentley *et al.* (1998), and noted that the lack of association between VCG and DNA fingerprint could also possibly indicate recombination. A lack of VCG and genotype association, however, was not evident within this study. The implication of recombination within some of the *Foc* lineages may be due to unobserved sexual reproduction in nature, parasexuality and heterokaryosis, a non-sexual mode of genetic exchange which are known to occur in *F. oxysporum* (Buxton, 1962; Kuhn *et al.*, 1995), or historical association with a sexual ancestor (Burt *et al.*, 2001). The mating-type assays performed also revealed the presence of both mating types within a single MLH and, in one case, a single VCG (VCG 0126). The presence of both mating types within groups support the hypothesis of sexual origins that could be more recent than initially anticipated, although sexual reproduction within *Foc* has not been demonstrated (Chapter 2).

In order to accurately identify species, one first needs to define what a species is. Bock (2004) argued that the existence of a single, clearly defined species concept applicable to all organisms is simply impossible. Leslie (2001) argued that some aspects of all species concepts (Sites & Marshall, 2004) should contribute towards a concept of what a species in *Fusarium* is. Within *F. oxysporum*, however, the absence of sexual recombination and the lack of morphological characters complicate the use of many of these concepts for inferring species. Results from this study, in combination with the previous multi-gene phylogenetic analyses (Chapter 2) clearly demonstrate the presence of multiple unrelated lineages. The fact these lineages are mostly genetically isolated with respect to one another suggests that the apparent lack of gene flow may potentially be used to demarcate the boundaries between cryptic species. However, a "*forma specialis*" designation does not necessarily form part of taxonomic hierarchy (Gordon & Martyn 1997). Therefore, in order to pinpoint species boundaries within *F. oxysporum,* one should not focus on a single agricultural crop. The true relationships among the isolates, VCGs, lineages and populations of *Foc* will only be revealed once the diversity of *F. oxysporum* is fully characterised. With regards to Fusarium Wilt of banana, however, it is clear that its causal agent constitutes a number of lineages, some of which potentially represents cryptic species that acquired their ability to cause disease towards banana independently (Chapter 2).

This study is one the first studies to use SSR markers was to detect genetic variation within *Foc*. My results support the finding of previous DNA-based studies using RAPDs (Bentley & Dale, 1995; Bentley & Bassam, 1996), DAF analysis (Bentley *et al.*, 1998) and AFLPs (Groenewald *et al.*, 2006). While these techniques shed light on the diversity and relationship among *Foc* VCGs as well as the clonal structure of the lineages within *Foc*, the current study investigated the origin of *Foc*, its reproductive strategy and population differentiation and structure. Knowledge of the origin and reproductive mode will help to clarify the overall evolution and movement of the fungus, whereas population differentiation and structure provides an understanding of the pathogen on a global scale. This information collectively provides a picture of the pathogen's possible history that may help to predict its future and to implement disease management strategies. SSRs could also be used to develop VCG-specific markers. For instance, in this study we were able to consistently identify VCGs 1210 and 01219, from the USA and Indonesia, respectively, due to the presence of unique alleles. This would be of great value for pathogen identification as pathogenicity testing is not always reliable and VCG assays are time consuming (Correll, 1991; Davis *et al.*, 1996).

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Table 1. Multi-gene sequence lineages, vegetative compatibility groups (VCG), origin and multi-locus haplotypes (genotypes) of a worldwide collection of *Fusarium oxysporum* f.sp. *cubense* isolates selected to analyse gene and genotypic diversity.

Lineage[1]	**VCG**	**Number**	**Origin**	**Microsatellite primers**									**MLH**	**Mating type**[2]
				G1 + MV15	**G2 + G3**	**G16 + G8**	**G20 + G21**	**G40 + G41**	**G42 + G43**	**MB11f + MB11r**	**MB14f + B14r**	**MB18f + MB18r**		
I	0126	CAV1050	Honduras	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV785	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV798	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV799	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV801	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV803	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV804	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV805	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV806	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV1048	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV1049	Indonesia	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV599	Philippines	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV613	Philippines	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV185	Philippines	d	a	a	a	a	a	a	a	a	1	Mat 2
I	0126	CAV795	Indonesia	c	a	a	a	a	a	a	a	a	2	Mat 1
I	0126	CAV796	Indonesia	c	a	a	a	a	a	a	a	a	2	Mat 2
I	0126	CAV797	Indonesia	c	a	a	a	a	a	a	a	a	2	Mat 2
I	01210	CAV788	USA	e	a	a	a	a	a	a	a	a	3	Mat 1
I	01210	CAV187	USA	e	a	a	a	a	a	a	a	a	3	Mat 1
I	01210	RP51	USA	e	a	a	a	a	a	a	a	a	3	Mat 1
I	01219	CAV603	Indonesia	f	a	a	a	d	a	a	a	a	4	Mat 1
I	01219	CAV848	Indonesia	f	a	a	a	a	a	a	a	a	5	Mat 1
I	01219	CAV195	Indonesia	f	a	a	a	a	a	a	a	a	5	Mat 1
II	0121	CAV180	Taiwan	a	c	a	a	c	b	a	a	c	6	Mat 2
II	0121	F9130	Taiwan	a	c	a	a	c	b	a	a	c	6	Mat 2
II	0121	RP8	Taiwan	a	c	a	a	c	b	a	a	c	6	Mat 2
II	0121	RP9	Taiwan	a	c	a	a	c	b	a	a	c	6	Mat 2

Lineage[1]	VCG	Number	Origin	Microsatellite primers									MLH	Mating type[2]
				G1 + MV15	G2 + G3	G16 + G8	G20 + G21	G40 + G41	G42 + G43	MB11f + MB11r	MB14f + B14r	MB18f + MB18r		
II	01216	CAV604	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV622	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV813	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV852	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV855	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV857	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV859	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV860	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV862	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV863	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV864	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV865	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV866	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV867	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV869	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01216	CAV870	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV899	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV808	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV811	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV812	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV300	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV301	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV302	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV303	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV304	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV305	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV307	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV310	Indonesia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV610	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213	CAV851	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1

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Lineage[1]	VCG	Number	Origin	Microsatellite primers									MLH	Mating type[2]
				G1 + MV15	G2 + G3	G16 + G8	G20 + G21	G40 + G41	G42 + G43	MB11f + MB11r	MB14f + B14r	MB18f + MB18r		
II	01213	CAV877	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213/16	CAV1065	Australia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213/16	CAV1072	Australia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	01213/16	CAV312	Malaysia	a	c	a	a	c	b	a	a	c	6	Mat 1
II	0121	RP7	Taiwan	a	a	a	a	c	b	a	a	c	7	Mat 2
II	01216	CAV815	Indonesia	a	c	a	a	d	b	a	a	c	8	Mat 1
II	01216	CAV816	Indonesia	a	c	a	a	d	b	a	a	c	8	Mat 1
II	01213	CAV306	Indonesia	a	c	a	a	d	b	a	a	c	8	Mat 1
II	01213	CAV309	Indonesia	a	c	a	a	d	b	a	a	c	8	Mat 1
II	01213	CAV878	Malaysia	a	c	a	a	d	b	a	a	c	8	Mat 1
II	01216	CAV814	Indonesia	a	c	a	a	c	b	a	a	b	9	Mat 1
II	01213	CAV807	Indonesia	a	c	a	a	c	b	a	a	c	10	Mat 1
II	01213	CAV1074	Australia	a	c	a	a	c	b	a	a	b	11	Mat 1
II	01213/16	CAV1076	Australia	a	c	a	a	c	b	a	a	a	12	Mat 1
II	01213/16	CAV1077	Australia	a	c	a	a	c	b	a	a	a	12	Mat 1
II	01213/16	CAV313	Malaysia	a	c	a	a	c	b	a	a	a	12	Mat 1
III	0129	CAV1122	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1133	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1145	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1168	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1169	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1170	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1087	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1089	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1090	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1101	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1113	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1129	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1143	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129	CAV1144	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2

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Lineage[1]	**VCG**	**Number**	**Origin**	**Microsatellite primers**									**MLH**	**Mating type[2]**
				G1 + MV15	**G2 + G3**	**G16 + G8**	**G20 + G21**	**G40 + G41**	**G42 + G43**	**MB11f + MB11r**	**MB14f + B14r**	**MB18f + MB18r**		
III	0120	CAV601	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1055	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1056	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1058	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1059	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1062	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1067	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1070	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1073	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1080	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1081	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1098	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1099	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1116	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1117	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV284	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV990	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV291	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV292	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV293	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV611	Costa Rica	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV1035	Honduras	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV294	Honduras	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV295	Honduras	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV296	Honduras	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV625	Indonesia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV290	Brazil	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV006	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV024	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV108	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2

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Lineage[1]	**VCG**	**Number**	**Origin**	**Microsatellite primers**									**MLH**	**Mating type**[2]
				G1 + MV15	**G2 + G3**	**G16 + G8**	**G20 + G21**	**G40 + G41**	**G42 + G43**	**MB11f + MB11r**	**MB14f + B14r**	**MB18f + MB18r**		
III	0120	CAV042	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV042	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV045	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV059	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV066	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV075	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV095	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV036	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV114	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV107	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV002	South Africa	a	a	a	a	a	a	a	a	a	13	Mat 2
III	01215	CAV612	Costa Rica	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV981	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV983	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV984	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV985	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV986	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV987	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV988	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV297	Brazil	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120/15	CAV298	Brazil	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0129/01211	CAV1107	Australia	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0121/15	CAV 982	Canary islands	a	a	a	a	a	a	a	a	a	13	Mat 2
III	0120	CAV289	Brazil	g	a	a	a	a	a	a	a	a	14	Mat 2
III	0120/15	CAV1061	Australia	a	a	a	a	c	a	a	a	a	15	Mat 2
III	0120/15	CAV299	Nigeria	a	a	a	a	a	a	a	a	n	16	Mat 2
III	0122	CAV605	Philippines	a	b	a	a	a	a	a	a	b	17	Mat 1
III	0122	CAV618	Philippines	a	b	a	a	a	a	a	a	b	17	Mat 1
IV	0123	CAV598	Philippines	b	d	b	a	b	b	a	a	b	18	Mat 2
IV	0123	CAV929	Philippines	b	d	c	a	b	b	a	a	b	19	Mat 2

Lineage[1]	**VCG**	**Number**	**Origin**	**Microsatellite primers**									**MLH**	**Mating type[2]**
				G1 + MV15	**G2 + G3**	**G16 + G8**	**G20 + G21**	**G40 + G41**	**G42 + G43**	**MB11f + MB11r**	**MB14f + B14r**	**MB18f + MB18r**		
IV	0123	CAV930	Philippines	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	0123	CAV931	Philippines	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	0123	CAV1036	Philippines	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	0123	CAV938	Thailand	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	0123	CAV939	Thailand	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	0123	RP19	Philippines	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	01217	CAV871	Malaysia	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	01217	CAV872	Malaysia	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	01217	CAV873	Malaysia	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	01217	CAV874	Malaysia	b	d	c	a	b	b	a	a	b	19	Mat 2
IV	01217	CAV193	Malaysia	b	d	c	a	b	b	a	a	b	19	Mat 2
V	0124	CAV609	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV976	Indonesia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1042	Jamaica	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1043	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1044	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1046	Nicaragua	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV890	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1136	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1139	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1140	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1153	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1155	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1157	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1159	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1176	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV1177	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV602	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV630	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV786	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2

Lineage[1]	VCG	Number	Origin	Microsatellite primers									MLH	Mating type[2]
				G1 + MV15	G2 + G3	G16 + G8	G20 + G21	G40 + G41	G42 + G43	MB11f + MB11r	MB14f + B14r	MB18f + MB18r		
V	0125	CAV1052	Jamaica	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1091	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1092	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1095	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1180	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV787	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1083	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1088	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1120	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1123	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1134	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV1135	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV973	India	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0125	CAV974	India	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV608	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1038	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1039	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1040	Malawi	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1112	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1126	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1154	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV967	Philippines	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1106	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1124	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV606	Thailand	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV961	Thailand	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5	CAV1041	USA	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	CAV893	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	CAV1097	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	CAV1121	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2

Lineage[1]	VCG	Number	Origin	Microsatellite primers									MLH	Mating type[2]
				G1 + MV15	G2 + G3	G16 + G8	G20 + G21	G40 + G41	G42 + G43	MB11f + MB11r	MB14f + B14r	MB18f + MB18r		
V	0128	CAV1125	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	CAV1131	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	22993	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0128	22994	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	01220	CAV1127	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	01220	CAV1128	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	01220	24211	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	01220	24219	Australia	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/22	CAV995	Tanzania	c	e	b	a	d	b	a	a	c	20	Mat 2
V	0124/22	CAV1000	Uganda	c	e	b	a	d	b	a	a	c	20	Mat 2
V	0124/5/8	CAV 996	Tanzania	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124/5/8	CAV 997	Tanzania	c	e	b	a	d	b	a	a	a	20	Mat 2
V	0124	CAV977	Brazil	c	e	b	a	c	b	a	a	a	21	Mat 2
V	0124	CAV631	Honduras	c	e	c	a	d	b	a	a	a	22	Mat 2
V	0124	CAV1111	Australia	c	e	c	a	d	b	a	a	a	22	Mat 2
V	0124	CAV1175	Australia	c	e	c	a	d	b	a	a	a	22	Mat 2
V	0124	CAV1079	Australia	c	e	c	a	d	b	a	a	a	22	Mat 2
V	0124	CAV614	Australia	c	e	b	a	b	b	a	a	a	23	Mat 2
V	0125	CAV1158	Australia	d	e	b	a	d	b	a	a	a	24	Mat 2
V	0125	CAV941	Thailand	c	e	b	a	d	b	a	a	a	24	Mat 2
V	0125	CAV1054	Zaire	c	e	b	a	d	b	a	a	a	24	Mat 2
V	0125	CAV994	Tanzania	g	e	b	a	d	b	a	a	a	25	Mat 2
V	0124/5	CAV968	India	c	e	b	a	d	b	a	a	b	26	Mat 2
V	0124/5	CAV970	India	c	e	b	a	d	b	a	a	b	26	Mat 2
V	0124/5	CAV972	India	c	e	b	a	d	b	a	a	b	26	Mat 2
V	0124/5	CAV969	India	c	e	b	a	d	b	a	a	c	27	Mat 2
V	0124/5	CAV1066	Australia	c	e	b	a	d	b	a	a	c	27	Mat 2
V	0124/5	CAV1105	Australia	c	e	a	a	d	b	a	a	a	28	Mat 2
V	0128	CAV1132	Australia	c	e	b	a	d	a	a	a	a	29	Mat 2
V	01212	RP58	Tanzania	g	e	b	a	d	b	a	a	b	30	Mat 2

Lineage[1]	VCG	Number	Origin	Microsatellite primers									MLH	Mating type[2]
				G1 + MV15	G2 + G3	G16 + G8	G20 + G21	G40 + G41	G42 + G43	MB11f + MB11r	MB14f + B14r	MB18f + MB18r		
V	01212	CAV188	Tanzania	g	e	b	a	d	b	a	a	b	30	Mat 2
V	01222	CAV998	Uganda	c	e	b	a	d	b	a	a	n	31	Mat 2

[1] *Foc* lineages as defined by multi-gene phylogenetic lineage association (Chapter 2).

[2] Mating type determined by PCR assays using primer pair Falpha 1 and Falpha 2 for MAT-1 (Arie *et al.*, 2000) and GFmat2c (Steenkamp *et al.*, 2000) and *Foc* FF1 (Visser, 2003) for MAT-2.

Table 2. Primer sequences, fluorescent labelling and annealing temperatures of the microsatellite primers used to analyse a worldwide population of *Fusarium oxysporum* f.sp. *cubense* isolates, as well as their allele configuration.

Primer	**Primer sequence 5'-3'**	**Annealing tempertature**	**Core sequence**	**Label**	**Allele and genotype configuration**
G1[1]	F: CTC GTC CTT TGC GAA TGA CC	58°C	$(GA)_{19}$	6-FAM	454 (a), 444 (b), 459 (c), 467 (d), 481 (e), 469 (f), 461 (g)
MV15[1]	R: GAC CAC CTC GGT GAT GGT GAG ACG G	58°C			
G2[1]	F: GAG CTG CTG GTC TCG ATG TGG	58°C	$(TGG)_7$	PET	346 (a), 354 (b), 349 (c), 344 (d), 343 (e)
G3[1]	R: GCA GCA TGT ACG TTA CTC AAT CTG GCG GC	58°C			
G16 [1]	F: GAG AAC TGG GCG TAT TTG TTA GAT CAC	58°C	$(GGC)_4(GAC)_3$	VIC	305 (a), 302 (b), 300 (c)
G8[1]	R: GCT TAG GCC GAG GGA GGC AGA G	58°C			
G20[1]	F: GTG GAG CAA CAG AAT GTG GAA GAG CC	58°C	$(GTT)_7(GAT)_2(GTT)_2(GA)_2$	6-FAM	285 (a)
G21[1]	R: GCG GCT CCA GGA GCT GCT CTG AAG TG	58°C			
G40[1]	F: CCA CGG CTT GTC CGA GCT AGT GGA G	58°C	$(GTT)_1(GT)_1(GGT)_7$	NED	210 (a), 216 (b), 222 (c), 219 (d)
G41[1]	R: CAA GCC GCT CTC CAC GGC GAA GGC G	58°C			
G42[1]	F: GGA GGT AAT GTT ACG CAA GAG G	58°C	$(CA)_8$	PET	207 (a), 205 (b)
G43[1]	R: CGA CAC TCA AGC AAG AGT GTG C	58°C			
MB11F[2]	F: GTG GAC GAA CAC CTG CAT C	68°C	$(GGC)_7$	6-FAM	175 (a)
MB11R[2]	R: AGA TCC TCC ACC TCC ACC TC	68°C			
MB14F[2]	F: CGT CTC TGA ACC ACC TTC CAT C	57°C	$(CCA)_5$	VIC	183 (a)
MB14R[2]	R: TTC CTC CGT CCA TCC TGA C	57°C			
MB18F[2]	F: GGT AGG AAA TGA CGA AGC TGA C	57°C	$(CAACA)_6$		294 (a), 289 (b), 279 (c)
MB18R[2]	R: TGA GCA CTC TAG CAC TCC AAA C	57°C		VIC	

[1] Primers designed by Visser (2003).

[2] Primers designed by Bogale *et al.* (2005).

Table 3. The relationship between multi-locus haplotype (MLH) and vegetative compatibility group (VCG) in a worldwide collection of *Fusarium oxysporum* f.sp. *cubense*.

Lineage[1]	**VCG**	**MLH**[2]	**Geographic origin**
I	0126	1, 2	Honduras, Indonesia
I	01210	3	USA
I	01219	4, 5	Indonesia
II	0121	6, 7	Taiwan
II	01216	6, 8, 9	Indonesia, Malaysia
II	01213	6, 8, 10, 11	Indonesia, Malaysia, Australia
II	01213/16	6, 12	Australia, Malaysia
III	0129	13	Australia
III	0120	13, 14	Australia, Canary Islands, Costa Rica, Honduras, Indonesia, Brazil, South Africa
III	01215	13	Costa Rica
III	0120/15	13, 15, 16	Canary Islands, Brazil, Australia, Nigeria
III	0122	17	Philippines
III	0129/01211	13	Australia
III	0121/15	13	Canary Islands
IV	0123	18, 19	Philippines, Thailand
IV	01217	19	Malaysia
V	0124	20, 21, 22, 23	Australia, Indonesia, Jamaica, Nicaragua, Brazil, Honsuras
V	0125	20, 24, 25	Australia, Jamaica, India, Thailand, Zaire, Tanzania
V	0124/5	20, 26, 27, 28	Malawi, Australia, Philippines, Thailand, USA, India
V	0128	20, 29	Australia
V	01220	20	Australia
V	0124/22	20	Tanzania, Uganda
V	01212	30	Tanzania
V	01222	31	Uganda
V	0124/5/8	20	Tanzania

[1] *Foc* lineage as defend from their mulit-gene phylogenetic lineage association.

[2] Multi locus haplotypes defined according to the presence of a specific allele per locus.

Table 4. Allele size (bp) and frequency of nine microsatellite markers for geographically-defined populations of *Fusarium oxysporum* f.sp. *cubense* determined by POPGENE.

Locus	Allele size[1]	Australia	Americas	Asia	Africa
G1/MV15	454 (a)	0.43	0.67	0.53	0.5
	444 (b)			0.14	
	459 (c)	0.56	0.19	0.15	0.4
	467 (d)	0.01	0.03	0.14	
	481 (e)		0.09		
	469 (f)			0.03	
	461 (g)		0.03		0.1
		h = 0.5[2]	h = 0.5[2]	h = 0.6[2]	h = 0.6[2]
G2/G3	346 (a)	0.37	0.81	0.23	0.5
	344 (b)			0.02	
	349 (c)	0.06		0.48	
	344 (d)			0.14	
	343 (e)	0.57	0.19	0.12	0.5
		h = 0.53[2]	h = 0.3[2]	h = 0.7[2]	h = 0.5[2]
G16/G8	305 (a)	0.44	0.81	0.74	0.5
	302 (b)	0.52	0.16	0.13	0.5
	300 (c)	0.03	0.03	0.13	
		h = 0.53[2]	h = 0.31[2]	h = 0.42[2]	h = 0.5[2]
G20/G21	285 (a)	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
G40/G41	210 (a)	0.36	0.81	0.23	0.5
	216 (b)	0.01		0.14	
	223 (c)	0.07	0.03	0.44	
	219 (d)	0.56	0.16	0.19	0.5
		h = 0.6[2]	h = 0.31[2]	h = 0.7[2]	h = 0.5[2]
G42/G43	207 (a)	0.38	0.81	0.24	0.5
	205 (a)	0.62	0.19	0.76	0.5
		h = 0.5[2]	h = 0.3[2]	h = 0.4[2]	h = 0.5[2]
MB11F/MB11R	175 (a)	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
MB14F/MB14R	183 (a)	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
MB18F/MB18R	294 (a)	0.95	1	0.31	0.8
	289 (b)	0.01		0.2	0.1
	279 (c)	0.03		0.5	0.1
		h = 0.1[2]	h = 0[2]	h = 0.6[2]	h = 0.3[2]

[1] DNA fragment sizes determined using Genescan 2.1 and GENEMAPPER software. A unique letter was assigned to each fragment sixe/allele per loci.

[2] H = Gene diversity (Nei, 1973).

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Table 5. Gene and genotypic diversity for geographically-defined populations of *Fusarium oxysporum* f.sp. *cubense*.

	Australia	**Americas**	**Asia**	**Africa**
Population size	86	32	91	30
Number of alleles	21	19	25	17
Unique alleles	0	0	4	0
Polymorphic loci	6	5	6	6
% Polymorphic loci	66.67%	55.56%	66.67%	66.67%
Number of genotypes	12	6	18	6
Unique genotypes	5	4	4	4
Gene diversity (H)[1]	0.3	0.2	0.4	0.3
Genotypic diversity (G_{ST})[2]	3.09	2.18	5.34	2.98
$\hat{G}$[3]	3.59%	6.80%	5.34%	9.93%

[1] H = Gene diversity (Nei, 1973).

[2] G_{ST} = Genotypic diversity (Stoddart & Taylor, 1988).

[3] $\hat{G}$ = Maximum percentage of diversity (Chen *et al.*, 1994).

Table 6. Allele size (bp) and frequency of nine microsatellite markers for populations of *Fusarium oxysporum* f.sp. *cubense* defined according to multi-gene phylogenetic lineage associations determined by POPGENE.

Locus	Allele size[1]	I	II	III	IV	V
G1/MV15	454 (a)		1	0.98		
	444 (b)				1	
	459 (c)	0.13				0.95
	467 (d)	0.61				0.01
	481 (e)	0.13				
	469 (f)	0.13				
	461 (g)			0.014		0.04
		h = 0.6[2]	h = 0[2]	h = 0.03[2]	h = 0[2]	h = 0.22[2]
G2/G3	346 (a)	1	0.02	0.9722		
	344 (b)			0.0278		
	349 (c)		0.98			
	344 (d)				1	
	343 (e)					1
		h = 0[2]	h = 0.04[2]	h = 0.05[2]	h = 0[2]	h = 0[2]
G16/G8	305 (a)	1	1	1		0.01
	302 (b)				0.08	0.94
	300 (c)				0.92	0.05
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0.14[2]	h = 0.26[2]
G20/G21	285 (a)	1	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
G40/G41	210 (a)	0.95		0.98		
	216 (b)				1	0.012
	223 (c)		0.9	0.01		0.012
	219 (d)	0.04	0.1			0.98
		h = 0.1[2]	h = 0.2[2]	h = 0.03[2]	h = 0[2]	h = 0.13[2]
G42/G43	207 (a)	1		1		0.01
	205 (b)		1		1	0.99
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]	h = 0.07[2]
MB11F/MB11R	175 (a)	1	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
MB14F/MB14R	183 (a)	1	1	1	1	1
		h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]	h = 0[2]
MB18F/MB18R	294 (a)	1	0.06	0.96		0.88
	289 (b)		0.04	0.03	0.92	0.06
	279 (c)		0.9		0.08	0.05
		h = 0[2]	h = 0.2[2]	h = 0.08[2]	h = 0.14[2]	h = 0.5[2]

[1] DNA fragment sizes determined using Genescan 2.1 and GENEMAPPER software. A unique letter was assigned to each fragment sixe/allele per loci.

[2] H = Gene diversity (Nei, 1973).

Table 7. Gene and genotypic diversity for populations of *Fusarium oxysporum* f.sp. *cubense* defined according to multi-gene phylogenetic lineage associations.

	I	**II**	**III**	**IV**	**V**
Population size	23	50	72	13	81
Number of alleles	13	13	13	11	18
Unique alleles	0	0	0	0	0
Polymorphic loci	2	3	4	2	5
% Polymorphic loci	22.22%	33.33%	44.44%	22.22%	55.55%
Number of genotypes	2	7	5	2	12
Unique genotypes	2	7	5	2	12z
Gene diversity (H)[1]	0.07	0.05	0.02	0.03	0.13
Genotypic diversity (G_{ST})[2]	2.41	1.7	1.14	1.2	2.02
$\hat{G}$[3]	10.50%	3.43%	1.59%	8.90%	2.49%

[1] H = Gene diversity (Nei, 1973).

[2] G_{ST} = Genotypic diversity (Stoddart & Taylor, 1988).

[3] $\hat{G}$ = Maximum percentage of diversity (Chen *et al.*, 1994).

Table 8. Population differentiation values (above the diagonal) and gene flow (number of migrants) (below the diagonal) for Lineages II, III and V from the Australian population of *Fusarium oxysporum* f.sp. *cubense*.

	II	**III**	**V**
II		0.75	0.68
III	0.17		0.72
V	0.23	0.19	

Population differentiation values according to Weir's theta (θ) under 1000 randomised datasets (Weir, 1996).

From θ, gene flow was calculated as $M = [(1/\theta) - 1]/2$ (Cockerham & Weir, 1993).

No significant values were reported.

Table 9. Population differentiation values (above the diagonal) and gene flow (number of migrants) (below the diagonal) for Asian populations of *Fusarium oxysporum* f.sp. *cubense*.

	India	**Indonesia**	**Malaysia**	**Philippines**	**Taiwan**	**Thailand**
India		0.4*	0.6*	0.2	0.8*	0.04
Indonesia	0.75		0.2*	0.06	0.15	0.2
Malaysia	0.3	2		0.2	-0.1	0.2
Philippines	2	7.8	2		0.2	-0.3
Taiwan	0.1	2.8	5.5	2		0.4
Thailand	12	2	2	2.1	0.75	

Population differentiation values according to Weir's theta (θ) under 1000 randomised datasets (Weir, 1996).

From θ, gene flow was calculated as $M = [(1/\theta) - 1]/2$ (Cockerham & Weir, 1993).

*Indicates significant values at $P < 0.05$.

Table 10. Population differentiation values (above the diagonal) and gene flow (number of migrants) (below the diagonal) for Asian lineage populations of *Fusarium oxysporum* f.sp. *cubense*.

	I	**II**	**III**	**IV**	**V**
I		0.72*	0.31	0.78	0.78
II	0.19		0.62	0.74	0.76*
III	1.1	0.3		0.6	0.8
IV	0.14	0.37	0.3		0.82
V	0.14	0.15	0.13	0.11	

Population differentiation values according to Weir's theta (θ) under 1000 randomised datasets (Weir, 1996).

From θ, gene flow was calculated as $M = [(1/\theta) - 1]/2$ (Cockerham & Weir, 1993).

*Indicates significant values at $P < 0.05$.

Figure 1. An unrooted parsiomony-based network of relationships among the 31 multi-locus haplotypes representing the 239 *Fusarium oxysporum* f.sp. *cubense* isolates used in this study. The network was inferred using PAUP* version 10b (Swofford, 1998) and the previously determined multi-gene phylogenetic lineage associations (Chapter 2) of the respective MLHs are indicated.

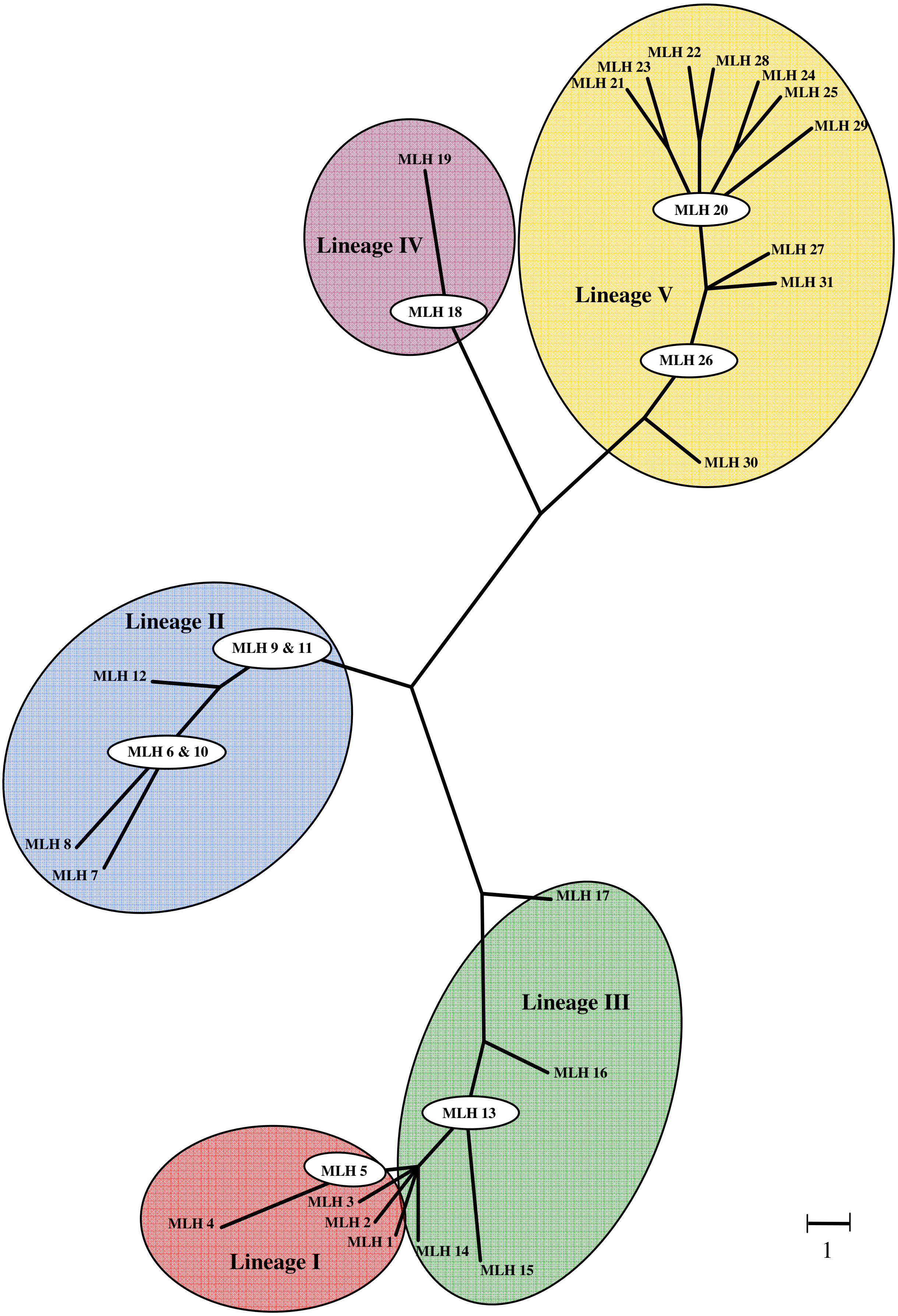

MLH 22
MLH 23
MLH 28
MLH 21
MLH 24
MLH 25
MLH 29
MLH 19
MLH 20
Lineage IV
MLH 27
MLH 31
Lineage V
MLH 18
MLH 26
MLH 30
Lineage II
MLH 9 & 11
MLH 12
MLH 6 & 10
MLH 8
MLH 7
MLH 17
Lineage III
MLH 16
MLH 13
MLH 5
MLH 3
MLH 4
MLH 2
MLH 1
MLH 14
MLH 15
Lineage I
1

Figure 2. An estimation of population structure of a population of *Fusarium oxysporum* f.sp. *cubense*. The highest plotted value on the graph of Ln P(D) verses K is estimated from the graph. This value represents the most likely *K*-value and therefore the most likely number of populations.

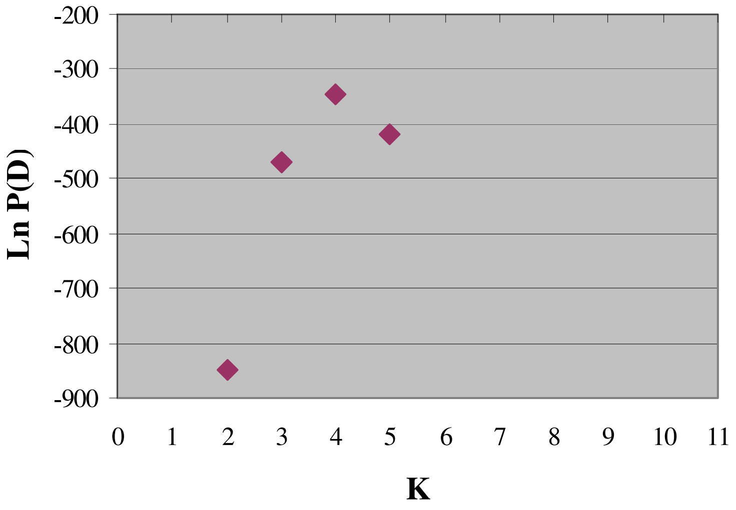


Figure 3. Multi-locus haplotypes compiled from nine SSR markers for geographically-defined populations of *Fusarium oxysporum* f.sp. *cubense.*

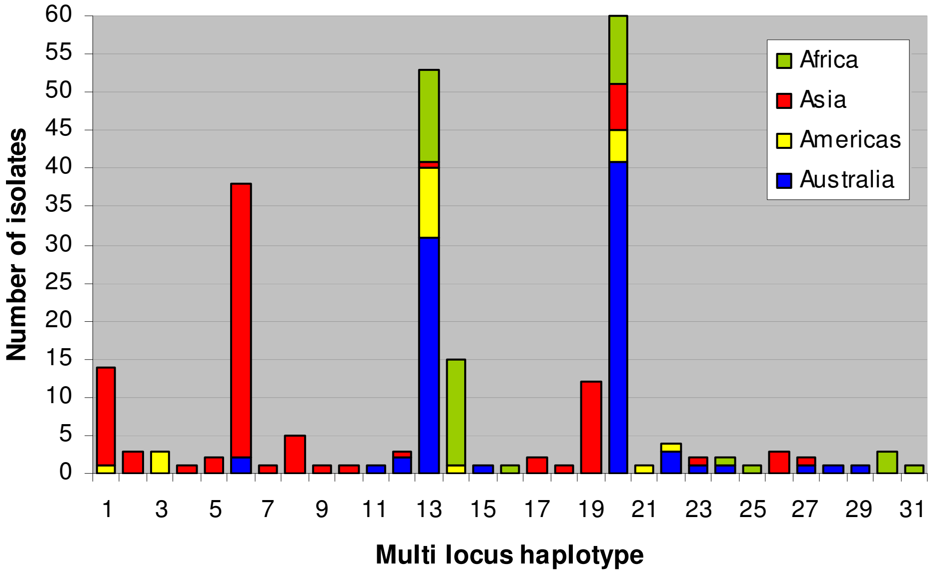


Figure 4. Multi-locus haplotypes compiled from nine SSR markers for populations of *Fusarium oxysporum* f.sp. *cubense* defined from phylogenetic analysis of various *Foc* and *F. oxysporum* isolates using nucleotide sequence information for genes encoding the translation elongation factor-1α and the mitochondrial small subunit ribosomal RNA.

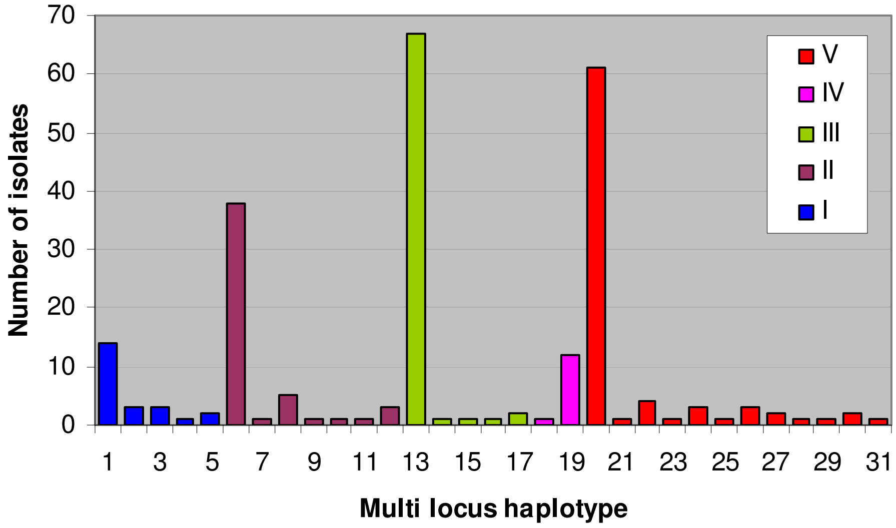
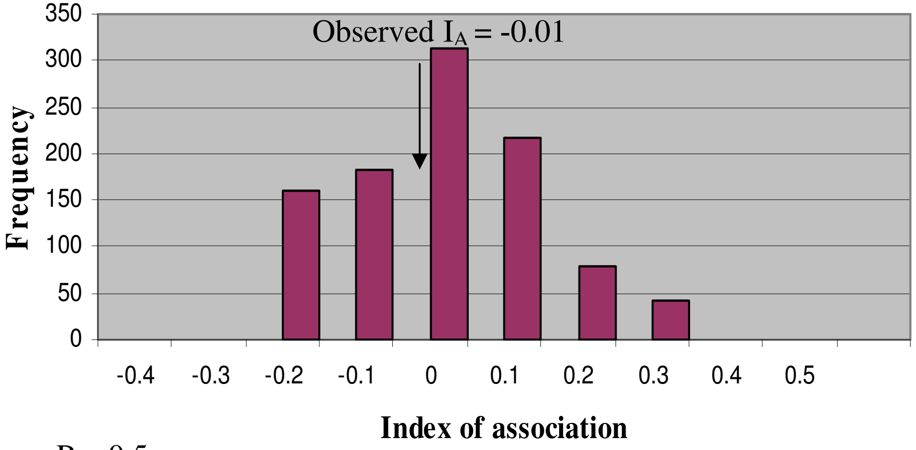
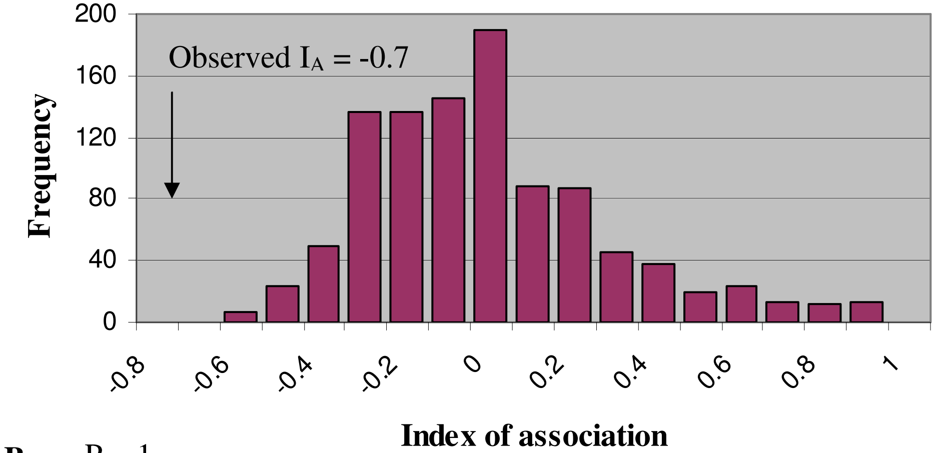


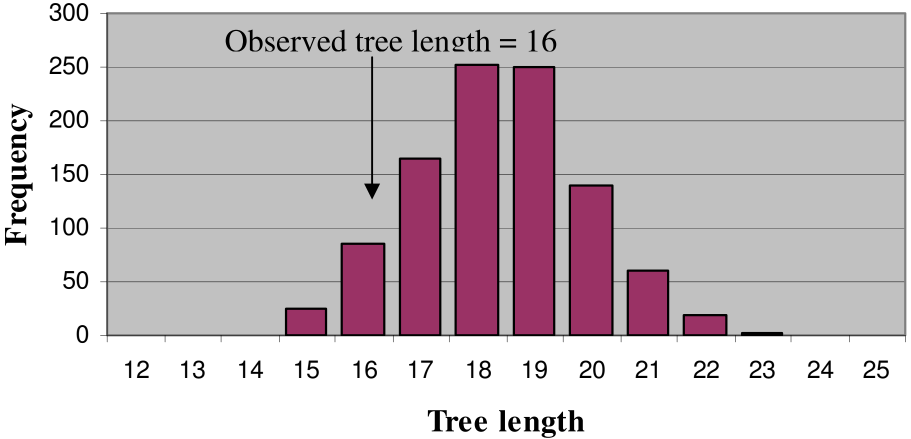
Figure 5. Estimation of the mode of reproduction of isolates of *Fusarium oxysporum* f.sp. *cubense* Clade V. **A**: Linkage disequilibrium (I_A) values, observed and expected of a clone-corrected population calculated with Multilocus® with 1000 randomisations. **B**: Linkage disequilibrium (I_A) values, observed and expected, of a uncorrected populations calculated with Multilocus® with 1000 randomisations. **C**: Tree length values, observed and expected, of a clone-corrected population, estimated with the Parsimony Tree Length Permutation Test (PTLPT). **D**: Tree length values, observed and expected, of a uncorrected population, estimated using the PTLPT.



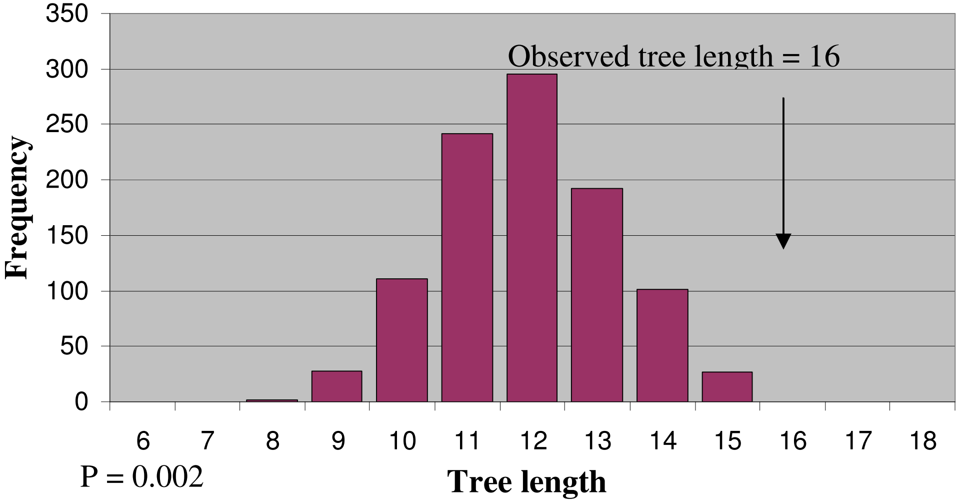
A P = 0.5



B P = 1



C P = 0.02



P = 0.002

SUMMARY

Fusarium oxysporum Schlecht. is a cosmopolitan species complex that consists of both pathogenic and non-pathogenic members. The pathogenic members are subdivided into *formae speciales*, based on virulence to specific host species. More than 150 *formae speciales* have been described, of which *F. oxysporum* f.sp. *cubense* (E.F.Smith) Snyder and Hansen (*Foc*), causal agent of Fusarium wilt of banana, is regarded as one of the economically most important and destructive. According to phenotypic and genotypic markers, *Foc* has been classified into three races and 24 vegetative compatibility groups, and can be divided into a number of clonal lineages that roughly correspond with VCG groupings. In this thesis, we investigated the evolutionary relationships among VCGs using multi-gene sequencing and *MAT* genotyping. A PCR-RFLP fingerprint discriminating the *Foc* lineages and a PCR primer that identified *Foc* 'subtropical' race 4 isolates, was developed. Nine microsatellite markers (SSRs) were applied to a global population of *Foc* in order to investigate diversity not always detectable using sequencing data.

Phylogenetic analysis of isolates representing *Foc*, various other *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* of the genes encoding the translation elongation factor-1α (TEF), the mitochondrial small subunit (MtSSU), ribosomal RNA (rRNA), the repeated region encoded on the mitochondrion (MtR) and the intergenic spacer (IGS) gene regions separated these isolates into four clades, two of which included *Foc*. Within these two clades, *Foc* separated into six lineages that broadly corresponded to VCGs, while the non-pathogenic isolates of *F. oxysporum* grouped together in only one of the two clades, with an unknown *Foc* VCG isolate. The mating type of all isolates was determined and crosses were attempted between isolates harbouring *MAT*-1 and *MAT*-2 genes, without success. Cultural, morphological and pathogenic variation among isolates of *Foc* was unable to identify lineages as species. The separation of *Foc* isolates into two clades suggested that the banana pathogen evolved during two unrelated events. Factors such as horizontal gene transfer, however, might also have played a part in the pathogen's evolution, as was evident from the divergent placement of some VCGs and lineages within the phylogenetic trees constructed. The inclusion of other *formae speciales* of *F. oxysporum* and non-pathogenic *F. oxysporum* isolates illustrated the great diversity that exists within the *F. oxysporum* complex. The inclusion of the *Foc* isolate of an unknown VCG suggests that the genetic diversity of

Foc might be far greater than what is known and what was revealed in this study. The opposite mating types found in the respective lineages indicate a sexual origin for the Fusarium wilt fungus that could account for its polyphyletic nature.

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Within South Africa, *Foc* ‘subtropical’ race 4 is regarded the most important constrain to banana production. Conventional control practices for Fusarium wilt of banana are ineffective, and disease management relies heavily on the use of clean planting material and the early detection and isolation of the pathogen, in order to restrict spread to unaffected areas. Identification of *Foc* typically involves vegetative compatibility assays and pathogenicity testing using a set of differential host cultivars. The development of a PCR-based method for the rapid and accurate identification of *Foc* ‘subtropical’ race 4 will, therefore, be of great importance. The lack of morphological variation between lineages of *Foc*, and between pathogenic and non-pathogenic members, as well as the unreliability in race identification in *Foc*, makes the use of molecular tools a viable alternative. Following DNA isolation, PCR and sequencing of the MtR, the DNA sequence data revealed an 8-bp insertion that was subsequently targeted for the design of a *Foc* ‘subtropical’ race 4-specific primer. Isolates were positively identified as *Foc* ‘subtropical’ race 4 with the amplification of an 800-pb fragment. The development of the *Foc* ‘subtropical’ race 4 primer will aid in rapid and accurate detection of the Fusarium wilt pathogen of banana.

The population structure defined according to SSR data of a global population of 239 *Foc* isolates resembled the structure defined according to multi-gene phylogeny, with some exceptions. Measures of gene and genotypic diversity unequivocally supported the opinion that Asia is the centre of origin of *Foc*. The presence of unique genotypes in all geographically-defined *Foc* populations could potentially indicate their evolution outside the centre of origin, although this is highly unlikely. The absence of certain genotypes from the Asian population was either due to insufficient and selective sampling, or it demonstrated the effects of clonal selection in combination with adaptation to the forces of geographic isolation and environmental changes over time. The worldwide collection of *Foc* mostly consisted of six over represented genotypes, thereby providing support for a clonal genetic structure. It was, however, not possible to reject the hypothesis of a recombining population for the populations representing isolates of Lineage V. The implication of recombination within some *Foc* lineages may be due to unobserved sexual reproduction in nature or the historical association with a sexual ancestor. When one considers diversity within and among genotypes, a specific genotype was mostly associated with only one or two *Foc* VCGs, therefore indicating that vegetative compatibility determination, in combination with phylogenetic analyses, is a powerful tool for characterizing isolates causing Fusarium wilt of banana. Results from this study, in combination with the multi-gene phylogeny, clearly indicated the presence of unrelated lineages that most probably represent cryptic species.

Appendix A

The nucleotide sequence information for genes encoding the translation elongation factor-1α and the mitochondrial small subunit ribosomal RNA of *Fusarium oxysporum* f.sp. *cubense.* ..'s represent similar sequence, -'s represents a gap in the alignment and n represent missing sequences. Alignments were constructed with the use of MAFFT software programme.

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                       ....|....|....|....|....|....|....|....|....|....|....|....|
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CAV793-0126            .......................................-....................
CAV794-0126            .......................................-....................
CAV1051-01210          .......................................-....................
CAV632-01210           .......................................-....................
CAV847-01219           .......................................-....................
CAV195-01219           .......................................-....................
RP7-0121               .......................................-....................
RP8-0121               .......................................-....................
RP9-0121               .......................................-....................
CAV810-01213           .......................................-....................
CAV811-01213           .......................................-....................
CAV300-01213           .......................................-....................
CAV814-01216           .......................................-....................
CAV815-01216           .......................................-....................
CAV604-01216           .......................................-....................
CAV312-01213/16        .......................................-....................
CAV313-01213/16        .......................................-....................
23510-0129             .......................................-....................
CAV1100-0129           .......................................-....................
CAV009-0120            .......................................-....................
CAV293-0120            .......................................-....................
CAV294-0120            .......................................-....................
CAV296-0120            .......................................-....................
CAV612-01215           .......................................-....................
CAV298-0120/15         .......................................-....................
CAV299-0120/15         .......................................-....................
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CAV933-0123            .......................................-....................
CAV871-01217           .......................................-....................
CAV194-01218           .......................................-....................
CAV189-01214           .......................................-.........A..........
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CAV609-0124            .......................................-....................
CAV786-0124            .......................................-....................
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NRRL22903              .................................C...T.G..G.................
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CAV847-01219        ............................................................
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RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
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CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
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22993-0128          T.............................T.........T...................
22994-0128          T.............................T.........T...................
24211-01220         T.............................T.........T...................
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CAV786-0124          ............................................................
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23487-0125           ............................................................
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RP9-0121            ............................................................
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CAV814-01216        ............................................................
CAV815-01216        ............................................................
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CAV312-01213/16     ............................................................
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CAV312-01213/16     ..........................................................--
CAV313-01213/16     ..........................................................--
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CAV189-01214        ..........................................................--
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CAV609-0124         ..........................................................-T
CAV786-0124         ..........................................................-T
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23487-0125          ..........................................................-T
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CAV195-01219        ......-...................................................-.
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RP8-0121            ......-...................................................-.
RP9-0121            ......-...................................................-.
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CAV811-01213        ......-...................................................-.
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CAV814-01216        ......-...................................................-.
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CAV604-01216        ......-...................................................-.
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CAV1100-0129        ......-...................................................-.
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CAV605-0122         ......-...................................................-.
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CAV1107-0129/11     ......-...................................................-.
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24219-01220         ......-..........C.....................-...A..............-T
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CAV794-0126         .....---..................--................................
CAV1051-01210       .....---..................--................................
CAV632-01210        .....---..................--................................
CAV847-01219        .....---..................--................................
CAV195-01219        .....---..................--................................
RP7-0121            .....---..................--.........................T......
RP8-0121            .....---..................--.........................T......
RP9-0121            .....---..................--.........................T......
CAV810-01213        .....---..................--.........................T......
CAV811-01213        .....---..................--.........................T......
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CAV815-01216        .....---..................--.........................T......
CAV604-01216        .....---..................--.........................T......
CAV312-01213/16     .....---..................--.........................T......
CAV313-01213/16     .....---..................--.........................T......
23510-0129          .....---........G.........--................................
CAV1100-0129        .....---........G.........--................................
CAV009-0120         .....---..................--................................
CAV293-0120         .....---..................--................................
CAV294-0120         .....---..................--................................
CAV296-0120         .....---..................--................................
CAV612-01215        .....---..................--................................
CAV298-0120/15      .....---..................--................................
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CAV605-0122         .....---..................--................................
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CAV957-0123         .....CTC..................--................................
CAV933-0123         .....CTC..................--................................
CAV871-01217        .....CTC..................--................................
CAV194-01218        .....CTC..................--................................
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CAV609-0124         .....CTC........G.........--................................
CAV786-0124         .....CTC........G.........--................................
8611-0125           .....CTC........G.........--..........A.....................
23487-0125          .....CTC........G.........--................................
22993-0128          .....CTC........G.........--................................
22994-0128          .....CTC........G.........--................................
24211-01220         .....CTC........G.........--................................
24219-01220         .....CTC........G.........--................................
RP58-01212          .....CTC........G.........--................................
NRRL22903           .....TCC..............C..A--.............C..................
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CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
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CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
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CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
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CAV612-01215        ............................................................
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CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
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CAV1107-0129/11     ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
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24211-01220         ............................................................
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RP58-01212          ............................................................
NRRL22903           .T..........................................................
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                           490       500       510       520       530       540
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CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV1107-0129/11     ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV194-01218        ............................................................
CAV189-01214        ..............................................N.............
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22994-0128          ............................................................
24211-01220         ............................................................
24219-01220         ............................................................
RP58-01212          ............................................................
NRRL22903           ............................................................
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                            550       560       570       580       590       600
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCGCTCATGCTTCATTCTACGTCTCT
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ......................................................T.....
CAV195-01219        ......................................................T.....
RP7-0121            ......................................................T.....
RP8-0121            ......................................................T.....
RP9-0121            ......................................................T.....
CAV810-01213        ......................................................T.....
CAV811-01213        ......................................................T.....
CAV300-01213        ......................................................T.....
CAV814-01216        ......................................................T.....
CAV815-01216        ......................................................T.....
CAV604-01216        ......................................................T.....
CAV312-01213/16     ......................................................T.....
CAV313-01213/16     ......................................................T.....
23510-0129          ......................................................T.....
CAV1100-0129        ......................................................T.....
CAV009-0120         .........................NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
CAV293-0120         ......................................................T.....
CAV294-0120         ......................................................T.....
CAV296-0120         ......................................................T.....
CAV612-01215        ......................................................T.....
CAV298-0120/15      ......................................................T.....
CAV299-0120/15      ......................................................T.....
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ......................................................T.....
CAV1107-0129/11     ......................................................T.....
CAV929-0123         ......................................................T.....
CAV957-0123         ......................................................T.....
CAV933-0123         ......................................................T.....
CAV871-01217        ......................................................T.....
CAV194-01218        ......................................................T.....
CAV189-01214        ......................................................T.....
CAV602-0124         ......................................................T.....
CAV609-0124         ......................................................T.....
CAV786-0124         ......................................................T.....
8611-0125           ......................................................T.....
23487-0125          ......................................................T.....
22993-0128          ......................................................T.....
22994-0128          ......................................................T.....
24211-01220         ......................................................T.....
24219-01220         ......................................................T.....
RP58-01212          ......................................................T.....
NRRL22903           ......................................................T.....
```

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                             610      620       630       640       650      660
                     ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126          TCGTACTAATATATCACTCAGACGCTCCCGGTCACCGTGGAACTGGCAACTTGGAGAAGT
CAV793-0126          ............................................................
CAV794-0126          ............................................................
CAV1051-01210        ............................................................
CAV632-01210         ............................................................
CAV847-01219         ............................................................
CAV195-01219         ............................................................
RP7-0121             ............................................................
RP8-0121             ............................................................
RP9-0121             ............................................................
CAV810-01213         ............................................................
CAV811-01213         ............................................................
CAV300-01213         ............................................................
CAV814-01216         ............................................................
CAV815-01216         ............................................................
CAV604-01216         ............................................................
CAV312-01213/16      ............................................................
CAV313-01213/16      ............................................................
23510-0129           .........C..................................................
CAV1100-0129         .........C..................................................
CAV009-0120          NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.....................
CAV293-0120          ............................................................
CAV294-0120          ............................................................
CAV296-0120          ............................................................
CAV612-01215         ............................................................
CAV298-0120/15       ............................................................
CAV299-0120/15       ............................................................
CAV607-0122          ............................................................
CAV605-0122          ............................................................
23631-01211          .........C..................................................
CAV1107-0129/11      .........C.........................................N........
CAV929-0123          .........C..................................................
CAV957-0123          .........C..................................................
CAV933-0123          .........C..................................................
CAV871-01217         .........C..................................................
CAV194-01218         .........C..................................................
CAV189-01214         ......NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.....................
CAV602-0124          .........C..................................................
CAV609-0124          .........C..................................................
CAV786-0124          .........C..................................................
8611-0125            .........C..................................................
23487-0125           .........C..................................................
22993-0128           .........C..................................................
22994-0128           .........C..................................................
24211-01220          .........C..................................................
24219-01220          .........C..................................................
RP58-01212           .........C..................................................
NRRL22903            .......G.C..................................................
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                             670       680       690       700       710      720
                     ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126          GGCAAGTC-TTCCAGTA--TGGGGAGCAAAA-CAGCTATG-GGTCAAGT-CCGATATCTT
CAV793-0126          ........-........--............-........-........-..........
CAV794-0126          ........-........--............-........-........-..........
CAV1051-01210        ........-........--............-........-........-..........
CAV632-01210         ........-........--............-........-........-..........
CAV847-01219         ........-........--............-........-........-..........
CAV195-01219         ........-........--............-........-........-..........
RP7-0121             ........-........--............-........-........-..........
RP8-0121             ........-........--............-........-........-..........
RP9-0121             ........-........--............-........-........-..........
CAV810-01213         ........-........--............-........-........-..........
CAV811-01213         ........-........--............-........-........-..........
CAV300-01213         ........-........--............-........-........-..........
CAV814-01216         ........-........--............-........-........-..........
CAV815-01216         ........-........--............-........-........-..........
CAV604-01216         ........-........--............-........-........-..........
CAV312-01213/16      ........-........--............-........-........-..........
CAV313-01213/16      ........-........--............-........-........-..........
23510-0129           ........-........--............-........-........-..........
CAV1100-0129         ........-........--............-........-........-..........
CAV009-0120          ........-........--............-........-........-..........
CAV293-0120          ........-........--............-........-........-..........
CAV294-0120          ........-........--............-........-........-..........
CAV296-0120          ........-........--............-........-........-..........
CAV612-01215         ........-........--............-........-........-..........
CAV298-0120/15       ........-........--............-........-........-..........
CAV299-0120/15       ........-........--............-........-........-..........
CAV607-0122          ........-........--............-........-........-..........
CAV605-0122          ........-........--............-........-........-..........
23631-01211          ........-........--............-........-........-..........
CAV1107-0129/11      ........-........--............-........-........-..........
CAV929-0123          ........-........--............-........-........-..........
CAV957-0123          ........-........--............-........-........-..........
CAV933-0123          ........-........--............-........-........-..........
CAV871-01217         ........-........--............-........-........-..........
CAV194-01218         ........-........--............-........-........-..........
CAV189-01214         ........-........--............-........-........-..........
CAV602-0124          ........-........--............-........-........-..........
CAV609-0124          ........-........--............-........-........-..........
CAV786-0124          ........-........--............-........-........-..........
8611-0125            ........-........--............-........-........-..........
23487-0125           ........-........--............-........-........-..........
22993-0128           ........-........--............-........-........-..........
22994-0128           ........-........--............-........-........-..........
24211-01220          ........-........--............-........-........-..........
24219-01220          ........-........--............-........-........-..........
RP58-01212           ........-........--............-........-........-..........
NRRL22903            ........-........--............-........-........-..........
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                           730       740       750       760       770       780
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TAGGAGAAG---------TCTTATTGTGAGGGCGAGTTATATAACA--CCATAGG---AC
CAV793-0126         .........---------............................--.......---..
CAV794-0126         .........---------............................--.......---..
CAV1051-01210       .........---------............................--.......---..
CAV632-01210        .........---------............................--.......---..
CAV847-01219        .........---------............................--.......---..
CAV195-01219        .........---------............................--.......---..
RP7-0121            .........---------............................--.......---..
RP8-0121            .........---------............................--.......---..
RP9-0121            .........---------............................--.......---..
CAV810-01213        .........---------............................--.......---..
CAV811-01213        .........---------............................--.......---..
CAV300-01213        .........---------............................--.......---..
CAV814-01216        .........---------............................--.......---..
CAV815-01216        .........---------............................--.......---..
CAV604-01216        .........---------............................--.......---..
CAV312-01213/16     .........---------............................--.......---..
CAV313-01213/16     .........---------............................--.......---..
23510-0129          .........---------............................--.......---..
CAV1100-0129        .........---------............................--.......---..
CAV009-0120         .........---------............................--.......---..
CAV293-0120         .........---------............................--.......---..
CAV294-0120         .........---------............................--.......---..
CAV296-0120         .........---------............................--.......---..
CAV612-01215        .........---------............................--.......---..
CAV298-0120/15      .........---------............................--.......---..
CAV299-0120/15      .........---------............................--.......---..
CAV607-0122         .........---------............................--.......---..
CAV605-0122         .........---------............................--.......---..
23631-01211         .........---------............................--.......---..
CAV1107-0129/11     .........---------............................--.......---..
CAV929-0123         .........---------............................--.......---..
CAV957-0123         .........---------............................--.......---..
CAV933-0123         .........---------............................--.......---..
CAV871-01217        .........---------............................--.......---..
CAV194-01218        .........---------............................--.......---..
CAV189-01214        .........---------........N...........NNN.....--.......---..
CAV602-0124         .........---------............................--.......---..
CAV609-0124         .........---------............................--.......---..
CAV786-0124         .........---------............................--.......---..
8611-0125           .........---------............................--.......---..
23487-0125          .........---------............................--.......---..
22993-0128          .........---------............................--.......---..
22994-0128          .........---------............................--.......---..
24211-01220         .........---------............................--.......---..
24219-01220         .........---------............................--.......---..
RP58-01212          .........---------............................--.......---..
NRRL22903           .........---------............................--.......---..
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```
                            790       800       810       820       830      840
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TGGCCGTCCCATATGAAAAGATTATATTAGAATTGAATGAAGCTTTGTTTATATATTGAT
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV1107-0129/11     ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV194-01218        ............................................................
CAV189-01214        .............................N..............................
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22994-0128          ............................................................
24211-01220         ............................................................
24219-01220         ............................................................
RP58-01212          ............................................................
NRRL22903           ............................................................
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                             850       860       870       880       890       900
                      ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126           AATGACAGTATATATATCGTGTCTTGACTAATTGC-GTGCCAGCAGTC----GCGGTAAT
CAV793-0126           ...................................-............----........
CAV794-0126           ...................................-............----........
CAV1051-01210         ...................................-............----........
CAV632-01210          ...................................-............----........
CAV847-01219          ...................................-............----........
CAV195-01219          ...................................-............----........
RP7-0121              ...................................-............----........
RP8-0121              ...................................-............----........
RP9-0121              ...................................-............----........
CAV810-01213          ...................................-............----........
CAV811-01213          ...................................-............----........
CAV300-01213          ...................................-............----........
CAV814-01216          ...................................-............----........
CAV815-01216          ...................................-............----........
CAV604-01216          ...................................-............----........
CAV312-01213/16       ...................................-............----........
CAV313-01213/16       ...................................-............----........
23510-0129            ...................................-............----........
CAV1100-0129          ...................................-............----........
CAV009-0120           ...................................-............----........
CAV293-0120           ...................................-............----........
CAV294-0120           ...................................-............----........
CAV296-0120           ...................................-............----........
CAV612-01215          ...................................-............----........
CAV298-0120/15        ...................................-............----........
CAV299-0120/15        ...................................-............----........
CAV607-0122           ...................................-............----........
CAV605-0122           ...................................-............----........
23631-01211           ...................................-............----........
CAV1107-0129/11       ...................................-............----........
CAV929-0123           ...................................-............----........
CAV957-0123           ...................................-............----........
CAV933-0123           ...................................-............----........
CAV871-01217          ...................................-............----........
CAV194-01218          ...................................-............----........
CAV189-01214          ..............N....................-............----........
CAV602-0124           ...................................-............----........
CAV609-0124           ...................................-............----........
CAV786-0124           ...................................-............----........
8611-0125             ...................................-............----........
23487-0125            ...................................-............----........
22993-0128            ...................................-............----........
22994-0128            ...................................-............----........
24211-01220           ...................................-............----........
24219-01220           ...................................-............----........
RP58-01212            ...................................-............----........
NRRL22903             ...................................-............----........
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                              910       920       930       940       950       960
                       ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126            ACGTAAGAGACTAGTGTTATTCATCTTAATTAGGTTTAAAGGGTACCCAGACGGTCAATA
CAV793-0126            ............................................................
CAV794-0126            ............................................................
CAV1051-01210          ............................................................
CAV632-01210           ............................................................
CAV847-01219           ............................................................
CAV195-01219           ............................................................
RP7-0121               ............................................................
RP8-0121               ............................................................
RP9-0121               ............................................................
CAV810-01213           ............................................................
CAV811-01213           ............................................................
CAV300-01213           ............................................................
CAV814-01216           ............................................................
CAV815-01216           ............................................................
CAV604-01216           ............................................................
CAV312-01213/16        ............................................................
CAV313-01213/16        ............................................................
23510-0129             ............................................................
CAV1100-0129           ............................................................
CAV009-0120            ............................................................
CAV293-0120            ............................................................
CAV294-0120            ............................................................
CAV296-0120            ............................................................
CAV612-01215           ............................................................
CAV298-0120/15         ............................................................
CAV299-0120/15         ............................................................
CAV607-0122            ............................................................
CAV605-0122            ............................................................
23631-01211            ............................................................
CAV1107-0129/11        ............................................................
CAV929-0123            ............................................................
CAV957-0123            ............................................................
CAV933-0123            ............................................................
CAV871-01217           ............................................................
CAV194-01218           ............................................................
CAV189-01214           ............................................................
CAV602-0124            ............................................................
CAV609-0124            ............................................................
CAV786-0124            ............................................................
8611-0125              ............................................................
23487-0125             ............................................................
22993-0128             ............................................................
22994-0128             ............................................................
24211-01220            ............................................................
24219-01220            ............................................................
RP58-01212             ............................................................
NRRL22903              ............................................................
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                            970       980       990      1000      1010    1020
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TAGCTTA-TAAAATGTTAGTACTTGACTAGAGTTTTATGTAAGAGGGCAGTACTTGAGGA
CAV793-0126         .......-....................................................
CAV794-0126         .......-....................................................
CAV1051-01210       .......-....................................................
CAV632-01210        .......-....................................................
CAV847-01219        .......-....................................................
CAV195-01219        .......-....................................................
RP7-0121            .......-........G...........................................
RP8-0121            .......-........G...........................................
RP9-0121            .......-........G...........................................
CAV810-01213        .......-........G...........................................
CAV811-01213        .......-........G...........................................
CAV300-01213        .......-........G...........................................
CAV814-01216        .......-........G...........................................
CAV815-01216        .......-........G...........................................
CAV604-01216        .......-........G...........................................
CAV312-01213/16     .......-........G...........................................
CAV313-01213/16     .......-........G...........................................
23510-0129          .......-........G...........................................
CAV1100-0129        .......-........G...........................................
CAV009-0120         .......-........G...........................................
CAV293-0120         .......-........G...........................................
CAV294-0120         .......-........G...........................................
CAV296-0120         .......-........G...........................................
CAV612-01215        .......-........G...........................................
CAV298-0120/15      .......-........G...........................................
CAV299-0120/15      .......-........G...........................................
CAV607-0122         .......-........G...........................................
CAV605-0122         .......-........G...........................................
23631-01211         .......-........G...........................................
CAV1107-0129/11     .......-........G...........................................
CAV929-0123         .......-....................................................
CAV957-0123         .......-....................................................
CAV933-0123         .......-....................................................
CAV871-01217        .......-....................................................
CAV194-01218        .......-....................................................
CAV189-01214        .......-........N...........................................
CAV602-0124         .......-....................................................
CAV609-0124         .......-....................................................
CAV786-0124         .......-....................................................
8611-0125           .......-....................................................
23487-0125          .......-....................................................
22993-0128          .......-....................................................
22994-0128          .......-....................................................
24211-01220         .......-....................................................
24219-01220         .......-....................................................
RP58-01212          .......-....................................................
NRRL22903           .......-....................................................
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                            1030      1040      1050      1060      1070     1080
                     ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126          GGAGAGATGAAATTTCGTGATACCAAAGGGACTCTGTAAAGGCGAAGGCAGCCCTCTATG
CAV793-0126          ............................................................
CAV794-0126          ............................................................
CAV1051-01210        ............................................................
CAV632-01210         ............................................................
CAV847-01219         ............................................................
CAV195-01219         ............................................................
RP7-0121             ............................................................
RP8-0121             ............................................................
RP9-0121             ............................................................
CAV810-01213         ............................................................
CAV811-01213         ............................................................
CAV300-01213         ............................................................
CAV814-01216         ............................................................
CAV815-01216         ............................................................
CAV604-01216         ............................................................
CAV312-01213/16      ............................................................
CAV313-01213/16      ............................................................
23510-0129           ............................................................
CAV1100-0129         ............................................................
CAV009-0120          ............................................................
CAV293-0120          ............................................................
CAV294-0120          ............................................................
CAV296-0120          ............................................................
CAV612-01215         ............................................................
CAV298-0120/15       ............................................................
CAV299-0120/15       ............................................................
CAV607-0122          ............................................................
CAV605-0122          ............................................................
23631-01211          ............................................................
CAV1107-0129/11      ............................................................
CAV929-0123          ............................................................
CAV957-0123          ............................................................
CAV933-0123          ............................................................
CAV871-01217         ............................................................
CAV194-01218         ............................................................
CAV189-01214         ............................................................
CAV602-0124          ............................................................
CAV609-0124          ............................................................
CAV786-0124          ............................................................
8611-0125            ............................................................
23487-0125           ............................................................
22993-0128           ............................................................
22994-0128           ............................................................
24211-01220          ............................................................
24219-01220          ............................................................
RP58-01212           ............................................................
NRRL22903            ............................................................
```

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                            1090      1100      1110      1120      1130    1140
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TAAAAACTGACGTTGAAGGACGAAGGCACAGAGAACAAACAGGATTAGATACCCAAGTAG
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ...........................C................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV1107-0129/11     ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV194-01218        ............................................................
CAV189-01214        ............................................................
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22994-0128          ............................................................
24211-01220         ............................................................
24219-01220         ............................................................
RP58-01212          ............................................................
NRRL22903           ............................................................
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                                1150      1160      1170      1180      1190    1200
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         TCTTTG-CAGTAAATGATGAATGCCATAGGTTAGATTAATCAAATATAGTCTAG------
CAV793-0126         ......-...............................................------
CAV794-0126         ......-...............................................------
CAV1051-01210       ......-...............................................------
CAV632-01210        ......-...............................................------
CAV847-01219        ......-...............................................------
CAV195-01219        ......-...............................................------
RP7-0121            ......-.....................................C.........------
RP8-0121            ......-.....................................C.........------
RP9-0121            ......-.....................................C.........------
CAV810-01213        ......-.....................................C.........------
CAV811-01213        ......-.....................................C.........------
CAV300-01213        ......-.....................................C.........------
CAV814-01216        ......-.....................................C.........------
CAV815-01216        ......-.....................................C.........------
CAV604-01216        ......-.....................................C.........------
CAV312-01213/16     ......-.....................................C.........------
CAV313-01213/16     ......-.....................................C.........------
23510-0129          ......-.....................................C.........------
CAV1100-0129        ......-.....................................C.........------
CAV009-0120         ......-.....................................C.........------
CAV293-0120         ......-.....................................C.........------
CAV294-0120         ......-.....................................C.........------
CAV296-0120         ......-.....................................C.........------
CAV612-01215        ......-.....................................C.........------
CAV298-0120/15      ......-.....................................C.........------
CAV299-0120/15      ......-.....................................C.........------
CAV607-0122         ......-.....................................C.........------
CAV605-0122         ......-.....................................C.........------
23631-01211         ......-.....................................C.........------
CAV1107-0129/11     ......-.....................................C.........------
CAV929-0123         ......-..............................G.....T..........------
CAV957-0123         ......-..............................G.....T..........------
CAV933-0123         ......-..............................G.....T..........------
CAV871-01217        ......-..............................G.....T..........------
CAV194-01218        ......-..............................G.....T..........------
CAV189-01214        ......-..............................R.....Nn.........------
CAV602-0124         ......-..............................G.....T..........------
CAV609-0124         ......-..............................G.....T..........------
CAV786-0124         ......-..............................G.....T..........------
8611-0125           ......-..............................G.....T..........------
23487-0125          ......-..............................G.....T..........------
22993-0128          ......-..............................G.....T..........------
22994-0128          ......-..............................G.....T..........------
24211-01220         ......-..............................G.....T..........------
24219-01220         ......-..............................G.....T..........------
RP58-01212          ......-..............................G.....T..........------
NRRL22903           ......-........................C....A.CCAGTTA..GT.TATAGTCTAA
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                             1210      1220      1230      1240      1250    1260
                     ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126          ------GGTTAGTCTAGCAAACTAATGACCTATACTATAATGAAA---AATATCTGGTCT
CAV793-0126          ------.......................................---............
CAV794-0126          ------.......................................---............
CAV1051-01210        ------.......................................---............
CAV632-01210         ------.......................................---............
CAV847-01219         ------.......................................---............
CAV195-01219         ------.......................................---............
RP7-0121             ------...............................C.......AAA............
RP8-0121             ------...............................C.......AAA............
RP9-0121             ------...............................C.......AAA............
CAV810-01213         ------...............................C.......AAA............
CAV811-01213         ------...............................C.......AAA............
CAV300-01213         ------...............................C.......AAA............
CAV814-01216         ------...............................C.......AAA............
CAV815-01216         ------...............................C.......AAA............
CAV604-01216         ------...............................C.......AAA............
CAV312-01213/16      ------...............................C.......AAA............
CAV313-01213/16      ------...............................C.......AAA............
23510-0129           ------...............................C.......AAA............
CAV1100-0129         ------...............................C.......AAA............
CAV009-0120          ------...............................C.......AAA............
CAV293-0120          ------...............................C.......AAA............
CAV294-0120          ------...............................C.......AAA............
CAV296-0120          ------...............................C.......AAA............
CAV612-01215         ------...............................C.......AAA............
CAV298-0120/15       ------...............................C.......AAA............
CAV299-0120/15       ------...............................C.......AAA............
CAV607-0122          ------...............................C.......AAA............
CAV605-0122          ------...............................C.......AAA............
23631-01211          ------...............................C.......AAA............
CAV1107-0129/11      ------...............................C.......AAA............
CAV929-0123          ------.....................C.....GA......TC..---............
CAV957-0123          ------.....................C.....GA......TC..---............
CAV933-0123          ------.....................C.....GA......TC..---............
CAV871-01217         ------.....................C.....GA......TC..---............
CAV194-01218         ------.....................C.....GA......TC..---............
CAV189-01214         ------.....................N.....GA..N...nnnnnnnnnnnnnnnnnnn
CAV602-0124          ------.....................C.....GA......TC..---............
CAV609-0124          ------.....................C.....GA......TC..---............
CAV786-0124          ------.....................C.....GA......TC..---............
8611-0125            ------.....................C.....GA......TC..---............
23487-0125           ------.....................C.....GA......TC..---............
22993-0128           ------.....................C.....GA......TC..---............
22994-0128           ------.....................C.....GA......TC..---............
24211-01220          ------.....................C.....GA......TC..---............
24219-01220          ------.....................C.....GA......TC..---............
RP58-01212           ------.....................C.....GA......TC..---............
NRRL22903            ---TAG......C................A..G.....CCATT..A--.....T......
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                              1270      1280      1290      1300      1310      1320
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         ATAAATGAAAGTGTAAGCATTTCACC-TCAAGAGTAATG-TGGCAACGCAGGAACTGAAA
CAV793-0126         ..........................-............-....................
CAV794-0126         ..........................-............-....................
CAV1051-01210       ..........................-............-....................
CAV632-01210        ..........................-............-....................
CAV847-01219        ..........................-............-..............NNNNNN
CAV195-01219        ..........................-............-............NNNNNNNN
RP7-0121            ..........................-............-....................
RP8-0121            ..........................-............-....................
RP9-0121            ..........................-............-....................
CAV810-01213        ..........................-............-....................
CAV811-01213        ..........................-............-....................
CAV300-01213        ..........................-............-....................
CAV814-01216        ..........................-............-....................
CAV815-01216        ..........................-............-....................
CAV604-01216        ..........................-............-....................
CAV312-01213/16     ..........................-............-....................
CAV313-01213/16     ..........................-............-....................
23510-0129          ..........................-............-....................
CAV1100-0129        ..........................-............-....................
CAV009-0120         ..........................-............-....................
CAV293-0120         ..........................-............-....................
CAV294-0120         ..........................-............-....................
CAV296-0120         ..........................-............-....................
CAV612-01215        ..........................-............-...........NNNNNNNNN
CAV298-0120/15      ..........................-............-....................
CAV299-0120/15      ..........................-............-....................
CAV607-0122         ..........................-............-....................
CAV605-0122         ..........................-............-....................
23631-01211         ..........................-............-....................
CAV1107-0129/11     ..........................-............-....................
CAV929-0123         ..........................-............-....................
CAV957-0123         ..........................-............-....................
CAV933-0123         ..........................-............-....................
CAV871-01217        ..........................-............-....................
CAV194-01218        ..........................-............-....................
CAV189-01214        nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
CAV602-0124         ..........................-............-....................
CAV609-0124         ..........................-............-....................
CAV786-0124         ..........................-............-....................
8611-0125           ..........................-............-....................
23487-0125          ..........................-............-....................
22993-0128          ..........................-............-....................
22994-0128          ..........................-............-....................
24211-01220         ..........................-............-....................
24219-01220         ..........................-............-....................
RP58-01212          ..........................-............-....................
NRRL22903           ..........................-............-....................
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                          ....|...
CAV613-0126               TCACTAGA
CAV793-0126               ........
CAV794-0126               ........
CAV1051-01210             ........
CAV632-01210              ........
CAV847-01219              NNNNNNNN
CAV195-01219              NNNNNNNN
RP7-0121                  ........
RP8-0121                  ..NNNNNN
RP9-0121                  ......NN
CAV810-01213              ........
CAV811-01213              ........
CAV300-01213              ..NNNNNN
CAV814-01216              ........
CAV815-01216              ..NNNNNN
CAV604-01216              ........
CAV312-01213/16           ..NNNNNN
CAV313-01213/16           ........
23510-0129                ........
CAV1100-0129              ........
CAV009-0120               ........
CAV293-0120               ........
CAV294-0120               ........
CAV296-0120               ........
CAV612-01215              NNNNNNNN
CAV298-0120/15            ........
CAV299-0120/15            ........
CAV607-0122               ........
CAV605-0122               ........
23631-01211               ........
CAV1107-0129/11           ........
CAV929-0123               ........
CAV957-0123               ........
CAV933-0123               ........
CAV871-01217              ........
CAV194-01218              ........
CAV189-01214              nnnnnnnn
CAV602-0124               ........
CAV609-0124               ..NNNNNN
CAV786-0124               ........
8611-0125                 ........
23487-0125                ........
22993-0128                ........
22994-0128                ........
24211-01220               ........
24219-01220               ........
RP58-01212                ........
NRRL22903                 ........
```

Appendix B

The nucleotide sequence information for gene encoding the rRNA intergenic spacer of *Fusarium oxysporum* f.sp. *cubense*. ..'s represent similar sequence, -'s represents a gap in the alignment and n represent missing sequences. Alignments were constructed with the use of MAFFT software programme.

```
                            10        20        30        40        50        60
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         tccggcccggccggggcccatcgcgagctgccgggtaggtaaaagtaaaaaagttgttaa
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ..........................t.................................
CAV1100-0129        ..........................t.................................
CAV009-0120         .........................n..........................n.......
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV1107-0129/11     ............................................................
CAV929-0123         ..........................t.................................
CAV957-0123         ..........t.................................................
CAV933-0123         ..........................t.................................
CAV871-01217        ..........t.................................................
CAV194-01218        ..........t.................................................
CAV189-01214        ..........................t.................................
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
23480-0125          ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22994-0128          ............................................................
24211-01220         ..........................t.................................
24219-01220         ..........................t.................................
RP58-01212          ..........................t.................................
FCC                 .......g..................c....a............................
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                         70        80        90       100       110       120
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gaggcgcggtgtcggcgtgcttgtatttgcgggagagaattatctggaggtgctgggtag
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       .........................................................c..
CAV1100-0129     .........................................................c..
CAV009-0120      n...........................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      .........................................................c..
CAV1107-0129/11  .........................................................c..
CAV929-0123      ...............................................g............
CAV957-0123      ...............................................g............
CAV933-0123      ...............................................g............
CAV871-01217     ...............................................g............
CAV194-01218     ...............................................g............
CAV189-01214     ...............................................g............
CAV602-0124      ...............................................g............
CAV609-0124      ...............................................g............
CAV786-0124      ...............................................g............
8611-0125        ...............................................g............
23480-0125       ...............................................g............
23487-0125       ...............................................g............
22993-0128       ...............................................g............
22994-0128       ...............................................g............
24211-01220      ...............................................g............
24219-01220      ...............................................g............
RP58-01212       ...............................................g............
FCC              ...............t.............a..a........t.......a-.g.a.aag.
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```
                            130       140       150       160       170       180
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         ccggga-agacttggacggatctgg-cccgggaatgggtctgggcctggattctggtgtg
CAV793-0126         ......-..................-..................................
CAV794-0126         ......-..................-..................................
CAV1051-01210       ......-.................-c..................................
CAV632-01210        ......-.................-c..................................
CAV847-01219        ......-..................-..................................
CAV195-01219        ......-..................-..................................
RP7-0121            ......-..................-..................................
RP8-0121            ......-..................-..................................
RP9-0121            ......-..................-..................................
CAV810-01213        ......-..................-..................................
CAV811-01213        ......-..................-..................................
CAV300-01213        ......-..................-..................................
CAV814-01216        ......-..................-..................................
CAV815-01216        ......-..................-..................................
CAV604-01216        ......-..................-..................................
CAV312-01213/16     ......-..................-..................................
CAV313-01213/16     ......-..................-..................................
23510-0129          ...c..-..................-..................................
CAV1100-0129        ...c..-..................-..................................
CAV009-0120         ......-..................-..............................n.n.
CAV293-0120         ......-..................-..................................
CAV294-0120         ......-..................-..................................
CAV296-0120         ......-..................-..................................
CAV612-01215        ......-..................-..................................
CAV298-0120/15      ......-..................-..................................
CAV299-0120/15      ......-..................-..................................
CAV607-0122         ......-.................-c..................................
CAV605-0122         ......-.................-c..................................
23631-01211         ...c..-..................-..................................
CAV1107-0129/11     ...c..-..................-..................................
CAV929-0123         ......-..................-..................................
CAV957-0123         ......-..................-..................................
CAV933-0123         ......-..................-..................................
CAV871-01217        ......-..................-..................................
CAV194-01218        ......-..................-..................................
CAV189-01214        ......-..................-..................................
CAV602-0124         ......-..................-..................................
CAV609-0124         ......-..................-..................................
CAV786-0124         ......-..................-..................................
8611-0125           ......-..................-..................................
23480-0125          ......-..................-..................................
23487-0125          ......-..................-..................................
22993-0128          ......-..................-..................................
22994-0128          ......-..................-..................................
24211-01220         ......-..................-..................................
24219-01220         ......-..................-..................................
RP58-01212          ......-..................-..................................
FCC                 g.a...-..................-t.......c..........tc.....t...g...
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```
                         190       200       210       220       230       240
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gt-----gta---ggg-taggcgtagatagatgagtggtc-tagggtaggtagagggtag
CAV793-0126      ..-----...---...-.......................-...................
CAV794-0126      ..-----...---...-.......................-...................
CAV1051-01210    ..-----...---..a-..a....................-........a..c.......
CAV632-01210     ..-----...---..a-..a....................-........a..c.......
CAV847-01219     ..-----...---...-.......................-...................
CAV195-01219     ..-----...---...-.......................-...................
RP7-0121         ..-----...---...-.......................-........a...t......
RP8-0121         ..-----...---...-.......................-........a...t......
RP9-0121         ..-----...---...-.......................-........a...t......
CAV810-01213     ..-----...---..a-..a....................-........a..c.......
CAV811-01213     ..-----...---..a-..a....................-........a..c.......
CAV300-01213     ..-----...---..a-..a....................-........a..c.......
CAV814-01216     ..-----...---..a-..a....................-........a..c.......
CAV815-01216     ..-----...---..a-..a....................-........a..c.......
CAV604-01216     ..-----...---..a-..a....................-........a..c.......
CAV312-01213/16  ..-----...---..a-..a....................-........a..c.......
CAV313-01213/16  ..-----...---..a-..a....................-........a..c.......
23510-0129       ..-----...---..a-..a....................-.-----------.......
CAV1100-0129     ..-----...---..a-..a....................-.-----------.......
CAV009-0120      ..-----...---..a-..a..a.................-.-----------.......
CAV293-0120      ..-----...---..a-..a..a.................-.-----------.......
CAV294-0120      ..-----...---..a-..a..a.................-.-----------.......
CAV296-0120      ..-----...---..a-..a..a.................-.-----------.......
CAV612-01215     ..-----...---..a-..a..a.................-.-----------.......
CAV298-0120/15   ..-----...---..a-..a..a.................-.-----------.......
CAV299-0120/15   ..-----...---..a-..a..a.................-.-----------.......
CAV607-0122      ..-----...---..a-..a....................-........a..c.......
CAV605-0122      ..-----...---..a-..a....................-........a..c.......
23631-01211      ..-----...---..a-..a....................-.-----------.......
CAV1107-0129/11  ..-----...---..a-..a....................-.-----------.......
CAV929-0123      ..-----...---...-.......................-........a..c.......
CAV957-0123      ..-----...---...-.......................-........a..c.......
CAV933-0123      ..-----...---...-.......................-........a..c.......
CAV871-01217     ..-----...---...-.......................-........a..c.......
CAV194-01218     ..-----...---...-.......................-........a..c.......
CAV189-01214     ..-----...---...-.......................-........a..c.......
CAV602-0124      ..-----...---...-.......................t........a..c.......
CAV609-0124      ..-----...---...-.......................t........a..c.......
CAV786-0124      ..-----...---...-.......................t........a..c.......
8611-0125        ..-----...---...-.......................t........a..c.......
23480-0125       ..-----...---...-.......................t........a..c.......
23487-0125       ..-----...---...-.......................t........a..c.......
22993-0128       ..-----...---...-.......................t........a..c.......
22994-0128       ..-----...---...-.......................t........a..c.......
24211-01220      ..-----...---...-.......................t........a..c.......
24219-01220      ..-----...---...-.......................t........a..c.......
RP58-01212       ..-----...---...-.......................t........a..c.......
FCC              ..-----.c.---...c.....agctt..c..a.ac....-c..........c.......
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                         250       260       270       280       290       300
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      cc------------agaagtctggtacatagtat--gg-gggtgtagggtaggtctggac
CAV793-0126      ..------------....................--..-.....................
CAV794-0126      ..------------....................--..-.....................
CAV1051-01210    ..------------............t.......-t..-.....................
CAV632-01210     ..------------............t.......-t..-.....................
CAV847-01219     ..------------....................--..-.....................
CAV195-01219     ..------------....................--..-.....................
RP7-0121         ..------------............t.......--..-.....................
RP8-0121         ..------------............t.......--..-.....................
RP9-0121         ..------------............t.......--..-.....................
CAV810-01213     ..------------............t.......-g..-.....................
CAV811-01213     ..------------............t.......-g..-.....................
CAV300-01213     ..------------............t.......-g..-.....................
CAV814-01216     ..------------............t.......-g..-.....................
CAV815-01216     ..------------............t.......-g..-.....................
CAV604-01216     ..------------............t.......-g..-.....................
CAV312-01213/16  ..------------............t.......-g..-.....................
CAV313-01213/16  ..------------............t.......-g..-.....................
23510-0129       ..------------............t.......-g..-.....................
CAV1100-0129     ..------------............t.......-g..-.....................
CAV009-0120      ..------------............t.......-g..-.....................
CAV293-0120      ..------------............t.......-g..-.....................
CAV294-0120      ..------------............t.......-g..-.....................
CAV296-0120      ..------------............t.......-g..-.....................
CAV612-01215     ..------------............t.......-g..-.....................
CAV298-0120/15   ..------------............t.......-g..-.....................
CAV299-0120/15   ..------------............t.......-g..-.....................
CAV607-0122      ..------------............t.......-t..-.....................
CAV605-0122      ..------------............t.......-t..-.....................
23631-01211      ..------------............t.......-g..-.....................
CAV1107-0129/11  ..------------............t.......-g..-.....................
CAV929-0123      ..------------...c........t.......--..-.....................
CAV957-0123      ..------------...c........t.......--..-.....................
CAV933-0123      ..------------...c........t.......--..-.....................
CAV871-01217     ..------------...c........t.......--..-.....................
CAV194-01218     ..------------............t.......--..-.....................
CAV189-01214     ..------------...c........t.......--..-.....................
CAV602-0124      ..------------...c........t.......--..-.....................
CAV609-0124      ..------------...c........t.......--..-.....................
CAV786-0124      ..------------...c........t.......--..-.....................
8611-0125        ..------------...c........t.......--..-.....................
23480-0125       ..------------...c........t.......--..-.....................
23487-0125       ..------------...c........t.......--..-.....................
22993-0128       ..------------...c........t.......--..-.....................
22994-0128       ..------------...c........t.......--..-.....................
24211-01220      ..------------...c........t.......--..-.....................
24219-01220      ..------------...c........t.......--..-.....................
RP58-01212       ..------------...c........t.......--..-.....................
FCC              g.acagggttaggt............t.......-g..c..............ct....t
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                            310       320       330       340       350       360
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         accgttttctacttcgcccttccctttactcga-gggaggacgatcttggctgggatgga
CAV793-0126         .................................-..........................
CAV794-0126         .................................-..........................
CAV1051-01210       .........c.......................-..........................
CAV632-01210        .........c.......................-..........................
CAV847-01219        .................................-..........................
CAV195-01219        .................................-..........................
RP7-0121            ....c....c.......................-............c...t....gc...
RP8-0121            ....c....c.......................-............c...t....gc...
RP9-0121            ....c....c.......................-............c...t....gc...
CAV810-01213        .........c.......................-..........................
CAV811-01213        .........c.......................-..........................
CAV300-01213        .........c.......................-..........................
CAV814-01216        .........c.......................-..........................
CAV815-01216        .........c.......................-..........................
CAV604-01216        .........c.......................-..........................
CAV312-01213/16     .........c.......................-..........................
CAV313-01213/16     .........c.......................-..........................
23510-0129          .........c.......................-..........................
CAV1100-0129        .........c.......................-..........................
CAV009-0120         .........c.......................-..........................
CAV293-0120         .........c.......................-..........................
CAV294-0120         .........c.......................-..........................
CAV296-0120         .........c.......................-..........................
CAV612-01215        .........c.......................-..........................
CAV298-0120/15      .........c.......................-..........................
CAV299-0120/15      .........c.......................-..........................
CAV607-0122         .........c.......................-..........................
CAV605-0122         .........c.......................-..........................
23631-01211         .........c.......................-..........................
CAV1107-0129/11     .........c.......................-..........................
CAV929-0123         .........c..................g....-......................c...
CAV957-0123         .........c..................g....-......................c...
CAV933-0123         .........c..................g....-......................c...
CAV871-01217        .........c..................g....-......................c...
CAV194-01218        .........c..................g....-......................c...
CAV189-01214        .........c..................g....-......................c...
CAV602-0124         .........c.....a............g....-......................c...
CAV609-0124         .........c.....a............g....-......................c...
CAV786-0124         .........c.....a............g....-......................c...
8611-0125           .........c.....a............g....-......................c...
23480-0125          .........c.....a............g....-......................c...
23487-0125          .........c.....a............g....-......................c...
22993-0128          .........c.....a............g....-......................c...
22994-0128          .........c.....a............g....-......................c...
24211-01220         .........c.....a............g....-......................c...
24219-01220         .........c.....a............g....-......................c...
RP58-01212          .........c.....a............g....-......................c...
FCC                 ........g.ttca.......a....gg.....-.aag....a........c.....c..
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                                370       380       390       400       410       420
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       ggtgtagggtaggcttaatttacgattacatgatctgtgt-cactctagggtaggtgaaa
CAV793-0126       ........................................-...................
CAV794-0126       ........................................-...................
CAV1051-01210     .................g..............g.......-.g.................
CAV632-01210      .................g..............g.......-.g.................
CAV847-01219      ........................................-...................
CAV195-01219      ........................................-...................
RP7-0121          ...........-..........a..c........t.c...-.............c.....
RP8-0121          ...........-..........a..c........t.c...-.............c.....
RP9-0121          ...........-..........a..c........t.c...-.............c.....
CAV810-01213      ................................g.......-.g.................
CAV811-01213      ................................g.......-.g.................
CAV300-01213      ................................g.......-.g.................
CAV814-01216      ................................g.......-.g.................
CAV815-01216      ................................g.......-.g.................
CAV604-01216      ................................g.......-.g.................
CAV312-01213/16   ................................g.......-.g.................
CAV313-01213/16   ................................g.......-.g.................
23510-0129        ...................c....................-...................
CAV1100-0129      ...................c....................-...................
CAV009-0120       ..................c.....................-...................
CAV293-0120       ..................c.....................-...................
CAV294-0120       ..................c.....................-...................
CAV296-0120       ..................c.....................-...................
CAV612-01215      ..................c.....................-...................
CAV298-0120/15    ..................c.....................-...................
CAV299-0120/15    ..................c.....................-...................
CAV607-0122       .................g..............g.......-.g.................
CAV605-0122       .................g..............g.......-.g.................
23631-01211       ...................c....................-...................
CAV1107-0129/11   ...................c....................-...................
CAV929-0123       ..................c.....................-...................
CAV957-0123       ..................c.....................-...................
CAV933-0123       ..................c.....................-...................
CAV871-01217      ..................c.....................-...................
CAV194-01218      ..................c.....................-...................
CAV189-01214      ..................c.....................-...................
CAV602-0124       ........................................-...................
CAV609-0124       ........................................-...................
CAV786-0124       ........................................-...................
8611-0125         ........................................-...................
23480-0125        ........................................-...................
23487-0125        ........................................-...................
22993-0128        ........................................-...................
22994-0128        ........................................-...................
24211-01220       ........................................-...................
24219-01220       ........................................-...................
RP58-01212        ........................................-...................
FCC               a..c...........g...g..t.....tg.a.g....a.-ag.............a...
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                            430       440       450       460       470       480
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         atcccatatatatctgatcaca-tttggtgaagagg-tggtttggctggtgagatggaca
CAV793-0126         ......................-.............-.......................
CAV794-0126         ......................-.............-.......................
CAV1051-01210       ......................-.............-.......................
CAV632-01210        ......................-.............-.......................
CAV847-01219        ......................-.............-.......................
CAV195-01219        ......................-.............-.......................
RP7-0121            .........g............-.............t........a..a...........
RP8-0121            .........g............-.............t........a..a...........
RP9-0121            .........g............-.............t........a..a...........
CAV810-01213        ......................-.............-.......................
CAV811-01213        ......................-.............-.......................
CAV300-01213        ......................-.............-.......................
CAV814-01216        ......................-.............-.......................
CAV815-01216        ......................-.............-.......................
CAV604-01216        ......................-.............-.......................
CAV312-01213/16     ......................-.............-.......................
CAV313-01213/16     ......................-.............-.......................
23510-0129          ......................-.............-.....................t.
CAV1100-0129        ......................-.............-.....................t.
CAV009-0120         ......................-.............-.......................
CAV293-0120         ......................-.............-.......................
CAV294-0120         ......................-.............-.......................
CAV296-0120         ......................-.............-.......................
CAV612-01215        ......................-.............-.......................
CAV298-0120/15      ......................-.............-.......................
CAV299-0120/15      ......................-.............-.......................
CAV607-0122         ......................-.............-.......................
CAV605-0122         ......................-.............-.......................
23631-01211         ......................-.............-.....................t.
CAV1107-0129/11     ......................-.............-.....................t.
CAV929-0123         .............a........-.............-.......................
CAV957-0123         .............a........-.............-.......................
CAV933-0123         .............a........-.............-.......................
CAV871-01217        ...........g.a........-.............-.......................
CAV194-01218        ...........g.a........-.............-.......................
CAV189-01214        .............a........-.............-.......................
CAV602-0124         ......................-.............-..............g........
CAV609-0124         ......................-.............-..............g........
CAV786-0124         ......................-.............-..............g........
8611-0125           ......................-.............-..............g........
23480-0125          ......................-.............-..............g........
23487-0125          ......................-.............-..............g........
22993-0128          ......................-.............-..............g........
22994-0128          ......................-.............-..............g........
24211-01220         ......................-.............-..............g........
24219-01220         ......................-.............-..............g........
RP58-01212          ......................-.............-..............g........
FCC                 ........c.a.......tgg.-a..........t.-..a.........a.........g
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                        490       500       510       520       530       540
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      aaa-gtgcaatgtagaattatatgtgattttgcaaaagtgggtgtgaaattgggaaatcg
CAV793-0126      ...-........................................................
CAV794-0126      ...-........................................................
CAV1051-01210    ...-........................................................
CAV632-01210     ...-........................................................
CAV847-01219     ...-........................................................
CAV195-01219     ...-........................................................
RP7-0121         ...-............g..g...a.............................a..g...
RP8-0121         ...-............g..g...a.............................a..g...
RP9-0121         ...-............g..g...a.............................a..g...
CAV810-01213     ...-........................................................
CAV811-01213     ...-........................................................
CAV300-01213     ...-........................................................
CAV814-01216     ...-........................................................
CAV815-01216     ...-........................................................
CAV604-01216     ...-........................................................
CAV312-01213/16  ...-........................................................
CAV313-01213/16  ...-........................................................
23510-0129       ...-......c............................a....................
CAV1100-0129     ...-......c............................a....................
CAV009-0120      ...-........................................................
CAV293-0120      ...-........................................................
CAV294-0120      ...-........................................................
CAV296-0120      ...-........................................................
CAV612-01215     ...-........................................................
CAV298-0120/15   ...-........................................................
CAV299-0120/15   ...-........................................................
CAV607-0122      ...-........................................................
CAV605-0122      ...-........................................................
23631-01211      ...-......c............................a....................
CAV1107-0129/11  ...-......c............................a....................
CAV929-0123      ...-.......a................................................
CAV957-0123      ...-.......a................................................
CAV933-0123      ...-.......a................................................
CAV871-01217     ...-.......a................................................
CAV194-01218     ...-.......a................................................
CAV189-01214     ...-.......a................................................
CAV602-0124      ...-.................................................a..g...
CAV609-0124      ...-.................................................a..g...
CAV786-0124      ...-.................................................a..g...
8611-0125        ...-.................................................a..g...
23480-0125       ...-.................................................a..g...
23487-0125       ...-.................................................a..g...
22993-0128       ...-.................................................a..g...
22994-0128       ...-.................................................a..g...
24211-01220      ...-.................................................a..g...
24219-01220      ...-.................................................a..g...
RP58-01212       ...-.................................................a..g...
FCC              n..-........c....c.g....................a....a..ga...a..g...
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                                 550       560       570       580       590       600
                          ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126               gttttcccgcacagatgagagcacgttcgaggtgccatgagatgcacctctccgagacga
CAV793-0126               ............................................................
CAV794-0126               ............................................................
CAV1051-01210             .......t...t...............ta...............................
CAV632-01210              .......t...t...............ta...............................
CAV847-01219              ............................................................
CAV195-01219              ............................................................
RP7-0121                  ..........g................t.................g............a.
RP8-0121                  ..........g................t.................g............a.
RP9-0121                  ..........g................t.................g............a.
CAV810-01213              .......t...t...............ta.............g.................
CAV811-01213              .......t...t...............ta.............g.................
CAV300-01213              .......t...t...............ta.............g.................
CAV814-01216              .......t...t...............ta.............g.................
CAV815-01216              .......t...t...............ta.............g.................
CAV604-01216              .......t...t...............ta.............g.................
CAV312-01213/16           .......t...t...............ta.............g.................
CAV313-01213/16           .......t...t...............ta.............g.................
23510-0129                ...........................t................................
CAV1100-0129              ...........................t................................
CAV009-0120               ...........................t........................t.......
CAV293-0120               ...........................t........................t.......
CAV294-0120               ...........................t........................t.......
CAV296-0120               ...........................t........................t.......
CAV612-01215              ...........................t........................t.......
CAV298-0120/15            ...........................t........................t.......
CAV299-0120/15            ...........................t........................t.......
CAV607-0122               .......t...t...............ta...............................
CAV605-0122               .......t...t...............ta...............................
23631-01211               ...........................t................................
CAV1107-0129/11           ...........................t................................
CAV929-0123               ...........................t................................
CAV957-0123               ...........................t................................
CAV933-0123               ...........................t................................
CAV871-01217              ...........................t.....t..........................
CAV194-01218              ...........................t.....t..........................
CAV189-01214              ...........................t................................
CAV602-0124               .......................a...t................................
CAV609-0124               .......................a...t................................
CAV786-0124               .......................a...t................................
8611-0125                 .......................a...t................................
23480-0125                .......................a...t................................
23487-0125                .......................a...t................................
22993-0128                .......................a...t................................
22994-0128                .......................a...t................................
24211-01220               .......................a...t................................
24219-01220               .......................a...t................................
RP58-01212                .......................a...t................................
FCC                       a.g......ag...g............t...ac.t...cg.....g....gt........
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                          610       620       630       640       650       660
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       cctcaacggtaccaccgatgtgttggtcgggctcctgtgcggccgtccagggcgggatat
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          t.c........t....c..........................................a
RP8-0121          t.c........t....c..........................................a
RP9-0121          t.c........t....c..........................................a
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV1107-0129/11   ............................................................
CAV929-0123       ................c..........................................a
CAV957-0123       ................c..........................................a
CAV933-0123       ................c..........................................a
CAV871-01217      ................c..........................................a
CAV194-01218      ................c..........................................a
CAV189-01214      ................c..........................................a
CAV602-0124       ................c..........................g...............a
CAV609-0124       ................c..........................g...............a
CAV786-0124       ................c..........................g...............a
8611-0125         ................c..........................g...............a
23480-0125        ................c..........................g...............a
23487-0125        ................c..........................g...............a
22993-0128        ................c..........................g...............a
22994-0128        ................c..........................g...............a
24211-01220       ................c..........................g...............a
24219-01220       ................c..........................g...............a
RP58-01212        ................c..........................g...............a
FCC               g.a.......gtag..c......gt.cac.a..........t.................a
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                          670       680       690       700       710       720
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       gtagagaatgtggtggtgtagggtaggtggccaggtccagggtaggttctccaaatccgt
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ...a.ag.....................a.at..a..............att.g......
RP8-0121          ...a.ag.....................a.at..a..............att.g......
RP9-0121          ...a.ag.....................a.at..a..............att.g......
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ...a........................................................
CAV299-0120/15    ...a........................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV1107-0129/11   .............................a...................g..........
CAV929-0123       ..................c..........a...................g..........
CAV957-0123       ..................c..........a...................g..........
CAV933-0123       ..................c..........a...................g..........
CAV871-01217      .............................a...................g..........
CAV194-01218      .............................a...................g..........
CAV189-01214      ..................c..........a...................g..........
CAV602-0124       .............................a...................g..........
CAV609-0124       .............................a...................g..........
CAV786-0124       .............................a...................g..........
8611-0125         .............................a...................g..........
23480-0125        .............................a...................g..........
23487-0125        .............................a...................g..........
22993-0128        .............................a...................g..........
22994-0128        .............................a...................g..........
24211-01220       .............................a...................g..........
24219-01220       .............................a...................g..........
RP58-01212        .............................a...................g..........
FCC               .....a..gtga.a..............c.t..cac...c.accc.a..a..tcg.....
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                          730       740       750       760       770       780
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       caatccggcttgaatcgaaggacaggtctagggtaggccagagtcgggtctagggtaggc
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ....t.......................................................
RP8-0121          ....t.......................................................
RP9-0121          ....t.......................................................
CAV810-01213      .................g..........................................
CAV811-01213      .................g..........................................
CAV300-01213      .................g..........................................
CAV814-01216      .................g..........................................
CAV815-01216      .................g..........................................
CAV604-01216      .................g..........................................
CAV312-01213/16   .................g..........................................
CAV313-01213/16   .................g..........................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ...................n........................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV1107-0129/11   ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV194-01218      ............................................................
CAV189-01214      ............................................................
CAV602-0124       ............................................................
CAV609-0124       ............................................................
CAV786-0124       ............................................................
8611-0125         ............................................................
23480-0125        ............................................................
23487-0125        ............................................................
22993-0128        ............................................................
22994-0128        ............................................................
24211-01220       ............................................................
24219-01220       ............................................................
RP58-01212        ............................................................
FCC               .g..t..t...t.tct.tg.....................a....a..............
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                          790       800       810       820       830       840
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       agctctcaccctcgaagtggtctacccggtagtcaacttcaatcgcctctcacggccgcc
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ....................c.......................................
RP8-0121          ....................c.......................................
RP9-0121          ....................c.......................................
CAV810-01213      ......a.....................................................
CAV811-01213      ......a.....................................................
CAV300-01213      ......a.....................................................
CAV814-01216      ......a.....................................................
CAV815-01216      ......a.....................................................
CAV604-01216      ......a.....................................................
CAV312-01213/16   ......a.....................................................
CAV313-01213/16   ......a.....................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV1107-0129/11   .a.c............................c...........................
CAV929-0123       .a.c............................c...........................
CAV957-0123       .a.c.........n..................c...........................
CAV933-0123       .a.c............................c...........................
CAV871-01217      .a.c............................c...........................
CAV194-01218      .a.c............................c...........................
CAV189-01214      .a.c............................c...........................
CAV602-0124       .a.c............................c...........................
CAV609-0124       .a.c............................c...........................
CAV786-0124       .a.c............................c...........................
8611-0125         .a.c............................c...........................
23480-0125        .a.c............................c...........................
23487-0125        .a.c............................c...........................
22993-0128        .a.c............................c...........................
22994-0128        .a.c............................c...........................
24211-01220       .a.c............................c...........................
24219-01220       .a.c............................c...........................
RP58-01212        .a.c............................c...........................
FCC               .a..tc..a....ag..ct.c........g..c...t.................c.tc..
```

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                            850       860       870       880       890       900
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         acggacctcgcatgacgacgggaccaccaccatcggatttgccttggtcgaaatagttgg
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       .........................................................c..
CAV632-01210        .........................................................c..
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ...................a...............a.c.c.................c..
RP8-0121            ...................a...............a.c.c.................c..
RP9-0121            ...................a...............a.c.c.................c..
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          .........................................................c..
CAV1100-0129        .........................................................c..
CAV009-0120         .........................................................c..
CAV293-0120         .........................................................c..
CAV294-0120         .........................................................c..
CAV296-0120         .........................................................c..
CAV612-01215        .........................................................c..
CAV298-0120/15      .........................................................c..
CAV299-0120/15      .........................................................c..
CAV607-0122         .........................................................c..
CAV605-0122         .........................................................c..
23631-01211         .........................................................c..
CAV1107-0129/11     ..........a.......t.....t............c.c.................c..
CAV929-0123         ..........a.............t..............c................c...
CAV957-0123         ..........a.............t..............c................c...
CAV933-0123         ..........a.............t..............c................c...
CAV871-01217        ..........a.............t..............c................c...
CAV194-01218        ..........a.............t..............c................c...
CAV189-01214        ..........a.............t..............c................c...
CAV602-0124         ........................t..............c................c...
CAV609-0124         ........................t..............c................c...
CAV786-0124         ........................t..............c................c...
8611-0125           ........................t..............c................c...
23480-0125          ........................t..............c................c...
23487-0125          ........................t..............c................c...
22993-0128          ........................t..............c................c...
22994-0128          ........................t..............c................c...
24211-01220         ........................t..............c................c...
24219-01220         ........................t..............c................c...
RP58-01212          ........................t..............c................c...
FCC                 ..a.........c..g..t.c............t.a.c.c.................c..
```

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                          910       920       930       940       950       960
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       tatatgcacttttgaaaaaatgcgtgcaaaatggttttgtggtttggtggtcgtgagtcg
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ........................g...................................
CAV195-01219      ........................g...................................
RP7-0121          .........-........................................c.........
RP8-0121          .........-........................................c.........
RP9-0121          .........-........................................c.........
CAV810-01213      ..................................................c.........
CAV811-01213      ..................................................c.........
CAV300-01213      ..................................................c.........
CAV814-01216      ..................................................c.........
CAV815-01216      ..................................................c.........
CAV604-01216      ..................................................c.........
CAV312-01213/16   ..................................................c.........
CAV313-01213/16   ..................................................c.........
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ..................................c.........................
CAV293-0120       ..................................c.........................
CAV294-0120       ..................................c.........................
CAV296-0120       ..................................c.........................
CAV612-01215      ..................................c.........................
CAV298-0120/15    ..................................c.........................
CAV299-0120/15    ..................................c.........................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV1107-0129/11   ............................................................
CAV929-0123       ..................................................c.........
CAV957-0123       ..................................................c.........
CAV933-0123       ..................................................c.........
CAV871-01217      ..................................................c.........
CAV194-01218      ..................................................c.........
CAV189-01214      ..................................................c.........
CAV602-0124       ..................................................c.........
CAV609-0124       ..................................................c.........
CAV786-0124       ..................................................c.........
8611-0125         ..................................................c.........
23480-0125        ..................................................c.........
23487-0125        ..................................................c.........
22993-0128        .................................t................c.........
22994-0128        .................................t................c.........
24211-01220       ................................................-.c.........
24219-01220       ................................................-.c.........
RP58-01212        ..................................................c.........
FCC               ....................a.............................c.........
```

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                          970       980       990      1000      1010      1020
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       attttttttgtttttcccatacaaatgaattttgcggaaaataaaaagtgg-cccacgaag
CAV793-0126       ..................................................-.........
CAV794-0126       ..................................................-.........
CAV1051-01210     .........-........................................-.........
CAV632-01210      .........-........................................-.........
CAV847-01219      .........-........................................-.........
CAV195-01219      .........-........................................-.........
RP7-0121          .........-........................................-.......g.
RP8-0121          .........-........................................-.......g.
RP9-0121          .........-........................................-.......g.
CAV810-01213      .........-........................................-.t.....g.
CAV811-01213      .........-........................................-.t.....g.
CAV300-01213      .........-........................................-.t.....g.
CAV814-01216      .........-........................................-.t.....g.
CAV815-01216      .........-........................................-.t.....g.
CAV604-01216      .........-........................................-.t.....g.
CAV312-01213/16   .........-........................................-.t.....g.
CAV313-01213/16   .........-........................................-.t.....g.
23510-0129        .........-........................................-.........
CAV1100-0129      .........-........................................-.........
CAV009-0120       .........-........................................-.........
CAV293-0120       .........-........................................-.........
CAV294-0120       .........-........................................-.........
CAV296-0120       .........-........................................-.........
CAV612-01215      .........-........................................-.........
CAV298-0120/15    .........-........................................-.........
CAV299-0120/15    .........-........................................-.........
CAV607-0122       ..................................................-.........
CAV605-0122       ..................................................-.........
23631-01211       .........-........................................-.........
CAV1107-0129/11   .........-........................................-.........
CAV929-0123       .........-........................................-.......g.
CAV957-0123       .........-........................................-.......g.
CAV933-0123       .........-........................................-.......g.
CAV871-01217      .........-........................................-.......g.
CAV194-01218      .........-........................................-.......g.
CAV189-01214      .........-........................................-.......g.
CAV602-0124       .........-........................................-.......g.
CAV609-0124       .........-........................................-.......g.
CAV786-0124       .........-........................................-.......g.
8611-0125         .........-........................................-.......g.
23480-0125        .........-........................................-.......g.
23487-0125        .........-........................................-.......g.
22993-0128        .........-........................................-.......g.
22994-0128        .........-........................................-.......g.
24211-01220       .........-........................................-.......g.
24219-01220       .........-........................................-.......g.
RP58-01212        .........-........................................-.......g.
FCC               .........-........................................-.......g.
```

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                          1030      1040      1050      1060      1070      1080
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       cagttctggcgtgcggcccactaaaacggtctcggagggtatatgagaagggagcaaatc
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ...c........................................................
RP8-0121          ...c........................................................
RP9-0121          ...c........................................................
CAV810-01213      ....c.......................................................
CAV811-01213      ....c.......................................................
CAV300-01213      ....c.......................................................
CAV814-01216      ....c.......................................................
CAV815-01216      ....c.......................................................
CAV604-01216      ....c.......................................................
CAV312-01213/16   ....c.......................................................
CAV313-01213/16   ....c.......................................................
23510-0129        ...c........................................................
CAV1100-0129      ...c........................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ...c........................................................
CAV1107-0129/11   ...c........................................................
CAV929-0123       ....c.......................................................
CAV957-0123       ....c.......................................................
CAV933-0123       ....c.......................................................
CAV871-01217      ....c.......................................................
CAV194-01218      ....c.......................................................
CAV189-01214      ....c.......................................................
CAV602-0124       ...c........................................................
CAV609-0124       ...c........................................................
CAV786-0124       ...c........................................................
8611-0125         ...c........................................................
23480-0125        ...c........................................................
23487-0125        ...c........................................................
22993-0128        ...c........................................................
22994-0128        ...c........................................................
24211-01220       ...c........................................................
24219-01220       ...c........................................................
RP58-01212        ...c........................................................
FCC               .g..........................................................
```

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                                1090      1100      1110      1120      1130      1140
                         ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126              cggccgagcctgaaagggtgaggacaaaccgggcgagcaacctctcagtatcagatcttg
CAV793-0126              ............................................................
CAV794-0126              ............................................................
CAV1051-01210            ........................................t...................
CAV632-01210             ........................................t...................
CAV847-01219             ............................................................
CAV195-01219             ............................................................
RP7-0121                 ............................................................
RP8-0121                 ............................................................
RP9-0121                 ............................................................
CAV810-01213             ............................................................
CAV811-01213             ............................................................
CAV300-01213             ............................................................
CAV814-01216             ............................................................
CAV815-01216             ............................................................
CAV604-01216             ............................................................
CAV312-01213/16          ............................................................
CAV313-01213/16          ............................................................
23510-0129               ............................................................
CAV1100-0129             ............................................................
CAV009-0120              ..................c.........................................
CAV293-0120              ..................c.........................................
CAV294-0120              ..................c.........................................
CAV296-0120              ..................c.........................................
CAV612-01215             ..................c.........................................
CAV298-0120/15           ..................c.........................................
CAV299-0120/15           ..................c.........................................
CAV607-0122              ........................................t...................
CAV605-0122              ........................................t...................
23631-01211              ............................................................
CAV1107-0129/11          ............................................................
CAV929-0123              ............................................................
CAV957-0123              ............................................................
CAV933-0123              ............................................................
CAV871-01217             ............................................................
CAV194-01218             ............................................................
CAV189-01214             ............................................................
CAV602-0124              ............................................................
CAV609-0124              ............................................................
CAV786-0124              ............................................................
8611-0125                ............................................................
23480-0125               ............................................................
23487-0125               ............................................................
22993-0128               ............................................................
22994-0128               ............................................................
24211-01220              ............................................................
24219-01220              ............................................................
RP58-01212               ............................................................
FCC                      ....--------------------------------------------------------
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                                 1150      1160      1170      1180      1190      1200
                         ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126              cagacttccactgcgtgtccctctgtacagctttgcaggctccggcctcggcagcggggg
CAV793-0126              ............................................................
CAV794-0126              ............................................................
CAV1051-01210            ............................................................
CAV632-01210             ............................................................
CAV847-01219             ............................................................
CAV195-01219             ............................................................
RP7-0121                 ............................................................
RP8-0121                 ............................................................
RP9-0121                 ............................................................
CAV810-01213             ............................................................
CAV811-01213             ............................................................
CAV300-01213             ............................................................
CAV814-01216             ............................................................
CAV815-01216             ............................................................
CAV604-01216             ............................................................
CAV312-01213/16          ............................................................
CAV313-01213/16          ............................................................
23510-0129               ............................................................
CAV1100-0129             ............................................................
CAV009-0120              ............................................................
CAV293-0120              ............................................................
CAV294-0120              ............................................................
CAV296-0120              ............................................................
CAV612-01215             ............................................................
CAV298-0120/15           ............................................................
CAV299-0120/15           ............................................................
CAV607-0122              ............................................................
CAV605-0122              ............................................................
23631-01211              ............................................................
CAV1107-0129/11          ............................................................
CAV929-0123              ............................................................
CAV957-0123              ............................................................
CAV933-0123              ............................................................
CAV871-01217             ............................................................
CAV194-01218             ............................................................
CAV189-01214             ............................................................
CAV602-0124              ............................................................
CAV609-0124              ............................................................
CAV786-0124              ............................................................
8611-0125                ............................................................
23480-0125               ............................................................
23487-0125               ............................................................
22993-0128               ............................................................
22994-0128               ............................................................
24211-01220              ............................................................
24219-01220              ............................................................
RP58-01212               ............................................................
FCC                      ------------------------------------------------------------
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                         1210      1220      1230      1240      1250      1260
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gttcatagtggtcgtcgacctccacgaaactgcacgctccggcgtgacagcgtactgggg
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      ............................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV1107-0129/11  ............................................................
CAV929-0123      ............................................................
CAV957-0123      ............................................................
CAV933-0123      ............................................................
CAV871-01217     ............................................................
CAV194-01218     ............................................................
CAV189-01214     ............................................................
CAV602-0124      ............................................................
CAV609-0124      ............................................................
CAV786-0124      ............................................................
8611-0125        ............................................................
23480-0125       ............................................................
23487-0125       ............................................................
22993-0128       ............................................................
22994-0128       ............................................................
24211-01220      ............................................................
24219-01220      ............................................................
RP58-01212       ............................................................
FCC              ------------------------------------------------------------
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                              1270      1280      1290      1300      1310      1320
                        ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126             atgcctgtgtagatgcagtccgggcttgcctggaccgctagcagatgggctctgtggatg
CAV793-0126             ............................................................
CAV794-0126             ............................................................
CAV1051-01210           ............................................................
CAV632-01210            ............................................................
CAV847-01219            ............................................................
CAV195-01219            ............................................................
RP7-0121                ............................................................
RP8-0121                ............................................................
RP9-0121                ............................................................
CAV810-01213            ............................................................
CAV811-01213            ............................................................
CAV300-01213            ............................................................
CAV814-01216            ............................................................
CAV815-01216            ............................................................
CAV604-01216            ............................................................
CAV312-01213/16         ............................................................
CAV313-01213/16         ............................................................
23510-0129              ............................................................
CAV1100-0129            ............................................................
CAV009-0120             ............................................................
CAV293-0120             ............................................................
CAV294-0120             ............................................................
CAV296-0120             ............................................................
CAV612-01215            ............................................................
CAV298-0120/15          ............................................................
CAV299-0120/15          ............................................................
CAV607-0122             ............................................................
CAV605-0122             ............................................................
23631-01211             ............................................................
CAV1107-0129/11         ............................................................
CAV929-0123             ............................................................
CAV957-0123             ............................................................
CAV933-0123             ............................................................
CAV871-01217            ............................................................
CAV194-01218            ............................................................
CAV189-01214            ............................................................
CAV602-0124             ............................................................
CAV609-0124             ............................................................
CAV786-0124             ............................................................
8611-0125               ............................................................
23480-0125              ............................................................
23487-0125              ............................................................
22993-0128              ............................................................
22994-0128              ............................................................
24211-01220             ............................................................
24219-01220             ............................................................
RP58-01212              ............................................................
FCC                     ------------------------------------------------------------
```

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                         1330      1340      1350      1360      1370      1380
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      actggccgctggctagacctgaaacctgagcaacgggaggtaacctc-tcgccgcgga-c
CAV793-0126      ...............................................-..........-.
CAV794-0126      ...............................................-..........-.
CAV1051-01210    ...............................................-..........-.
CAV632-01210     ...............................................-..........-.
CAV847-01219     ...............................................-..........-.
CAV195-01219     ...............................................-..........-.
RP7-0121         ...............................................-..........-.
RP8-0121         ...............................................-..........-.
RP9-0121         ...............................................-..........-.
CAV810-01213     ...............................................-..........-.
CAV811-01213     ...............................................-..........-.
CAV300-01213     ...............................................-..........-.
CAV814-01216     ...............................................-..........-.
CAV815-01216     ...............................................-..........-.
CAV604-01216     ...............................................-..........-.
CAV312-01213/16  ...............................................-..........-.
CAV313-01213/16  ...............................................-..........-.
23510-0129       ...............................................-..........-.
CAV1100-0129     ...............................................-..........-.
CAV009-0120      ...............................................-..........-.
CAV293-0120      ...............................................-..........-.
CAV294-0120      ...............................................-..........-.
CAV296-0120      ...............................................-..........-.
CAV612-01215     ...............................................-..........-.
CAV298-0120/15   ...............................................-..........-.
CAV299-0120/15   ...............................................-..........-.
CAV607-0122      ...............................................-..........-.
CAV605-0122      ...............................................-..........-.
23631-01211      ...............................................-..........-.
CAV1107-0129/11  ...............................................-..........-.
CAV929-0123      ...............................................-..........-.
CAV957-0123      ...............................................-..........-.
CAV933-0123      ...............................................-..........-.
CAV871-01217     ...............................................-..........-.
CAV194-01218     ...............................................-..........-.
CAV189-01214     ...............................................-..........-.
CAV602-0124      ...............................................-..........-.
CAV609-0124      ...............................................-..........-.
CAV786-0124      ...............................................-..........-.
8611-0125        ...............................................-..........-.
23480-0125       ...............................................-..........-.
23487-0125       ...............................................-..........-.
22993-0128       ...............................................-..........-.
22994-0128       ...............................................-..........-.
24211-01220      ...............................................-..........-.
24219-01220      ...............................................-..........-.
RP58-01212       ...............................................-..........-.
FCC              ------------------------------------------------------------
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                                 1390      1400      1410      1420      1430      1440
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      accggaatggtagaagcggcgtgctgcgtcctcctcttggggcccctaagccacacctcc
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      ............................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV1107-0129/11  ............................................................
CAV929-0123      ............................................................
CAV957-0123      ............................................................
CAV933-0123      ............................................................
CAV871-01217     ............................................................
CAV194-01218     ............................................................
CAV189-01214     ............................................................
CAV602-0124      ............................................................
CAV609-0124      ............................................................
CAV786-0124      ............................................................
8611-0125        ............................................................
23480-0125       ............................................................
23487-0125       ............................................................
22993-0128       ............................................................
22994-0128       ............................................................
24211-01220      ............................................................
24219-01220      ............................................................
RP58-01212       ............................................................
FCC              ------------------------------------------------------------
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                         1450      1460      1470      1480      1490      1500
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      catagcgggttcggtgcggcggacggacgccctggggaatttagagggggaaagcggatt
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      ............................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV1107-0129/11  ............................................................
CAV929-0123      ..c.........................................................
CAV957-0123      ..c.........................................................
CAV933-0123      ..c.........................................................
CAV871-01217     ..c.........................................................
CAV194-01218     ..c.........................................................
CAV189-01214     ..c.........................................................
CAV602-0124      ..c.........................................................
CAV609-0124      ..c.........................................................
CAV786-0124      ..c.........................................................
8611-0125        ..c.........................................................
23480-0125       ..c.........................................................
23487-0125       ..c.........................................................
22993-0128       ..c.........................................................
22994-0128       ..c.........................................................
24211-01220      ..c.........................................................
24219-01220      ..c.........................................................
RP58-01212       ..c.........................................................
FCC              ------------------------------------------------------------
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                         1510      1520      1530      1540      1550      1560
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gccctagcggtgttgttggccctgccgacctcactgcgaaaggcgcgacttcaccgtc-g
CAV793-0126      ..........................................................-.
CAV794-0126      ..........................................................-.
CAV1051-01210    ..........................................................-.
CAV632-01210     ..........................................................-.
CAV847-01219     ..........................................................-.
CAV195-01219     ..........................................................-.
RP7-0121         ..........................................................-.
RP8-0121         ..........................................................-.
RP9-0121         ..........................................................-.
CAV810-01213     ..........................................................-.
CAV811-01213     ..........................................................-.
CAV300-01213     ..........................................................-.
CAV814-01216     ..........................................................-.
CAV815-01216     ..........................................................-.
CAV604-01216     ..........................................................-.
CAV312-01213/16  ..........................................................-.
CAV313-01213/16  ..........................................................-.
23510-0129       ..........................................................-.
CAV1100-0129     ..........................................................-.
CAV009-0120      ................n...................................nnnnnn-n
CAV293-0120      ..........................................................-.
CAV294-0120      ..........................................................-.
CAV296-0120      ..........................................................-.
CAV612-01215     ..........................................................-.
CAV298-0120/15   ..........................................................-.
CAV299-0120/15   ..........................................................-.
CAV607-0122      ..........................................................-.
CAV605-0122      ..........................................................-.
23631-01211      .........................................................n-n
CAV1107-0129/11  ..........................................................-.
CAV929-0123      ..........................................................-.
CAV957-0123      ..........................................................-.
CAV933-0123      ..........................................................-.
CAV871-01217     ..........................................................-.
CAV194-01218     ..........................................................-.
CAV189-01214     ..........................................................-.
CAV602-0124      ..........................................................-.
CAV609-0124      ..........................................................-.
CAV786-0124      ..........................................................-.
8611-0125        ..........................................................-.
23480-0125       ..........................................................-.
23487-0125       ..........................................................-.
22993-0128       ..........................................................-.
22994-0128       ..........................................................-.
24211-01220      ..........................................................-.
24219-01220      ..........................................................-.
RP58-01212       ..........................................................-.
FCC              ------------------------------------------------------------
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                         1570
                 ....|....|....|..
CAV613-0126      ccacccagtaacttgtc
CAV793-0126      .................
CAV794-0126      .................
CAV1051-01210    .................
CAV632-01210     .................
CAV847-01219     .................
CAV195-01219     .................
RP7-0121         .................
RP8-0121         .................
RP9-0121         .................
CAV810-01213     .................
CAV811-01213     .................
CAV300-01213     .................
CAV814-01216     .................
CAV815-01216     .................
CAV604-01216     .................
CAV312-01213/16  ................a
CAV313-01213/16  ................a
23510-0129       .............nnnn
CAV1100-0129     .............nnnn
CAV009-0120      nnnnnnnnnnnnnnnnn
CAV293-0120      .................
CAV294-0120      .................
CAV296-0120      .................
CAV612-01215     .................
CAV298-0120/15   .................
CAV299-0120/15   .................
CAV607-0122      .................
CAV605-0122      .................
23631-01211      nnnnnnnnnnnnnnnnn
CAV1107-0129/11  .................
CAV929-0123      .................
CAV957-0123      .................
CAV933-0123      .................
CAV871-01217     ......nnnnnnnnnnn
CAV194-01218     .................
CAV189-01214     .................
CAV602-0124      .................
CAV609-0124      .................
CAV786-0124      .................
8611-0125        .................
23480-0125       .................
23487-0125       .................
22993-0128       .................
22994-0128       .................
24211-01220      .................
24219-01220      .................
RP58-01212       .......nnnnnnnnnn
FCC              -----------------
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Appendix C

The nucleotide sequence information for gene encoding a repeated region encoded on the mitochondrion of *Fusarium oxysporum* f.sp. *cubense.* ..'s represent similar sequence, -'s represents a gap in the alignment and n represent missing sequences. Alignments were constructed with the use of MAFFT software programme.

```
                           10        20        30        40        50        60
                   ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126        gaatatgaagagtgtacggcttcgccgctatttgaagcttcagacatattaacttctata
CAV793-0126        ............................................................
CAV794-0126        ............................................................
CAV1051-01210      ............................................................
CAV632-01210       ............................................................
CAV847-01219       ............................................................
CAV195-01219       ............................................................
RP7-0121           ............................................................
RP8-0121           ............................................................
RP9-0121           ............................................................
CAV810-01213       ............................................................
CAV811-01213       ............................................................
CAV300-01213       ............................................................
CAV814-01216       ............................................................
CAV815-01216       ............................................................
CAV604-01216       ............................................................
CAV312-01213/16    ............................................................
CAV313-01213/16    ............................................................
23510-0129         ............................................................
CAV1100-0129       nnnnnnnnnnnnnnnnnnnnnnnn....................................
CAV009-0120        ...............c............................................
CAV293-0120        ............................................................
CAV294-0120        ............................................................
CAV296-0120        ............................................................
CAV612-01215       ............................................................
CAV298-0120/15     ............................................................
CAV299-0120/15     ............................................................
CAV607-0122        ............................................................
CAV605-0122        ............................................................
23631-01211        ............................................................
CAV929-0123        .......t.....c..............................................
CAV957-0123        .......t.....c..............................................
CAV933-0123        .......t.....c..............................................
CAV871-01217       .......t.....c..............................................
CAV791-01218       .......t.....c..............................................
CAV189-01214       .......t.....c..............................................
CAV602-0124        .......t.....c..............................................
CAV609-0124        .......t.....c..............................................
CAV786-0124        .......t.....c..............................................
8611-0125          .......t.....c..............................................
12480-0125         .......t.....c..............................................
23487-0125         .......t.....c..............................................
22993-0128         .......t.....c..............................................
22993-0128         .......t.....c..............................................
24211-01220        .......t.....c..............................................
24219-01220        .......t.....c..............................................
RP58-01212         .......t.....c..............................................
CAV1107-0129/11    ............................................................
M.FCC              ....................................c....................c..
```

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                          70        80        90       100       110       120
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gcttgggaggctgaagaagagatccggcttactctttctaccgaattagatgggggtccc
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      ............................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV929-0123      ............................................................
CAV957-0123      ............................................................
CAV933-0123      ............................................................
CAV871-01217     ............................................................
CAV791-01218     ............................................................
CAV189-01214     ............................................................
CAV602-0124      ............................................................
CAV609-0124      ............................................................
CAV786-0124      ............................................................
8611-0125        ............................................................
12480-0125       ............................................................
23487-0125       ............................................................
22993-0128       ............................................................
22993-0128       ............................................................
24211-01220      ............................................................
24219-01220      ............................................................
RP58-01212       ............................................................
CAV1107-0129/11  ............................................................
M.FCC            .......................a..a...t..a.c.......t....a.a..a.a....
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                            130       140       150       160       170       180
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         tcattattataattattgagagaaatattcgggctatttactaactctttatcatctatt
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ......................................................c.....
CAV1100-0129        ......................................................c.....
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV929-0123         .................................t..........................
CAV957-0123         .................................t..........................
CAV933-0123         .................................t..........................
CAV871-01217        .................................t..........................
CAV791-01218        .................................t..........................
CAV189-01214        .................................t..........................
CAV602-0124         .................................t..........................
CAV609-0124         .................................t..........................
CAV786-0124         .................................t..........................
8611-0125           .................................t..........................
12480-0125          .................................t..........................
23487-0125          .................................t..........................
22993-0128          .................................t..........................
22993-0128          .................................t..........................
24211-01220         .................................t..........................
24219-01220         .................................t..........................
RP58-01212          .................................t..........................
CAV1107-0129/11     ............................................................
M.FCC               .................................t.........c.........t......
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```
                          190       200       210       220       230       240
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       ggttctgaaaatgagaaagaggaccgttctatattatttgaccgttctatgttatttgac
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ............................................................
RP8-0121          ............................................................
RP9-0121          ............................................................
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV791-01218      ..........................................t.................
CAV189-01214      ..........................................t.................
CAV602-0124       .................................------------------.........
CAV609-0124       .................................------------------.........
CAV786-0124       .................................------------------.........
8611-0125         .................................------------------.........
12480-0125        .................................------------------.........
23487-0125        .................................------------------.........
22993-0128        .................................------------------.........
22993-0128        .................................------------------.........
24211-01220       .................................------------------.........
24219-01220       .................................------------------.........
RP58-01212        .................................------------------.........
CAV1107-0129/11   ............................................................
M.FCC             ...........................................c................
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                         250       260       270       280       290       300
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      ccttctagattattcgggtgaatataatcttctaaaaacaccaattcaaagaaattaaaa
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      .......a....................................................
CAV293-0120      .......a....................................................
CAV294-0120      .......a....................................................
CAV296-0120      .......a....................................................
CAV612-01215     .......a....................................................
CAV298-0120/15   .......a....................................................
CAV299-0120/15   .......a....................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV929-0123      .................t..........................................
CAV957-0123      .................t..........................................
CAV933-0123      .................t..........................................
CAV871-01217     .................t..........................................
CAV791-01218     ............................................................
CAV189-01214     ............................................................
CAV602-0124      ............................................................
CAV609-0124      ............................................................
CAV786-0124      ............................................................
8611-0125        ............................................................
12480-0125       ............................................................
23487-0125       ............................................................
22993-0128       ............................................................
22993-0128       ............................................................
24211-01220      ............................................................
24219-01220      ............................................................
RP58-01212       ............................................................
CAV1107-0129/11  ............................................................
M.FCC            .......t...........a........................................
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                          310       320       330       340       350       360
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       taatacacaggattttctggatccccttctggcaaattctctataccatctggatatcct
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ............................................................
RP8-0121          ............................................................
RP9-0121          ............................................................
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV791-01218      ......................................a.....................
CAV189-01214      ............................................................
CAV602-0124       ............................................................
CAV609-0124       ............................................................
CAV786-0124       ............................................................
8611-0125         ............................................................
12480-0125        ............................................................
23487-0125        ............................................................
22993-0128        ............................................................
22993-0128        ............................................................
24211-01220       ............................................................
24219-01220       ............................................................
RP58-01212        ............................................................
CAV1107-0129/11   ............................................................
M.FCC             ........................................................c..c
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                          370       380       390       400       410       420
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       tgggcagcatcggatagatccacactatccacacccggaggtagataaagagtaacatca
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ............................................................
RP8-0121          ............................................................
RP9-0121          ............................................................
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV791-01218      ............................................................
CAV189-01214      ............................................................
CAV602-0124       ............................................................
CAV609-0124       ............................................................
CAV786-0124       ............................................................
8611-0125         ............................................................
12480-0125        ............................................................
23487-0125        ............................................................
22993-0128        ............................................................
22993-0128        ............................................................
24211-01220       ............................................................
24219-01220       ............................................................
RP58-01212        ............................................................
CAV1107-0129/11   ............................................................
M.FCC             ................t..t...g..t................t............c...
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                            430       440       450       460       470       480
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         tgatgattaacatgcaaatccggcacttctggaatatctcaggggaaaactcggtaatta
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV791-01218        ............................................................
CAV189-01214        ............................................a...............
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
12480-0125          ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22993-0128          ............................................................
24211-01220         ............................................................
24219-01220         ............................................................
RP58-01212          ............................................................
CAV1107-0129/11     ............................................................
M.FCC               ...........g............................ca..................
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                          490       500       510       520       530       540
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       gtatcaatagtcatctcgacataataacacctattattttctacacctagcaaacgcggc
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     ............................................................
CAV632-01210      ............................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ............................................................
RP8-0121          ............................................................
RP9-0121          ............................................................
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV791-01218      ............................................................
CAV189-01214      ............................................................
CAV602-0124       ............................................................
CAV609-0124       ............................................................
CAV786-0124       ............................................................
8611-0125         ............................................................
12480-0125        ............................................................
23487-0125        ............................................................
22993-0128        ............................................................
22993-0128        ............................................................
24211-01220       ............................................................
24219-01220       ............................................................
RP58-01212        ............................................................
CAV1107-0129/11   ............................................................
M.FCC             ............g........c..........g................c..........
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                           550       560       570       580       590       600
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         tctattccacccctaaccggggtcataactgctaacattctatcaatagctacagtcaag
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV791-01218        ............................................................
CAV189-01214        ............................................................
CAV602-0124         ............................................................
CAV609-0124         ............................................................
CAV786-0124         ............................................................
8611-0125           ............................................................
12480-0125          ............................................................
23487-0125          ............................................................
22993-0128          ............................................................
22993-0128          ............................................................
24211-01220         ............................................................
24219-01220         ............................................................
RP58-01212          ............................................................
CAV1107-0129/11     ............................................................
M.FCC               a....c....t..c..............t...............................
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                         610       620       630       640       650       660
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      tacttatatcccctttcacggtgaatagcttcggctacatatcgtaaattatcgtcatac
CAV793-0126      ............................................................
CAV794-0126      ............................................................
CAV1051-01210    ............................................................
CAV632-01210     ............................................................
CAV847-01219     ............................................................
CAV195-01219     ............................................................
RP7-0121         ............................................................
RP8-0121         ............................................................
RP9-0121         ............................................................
CAV810-01213     ............................................................
CAV811-01213     ............................................................
CAV300-01213     ............................................................
CAV814-01216     ............................................................
CAV815-01216     ............................................................
CAV604-01216     ............................................................
CAV312-01213/16  ............................................................
CAV313-01213/16  ............................................................
23510-0129       ............................................................
CAV1100-0129     ............................................................
CAV009-0120      ............................................................
CAV293-0120      ............................................................
CAV294-0120      ............................................................
CAV296-0120      ............................................................
CAV612-01215     ............................................................
CAV298-0120/15   ............................................................
CAV299-0120/15   ............................................................
CAV607-0122      ............................................................
CAV605-0122      ............................................................
23631-01211      ............................................................
CAV929-0123      ............................................................
CAV957-0123      ............................................................
CAV933-0123      ............................................................
CAV871-01217     ............................................................
CAV791-01218     ............................................................
CAV189-01214     ............................................................
CAV602-0124      ............................................................
CAV609-0124      ............................................................
CAV786-0124      ............................................................
8611-0125        ............................................................
12480-0125       ............................................................
23487-0125       ............................................................
22993-0128       ............................................................
22993-0128       ............................................................
24211-01220      ............................................................
24219-01220      ............................................................
RP58-01212       ............................................................
CAV1107-0129/11  ............................................................
M.FCC            ....................c...........c.................c.........
```

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                            670       680       690       700       710       720
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         gcctccggagaaggagcctcactagaactatcatctgatgatataataatagcctcagga
CAV793-0126         ............................................................
CAV794-0126         ............................................................
CAV1051-01210       ............................................................
CAV632-01210        ............................................................
CAV847-01219        ............................................................
CAV195-01219        ............................................................
RP7-0121            ............................................................
RP8-0121            ............................................................
RP9-0121            ............................................................
CAV810-01213        ............................................................
CAV811-01213        ............................................................
CAV300-01213        ............................................................
CAV814-01216        ............................................................
CAV815-01216        ............................................................
CAV604-01216        ............................................................
CAV312-01213/16     ............................................................
CAV313-01213/16     ............................................................
23510-0129          ............................................................
CAV1100-0129        ............................................................
CAV009-0120         ............................................................
CAV293-0120         ............................................................
CAV294-0120         ............................................................
CAV296-0120         ............................................................
CAV612-01215        ............................................................
CAV298-0120/15      ............................................................
CAV299-0120/15      ............................................................
CAV607-0122         ............................................................
CAV605-0122         ............................................................
23631-01211         ............................................................
CAV929-0123         ............................................................
CAV957-0123         ............................................................
CAV933-0123         ............................................................
CAV871-01217        ............................................................
CAV791-01218        ...................................a........................
CAV189-01214        ............................................................
CAV602-0124         .......a....................................................
CAV609-0124         .......a....................................................
CAV786-0124         .......a....................................................
8611-0125           .......a....................................................
12480-0125          .......a....................................................
23487-0125          .......a....................................................
22993-0128          .......a....................................................
22993-0128          .......a....................................................
24211-01220         .......a....................................................
24219-01220         .......a....................................................
RP58-01212          .......a....................................................
CAV1107-0129/11     ............................................................
M.FCC               ............n.....c.........................................
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                          730       740       750       760       770       780
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       tctcccacttctactggtgcaggaggagaag------------aagtatcctcactagaa
CAV793-0126       ...............................------------.................
CAV794-0126       ...............................------------.................
CAV1051-01210     ...............................------------.................
CAV632-01210      ...............................------------.................
CAV847-01219      ...............................------------.................
CAV195-01219      ...............................------------.................
RP7-0121          ...............................------------.................
RP8-0121          ...............................------------.................
RP9-0121          ...............................------------.................
CAV810-01213      ...............................------------.................
CAV811-01213      ...............................------------.................
CAV300-01213      ...............................------------.................
CAV814-01216      ...............................------------.................
CAV815-01216      ...............................------------.................
CAV604-01216      ...............................------------.................
CAV312-01213/16   ...............................------------.................
CAV313-01213/16   ...............................------------.................
23510-0129        ..............................a------------.................
CAV1100-0129      ...............................------------.................
CAV009-0120       ...............................------------.................
CAV293-0120       ...............................------------.................
CAV294-0120       ...............................------------.................
CAV296-0120       ...............................------------.................
CAV612-01215      ...............................------------.................
CAV298-0120/15    ...............................------------.................
CAV299-0120/15    ...............................------------.................
CAV607-0122       ...............................------------.................
CAV605-0122       ...............................------------.................
23631-01211       ...............................------------.................
CAV929-0123       ...............................------------.................
CAV957-0123       ...............................------------.................
CAV933-0123       ...............................------------.................
CAV871-01217      ...............................------------.................
CAV791-01218      ...............................------------.................
CAV189-01214      ..........................----------------------------------
CAV602-0124       ...............................------------.................
CAV609-0124       ...............................------------.................
CAV786-0124       ...............................------------.................
8611-0125         ...............................------------.................
12480-0125        ...............................------------.................
23487-0125        ...............................------------.................
22993-0128        ...............................------------.................
22993-0128        ...............................------------.................
24211-01220       ...............................------------.................
24219-01220       ...............................------------.................
RP58-01212        ...............................------------.................
CAV1107-0129/11   ...............................------------.................
M.FCC             ..c...g..................t.t...tattctcactag..c..............
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                          790       800       810       820       830       840
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       ctatcatctgatgatataataataacctcaggatctctcacttctactgctgccagagga
CAV793-0126       ............................................................
CAV794-0126       ............................................................
CAV1051-01210     .........t..................................................
CAV632-01210      .........t..................................................
CAV847-01219      ............................................................
CAV195-01219      ............................................................
RP7-0121          ............................................................
RP8-0121          ............................................................
RP9-0121          ............................................................
CAV810-01213      ............................................................
CAV811-01213      ............................................................
CAV300-01213      ............................................................
CAV814-01216      ............................................................
CAV815-01216      ............................................................
CAV604-01216      ............................................................
CAV312-01213/16   ............................................................
CAV313-01213/16   ............................................................
23510-0129        ............................................................
CAV1100-0129      ............................................................
CAV009-0120       ............................................................
CAV293-0120       ............................................................
CAV294-0120       ............................................................
CAV296-0120       ............................................................
CAV612-01215      ............................................................
CAV298-0120/15    ............................................................
CAV299-0120/15    ............................................................
CAV607-0122       ............................................................
CAV605-0122       ............................................................
23631-01211       ............................................................
CAV929-0123       ............................................................
CAV957-0123       ............................................................
CAV933-0123       ............................................................
CAV871-01217      ............................................................
CAV791-01218      ............................................................
CAV189-01214      ------------------------------------------------------------
CAV602-0124       ..............................t.............................
CAV609-0124       ..............................t.............................
CAV786-0124       ..............................t.............................
8611-0125         ..............................t.............................
12480-0125        ..............................t.............................
23487-0125        ..............................t.............................
22993-0128        ..............................t.............................
22993-0128        ..............................t.............................
24211-01220       ..............................t.............................
24219-01220       ..............................t.............................
RP58-01212        ..............................t.............................
CAV1107-0129/11   ............................................................
M.FCC             ...................c......................c....ca.g...g.....
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                          850       860       870       880       890       900
                  ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126       ataaagggatcaatagttttcacagaagcctcttcctcctttgaaa---------tatct
CAV793-0126       ..............................................---------.....
CAV794-0126       ..............................................---------.....
CAV1051-01210     ..............................................---------.....
CAV632-01210      ..............................................---------.....
CAV847-01219      ..............................................---------.....
CAV195-01219      ..............................................---------.....
RP7-0121          .......................................a......---------.....
RP8-0121          .......................................a......---------.....
RP9-0121          .......................................a......---------.....
CAV810-01213      .......................................a......---------.....
CAV811-01213      .......................................a......---------.....
CAV300-01213      .......................................a......---------.....
CAV814-01216      .......................................a......---------.....
CAV815-01216      .......................................a......---------.....
CAV604-01216      .......................................a......---------.....
CAV312-01213/16   .......................................a......---------.....
CAV313-01213/16   .......................................a......---------.....
23510-0129        .............................................ggcgcaagctct...
CAV1100-0129      .............................................ggcgcaagctct...
CAV009-0120       .............................................ggcgcaagctct...
CAV293-0120       .............................................ggcgcaagctct...
CAV294-0120       .............................................ggcgcaagctct...
CAV296-0120       .............................................ggcgcaagctct...
CAV612-01215      .............................................ggcgcaagctct...
CAV298-0120/15    .............................................ggcgcaagctct...
CAV299-0120/15    .............................................ggcgcaagctct...
CAV607-0122       .............................................ggcgcaagctct...
CAV605-0122       .............................................ggcgcaagctct...
23631-01211       .............................................ggcgcaagctct...
CAV929-0123       ..............................................---------.....
CAV957-0123       ..............................................---------.....
CAV933-0123       ..............................................---------.....
CAV871-01217      ..............................................---------.....
CAV791-01218      ..............................................---------.....
CAV189-01214      ..............................................---------.....
CAV602-0124       ..............................................---------.....
CAV609-0124       ..............................................---------.....
CAV786-0124       ..............................................---------.....
8611-0125         ..............................................---------.....
12480-0125        ..............................................---------.....
23487-0125        ..............................................---------.....
22993-0128        ..............................................---------.....
22993-0128        ..............................................---------.....
24211-01220       ..............................................---------.....
24219-01220       ..............................................---------.....
RP58-01212        ..............................................---------.....
CAV1107-0129/11   .............................................ggcgcaagctct...
M.FCC             .............g.........g..................a..ggcgcgagctct..c
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                         910       920       930       940       950       960
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      t-----------------------------------------------------------
CAV793-0126      .-----------------------------------------------------------
CAV794-0126      .-----------------------------------------------------------
CAV1051-01210    .-----------------------------------------------------------
CAV632-01210     .-----------------------------------------------------------
CAV847-01219     .-----------------------------------------------------------
CAV195-01219     .-----------------------------------------------------------
RP7-0121         .-----------------------------------------------------------
RP8-0121         .-----------------------------------------------------------
RP9-0121         .-----------------------------------------------------------
CAV810-01213     .-----------------------------------------------------------
CAV811-01213     .-----------------------------------------------------------
CAV300-01213     .-----------------------------------------------------------
CAV814-01216     .-----------------------------------------------------------
CAV815-01216     .-----------------------------------------------------------
CAV604-01216     .-----------------------------------------------------------
CAV312-01213/16  .-----------------------------------------------------------
CAV313-01213/16  .-----------------------------------------------------------
23510-0129       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV1100-0129     .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV009-0120      .-----------------------------------------------------------
CAV293-0120      .-----------------------------------------------------------
CAV294-0120      .-----------------------------------------------------------
CAV296-0120      .-----------------------------------------------------------
CAV612-01215     .-----------------------------------------------------------
CAV298-0120/15   .-----------------------------------------------------------
CAV299-0120/15   .-----------------------------------------------------------
CAV607-0122      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV605-0122      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
23631-01211      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV929-0123      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
CAV957-0123      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
CAV933-0123      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
CAV871-01217     .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
CAV791-01218     .-----------------------------------------------------------
CAV189-01214     .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggaaagtgga
CAV602-0124      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV609-0124      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV786-0124      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
8611-0125        .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
12480-0125       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
23487-0125       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
22993-0128       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
22993-0128       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
24211-01220      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
24219-01220      .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
RP58-01212       .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
CAV1107-0129/11  .ggttaaatatttcttgctctaatttagacagttcttcatctcctatactggagagtgga
M.FCC            c-----------------------------------------------------------
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                             970       980       990      1000      1010      1020
                    ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126         ------------------------------------------------------------
CAV793-0126         ------------------------------------------------------------
CAV794-0126         ------------------------------------------------------------
CAV1051-01210       ------------------------------------------------------------
CAV632-01210        ------------------------------------------------------------
CAV847-01219        ------------------------------------------------------------
CAV195-01219        ------------------------------------------------------------
RP7-0121            ------------------------------------------------------------
RP8-0121            ------------------------------------------------------------
RP9-0121            ------------------------------------------------------------
CAV810-01213        ------------------------------------------------------------
CAV811-01213        ------------------------------------------------------------
CAV300-01213        ------------------------------------------------------------
CAV814-01216        ------------------------------------------------------------
CAV815-01216        ------------------------------------------------------------
CAV604-01216        ------------------------------------------------------------
CAV312-01213/16     ------------------------------------------------------------
CAV313-01213/16     ------------------------------------------------------------
23510-0129          ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV1100-0129        ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV009-0120         ------------------------------------------------------------
CAV293-0120         ------------------------------------------------------------
CAV294-0120         ------------------------------------------------------------
CAV296-0120         ------------------------------------------------------------
CAV612-01215        ------------------------------------------------------------
CAV298-0120/15      ------------------------------------------------------------
CAV299-0120/15      ------------------------------------------------------------
CAV607-0122         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV605-0122         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
23631-01211         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV929-0123         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV957-0123         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV933-0123         ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV871-01217        ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
CAV791-01218        ------------------------------------------------------------
CAV189-01214        ctatcttcatccacaggtgcgtcttctttccctgaaggggtgcttcccccttccttccct
CAV602-0124         ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
CAV609-0124         ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
CAV786-0124         ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
8611-0125           ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
12480-0125          ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
23487-0125          ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
22993-0128          ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
22993-0128          ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
24211-01220         ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
24219-01220         ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
RP58-01212          ctatcttcatccccaggtgcgtcttctttccctgagggggtgcttcccccttccttccca
CAV1107-0129/11     ctatcttcatccccaggtgcgtcttctttccctgaaggggtgcttcccccttccttccca
M.FCC               ------------------------------------------------------------
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                             1030      1040      1050      1060      1070      1080
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      -------------------------------------ggttaaatatttcttgctctaat
CAV793-0126      -------------------------------------.......................
CAV794-0126      -------------------------------------.......................
CAV1051-01210    -------------------------------------.......................
CAV632-01210     -------------------------------------.......................
CAV847-01219     -------------------------------------.......................
CAV195-01219     -------------------------------------.......................
RP7-0121         -------------------------------------.......................
RP8-0121         -------------------------------------.......................
RP9-0121         -------------------------------------.......................
CAV810-01213     -------------------------------------.......................
CAV811-01213     -------------------------------------.......................
CAV300-01213     -------------------------------------.......................
CAV814-01216     -------------------------------------.......................
CAV815-01216     -------------------------------------.......................
CAV604-01216     -------------------------------------.......................
CAV312-01213/16  -------------------------------------.......................
CAV313-01213/16  -------------------------------------.......................
23510-0129       gaagcctcttcctccattgaaggcgcaagctcttctt.......................
CAV1100-0129     gaagcctcttcctccattgaaggcgcaagctcttctt.......................
CAV009-0120      -------------------------------------.......................
CAV293-0120      -------------------------------------.......................
CAV294-0120      -------------------------------------.......................
CAV296-0120      -------------------------------------.......................
CAV612-01215     -------------------------------------.......................
CAV298-0120/15   -------------------------------------.......................
CAV299-0120/15   -------------------------------------.......................
CAV607-0122      gaagcctcttcctccattgaaggcgcaagctcttctc.......................
CAV605-0122      gaagcctcttcctccattgaaggcgcaagctcttctt.......................
23631-01211      gaagcctcttcctccattgaaggcgcaagctcttctc.......................
CAV929-0123      gaagcctcttcctcctttgaaatatctc---------......................c
CAV957-0123      gaagcctcttcctcctttgaaatatctc---------......................c
CAV933-0123      gaagcctcttcctcctttgaaatatctc---------......................c
CAV871-01217     gaagcctcttcctcctttgaaatatctc---------......................c
CAV791-01218     -------------------------------------.......................
CAV189-01214     gaagcctcttcctccattgaaatatctc---------.......................
CAV602-0124      gaagcctcttcctccattgaaatatctc---------.......................
CAV609-0124      gaagcctcttcctccattgaaatatctc---------.......................
CAV786-0124      gaagcctcttcctccattgaaatatctc---------.......................
8611-0125        gaagcctcttcctccattgaaatatctc---------.......................
12480-0125       gaagcctcttcctccattgaaatatctc---------.......................
23487-0125       gaagcctcttcctccattgaaatatctc---------.......................
22993-0128       gaagcctcttcctccattgaaatatctc---------.......................
22993-0128       gaagcctcttcctccattgaaatatctc---------.......................
24211-01220      gaagcctcttcctccattgaaatatctc---------.......................
24219-01220      gaagcctcttcctccattgaaatatctc---------.......................
RP58-01212       gaagcctcttcctccattgaaatatctc---------.......................
CAV1107-0129/11  gaagcctcttcctccattgaaggcgcaagctcttctt.......................
M.FCC            -------------------------------------.....t.................
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                               1090      1100      1110      1120      1130      1140
                      ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126           ttagacagttcttcatctcctatactggagagtggactatcttcagcttctactggctcc
CAV793-0126           ............................................................
CAV794-0126           ............................................................
CAV1051-01210         ............................................................
CAV632-01210          ............................................................
CAV847-01219          ............................................................
CAV195-01219          ............................................................
RP7-0121              ............................................................
RP8-0121              ............................................................
RP9-0121              ............................................................
CAV810-01213          ............................................................
CAV811-01213          ............................................................
CAV300-01213          ............................................................
CAV814-01216          ............................................................
CAV815-01216          ............................................................
CAV604-01216          ............................................................
CAV312-01213/16       ............................................................
CAV313-01213/16       ............................................................
23510-0129            ............................................................
CAV1100-0129          ............................................................
CAV009-0120           ............................................................
CAV293-0120           ............................................................
CAV294-0120           ............................................................
CAV296-0120           ............................................................
CAV612-01215          ............................................................
CAV298-0120/15        ............................................................
CAV299-0120/15        ............................................................
CAV607-0122           ............................................................
CAV605-0122           ............................................................
23631-01211           ............................................................
CAV929-0123           ......................................g..............a......
CAV957-0123           ......................................g..............a......
CAV933-0123           ......................................g..............a......
CAV871-01217          ......................................g..............a......
CAV791-01218          .....................................................a......
CAV189-01214          .....................................................a......
CAV602-0124           .....................................................a......
CAV609-0124           .....................................................a......
CAV786-0124           .....................................................a......
8611-0125             .....................................................a......
12480-0125            .....................................................a......
23487-0125            .....................................................a......
22993-0128            .....................................................a......
22993-0128            .....................................................a......
24211-01220           .....................................................a......
24219-01220           .....................................................a......
RP58-01212            .....................................................a......
CAV1107-0129/11       ............................................................
M.FCC                 ...c...........................a..a............c..c.......g.
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                         1150      1160      1170      1180      1190      1200
                 ....|....|....|....|....|....|....|....|....|....|....|....|
CAV613-0126      gg-attacttggtaggatagggagaagtaggctaggatctatagtttc-tactactgttt
CAV793-0126      ..-.............................................-...........
CAV794-0126      ..-.............................................-...........
CAV1051-01210    ..-.............................................-...........
CAV632-01210     ..-.............................................-...........
CAV847-01219     ..-.............................................-...........
CAV195-01219     ..-.............................................-...........
RP7-0121         ..-............................a................-...........
RP8-0121         ..-............................a................-...........
RP9-0121         ..-............................a................-...........
CAV810-01213     ..-............................a................-...........
CAV811-01213     ..-............................a................-...........
CAV300-01213     ..-............................a................-...........
CAV814-01216     ..-............................a................-...........
CAV815-01216     ..-............................a................-...........
CAV604-01216     ..-............................a................-...........
CAV312-01213/16  ..-............................a................-...........
CAV313-01213/16  ..-............................a................-...........
23510-0129       ..-............................a...........c....-...........
CAV1100-0129     ..-............................a...........nnnnn-nnnnnnnnnnn
CAV009-0120      ..-............................a...........c....-...........
CAV293-0120      ..-............................a...........c....-...........
CAV294-0120      ..-............................a...........c....-...........
CAV296-0120      ..-............................a...........c....-...........
CAV612-01215     ..-............................a...........c....-...........
CAV298-0120/15   ..-............................a...........c....-...........
CAV299-0120/15   ..-............................a...........c....-...........
CAV607-0122      ..-............................a...........c....-...........
CAV605-0122      ..-............................a...........c....-...........
23631-01211      ..-............................a...........c....-...........
CAV929-0123      ..-..................a.........a................-...........
CAV957-0123      ..-..................a.........a................-...........
CAV933-0123      ..-..................a.........a................-...........
CAV871-01217     ..-..g...c.....................a...............g-.........a.
CAV791-01218     ..-..g.........................a................g..a........
CAV189-01214     ..-........a...................a................-...........
CAV602-0124      ..-...............c..........t.a................-.......a...
CAV609-0124      ..-...............c..........t.a................-.......a...
CAV786-0124      ..-...............c..........t.a................-.......a...
8611-0125        ..-...............c..........t.a................-.......a...
12480-0125       ..-...............c..........t.a................-.......a...
23487-0125       ..-...............c..........t.a................-.......a...
22993-0128       ..-...............c..........t.a................-.......a...
22993-0128       ..-...............c..........t.a................-.......a...
24211-01220      ..-...............c..........t.a................-.......a...
24219-01220      ..-...............c..........t.a................-.......a...
RP58-01212       ..-...............c..........t.a................-.......a...
CAV1107-0129/11  ..-............................a...........c....-...........
M.FCC            ..-...ga.a..ct.a.....a.t......a.................-......a.c..
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                          1210      1220
                  ....|....|....|....|....
CAV613-0126       ggtta-cctaaatccgcattcatc
CAV793-0126       .....-..................
CAV794-0126       .....-..................
CAV1051-01210     .....-..................
CAV632-01210      .....-..................
CAV847-01219      .....-..................
CAV195-01219      .....-..................
RP7-0121          .....-..................
RP8-0121          .....-..................
RP9-0121          .....-..................
CAV810-01213      .....-..................
CAV811-01213      .....-..................
CAV300-01213      .....-..................
CAV814-01216      .....-..................
CAV815-01216      .....-..................
CAV604-01216      .....-..................
CAV312-01213/16   .....-..................
CAV313-01213/16   .....-..................
23510-0129        .....-......c...........
CAV1100-0129      nnnnn-nnnnnnnnnnnnnnnnnn
CAV009-0120       .....-......c...........
CAV293-0120       .....-......c...........
CAV294-0120       .....-......c...........
CAV296-0120       .....-......c...........
CAV612-01215      .....-......c...........
CAV298-0120/15    .....-......c...........
CAV299-0120/15    .....-......c...........
CAV607-0122       .....-......c...........
CAV605-0122       .....-......c...........
23631-01211       .....-......c...........
CAV929-0123       ...c.-.......t..........
CAV957-0123       ...c.-.......t..........
CAV933-0123       ...c.-.......t..........
CAV871-01217      ...c.-.....c.tt.........
CAV791-01218      ...c.c.......t..........
CAV189-01214      ...c.-.......t..........
CAV602-0124       ...c.-...............t..
CAV609-0124       ...c.-...............t..
CAV786-0124       ...c.-...............t..
8611-0125         ...c.-...............t..
12480-0125        ...c.-...............t..
23487-0125        ...c.-...............t..
22993-0128        ...c.-...............t..
22993-0128        ...c.-...............t..
24211-01220       ...c.-...............t..
24219-01220       ...c.-...............t..
RP58-01212        ...c.-...............t..
CAV1107-0129/11   .....-......c...........
M.FCC             .....-......cta..g......
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