CHAPTER 7

A MOLECULAR AND MORPHOLOGICAL COMPARISON OF *FUSARIUM* SPECIES REPRESENTING *F. SUBGLUTINANS SENSU LATO*

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ABSTRACT

Fusarium subglutinans forms part of the *Gibberella fujikuroi* complex. Research on the phylogeny of *F. subglutinans* has indicated that it is a polyphyletic taxon (= *F. subglutinans sensu lato*) representing several discreet species. Many of these are important plant pathogens and some are well-known for their capacity to produce toxic secondary metabolites. However, the fungi comprising *F. subglutinans sensu lato* are morphologically cryptic, which has hampered their identification using morphological characters. Although a number of morphological characters have recently been reported as taxonomically useful, these have not been tested on all the known species of *F. subglutinans sensu lato*. The aim of this study was to compare and evaluate the diagnostic value of the morphological characters reported previously, on a larger set of species representing *F. subglutinans sensu lato*. Furthermore, we attempted to compare and distinguish these fungi using DNA-based techniques. From the morphological comparisons, we identified five apparently useful diagnostic characters. These were the origin of conidiophores with respect to hyphae on the substrate surface, conidiophore branching, the number of conidiogenous openings on polyphialides, macroconidial septation and the presence of sterile coiled hyphae. Using these characters, it was possible to distinguish most of the species from each other. For the molecular comparison of the species representing *F. subglutinans sensu lato*, we used DNA sequence from the genes encoding calmodulin, β-tubulin, translation elongation factor 1α (EF-1α) and mitochondrial ribosomal RNA small subunit. These sequences were subjected to restriction analyses using the internet-based programme 'Webcutter'. Four diagnostic restriction enzymes were identified in the EF-1α gene sequences of these fungi. Using the recognition sites for these restriction enzymes, we generated unique EF-1α restriction maps for each of the species that can now be used for diagnostic purposes. The use of these morphological and molecular characters allows accurate identification of all the species studied.

INTRODUCTION

Fungi identified as *Fusarium subglutinans* (Wollew. & Reinking) Nelson, Toussoun & Marasas using morphology have traditionally been classified in the *Fusarium* Section *Liseola* (1, 10, 23, 40). DNA-based approaches to classify these *Fusaria* have revealed the artificial nature of this section (25, 26). As a result *F. subglutinans* is currently classified in the more natural monophyletic *Gibberella fujikuroi* (Sawada) Wollenw. species complex (25, 26). This species complex encompasses more than 30 distinct *Fusarium* lineages without teleomorphs (26, 27, 29, Chapters 4 and 6 of this thesis). In addition, this complex also includes eight biological species or mating populations with teleomorphs in the genus *Gibberella* (2, 11, 13, 14, 16, 17, 24).

Phylogenetic studies have indicated that *F. subglutinans* is a polyphyletic taxon (= *F. subglutinans sensu lato*), representing at least 13 distinct species (25, 26, 28, 29, Chapters 4 and 6 of this thesis). Three of these (mating populations B, E and H) are associated with teleomorphs (2, 15, 16, 19). Most of the species comprising *F. subglutinans sensu lato* further appear to be associated with specific plant hosts, where they may cause serious disease (16, 19, 24, 28, 32, 34-36). As a result, *formae speciales* have been proposed for some of these plant-host associations (5, 35). In addition to the debilitating effect these fungi have on plants, they also pose a threat to human and animal health. This is because some of these species are capable of producing significant levels of toxic secondary metabolites (mycotoxins) (20-22, 36).

Implementation of disease management strategies, as well as preventing the spread of these fungi to new geographic regions, is difficult. This is mainly due to the lack of reliable identification protocols for the species representing *F. subglutinans sensu lato*. These species are all characterized by morphological characters typical of *F. subglutinans* (23). The traditionally used morphological classification systems and the molecular evolution of these fungi are thus incongruent (24, 26, 28, 29, Chapters 4 and 6 of this thesis). Currently, the only morphology-based classification scheme that appears to reflect the evolutionary histories of the fungi in the *G. fujikuroi* complex, is that proposed by Nirenberg and O'Donnell (24). However, this classification enables the differentiation of some, but not all of the species comprising *F. subglutinans sensu lato*. Furthermore, this classification was introduced relatively recently and has not been tested extensively. For this reason many researchers are reluctant to use it and prefer the older, more established classification protocols such as those proposed by Nelson et al. (23). Reliable and unambiguous identification protocols for implementing quarantine measures for this group of fungi are, therefore, lacking.

The objective of this study was to resolve some of the problems associated with identification of *Fusarium* isolates that display morphological traits characteristic of *F.*

subglutinans. For this purpose we considered 12 of the 13 *Fusarium* species in this group. Representative isolates for the species reported by O'Donnell et al (28) that were isolated from *Bidens pilosa* were not available and thus not included. The 12 species examined included all eight of the formally described species and four undescribed taxa that are recognized based on sequence data (26, 28-31, Chapters 2 and 4 of this thesis). The one species that we did not include was tOur aim was to compare these taxa using morphological and molecular characters and to identify diagnostic characters to differentiate between them. For this purpose we used the morphological characters reported by Nirenberg and O'Donnell (24). For the molecular comparisons, we used previously published (26, 28) DNA sequences for the three unlinked nuclear genes calmodulin, β-tubulin and translation elongation factor 1α (EF-1α), as well as sequence from the mitochondrial ribosomal RNA small subunit (mtSSU). The gene sequence for the most variable of these genes, EF-1α, was further analyzed for the presence of diagnostic restriction enzyme recognition sites, which could potentially be used to distinguish the different *Fusarium* species representing *F. subglutinans sensu lato*.

MATERIAL AND METHODS

Fungal isolates. In this study we included representative isolates for 12 taxa recognized as residing in *F. subglutinans sensu lato* (Table 1). These included the ex holotype strains of the six species described by Nirenberg and O'Donnell (24), i.e. *F. begoniae* Nirenberg et O'Donnell, *F. circinatum* Nirenberg et O'Donnell (= *G. fujikuroi* mating population H), *F. concentricum* Nirenberg et O'Donnell, *F. guttiforme* Nirenberg et O'Donnell, *F. pseudocircinatum* Nirenberg et O'Donnell and *F. bulbicola* Nirenberg et O'Donnell. In addition one of the mating tester strains from each of the *G. fujikuroi* mating populations B [= *F. sacchari* (Butler) W. Gams] and E (= *F. subglutinans sensu stricto*) were included. We further included isolates representing four taxa recognized as belonging in *F. subglutinans sensu lato* based on sequence data (26, 28-31, Chapters 2 and 4 of this thesis), but that have yet to be described. These taxa were designated as *Fusarium* sp. 1, 2, 3 and 4. *Fusarium* sp. 1 is associated with *Zea* spp. and is believed to form part of mating population E (28, 30, Chapter 6 of this thesis). *Fusarium* sp. 2 and 3 are associated with mango malformation (27, 29, Chapter 4 of this thesis) and *Fusarium* sp. 4 is associated with ornamental grasses and reeds (27, 31, Chatpter 2 of this thesis).

Morphological comparisons. All isolates were inoculated onto carnation leaf agar (8) and incubated in the dark for at least two weeks at 20 to 24°C (24). After incubation, the fungal cultures were examined microscopically. The morphological characters reported by Nelson et al. (23) and Nirenberg and O'Donnell (24) were identified, compared among isolates and evaluated for their value in identification.

Identification of diagnostic restriction enzyme recognition sites. The DNA sequences for calmodulin, β-tubulin, EF-1α and mtSSU from each of the eight *Fusarium* species and four undescribed taxa were used. These sequences were obtained from GenBank and were subjected to restriction analyses using Webcutter (http://www.medkem.gu.se/cutter). For these analyses, only the restriction sites for commonly available enzymes were included. Diagnostic restriction enzymes were identified and used to construct restriction maps for each of the 12 taxa that make up *F. subglutinans sensu lato*.

Phylogenetic analyses. The DNA sequences for the four regions studied, mtSSU, calmodulin, β-tubulin and EF-1α in the 12 taxa were used. For comparative purposes, the corresponding sequences for the mating tester strains for *G. fujikuroi* mating populations A, C, D, F and G were also included (see appendix 6 for aligned sequences). Phylogenetic analyses using parsimony were performed with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b1 (33) using the combined information for the four genes, as described previously (28).

RESULTS

Morphological comparisons. Using the morphological characters suggested by Nelson et al. (23), all 12 of the taxa studied, were found to be very similar. Each was characterized by abundant microconidia that were produced on branched and unbranched mono- and polyphialides. These conidia were always produced in false heads and never in chains. Macroconidia were abundant and straight to slightly sickle-shaped. No chlamydospores were observed in any of the taxa examined.

Using the characters proposed by Nirenberg and O'Donnell (24), we were able to distinguish between 11 of the 12 taxa in 'blind tests'. *Fusarium* sp. 1 and *F. subglutinans sensu stricto* could not be differentiated using morphology. Five discriminating characters were identified (Table 2). These characters did not included micro- and macroconidial dimensions and spore types. These latter traits were inordinately variable to be useful taxonomic characters.

The origin of the conidiophores on the aerial mycelium was identified as a valuable diagnostic character and three groups could be distinguished. One group of taxa had conidiophores that emerged directly from the hyphae on the substrate surface. *Fusarium* sp. 4, *F. bulbicola* and *F. circinatum* all produced these 'erect' conidiophores. A second group of taxa had conidiophores that emerged from hyphae that extended horizontally above the substrate surface. *F. begoniae*, *F. concentricum*, *F. pseudocircinatum* and *F. sacchari* produced these 'prostrate' conidiophores. The third group of species was characterized by the presence of both erect and prostrate conidiophores, and included *F. guttiforme* and *F. subglutinans sensu stricto*, as well as *Fusarium* spp. 1, 2 and 3.

The branching pattern of the conidiophores was another character that was useful in differentiating between the taxa. Using this character, three groups of taxa could be identified. The conidiophores of *Fusarium* sp. 4, *F. begoniae* and *F. concentricum* were rarely branched, whereas those of *F. bulbicola* and *F. guttiforme* were strongly branched (Fig. 1). The third group of species was characterized by the production of branched conidiophores with intercalary phialides (referred to as 'proliferating') (Fig. 1). *Fusarium* spp. 1, 2 and 3, as well as *F. circinatum*, *F. pseudocircinatum*, *F. sacchari* and *F. subglutinans sensu stricto* displayed these branched proliferating conidiophores.

The number of conidiogenous openings on the polyphialides was another useful diagnostic character (Fig. 1). Two groups of taxa could be distinguished. The polyphialides of *Fusarium* sp. 1 and 4, as well as *F. begoniae*, *F. bulbicola* and *F. subglutinans sensu stricto* usually had less than three openings. A second group of taxa including *F. circinatum*, *F. concentricum*, *F. guttiforme*, *F. pseudocircinatum*, *F. sacchari* and *Fusarium* spp. 2 and 3 were all characterized by polyphialides with more than three conidiogenous openings.

Macroconidial septation (Fig. 1) could be used to separate the 12 taxa into two groups. One group of taxa had macroconidia with three septa and included *F. begoniae*, *F. circinatum*, *F. guttiforme*, *F. pseudocircinatum*, *F. subglutinans sensu stricto* and *Fusarium* sp. 1. The second group of taxa included *F. bulbicola* and *F. concentricum*, as well as *Fusarium* spp. 2, 3 and 4, which all produced macroconidia with three to five septa. The ability to produce sterile coiled hyphae was also an important diagnostic character. Of the 12 taxa examined, only three were able to produce these coils. They were *Fusarium* sp. 2, *F. pseudocircinatum* and *F. circinatum* (Fig. 1).

Identification of diagnostic restriction enzyme recognition sites. Webcutter generated restriction maps for each of the 12 taxa studied. Comparisons of these maps revealed that the sequence for EF-1α contained sufficient polymorphisms to allow for the identification of diagnostic restriction enzymes. There were too few polymorphic restriction sites in the calmodulin, mtSSU and β-tubulin sequences to allow for differentiation between all the fungi included. The diagnostic restriction enzyme recognition sites identified in the EF-1α DNA sequence are those for the enzymes *Bgl*1, *Rsa*1, *Sau*3A1 and *Mse*1. Unique EF-1α restriction maps for each of the eight species and four undescribed taxa representing *F. subglutinans sensu lato* were generated with Webcutter using these recognition sites (Fig. 2).

Based on the presence of *Sau*3A1 restriction sites in the EF-1α gene for the taxa studied, three groups could be identified (Fig. 2). The first group of taxa was characterized by *Sau*3A1 recognition sites at ~150 base pairs (bp) and ~220 bp, and included *F. begoniae, F. bulbicola*, *F. subglutinans sensu stricto*, *F. guttiforme*, *F. pseudocircinatum*, *F. sacchari*, and *Fusarium* sp. 1. The second group included *F. circinatum* and *F. concentricum*, as well as *Fusarium* spp. 3 and 4. They all had *Sau*3A1 recognition sites at ~90 bp, ~150 bp and ~220 bp. *Fusarium* sp. 2 represented the third group. The EF-1α gene of this taxon was characterized by *Sau*3A1 recognition sites at 92 bp and 150 bp.

Among the 12 taxa three groups could be identified using the *Mse*1 recognition sites (Fig. 2). The first group included those taxa that did not harbor any recognition sites for this enzyme, and included *F. subglutinans sensu stricto*, *F. pseudocircinatum*, *F. sacchari* and *F. concentricum*, as well as *Fusarium* spp. 2 and 3. The second group of taxa included *F. begoniae*, *F. bulbicola*, *F. guttiforme, F. circinatum* and *Fusarium* sp. 4. Their EF-1α sequences were all characterized by the presence of a single *Mse*1 recognition sites at ~400 bp. *Fusarium* sp. 1 represented the third group and was characterized by two (402 bp and 599 bp) *Mse*1 recognition sites.

Based on the presence of *Rsa*1 recognition sites in the EF-1α gene, four groups of taxa could be identified. The first group included *F. guttiforme* and *F. pseudocircinatum*, which both had *Rsa*1 recognition sites at 47 bp and ~460 bp. The second group included *F. bulbicola*, *F.*

subglutinans sensu stricto, *F. concentricum* and *F. circinatum*, as well as *Fusarium* spp. 1, 2, 3 and 4. Their EF-1α sequences all harbored *Rsa*1 recognition sites at 47 bp, ~460 bp and ~610 bp. The two remaining groups were both comprised of single species. One was represented by *F. begoniae* with a *Rsa*1 recognition site at 47 bp, while the other was represented by *Fusarium* sp. 1 with *Rsa*1 recognition sites at 47 bp, 240 bp, 470 bp and 611 bp.

Restriction analysis with Webcutter using the recognition sites for the enzyme *Bgl*1 separated the 12 taxa into two groups. The one group comprised of taxa that had no *Bgl*1 restriction sites in their EF-1α sequences. They were *F. begoniae*, *F. bulbicola*, *F. subglutinans sensu stricto*, *F. guttiforme*, *F. pseudocircinatum*, *F. sacchari*, *F. circinatum*, as well as *Fusarium* spp. 1 and 3. The EF-1α sequences for *Fusarium* spp. 2 and 4, as well as *F. concentricum* were all characterized by the presence of a single *Bgl*1 recognition site at 92 bp.

Phylogenetic analyses. From phylogenetic analyses using parsimony of the combined sequence data sets, five most parsimonious trees with similar topologies were generated (Fig. 3). In all the analyses, three major clades, resembling the so-called 'American', 'Asian' and 'African' clades of O'Donnell et al. (26) were present. Among the 12 taxa constituting *F. subglutinans sensu lato*, three taxa, *Fusarium* sp. 3, *F. concentricum* and *F. sacchari*, clustered together with representatives of the C- and D-mating populations in the 'Asian' clade. Only one species, *F. pseudocircinatum*, clustered with representatives of mating populations A, F and G in the 'African' clade. The remaining eight taxa clustered in the so-called 'American' clade.

DISCUSSION

The primary goal of this study was to compare the different *Fusarium* species displaying morphological characters typical of *F. subglutinans*. From the morphological comparisons we identified five distinguishing diagnostic traits (Table 2). From comparisons of the EF-1α gene sequences, we further identified four restriction enzymes, from which unique restriction maps for each of the 12 taxa representing *F. subglutinans sensu lato* were constructed (Fig. 2). The use of these morphological and molecular characters allows for differentiation between the 12 best known species comprising *F. subglutinans sensu lato*.

Many previous studies have focussed on the different species representing *F. subglutinans sensu lato* (4, 7, 12, 16, 17, 24-26, 28, 31, 37-39, Chapter 2 of this thesis). Some considered only the phylogeny of these species (12, 25, 26, 28, 31, 37-39, Chapter 2 of this thesis), while others (4, 7, 12, 31, 37, 38, Chapter 2 of this thesis) also attempted species differentiation. However, the methods employed in most of these studies are unsuitable for diagnostic purposes, since they involve methods with low repeatability [e.g. random amplified polymorphic DNAs (RAPDs) (7, 37, 38)] or methods that are technically complicated [e.g. isolation of mitochondrial DNAs for restriction fragment length polymorphisms (RFLP)(4)]. Furthermore, some of these techniques, for example isozyme analyses (12), cannot be used to resolve all the known species comprising *F. subglutinans sensu lato*.

Two previous studies have provided simple diagnostic approaches for differentiating most of the species comprising *F. subglutinans sensu lato* (24, 31, Chapter 2 of this thesis). One of these employed morphological characters (24), whereas the other was DNA-based (31, Chapter 2 of this thesis). However, neither of these studies included all the known species of *F. subglutinans sensu lato*. The diagnostic key of Nirenberg and O'Donnell (24) that is based on morphology, included only the eight formally described species. The histone *H3* PCR-RFLP technique was tested on only six species (31, Chapter 2 of this thesis). The current study is, therefore, the first to attempt to differentiate between most of the species representing *F. subglutinans sensu lato* at both the morphological and molecular levels.

The morphological characters used to distinguish the different *Fusarium* species in this study, were primarily those identified by Nirenberg and O'Donnell (24). The major difference between these characters and those used in the older classifications, is that the traits used to distinguish the different species of *F. subglutinans sensu lato*, allow for a greater level of discrimination. For example, Nelson et al. (23) regard both branched and unbranched conidiophores as a typical feature of *F. subglutinans*. However, distinguishing between branched and unbranched conidiophores allows for differentiation between certain species (Table 2).

Using the diagnostic molecular characters reported here, it was possible to distinguish between all the taxa of *F. subglutinans sensu lato*, included in this study (Fig. 2). This is a major advantage, since not all these species are distinguishable using morphology (Table 2). The two taxa, *Fusarium* sp. 1 and *F. subglutinans sensu stricto*, were indistinguishable using morphology. Another advantage of using these molecular characters rather than the morphological characters, is that using the molecular characters accelerates the identification process. For example, an isolate displaying morphological characters typical of *F. subglutinans* would immediately be diagnosed as *F. begoniae* if its EF-1α gene harbored a single *Rsa*1 restriction site. The morphological characters for identifying this species would only emerge after at least two weeks of incubation in culture.

Two additional characteristics that can be used to distinguish between some the species making up *F. subglutinans sensu lato*, are host range and sexual compatibility. Four of the 12 taxa studied will produce teleomorphs in culture (Table 2). This makes it possible to use mating studies to differentiate species. These species include *F. circinatum* (mating population H) (2), *F. sacchari* (mating population B) (11), *F. subglutinans sensu stricto* (3, 17, 18) and *Fusarium* sp. 1 (31, Chapter 5 of this thesis). However, some of these species are able interact sexually across the species barrier (6, 31, Chapter 2, 5 and 6 of this thesis) and this can cause confusion.

Nine of the 12 taxa included in this study appear to be associated with a specific plant host (Table 2) (5, 9, 16-18, 24, 28, 29, 31, 35, 36, 41, Chapters 2, 4, 5 and 6 of this thesis). However, more than one species may be associated with a single host (5, 16, 24, Chapters 4, 5 and 6 of this thesis). For example, two of the 12 taxa included are associated with pine. One is the pitch canker fungus, *F. circinatum*, while the other is *F. pseudocircinatum* (5, 24). In order to differentiate between the species comprising *F. subglutinans sensu lato*, host range and sexual compatibility should, therefore, both be used with caution.

The molecular phylogenetic work conducted by O'Donnell et al. (26) has indicated that species in the *G. fujikuroi* complex can be separated into three distinct groups. Based on the phylogenetic clustering patterns of these species and the geographic origins of their hosts, O'Donnell et al. (26) proposed a phylogeography hypothesis. According to this hypothesis the evolutionary histories of *Fusarium* spp. in this complex are consistent with species radiations in Africa, South America and Asia, following the fragmentation of the ancient super-continent Gondwana (26). The phylogenetic placement of eight of 12 taxa studied, in any one of the so-called 'African', 'American' or 'Asian' clades (Fig. 3), fits this model (24, 26, 28, 29, Chapter 4 of this thesis). The phylogenetic placement of the remaining four taxa (*F. bulbicola*, *F. pseudocircinatum*, *Fusarium* sp. 3 and *Fusarium* sp. 4) is, however, incongruent with this hypothesis. It has subsequently been suggested that human introduction of plant hosts into new geographic areas, has resulted in the presence of, for example, an 'American' fungus on the 'African' continent (24, 26, 28,

29, Chapter 4 of this thesis). From our study and those of others (24, 28, 29, Chapter 4 of this thesis) it is thus clear that the phylogeography hypothesis (26) needs further verification and refinement.

Application of the diagnostic characters reported in this study, enables differentiation between most of the known *Fusarium* species comprising *F. subglutinans sensu lato* (Table 2 and Fig. 2). From this point of view, the current study is the first to propose both morphological and molecular approaches to identify these fungi. This study will also have a significant impact on agricultural practices since many of the fungi displaying morphological characters typical of *F. subglutinans,* are economically important plant pathogens (5, 9, 35, 36). It will now be possible to correctly identify the fungal pathogen and implement appropriate management strategies.

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Table 1. Hosts, geographic origins, and source of the 12 taxa representing *Fusarium subglutinans sensu lato* included in this study.

Species[1]	Host	Geographic origin	Source	Isolate[2]	Reference
F. circinatum (ex T)	*Pinus radiata*	United States	BBA	MRC 7541; BBA 69720; NRRL 25331	(24)
F. concentricum (ex T)	*Musa sapientum*	Costa Rica	BBA	MRC7540; BBA 64354; NRRL 25181	(24)
F. begoniae (ex T)	*Begonia* hybrid	Germany	BBA	MRC 7542; BBA 67781; NRRL 25315	(24)
F. bulbicola (ex T)	*Nerine bowdenii*	Germany	BBA	MRC 7534; BBA 63628; NRRL 13618	(24)
F. guttiforme (ex T)	Ananas comosus	Brazil	BBA	MRC 7539; BBA 69661; NRRL 25295	(24)
F. pseudocircinatum (ex T)	*Solanum* sp.	Ghana	BBA	MRC 7536; BBA 69636	(24)
F. sacchari	Laboratory cross		J.F. Leslie	MRC 6525; KSU 3853; M 6866	(2, 12, 41)
F. subglutinans sensu stricto	*Zea mays*	United States	J.F. Leslie	MRC 6512; KSU 2129; M 3693; NRRL 22016	(2, 12, 41)
Fusarium sp.1	*Zea mays*	South Africa	W.F.O. Marasas	MRC 1077; NRRL 25622	(28, 31, Chapter 2 of this thesis)
Fusarium sp.2	*Mangifera indica*	South Africa	W.F.O. Marasas	MRC 2802; NRRL 25623	(28, 29, Chapter 4 of this thesis)
Fusarium sp.3	*Mangifera indica*	South Africa	W.F.O. Marasas	MRC 2730	(26, 29, Chapter 4 of this thesis)
Fusarium sp.4	Ornamental grass	South Africa	W.F.O. Marasas	MRC 6747; NRRL 26756	(28, 30)

[1] Ex T = ex holotype strain.
[2] Culture collections: MRC = W. F. O. Marasas, Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, Tygerberg, South Africa; KSU = J. F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, Kansas; BBA = Biologishe Bundesanstalt für Land- und Forstwirtschaft, Berlin, Germany; NRRL = Northern Regional Research Laboratory, NCAUR, Preoria, Illinois.

Table 2. Summary of the diagnostic characters used to differentiate the 12 taxa representing *Fusarium subglutinans sensu lato* included in this study.

Species	Diagnostic characters [1]											
	Host specific [2]	Sexual stage	Conidiophore origin		Conidiophore branching			Conidiogenous openings		Macroconidia		Sterile coils
			Prostrate	Erect	Rarely branched	Branched	Proliferate	≥ 3	≤ 3	3 septa	3-5 septa	
F. begoniae	*Begonia* spp.		+		+				+	+		
F. bulbicola	Bulbous plants			+		+			+		+	
F. circinatum	*Pinus* spp.	+		+		+	+	+		+		+
F. concentricum	-		+		+			+			+	
F. guttiforme	*A. comosus*		+	+		+		+		+		
F. pseudocircinatum	-		+			+	+	+		+		+
F. sacchari	-	+	+			+	+	+		+		
F. subglutinans sensu stricto	*Zea* spp.	+	+	+		+	+		+	+		
Fusarium sp.1	*Zea* spp.	+	+	+		+	+		+	+		
Fusarium sp.2	*M. indica*		+	+		+	+	+			+	+
Fusarium sp.3	*M. indica*		+	+		+	+	+			+	
Fusarium sp.4	Grass and reed			+	+				+		+	

[1] The presence of a character is indicated by a plus sign (+).
[2] Each specific host is indicated and a minus sign (-) indicate that the fungus is associated with more than one host.

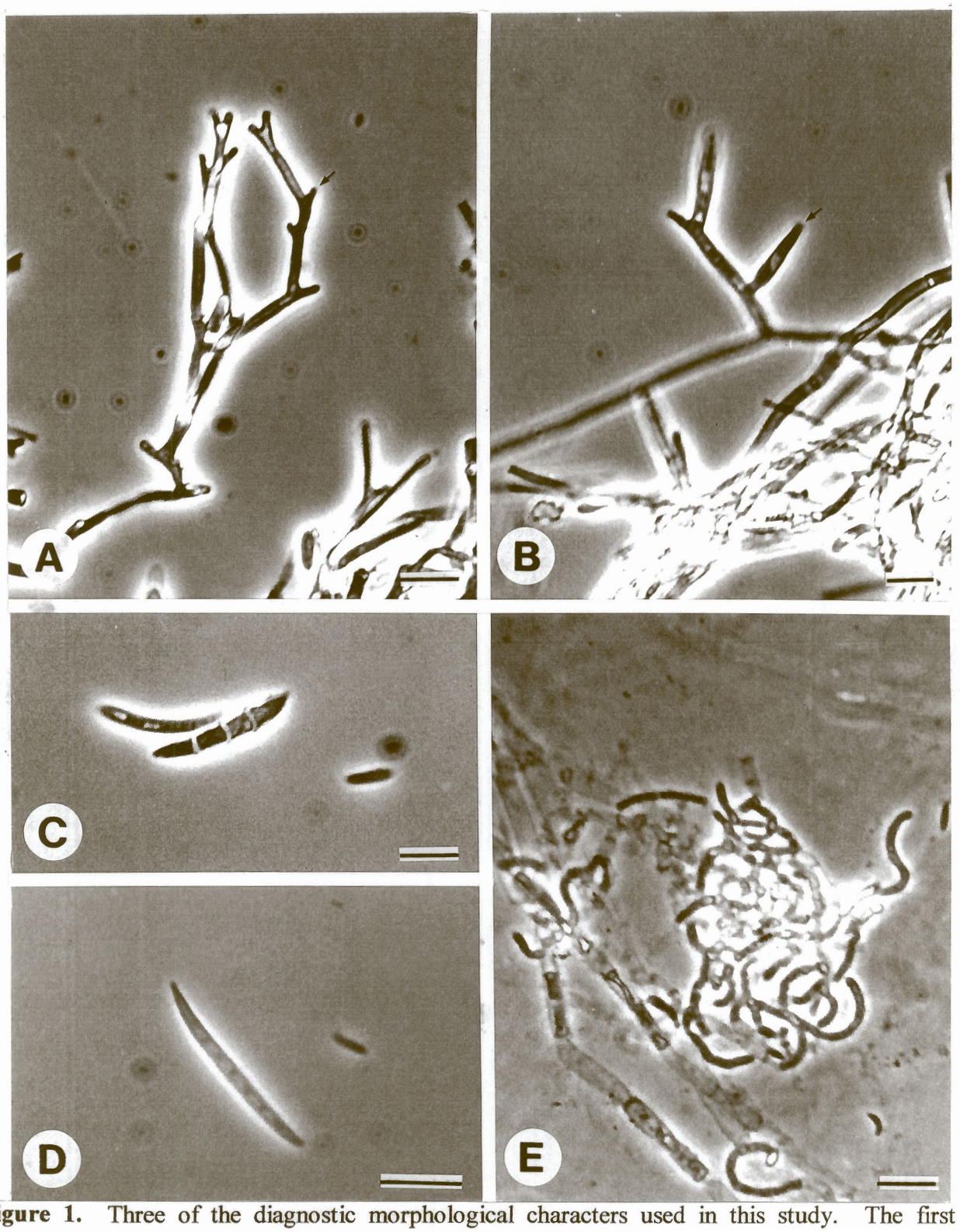

Figure 1. Three of the diagnostic morphological characters used in this study. The first is conidiophore branching patterns where **A** represents a branched conidiophore bearing polyphialides (arrow indicates an intercalary phialide) and **B** represents an unbranched conidiophore bearing two monophialides (arrow indicates the single conidiogenous opening of one of the monophialides). The second character is the number of macroconidial septa, where two groups are identified: 3-septate (**C**) and 5-septate (**D**) macroconidia. The third morphological character is sterile coiled hyphae (**E**). Scale bars = 10µm.

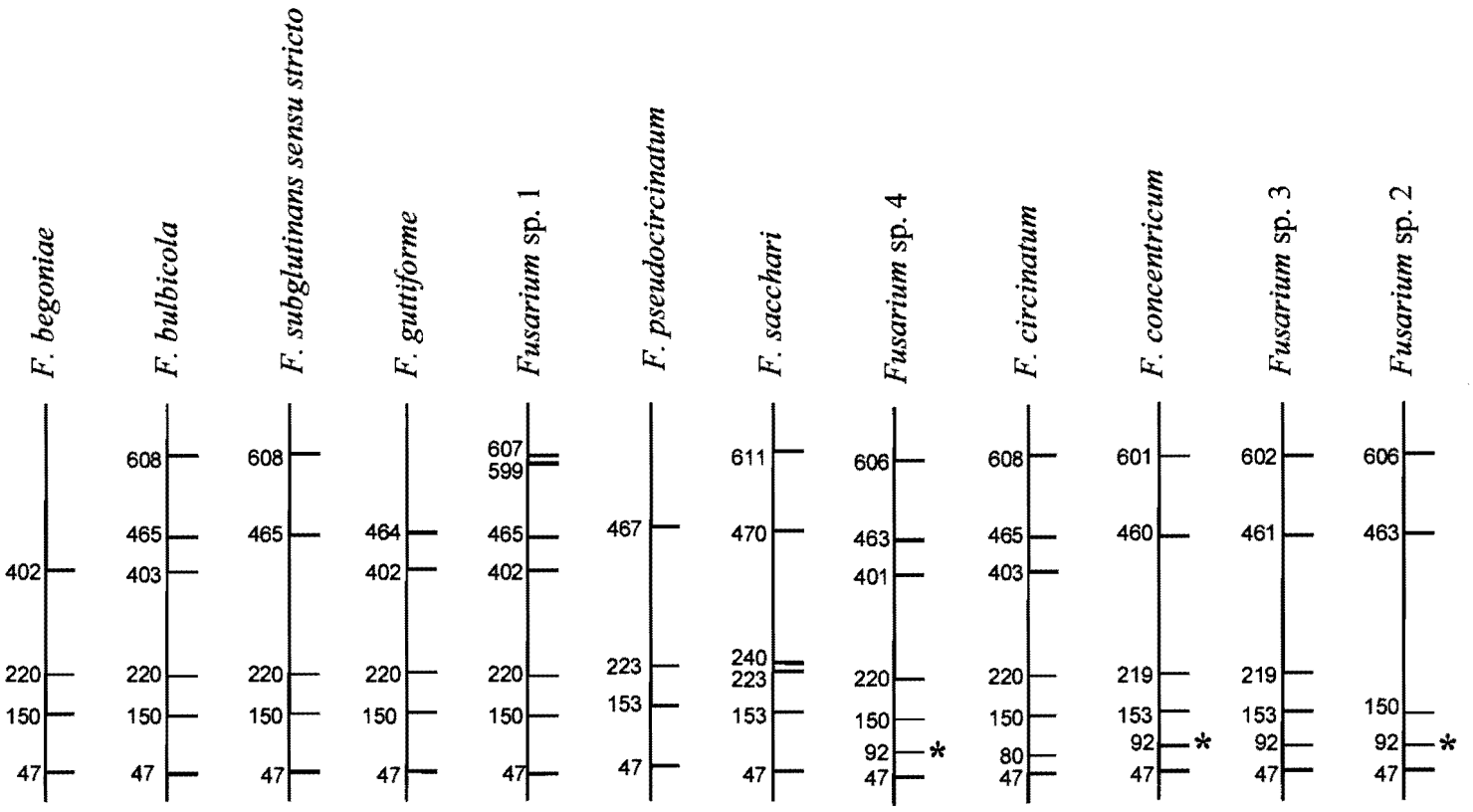

Figure 2. EF-1α restriction maps for the 12 taxa representing *F. subglutinans sensu lato*, generated with Webcutter using the recognition sites for the enzymes *Mse*1, *Bgl*1, *Rsa*1 and *Sau*3A1. In each case ~640 base pairs of the EF-1α gene are represented by a vertical line. The restriction sites are indicated as colored horizontal lines, with the restriction sites for *Mse*1 (red), *Rsa*1 (blue) and *Sau*3A1 (green) indicated in base pairs. The restriction site for the enzyme *Bgl*1 corresponds to the *Sau*3A1 site at position 92 in three of the taxa and are indicated with an asterisk (*).

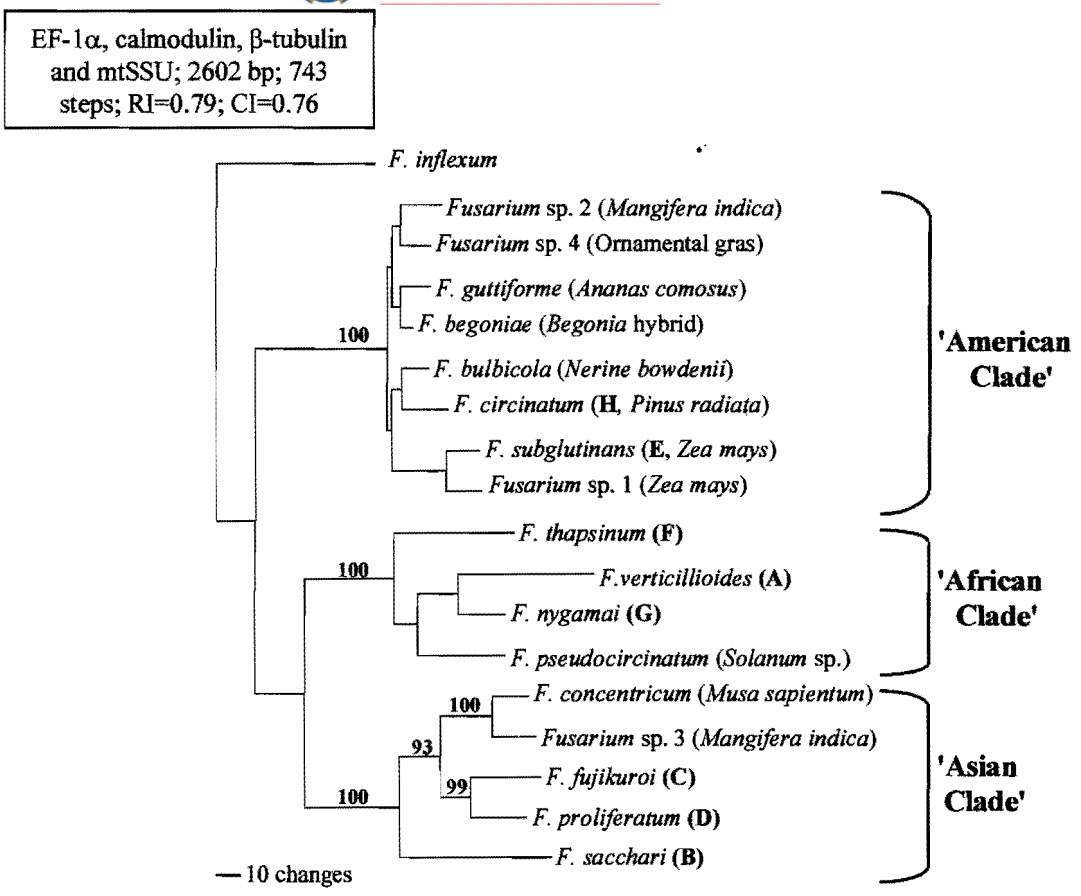

Figure 3. One of five most parsimonious trees generated from the combined sequence data sets for the four loci, mtSSU, calmodulin, EF-1α, and β-tubulin. The host for each of the 12 species representing *F. subglutinans sensu lato* are indicated in parnetheses. The *G. fujikuroi* mating populations A to H are indicated in bold letters. This tree is rooted to *F. inflexum* and bootstrap values are based on 1,000 replications. See O'Donnell et al. (28) for GenBank Accession numbers.