CHAPTER 6

CRYPTIC SPECIATION IN *GIBBERELLA FUJIKUROI* MATING POPULATION E

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ABSTRACT

Fusarium spp. that form part of the *Gibberella fujikuroi* complex have been classified using either a biological, morphological or phylogenetic species concept. Most problems with the taxonomy of *Fusarium* spp. in this complex are, however, experienced when the morphological species concept alone is applied. To solve this problem, the morphological species concept has often been applied in combination with the biological species concept. However, the most accurate identifications are obtained when the phylogenetic species concept has been used. Results from recent studies have suggested discordance between the biological and phylogenetic species concepts. A group of *F. subglutinans* isolates, apparently belonging to *G. fujikuroi* mating population E, could be sub-divided into more than one phylogenetic lineage. The aim of this study was to determine whether it represented a speciation event. For this purpose, we included 29 *F. subglutinans* isolates belonging to the E-mating population, that were collected from a wide geographic range. DNA sequence data for six nuclear regions in each of these isolates were obtained and used in phylogenetic analyses. These analyses showed that the E-mating population of the *G. fujikuroi* complex is divided into two reproductively isolated groups, designated Groups 1 and 2. The lack of shared polymorphisms between Groups 1 and 2 further suggested that they represent separate species. The taxonomy of all fungi previously identified as belonging to the E-mating population using the biological species concept should, therefore, be re-evaluated. We also suggest that the biological species concept should be used with caution when dealing with these and similar fungi. Of the three available species concepts, only the phylogenetic species concept allows for unequivocal identification and classification of species in the *G. fujikuroi* complex.

INTRODUCTION

Gibberella fujikuroi (Sawada) Wollenw. is a species complex that encompasses many separate *Fusarium* species (28-30, 34, Chapter 4 of this thesis). In the global setting, species in this complex are important, because of their association with diseases on agronomically important plants (6, 17, 20, 36, 40, 41). These fungi also affect human and animal health, since many species in this complex produce extremely toxic secondary metabolites such as moniliformin, beauvericin, fumonisin and fusaric acid (21-24, 42).

The taxonomy of *Fusarium* species in the *G. fujikuroi* complex has been subject to much controversy (17). This is mainly due to a lack of consensus among researchers on how to define a morphological species concept for *Fusaria* in this complex (11, 26, 27, 33). In an attempt to solve this problem, a biological species concept was introduced, whereby eight biological species, designated as mating populations A to H, have been identified (3, 13, 14, 16-19). The eight biological species, however, exclude more than 80% of the species in this complex, since many have no apparent sexual reproductive cycle or they represent sterile populations. Currently, the only method for classifying all the fungal strains in the *G. fujikuroi* complex is through the application of a so-called phylogenetic species concept (29). With this method, fungi in the *G. fujikuroi* complex are classified into at least 37 different phylogenetically distinct lineages, each constituting a separate species (29, 34, Chapter 4 of this thesis).

In a recent study, ten new and phylogenetically distinct species in the *G. fujikuroi* complex were reported (32). Among these was a *F. subglutinans* (Wollew. & Reinking) Nelson, Toussoun & Marasas strain associated with maize in South Africa. Using gene sequences, this strain was found to be very closely related, but not identical to *G. fujikuroi* mating population E. However, based on the biological species concept, the lineage reported on maize was classified as belonging to the existing E-mating population (35, Chapter 2 of this thesis). This strain was able to produce fertile progeny in a cross with another strain from mating population E (35, Chapter 2 of this thesis). This apparent inconsistency between DNA sequence data and the biological species concept was also highlighted in a recent phylogenetic study of *F. subglutinans* strains associated with the wild relatives of maize (Chapter 5 of this thesis). Strains that were apparently capable of interbreeding formed more than one phylogenetic lineage (Chapter 5 of this thesis).

All of the studies concerning the classification of *Fusarium* strains associated with maize and *G. fujikuroi* mating population E have been based on a small number of isolates (32, 35, Chapters 2 and 5 of this study). No significant conclusions could therefore, be made regarding the possible diversity within the E-mating population or how the phylogenetic species concept might influence our interpretation of the biological species concept. The aim of this study was, therefore,

to address these issues by (i) including several *Fusarium* strains from mating population E that are associated with maize and its wild relatives from a wide geographic range; (ii) to obtain DNA sequence data from six nuclear regions for these strains; and (iii) to use phylogenetic tools (10, 15, 38, 39) to determine whether these isolates are interbreeding in nature and, if not, to define sub-populations within *G. fujikuroi* mating population E.

MATERIAL AND METHODS

Fungal isolates. Twenty-nine *F. subglutinans* isolates associated with maize and teosintes in South Africa, the United States, Mexico and Guatemala were included in this study (Table 1). For outgroup purposes we also included two isolates from *G. fujikuroi* mating populations H.

DNA isolation, PCR amplification and sequencing. DNA was isolated using a CTAB (*N*-cetyl-*N,N,N*-trimethyl-ammonium bromide) extraction method (35, Chapter 2 of this thesis).

A portion of three nuclear genes, histone *H3*, calmodulin and β-tubulin, were amplified from all the isolates included in this study. The primers used for amplifying a region of the calmodulin gene were CAL-228F (5'-gagttcaaggaggccttctccc-3') and CAL-737R (5'-catctttctggccatcatgg-3') (5) and the primers Bt1-a (5'-ttcccccgtctccacttcttcatg-3') and Bt1-b (5'-gacgagatcgttcatgttgaactc-3') (12) were used for amplifying a portion of the β-tubulin gene. A section of the histone *H3* gene was amplified using the primers H3-1a (5'-actaagcagaccgcccgcagg-3') and H3-1b (5'-gcgggcgagctggatgtcctt-3') (12). We also used an additional set of primers that amplify three unlinked nuclear regions of unknown function (H. Britz, unpublished data). The first primer set is HB9-a (5'-tcaatacccctcgcctagaa-3') and HB9-b (5'-gaccacagcctcgagaacat-3'), the second is HB14-a (5'-ttccaccatgagaggaaaccc-3') and HB14-b (5'-ccattgccaatcttgatcct-3'), and the third HB26-a (5'-gacttgagtatctgcactgc-3') and HB26-b (5'-gaatgtactactcgacgtcg-3').

For amplification of all these loci, the PCR mixture contained 1 mM deoxynucleotide triphosphates (0.25 mM each), 2.5 mM $MgCl_2$, 0.2 μM of each primer, 0.25 ng/μl DNA, 0.05 U/μl of Super-Therm DNA polymerase [Southern Cross biotechnology (Pty.) Ltd., Cape Town, South Africa] and 1 x Super-Therm reaction buffer. PCR-cycling conditions were as follows: denaturation at 92°C for 20 s, annealing for 20 s at 55°C (calmodulin, tubulin and histone) or 47°C (HB9, HB14 and HB26), and elongation for 20 s at 72°C. This was repeated 30 times and was preceded by an initial denaturation at 92°C for 1 min and followed by a final elongation step at 72°C for 5 min.

After PCR, the products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and sequenced in both directions with the respective primers. Reactions were performed on an ABI PRISM™ 377 automated DNA sequencer, with an ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Warrington, United Kingdom). Sequences were analyzed with Sequence Navigator version 1.0.1.™ (Perkin Elmer Applied BioSystems, Inc., Foster City, CA).

Phylogenetic analyses. The data sets obtained for each primer set were aligned manually by inserting gaps (See Appendix 5 for aligned sequences). Phylogenetic analyses were performed with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b1 (37) where gaps were treated

as fifth characters in heuristic parsimony searches, with tree-bisection-reconnection (TBR) branch swapping and MULTREES (saving of all optimal trees) effective. For bootstrap analyses 1,000 replications were performed.

RESULTS

PCR amplification and sequencing. With the primers used, we were able to amplify and sequence 480 base pairs (bp), 456 bp and 332 bp of the calmodulin, β-tubulin and histone *H3* genes, respectively. For the three regions of unknown function, 250 bp, 235 bp and 236 bp were sequenced for HB9, HB14 and HB26, respectively. Out of the sequenced 1989 nucleotides (nc), 17 nc (0.9%) were polymorphic and none of these had more than two possible alleles. Among the different *F. subglutinans* strains associated with maize and teosinte, between one and six polymorphic nucleotides in each of the six regions were identified (Table 2). Upon combination of the polymorphisms for each individual at all these loci, we recognized seven different genotypes within the set of 29 *F. subglutinans* isolates studied (Table 2). The most frequently sampled genotype was 2-1, which was represented by eight strains associated with maize in South Africa and the United States and teosinte in Mexico and Guatemala. Seven strains displayed genotype 2-3, and were associated with maize in the United States and South Africa, as well as teosinte in Mexico. Five isolates displayed genotype 1-1 and were collected from maize in South Africa and Mexico, as well as Mexican teosinte. Genotype 1-4 was also represented by five strains, all of which were isolated from maize in South Africa and Mexico. Genotype 2-2 was represented by two strains associated with maize in the United States and both genotypes 1-2 and 1-3 were represented by single strains that were isolated from teosinte in Mexico.

Phylogenetic analyses. The number of parsimony informative characters in the six data sets ranged from 1 for HB27 to six for histone *H3* (Table 3). In all the data sets, only the β-tubulin sequence harbored a parsimony uninformative character (Table 3, Fig. 1). This variable character was present only in isolate Fst 26. Phylogenetic analyses based on the uninformative, as well as the informative characters generated unique gene genealogies for each of the individual data sets (Fig. 1). In each case, a single reconstruction was generated and the consistency (CI) and retention (RI) indices for each were 1.00 and 1.00, respectively. No homoplastic characters were present in any of the six individual data sets. As a result, all of the single-gene genealogies were of minimal length, i.e. equal to the number of parsimony informative sites. A single most parsimonious tree was also obtained from the combined data sets (Fig. 2). The length of this tree was equal to the number of parsimony informative characters (Table 3), since homoplastic characters were also absent in the combined data sets (CI = 1.00, RI = 1.00). The length of this tree was equal to the summed lengths of the individual gene genealogies (Table 3), which is a distinctive feature of absolute congruence among individual gene genealogies (9, 10, 15, 25, 38).

Clustering within the different genealogies was very similar, but not always identical. For the loci HB9, HB14 and HB27 the isolates were all clustered into two groups that always included

the same isolates (Fig. 1). Both the β-tubulin and calmodulin genealogies consisted of three clusters of isolates, but although there was some overlap, these two groups were different for each gene. The genealogy generated from the histone *H3* data generated four groups of isolates, some of which showed some resemblance to those generated for the other loci.

Phylogenetic analysis of the combined data sets from all the isolates included in this study revealed the presence of two distinct groups among the isolates associated with maize and teosinte. They were designated as Group 1 and 2 (Fig. 3). The genotypes 1-1, 1-2, 1-3 and 1-4 were present in Group 1 and the genotypes 2-1, 2-2 and 2-3 were present in Group 2 (Fig. 2 and 3). Although this clustering was not immediately detectable from the individual gene genealogies, the combined gene genealogy was not discordant with any of them. Strains from Group 1 never clustered with strains from Group 2 and vice versa.

DISCUSSION

In this study, we set out to use phylogenetic tools to answer what appeared to be a population level question. Although perhaps unusual, this approach is not without precedence and a number of researchers have reported on the value of using such methods (4, 10, 15, 31, 38, 39). Based on previous work (35, Chapters 2 and 5 of this thesis), our null hypothesis was that the set of *F. subglutinans* isolates associated with maize and teosinte, from a wide geographic range, would form part of the existing E-mating population of the *G. fujikuroi* complex. However, based on the data generated in this study, we had to reject this hypothesis. The results clearly show that mating population E is separated into two reproductively isolated populations that most probably constitute sibling, but separate species.

The use of phylogenetic analyses of DNA sequences from multiple loci to identify genetically isolated and recombining populations has been reported by several authors (9, 10, 15, 25, 38, 39). The basic rational behind these studies involves the detection of congruence or the lack there of among different gene trees. Incongruence among gene trees from different loci indicates interbreeding among individuals, since sexual recombination 'reshuffles' their genomes. The evolutionary histories of the genes in an individual from an interbreeding population are therefore, unique. However, the genes of individuals from this interbreeding population will have many shared characters or polymorphisms, because of gene flow via sexual reproduction. Individuals from a genetically isolated population will thus lack these shared polymorphisms. This appears to be true for the E-mating population isolates that are separated into Groups 1 and 2 (Fig. 3). The fact that they lack shared polymorphisms (Table 2) is reflected in perfectly concordant gene trees (Figs. 2 and 3). These results thus suggest that Groups 1 and 2 do not interbreed in nature.

Apart from indicating the separation of mating population E into two reproductively isolated populations, our results also suggest that these groups constitute separate species. Evidence for this can be found in the fact that 13 and 16 of the 18 polymorphic sites, identified in this study, are fixed in Groups 1 and 2, respectively (Table 2). Groups 1 and 2 also do not have any shared polymorphisms, which is the normal outcome of speciation events (1, 2, 39). Early in the speciation process, two populations would become genetically isolated, but still share several polymorphisms. Later in this process, shared polymorphisms would be lost and ultimately, the genetically isolated groups would display fixed polymorphisms. At this stage, they no longer represent reproductively isolated populations, but separate species. Our results, therefore, clearly show that Groups 1 and 2 of *G. fujikuroi* mating population E represent distinct species.

Many strains belonging to the E-mating population are sexually incompatible (7, 35, Chapters 2 and 5 of this thesis). A factor that could definitely influence sexual interactions among

these fungi, is the fact that they represent more than one species. This is especially true for the *F. subglutinans* strains that were collected from maize and teosinte in Mexico and Central America (7). For testing the biological species concept in these *F. subglutinans* strains, they were crossed to *F. subglutinans* strains from the E-mating population in the United States. There were, however, no fertile interactions. The results from the current study show that this might have been expected, since the available E-mating population isolates from the United States belongs to Group 2. In contrast, those from Mexico and Central America belongs to both Groups 1 and 2. However, the fact that most of the isolates collected from Mexico and Central America are capable of fertile sexual interaction, even though they represent separate species, cannot be easily explained. Steenkamp et al. (35, Chapter 2 of this thesis) also reported on two South African *F. subglutinans* isolates from Group 1 (MRC 1077 and MRC 837) that were able to interact sexually with a Group 2 isolate (MRC 6483 from the United States).

There are no apparent links between geographic origin or host and the group (Group 1 or 2) to which these *F. subglutinans* isolates from maize and teosinte belong. All the isolates from the United States, however, can be accommodated in Group 1. Two isolates from maize in South Africa and five from teosinte in Mexico and Guatemala also belonged to Group 1. Group 2 isolates were characterized by four South African isolates associated with maize and eight isolates associated with maize and teosinte in Mexico. Both groups can thus be found on teosinte and maize. It is, however, unclear why maize in the United States is apparently only associated with one of these groups.

It is possible that the *F. subglutinans* isolates belonging to mating population E could have evolved from a population resembling isolates found on maize and teosinte in Mexico and Central America. This would be consistent with the fact that both Groups 1 and 2 are present in the population of fungi collected in Central America and Mexico. This also supports the view that the ancestors of domestic maize (*Z. mays* ssp. *mays*) were teosinte-like plants, which most probably evolved in this geographic region (8). It is, therefore, also possible that *F. subglutinans* isolates found on teosintes and maize in this area, resemble the ancestral Groups 1 and 2 of *G. fujikuroi* mating population E.

Group 1 isolates of the E-mating population appear to be more diverse than those representing Group 2. However, the number of isolates representing these groups and the number of polymorphic nucleotides used in this study, are clearly insufficient to draw robust conclusions regarding their population structures. Nevertheless, among the isolates studied, Group 1 is represented by four genotypes, whereas three represented those in Group 2. Group 1 also displayed five non-fixed polymorphisms and Group 2 had only two such polymorphisms. Isolates from Group 1 are also unique in that certain individuals can interact across the species barrier with

isolates in *G. fujikuroi* mating population H (7). All these unique qualities displayed by isolates in Group 1 may suggest that fungi that are much like the modern Group 1 isolates, may have been the "founders" of the E-mating population. It would be interesting to test this hypothesis using a significantly larger set of isolates representing Groups 1 and 2 from as many geographical regions as possible. Such a collection is currently not available to us, but will be assembled in coming years.

The inconsistency between the biological and phylogenetic species concepts illustrated in this study, introduces serious complications for the classification and taxonomy of fungi in the *G. fujikuroi* species complex. This is especially true, when recognizing the fact that many researchers do not have direct access to DNA sequencing facilities. They must thus rely on identifying *Fusarium* spp. by using the morphological and biological species concepts. Problems with using the morphological species concept have been reported by many workers (29, 32, 35, Chapters 2 and 6 of this thesis), but the current study is the first report of disparities using the biological species concept. As we have indicated, the application of the biological species concept can obscure the true phylogenetic relationships among closely related species. The results of sexual compatibility studies using known mating tester strains should thus be interpreted with caution. Although we acknowledge the importance of both morphology and the biological species concept, we recommend the use of the phylogenetic species concept for the unambiguous identification of *Fusarium* species in the *G. fujikuroi* complex.

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Table 1. Hosts, geographic origins and sources of the *Fusarium subglutinans* isolates from mating populations E and H used in this study.

Isolate[1]	Host[2]	Geographic origin[3]	Source
MRC 115	*Z. mays*	Eastern Cape, South Africa	W.F.O. Marasas
MRC 714	*Z. mays*	Northern Province, South Africa	W.F.O. Marasas
MRC 756	*Z. mays*	Mpumalanga, South Africa	W.F.O. Marasas
MRC 837	*Z. mays*	Eastern Cape, South Africa	W.F.O. Marasas
MRC 1077	*Z. mays*	Eastern Cape, South Africa	W.F.O. Marasas
MRC 1084	*Z. mays*	Eastern Cape, South Africa	W.F.O. Marasas
MRC 6483, M 3696, KSU 990	*Z. mays*	Illinois United States	J.F. Leslie
MRC 6512, M 3693, KSU 2192	*Z. mays*	Illinois United States	J.F. Leslie
KSU 434, M 6496	*Z. mays*	Kansas, United States	J.F. Leslie
KSU 507, M 5119	*Z. mays*	Kansas, United States	J.F. Leslie
KSU 731, M 5126	*Z. mays*	Kansas, United States	J.F. Leslie
KSU 993, M 3698	*Z. mays*	Illinois United States	J.F. Leslie
KSU 1257	*Z. mays*	Kansas, United States	J.F. Leslie
KSU 1417	*Z. mays*	Kansas, United States	J.F. Leslie
KSU 2921, M 3763	*Z. mays*	Ohio, United States	J.F. Leslie
KSU 3815, M 851	N/a	N/a	J.F. Leslie
M 3935	*Z. mays* ssp. *mays*	N/a, Mexico	A.E. Desjardins
M 3869	*Z. mays* ssp. *mays*	N/a, Mexico	A.E. Desjardins
Fst 9	*Z. diploperennis*	Jalisco, Mexico	A.E. Desjardins
Fst 10	*Z. diploperennis*	Jalisco, Mexico	A.E. Desjardins
Fst 13, M 7794	*Z. luxurians*	Chiquimula, Guatemala	A.E. Desjardins
Fst 17, M 7799	*Z. mays* ssp. *huehuetenangensis*	Huehuetenen, Guatemala	A.E. Desjardins
Fst 22	*Z. mays* ssp. *mexicana*	Durango, Mexico	A.E. Desjardins
Fst 26	*Z. mays* ssp. *mexicana*	Michoacan, Mexico	A.E. Desjardins
Fst 40	*Z. mays* ssp. *parviglumis*	Guerrero, Mexico	A.E. Desjardins
Fst 51, M 8372	*Z. mays* ssp. *mexicana*	Texcoco, Mexico	A.E. Desjardins
Fst 54, M 8375	*Z. mays* ssp. *mays*	Texcoco, Mexico	A.E. Desjardins
Fst 58, M 8377	*Z. mays* ssp. *mays*	Texcoco, Mexico	A.E. Desjardins
Fst 69, M 8380	*Z. mays* ssp. *mays*	Texcoco, Mexico	A.E. Desjardins
Fsp 34	*Pinus* spp.	California, United States	T.R. Gordon
MR C6213	*Pinus* spp	Mpumalanga, South Africa	A. Viljoen

[1] 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; Fst = A. E. Desjardins, National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, Illinois, Fsp = T. R. Gordon, Department of Plant Pathology, University of California, Davis, California; M = D. M. Geiser, Fusarium Research Center, Pennsylvania State University, University park, Pennsylvania.

[2] N/a, information on the plant host from which the strain were isolated is not available.

[3] The exact location of isolation of strains, are not known.

Table 2. Summary of the polymorphic alleles or nucleotides (nc) in the six nuclear loci (calmodulin, β-tubulin, histone *H3*, HB9, HB14 and HB26) uncovered among the *Fusarium subglutinans* isolates associated with maize and teosinte.

Isolates	Polymorphisms at six nuclear loci (nc)[1]																		Genotype
	Calmo-dulin		β-tubulin			Histone *H3*						HB14				HB26	HB9		
	33	377	144	204	285	27	99	275	286	351	426	99	230	231	233	69	101	204	
Fst 51, Fst 54, MRC 837, MRC 1077, MRC 1084	T	C	**a**	T	**t**	C	**c**	**t**	G	C	**t**	T	-	-	-	A	C	C	1-1
Fst 9	T	C	**a**	T	**t**	C	**t**	**c**	G	C	**c**	T	-	-	-	A	C	C	1-2
Fst 26	T	C	**a**	T	**c**	C	**t**	**c**	G	C	**c**	T	-	-	-	A	C	C	1-3
M 3869, M 3935, Fst 58, Fst 69, MRC 756	T	C	**g**	T	**t**	C	**c**	**t**	G	C	**t**	T	-	-	-	A	C	C	1-4
Fst 10, Fst 13, Fst 17, Fst 22, MRC 115, KSU 1257, KSU 1417, KSU 3815	C	**c**	A	C	T	**c**	C	T	A	T	T	C	T	A	A	G	T	G	2-1
KSU 434, KSU 731	C	**t**	A	C	T	**t**	C	T	A	T	T	C	T	A	A	G	T	G	2-2
Fst 40, MRC 714, MRC 6512, MRC 6483, KSU 507, KSU 993, KSU 2921	C	**t**	A	C	T	**c**	C	T	A	T	T	C	T	A	A	G	T	G	2-3

[1] Alleles that are fixed in either or both populations are indicated in capital letters. Polymorphisms that are not fixed are indicated in bold lower case letters. Horizontal lines (-) indicate deleted nucleotides.

Table 3. Number of polymorphic and parsimony informative characters, as well as the actual length of trees generated from the individual and combined sequence data sets for each of the six loci used.

Locus	Informative characters	Polymorphic characters	Tree length[1]
Calmodulin	2	2	2
Histone *H3*	6	6	6
β-tubulin	2	3	2
HB9	2	2	2
HB14	4	4	4
HB26	1	1	1
Combined	17	18	17

[1] Note that the actual and expected tree lengths are similar due to the lack of homoplasy or polymorphisms that represent reversals, parallelisms or convergences.

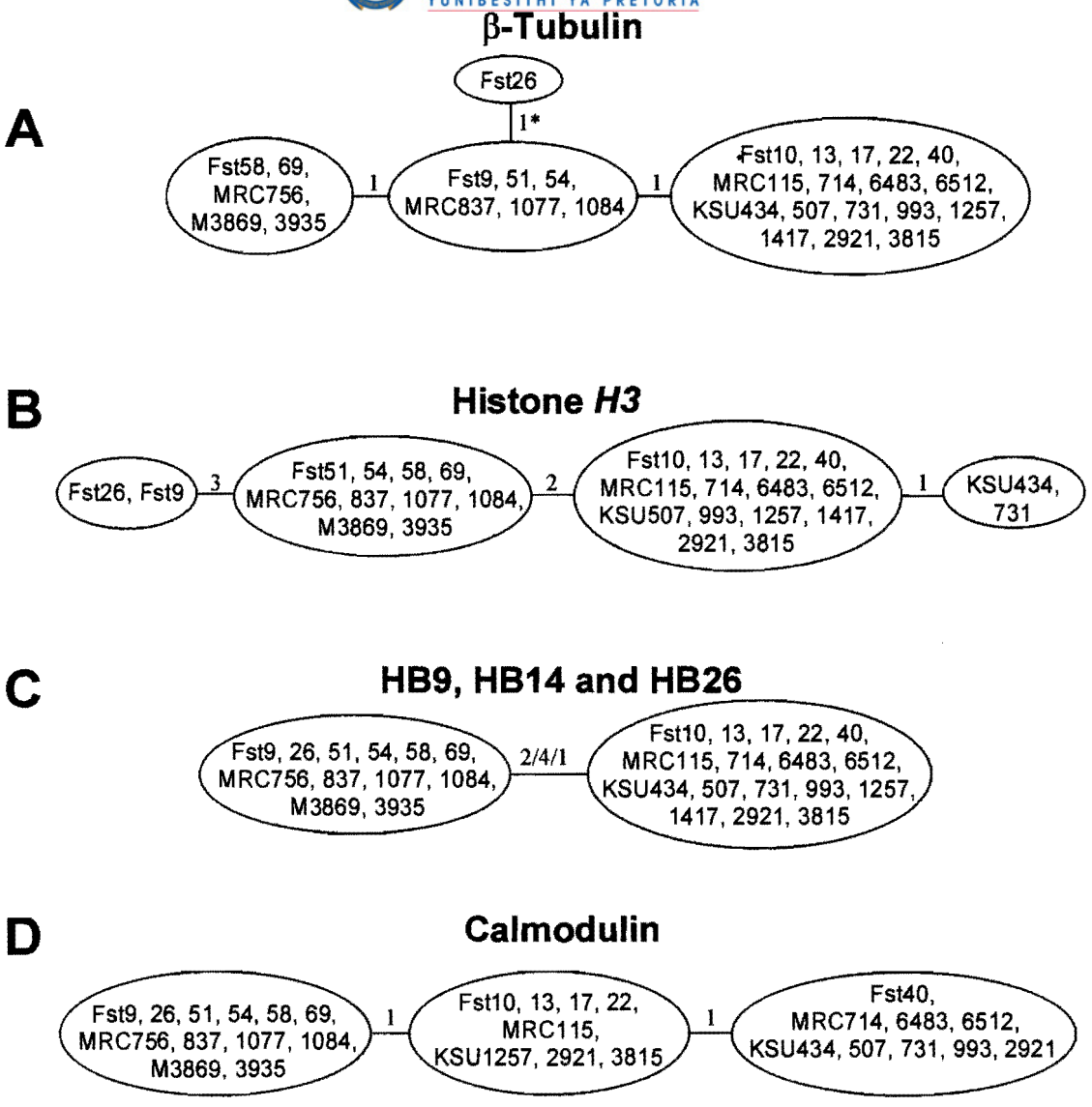

Figure 1. Single-gene genealogies generated from the sequence data sets for the six different loci studied in the 29 *F. subglutinans* strains associated with maize and teosinte. In each case only the informative characters were used and one most parsimonious reconstruction was obtained. The branch associated with the single parsimony uninformative character in the β-tubulin data set is indicated with an asterisk (*). The consistency (CI) and retention (RI) indices for each was 1.00 and 1.00, respectively. **A:** β-tubulin gene genealogy consisting of 2 parsimony informative characters and 2 steps. **B:** Histone *H3* genealogy consisting of 6 parsimony informative characters and 6 steps. **C:** The single-gene genealogy for each of the HB9, HB14 and HB27 nuclear regions. Because the clustering for each of these regions are identical, they are represented by a single tree with lengths 2, 4 and 1, respectively. **D:** Calmodulin gene genealogy consisting of 2 steps.

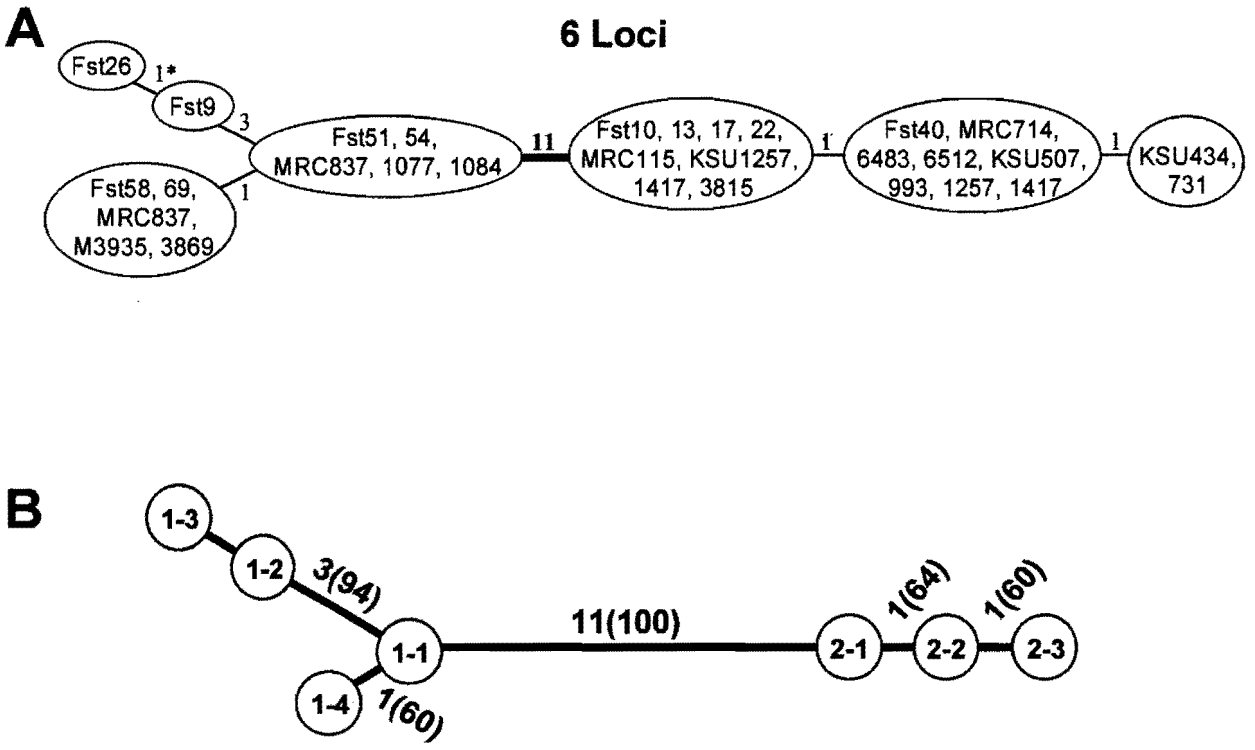

Figure 2. **A:** Genealogy generated from the combined data sets using all the parsimony informative characters (37). The branch associated with the single parsimony uninformative character in the β-tubulin data set is indicated with an asterisk (*). One single most parsimonious reconstruction with a length of 17 steps were obtained (CI = 1.00; RI = 1.00). **B:** The individuals included in each of the seven clusters corresponds with the individuals displaying each of six genotypes. Inclusion of the single uninformative character present in the β-tubulin sequence data set, allows the separation of genotypes 1-2 and 1-3. Bootstrap values are indicated in parentheses.

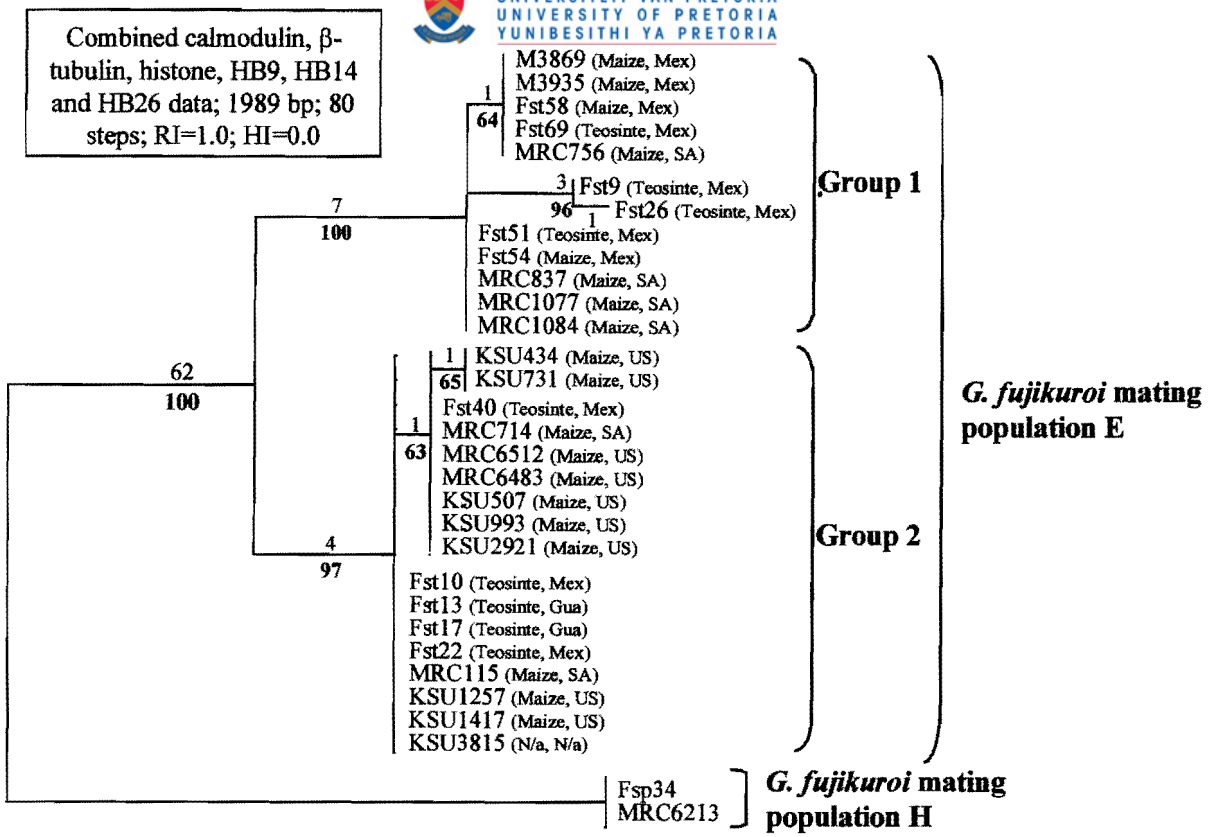

Figure 3. A single most parsimonious phylogram generated from the combined sequence data sets obtained from this study. Parsimony informative, as well as parsimony uninformative characters were included. Isolates representing both Groups 1 and 2 of the E-mating population, are included. The host and geographic origin are indicated in parentheses (Mex. = Mexico; SA = South Africa; US = United States; Gua = Guatemala; N/a = not available). The tree is rooted to *G. fujikuroi* mating population H. Branch lengths are indicated above the branches and bootstrap values based on a 1,000 replications, are indicated as bold digits below the branches.