Supplemental Table S1: Results of Codeml- Maximum likelihood analysis for estimation synonymous site divergence (*dS*) for primary-metabolism (PM) and *FUM* genes in individual species of *Fusarium*.

Taxon 1	Taxon 2	*dS FUM*	*dS* PM	D[a]	Probability[b]
F. phyllophilum	*F. nygamai*	0.057	0.108	-0.051	< 0.001
F. ramigenum	*F. nygamai*	0.089	0.103	-0.014	
F. verticillioides	*F. nygamai*	0.092	0.105	-0.013	
F. ramigenum	*F. phyllophilum*	0.107	0.136	-0.029	< 0.01
F. verticillioides	*F. phyllophilum*	0.099	0.137	-0.038	< 0.001
F. verticillioides	*F. ramigenum*	0.072	0.101	-0.029	< 0.001
F. globosum	*F. fujikuroi*	0.071	0.045	0.026	< 0.001
F. proliferatum	*F. fujikuroi*	0.057	0.051	0.006	
F. proliferatum	*F. globosum*	0.061	0.025	0.036	< 0.001

[a] D, (*dS FUM* – *dS* PM); s(D)

[b] Probability shown if *dS FUM* is significantly different ($P < 0.05$) than *dS* PM.

Supplemental Table 2: Oligonucleotide primers used for PCR and sequencing.

Target Gene[a]	Primer Designation	Nucleotide Sequence[b] (5' → 3')
CAL1	CL1	GARTWCAAGGAGGCCTTCTC
	CL2	TTTTGCATCATGAGTTGGAC
CPR1	1825	CTCTCACAACCCCTACATTGCCCCTAT
	1826	CTTACCTGCCAYTCCTTYTSGTACATGA
FLB1	3450	GCTATCCACAACCTCGGCAATCTC
	3451	CCCGAATGATCTCRTCAGCCTGCT
FUM1	32	ACAAGTGTCCTTGGGGTCCAGG
	33	GATGCTCTTGGAAGTGGCCTACG
FUM3	3026	ATGAAYAARGAAAAGGTTCCCCAGA
	3031	GATGYAAAGCMARTTCAGAGTAGTC
FUM6	2749	ACCGAGATYATGGTGACGAGCAGAGA
	2751	GTGTCCTYGATGAAGTCCCATYGCTA
FUM7	2753	CCACCMRGCAAACTCCCGGAACYCTA
	2756	GCCATGTACAGAATCTCAACTAC
FUM8	679	CGTAGTAGGAATGAGAAGGATG
	680	GCAAGCTTTGTGGCTGATTGTC
FUM10	3034	CATTGGCMTTGCAGGGAGTTTGTTG
	3037	GTTCTTCTTGGCTTKCCRGTCCGAT
FUM13	3417	GTGGGCTATGCTGTGCTKGTCA
	3422	CTCYGGCTTRAACTTGACGCCTAG
FUM14	2059	CARTCATSATAGAGGACGACACTG
	2061	CTCTTGAACTCATACTGCTGCAA
FUM19	1935	GTCTCCCAACGCCCTGCCTATC
	1937	GACAGCAGAACTAGGCTCATCGAGT
	2270	CTSAGCTYCTGGAAKCGAAAGAG
FUM19-flank	2271	CCTGCGCAATGTCTAGAATAATG
FUM19-flank	2276	TAGGCCTGTTCAGAGTCTTATCC

FUM21	1944	GTAATGGCWCAAACCCTTGCAATCA
	1949	GTCTGGRCGCAAAMGGCKGCATC
HIS3	1701	CAATGGCTCGCACTAAGCAGAC
	1702	CTCRTGAACGCCTTGATTATCC
LAE1	3442	TGGTTGTRATGCCTCCTCAAAACAGGT
	3444	CTTCCTRGCCGTCCAGACATGACT
RED1	3431	TCTCACCMACAAGACCRGAGCTCT
	3432	TCCCARTTYGCCCAGACRAACTT
RPB1	3434	CAYAARGARTCYATGATGGGWC
	3435	GTCATYTGDGTDGCDGGYTCDCC
	3436 [c]	CAATGAGACCTTCTCGACCAGC
	3437 [c]	ATGGGTATYGTCCAGGAYTC
	3438 [c]	CRACACAGAAGAGTTTGAAGG
	3439 [c]	TTCTTCCACGCCATGGCTGGTCG
RPB2-A	5F2	GGGGWGAYCAGAAGAAGGC
	7cR	CCCATRGCTTGYTTRCCCAT
RPB2-B	7cF	ATGGGYAARCAAGCYATGGG
	11aR	GCRTGGATCTTRTCRTCSACC
TEF1	ef1	ATGGGTAAGGARGACAAGAC
	ef2	GGARGTACCAGTSATCATGTT
	ef22[c]	AGGAACCCTTACCGAGCTC
TPS1	3452	CTGTCAGAGAAGCACTCCTTACTG
	3453	CTTCTCAAGATCAGCGACAGCCTGT
TUB2	T1	AACATGCGTGAGATTGTAAGT
	T11[c]	AATTGGTGCTGCTTTCTGGCA
	T21[c]	GGTTTGCCAGAAAGCAGCACC
	T22	TCTGGATGTTGTTGGGAATCC
	T121[c]	CCACCTGTCTCCGTTTCCCCG
	T222 [c]	GACCGGGGAAACGGAGACAGG
ZBD1	3446	CCACTCGAACTCTTGTTGCACCA
	3447	ACCATRTCCGCCACAACCTCCAT

[a] For each primary-metabolism gene, the name of the predicted protein, the *F. verticillioides* gene model designation from the *Fusarium* Comparative Database, and the *F. verticillioides* chromosome (chr) on which the gene is located are as follows: *CAL1*, calmodulin FVEG_07362 chr 8; *CPR1*, NADPH-cytochrome P450 reductase FVEG_06400 chr 2; *FLB1*, regulator of G protein signaling FVEG_08855 chr 1); *HIS3*, histone H3 FVEG_11374 chr 9; *LAE1*, Velvet complex methyltransferase FVEG_00539 chr 1; *RED1*, short chain dehydrogenase/reductase FVEG_13368 chr 8; *RPB1*, RNA polymerase II largest subunit FVEG_00683 chr 1; *RPB2*, RNA polymerase II second largest subunit FVEG_09286 chr 5; *TEF1* (also *EF-1*α), translation elongation factor 1α FVEG_02381 chr 6; *TPS1*, α,α-trehalose-phosphate synthase FVEG_04683 chr 4; *TUB2*, β-tubulin FVEG_04081 chr 2; and *ZBD1*, zinc-binding dehydrogenase FVEG_00314 chr 1.

[b] In nucleotide sequences, K = G or T, M = A or C, S = C or G, R = A or G, W = A or T, and Y = C or T.

[c] Primers used for sequence analysis only.

Supplemental Figure Legends

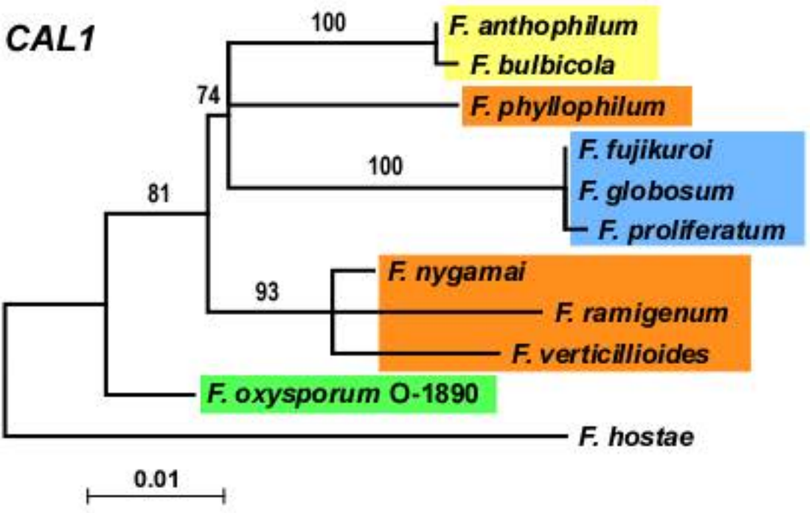
Supplemental Figure S1: Maximum likelihood trees generated with sequences of individual PM (A) and *FUM* (B) genes amplified from nine fumonisin producing species of *Fusarium* in the FFSC and *F. oxysporum* FRC O-1890. Numbers near branches indicate bootstrap values based on 1000 pseudoreplicates.

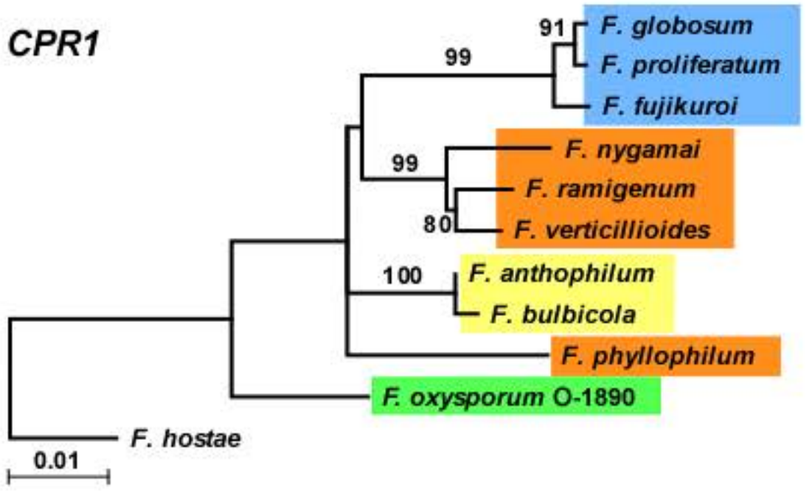
Supplemental Figure S2: Maximum likelihood trees generated with sequences of individual *FUM* genes amplified from multiple strains of nine fumonisin producing species of *Fusarium* in the *F. fujikuroi* species complex (FFSC). Numbers near branches indicate bootstrap values based on 1000 pseudoreplications.

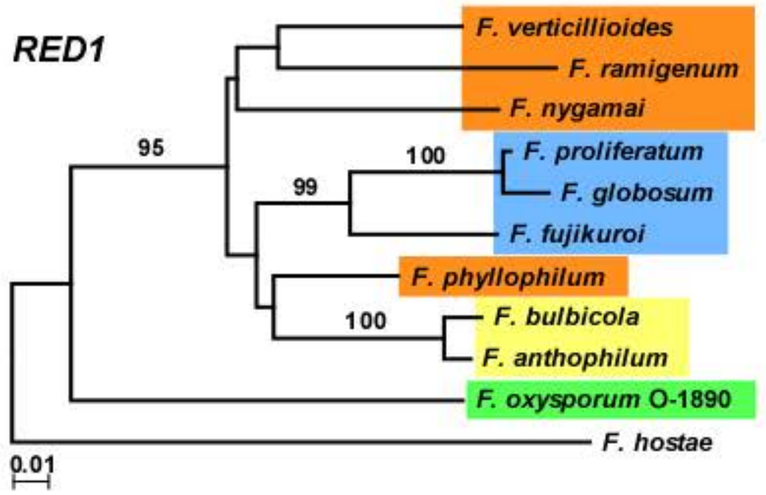
Supplemental Figure S3: Presence and absence of the *FUM* cluster mapped onto a species phylogeny for the *F. fujikuroi* species complex (FFSC). The tree was adapted from Figure 2 in (Kvas *et al.*, 2009). Blue branches indicate presence of the cluster, black branches indicate its absence, and rust-coloured branches indicate that the presence/absence of the cluster has not been determined. A change from blue to black indicates loss of the cluster. Yellow highlighting indicates species that have been examined by Southern blot, PCR and sequence, and/or genome sequence analyses for the presences of *FUM* genes in previous studies (Glenn *et al.*, 2008;Mirete *et al.*, 2004;Proctor *et al.*, 2004;Stepien *et al.*, 2010;Van Hove *et al.*, 2011) and/or in the current study. For this analysis, we assumed that the *FUM* cluster was present in the common ancestor of the FFSC and *F. oxysporum* as per the divergence data for *FUM* versus PM genes (Table 3) and the birth-and-death model of *FUM* cluster evolution (Figure 5B). Although there are reports of *FUM* genes in *F. subglutinans* and *F. temperatum* (e.g. (Stepien *et al.*, 2010)), we were unable

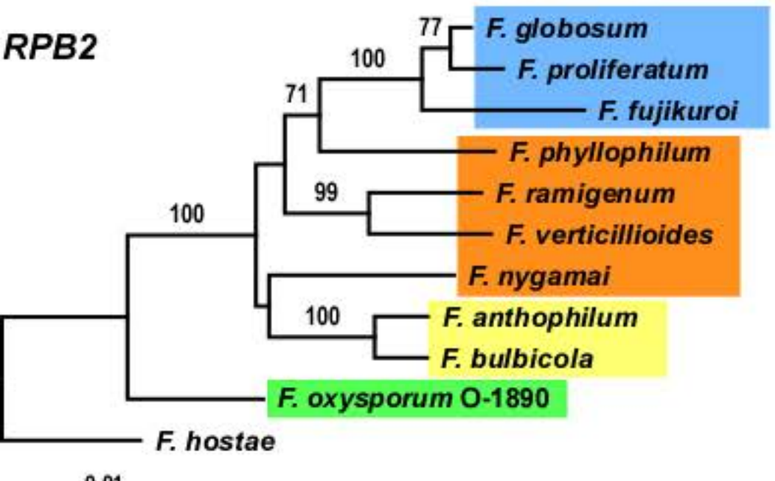
to confirm their presence by Southern blot and/or PCR analyses (data not shown) using some of the same strains employed by Stepien *et al.* (2010). Therefore, the figure indicates that the *FUM* cluster is absent in these two species.

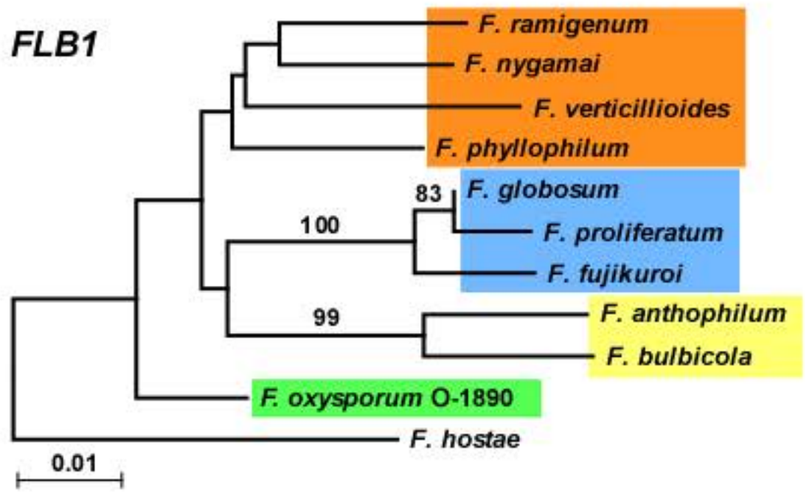
Supplemental Figure S1A

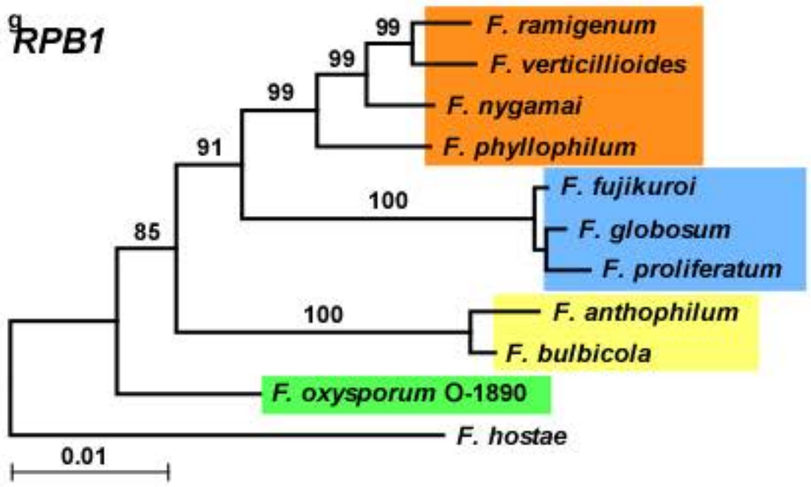


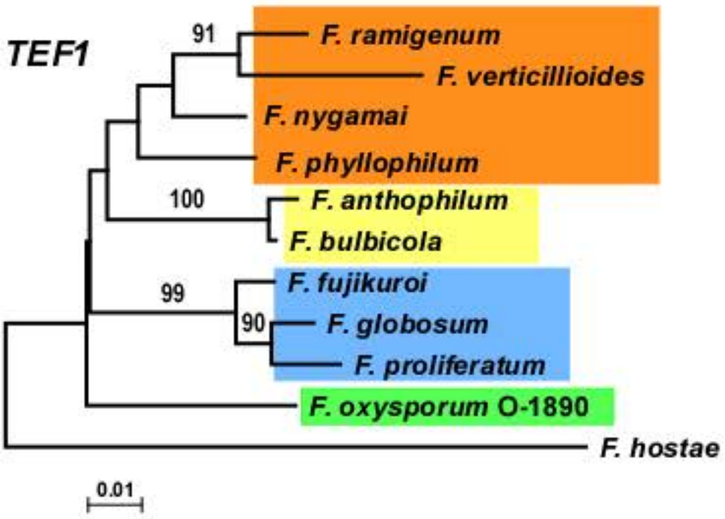


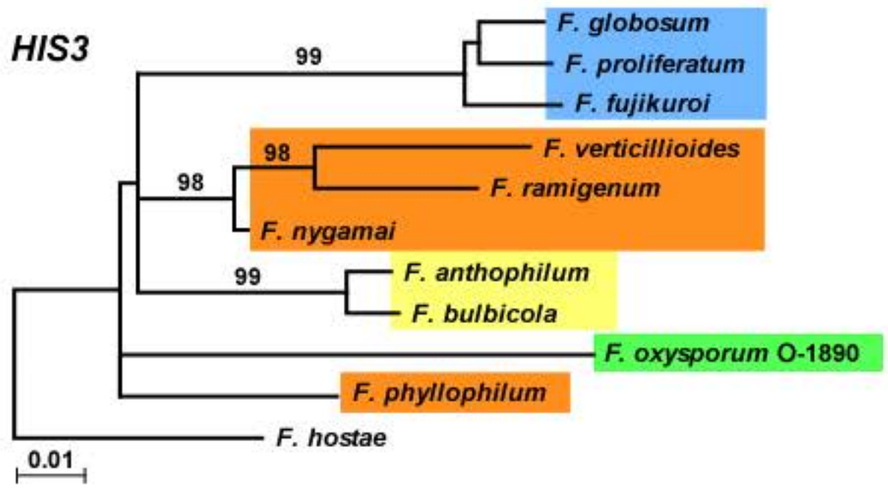


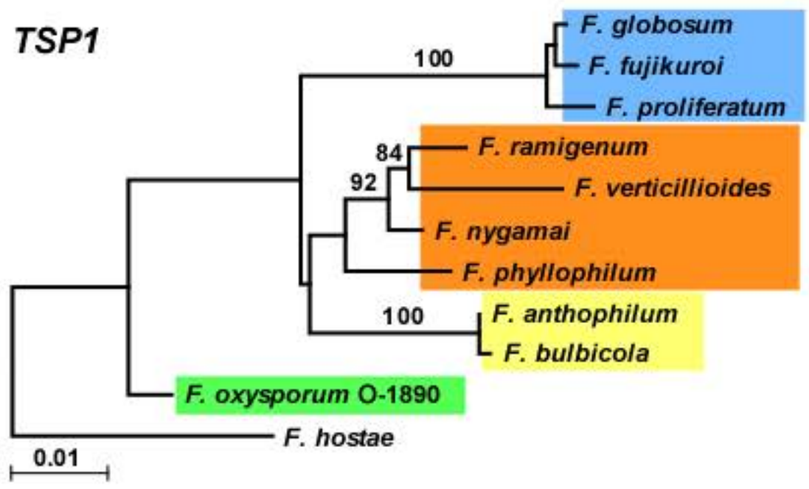


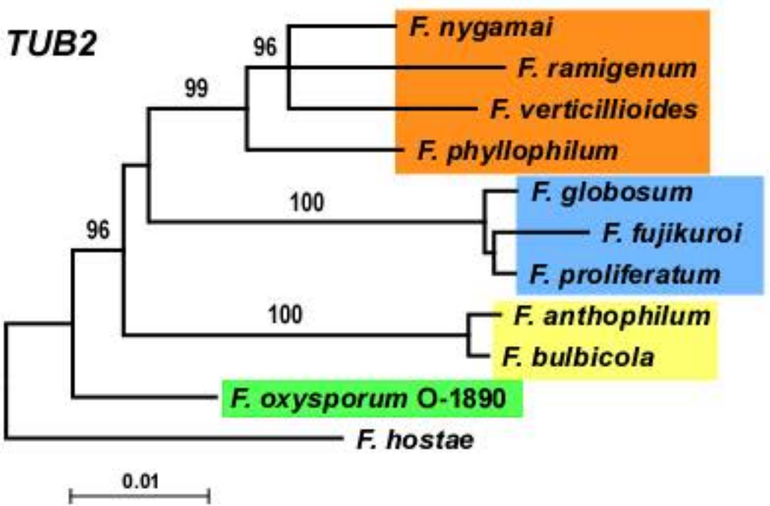


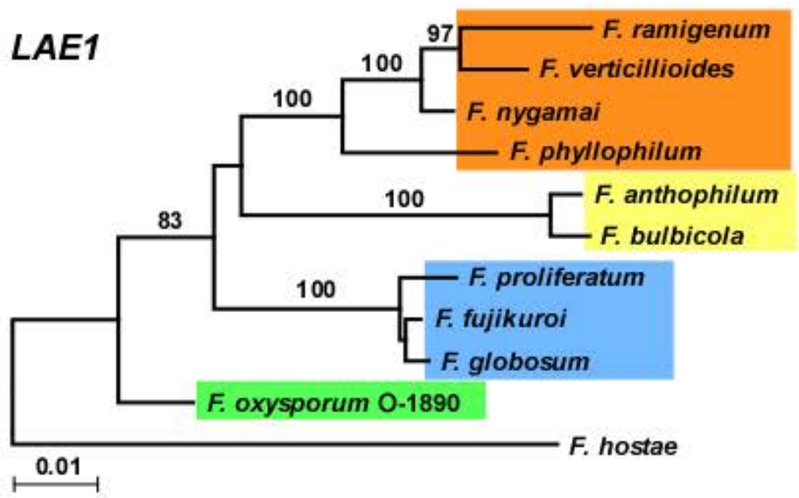


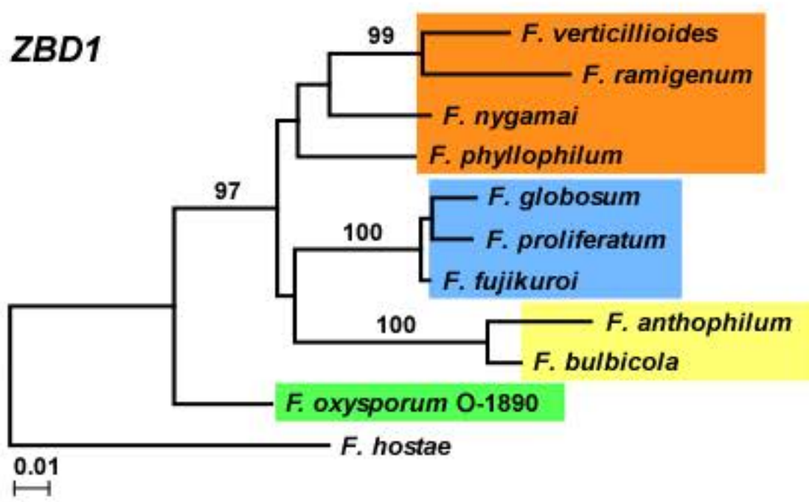




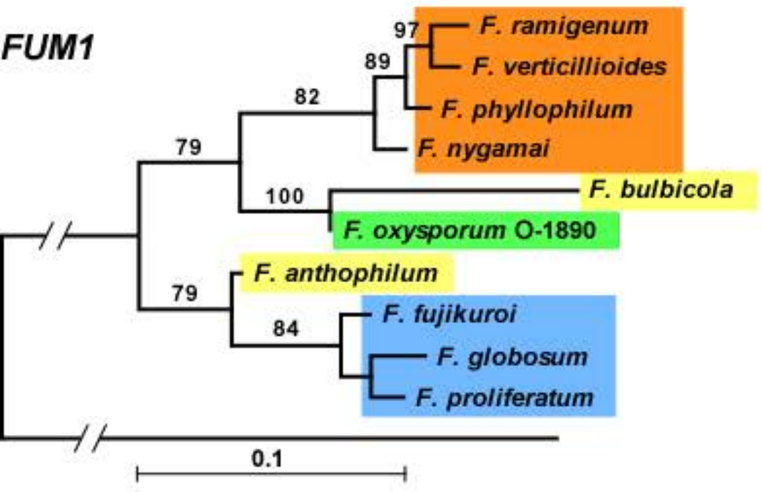


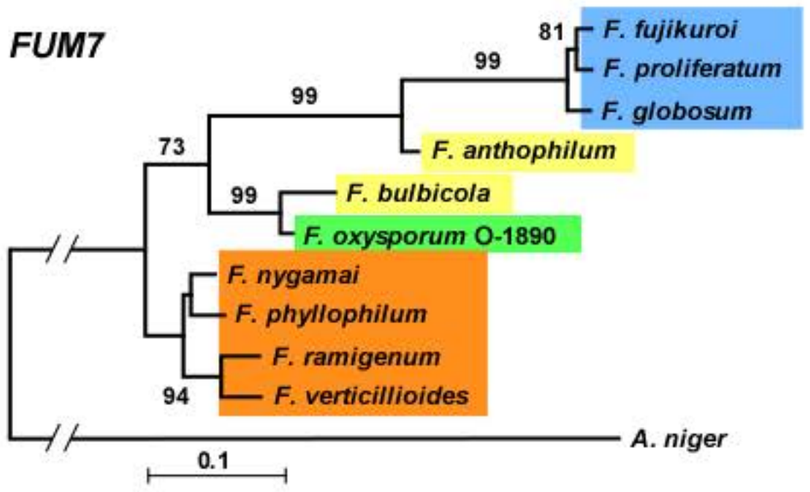


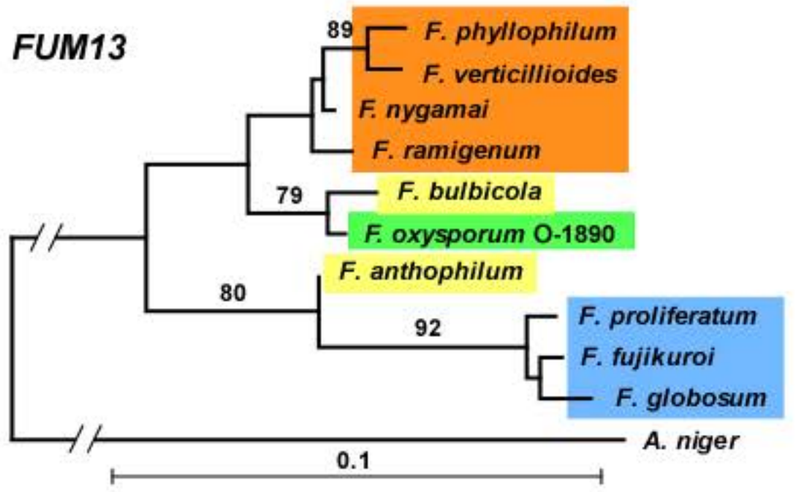


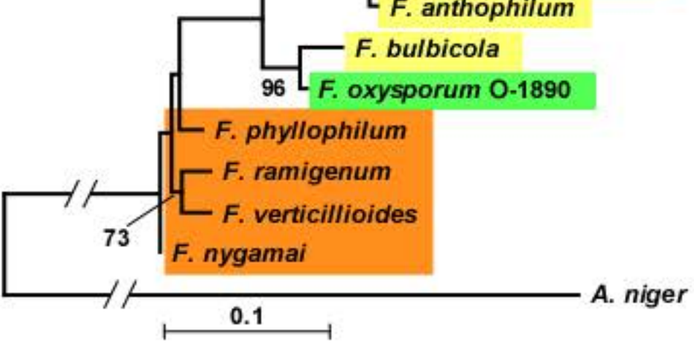


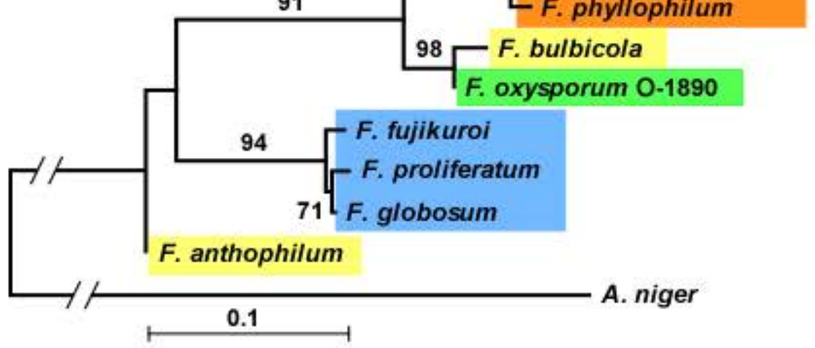
Supplemental Figure S1B

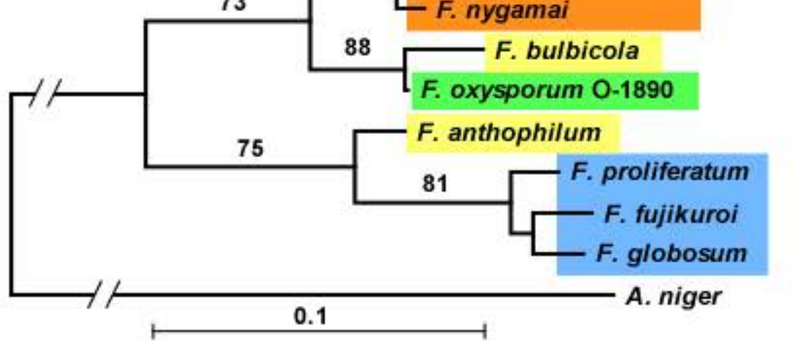


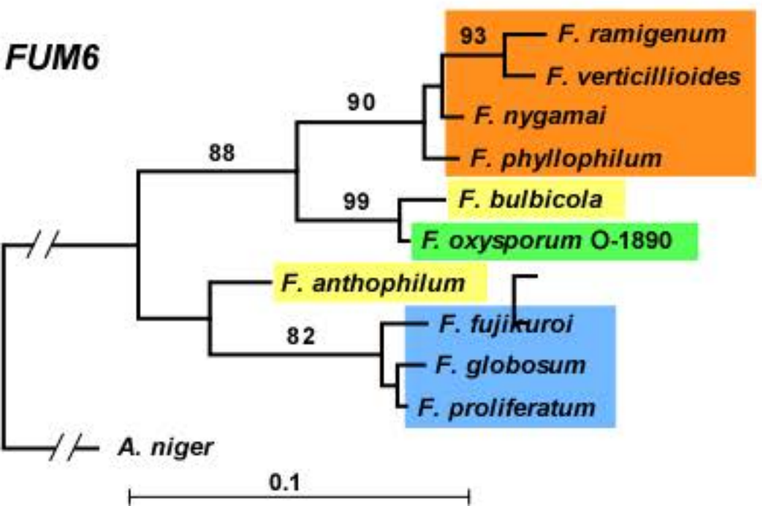


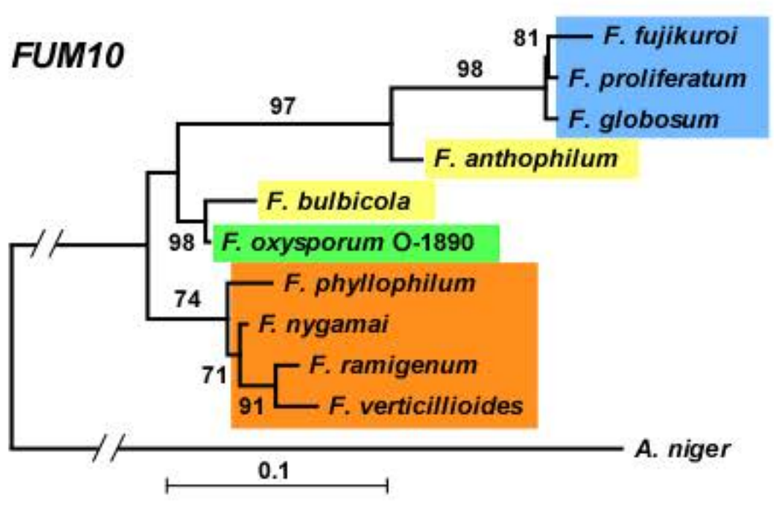


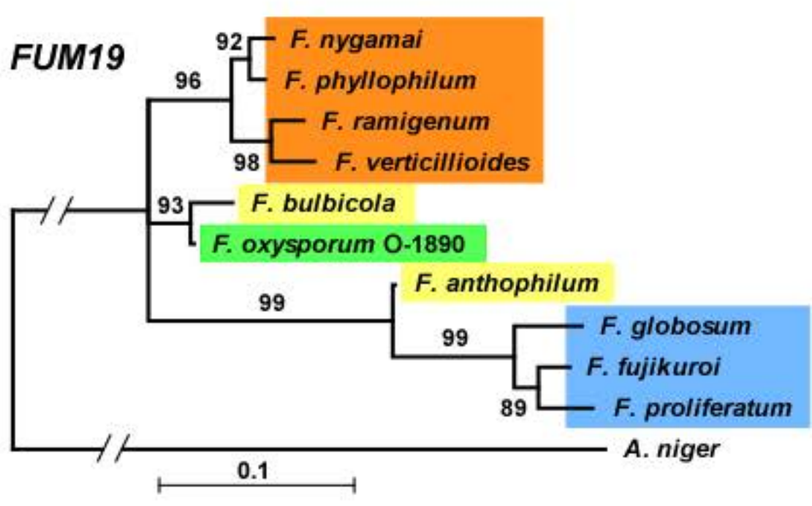




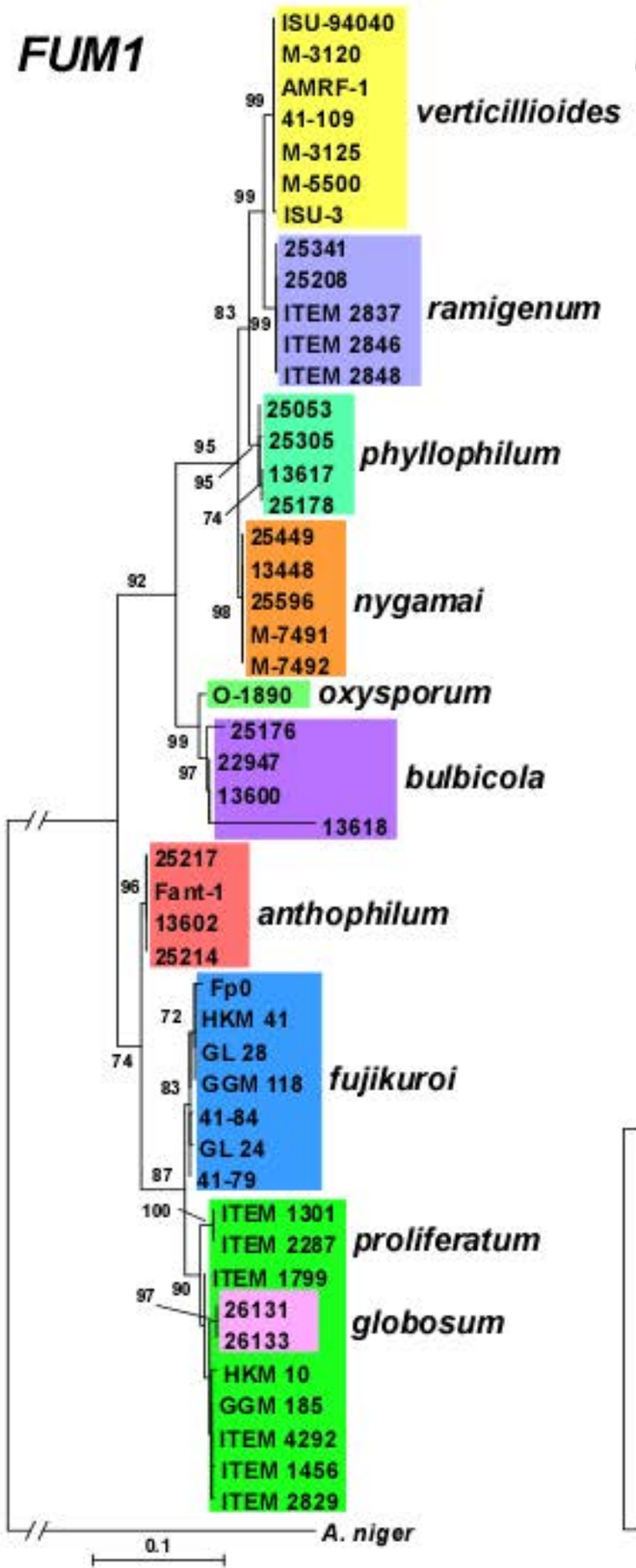


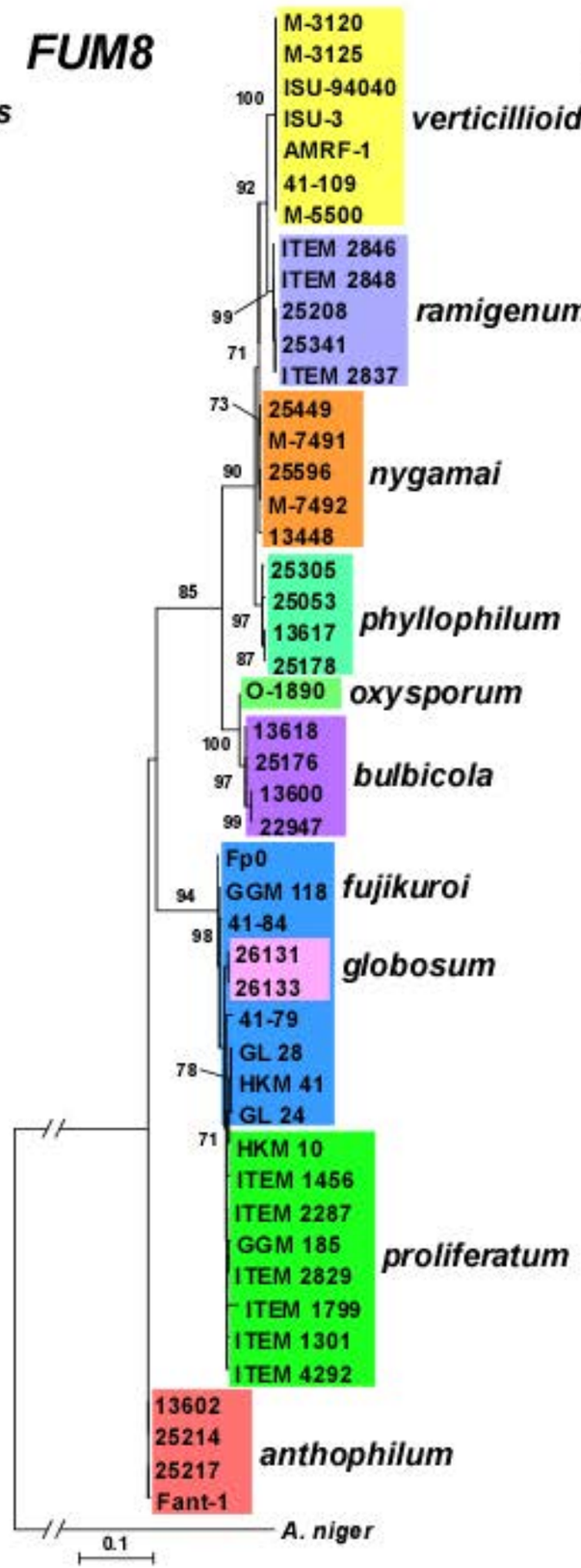


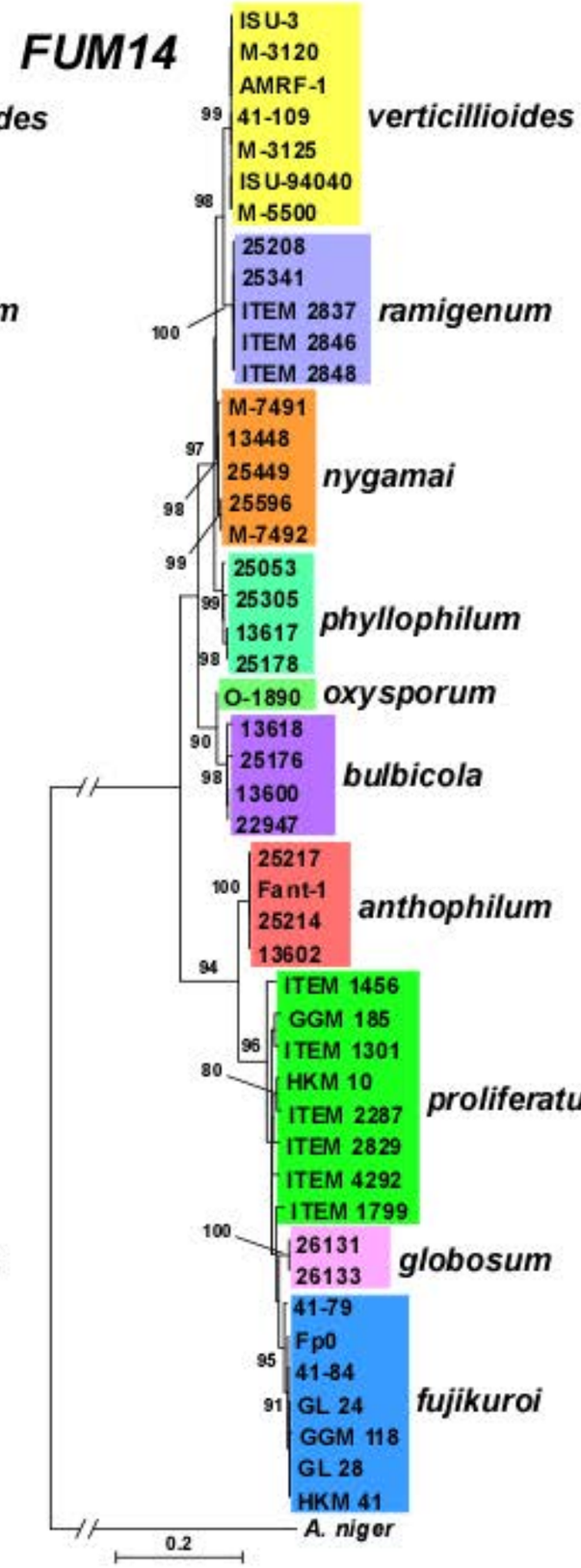


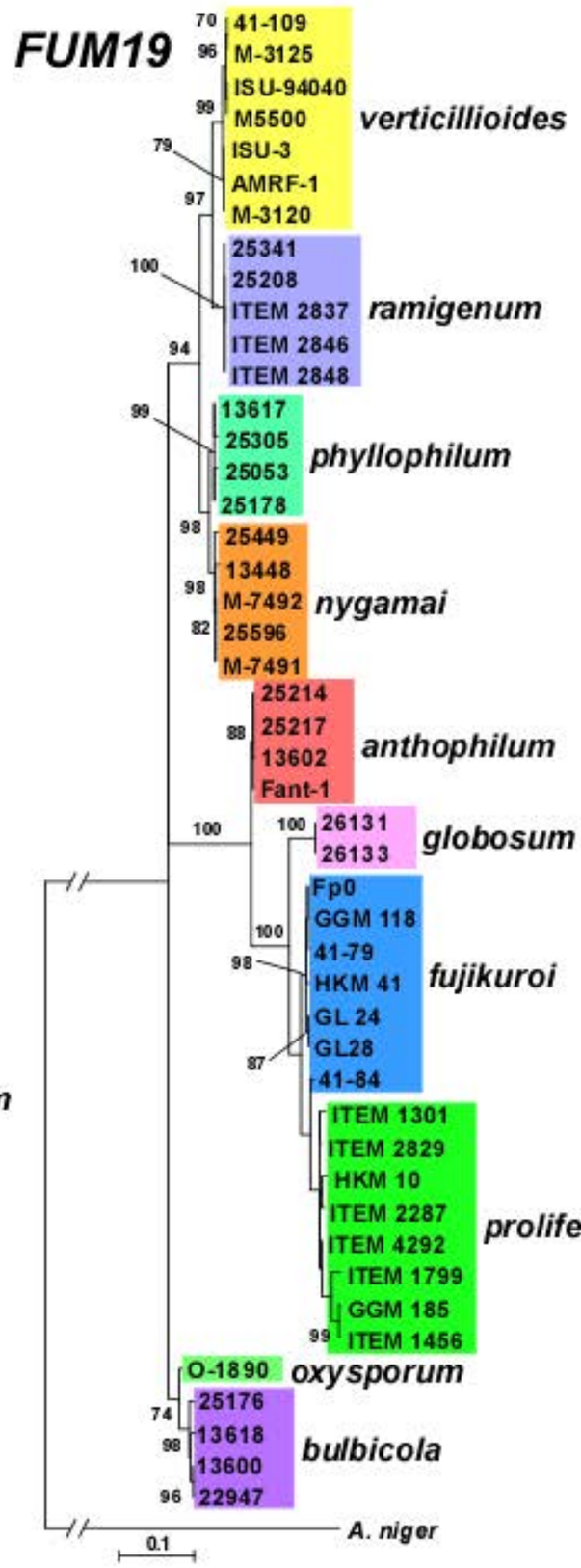


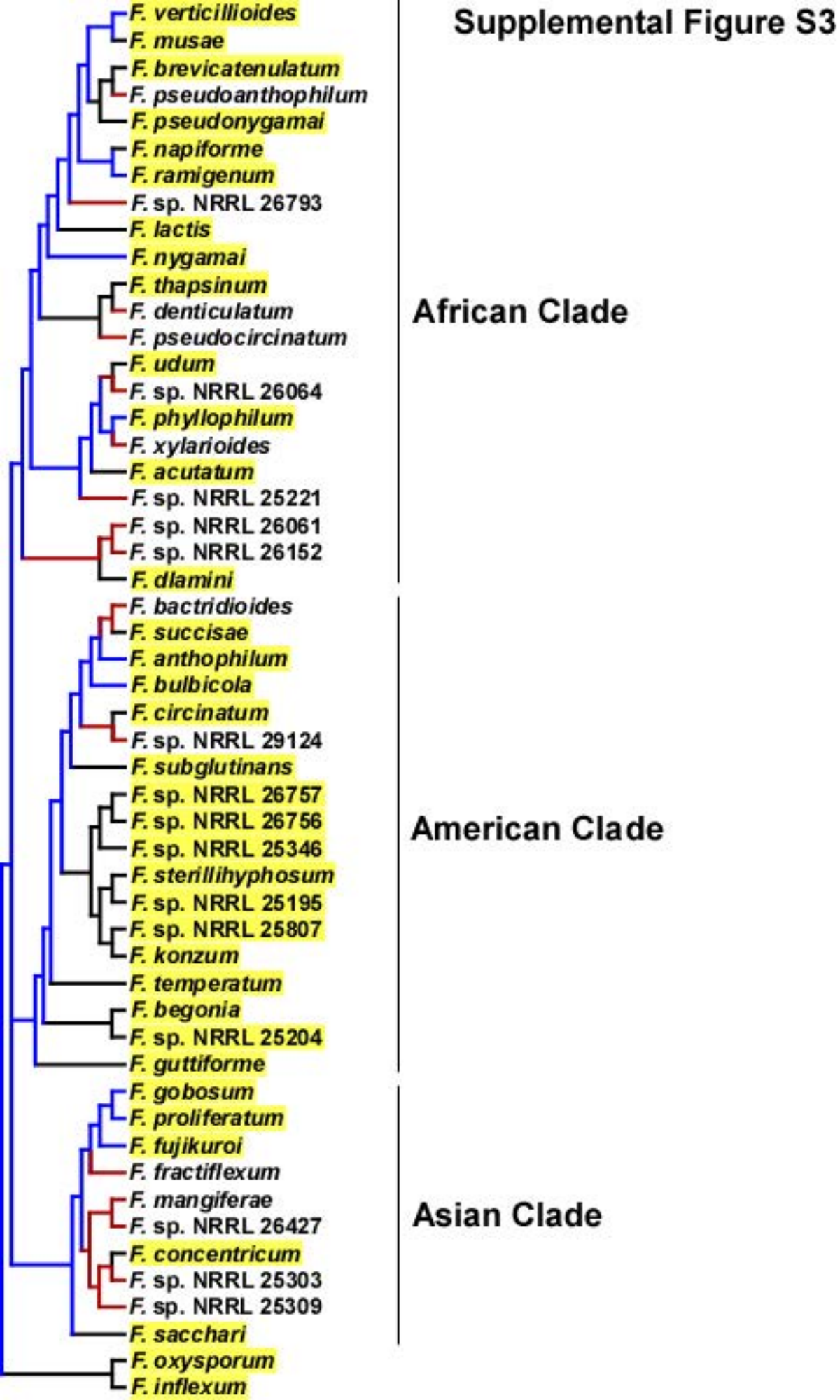
Supplemental Figure S2










Supplemental Figure S3
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F. pseudonygamai
F. napiforme
F. ramigenum
F. sp. NRRL 26793
F. lactis
F. nygamai
F. thapsinum
F. denticulatum
F. pseudocircinatum
F. udum
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F. bactridioides
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F. sacchari
F. oxysporum
F. inflexum
African Clade
American Clade
Asian Clade