



Thank to organizer committee

Re-classification of Fusarium species by taxonomy and phylogeny molecular using micro-sequences of DNA of orthologous genes.



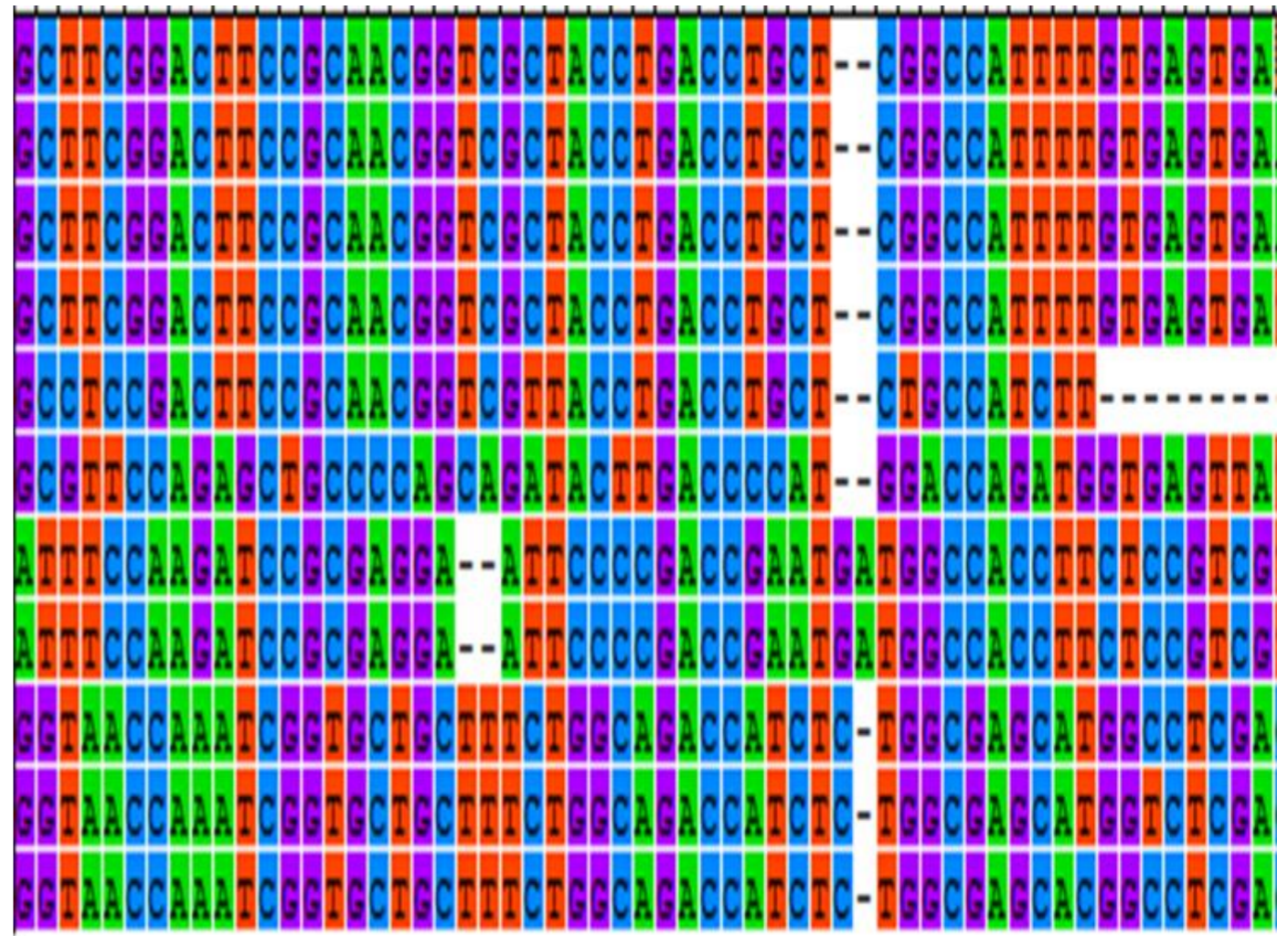
Re-classification of *Fusarium* species by taxonomy and phylogeny molecular using micro-sequences of DNA of orthologous genes.

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Juan, D. Henao, Ángela Niño N.

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Vs.



My plant has
Fusarium

Which ones?



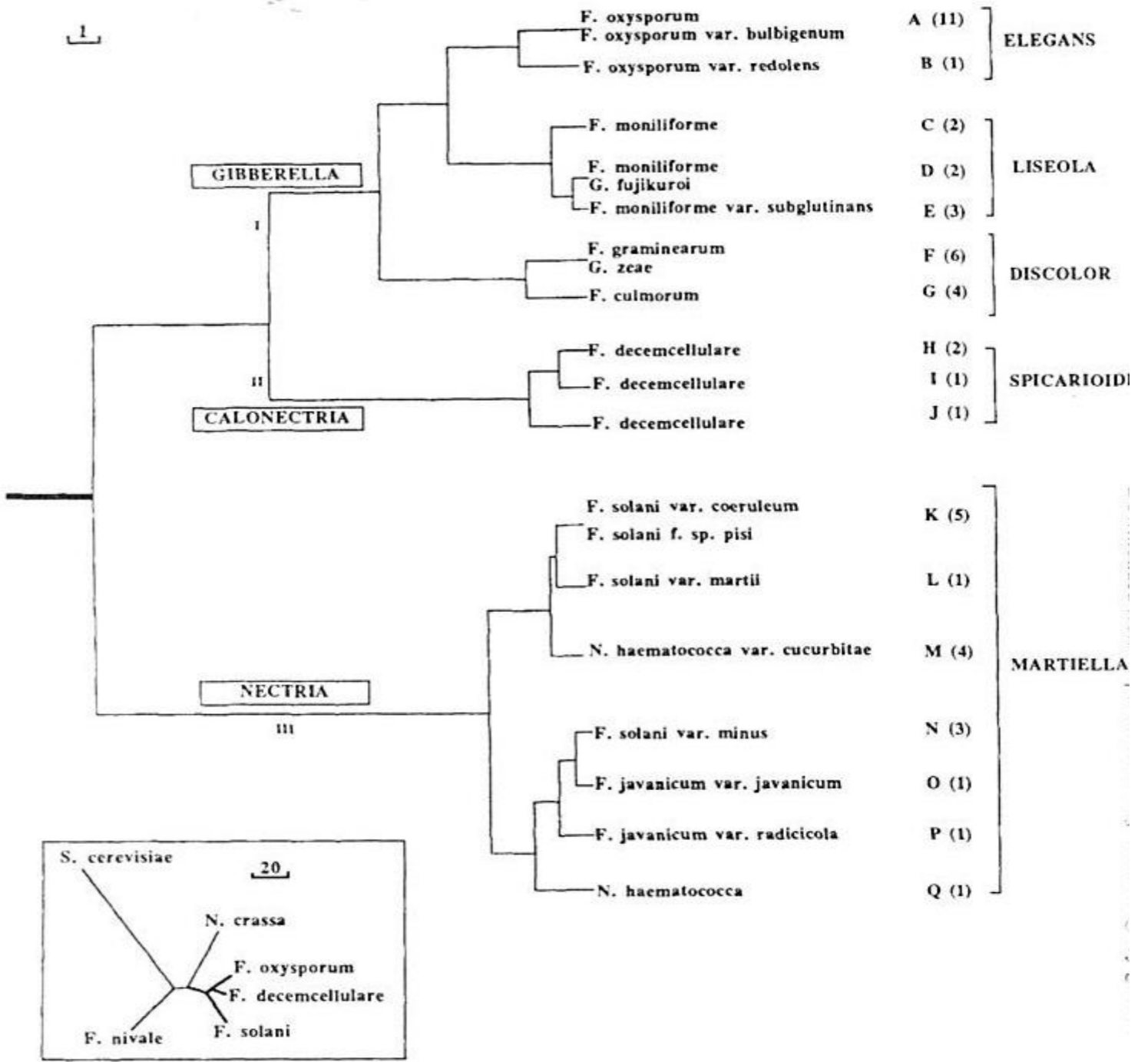


The plant pathologist and mycologist are confronted continually with the problem of identifying the widely distributed members of the genus *Fusarium*, so frequently associated with the disorders of plants.

Some of History

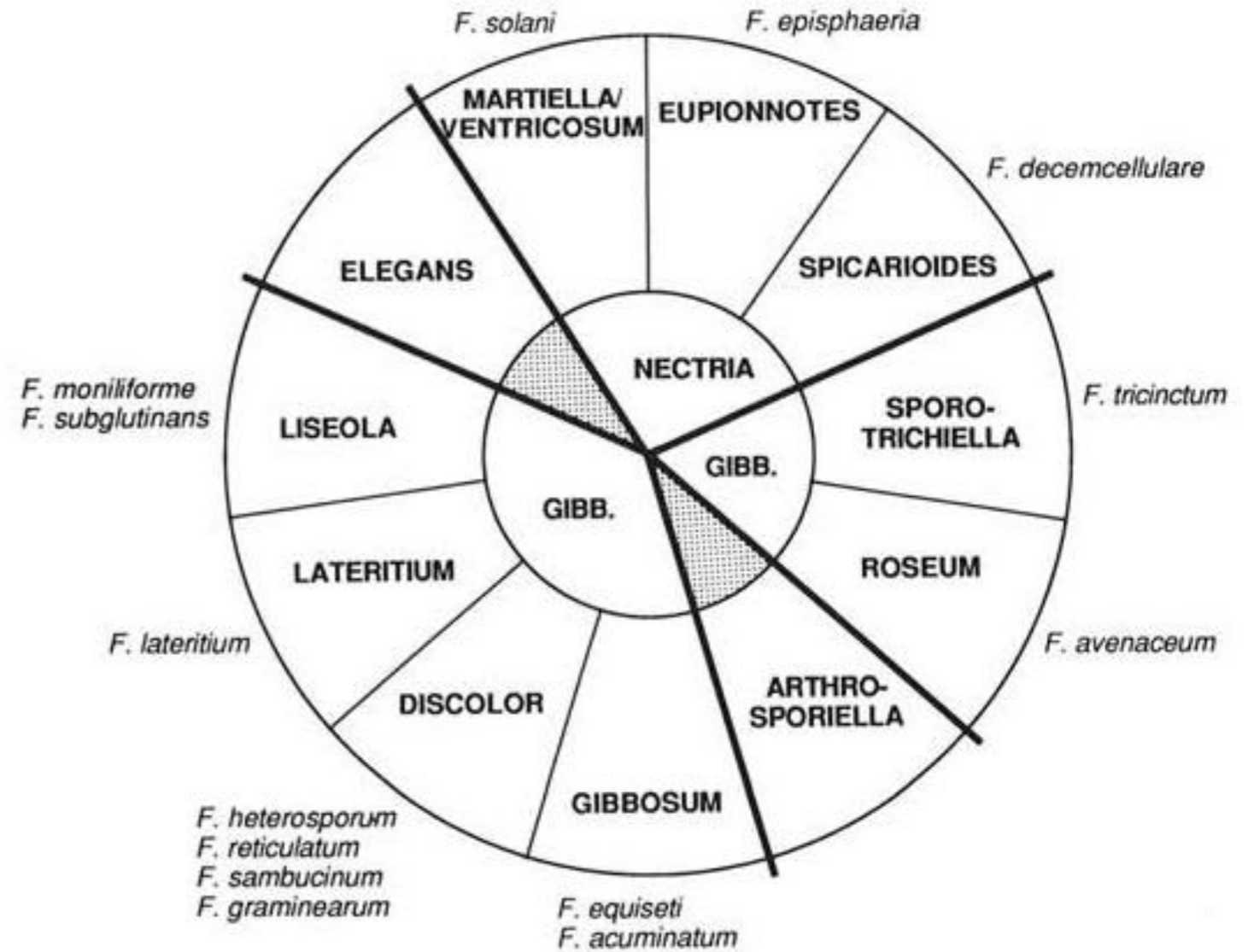
- 1809: Link create the genus *Fusarium*
- 1910: Appel and Wollenweber grouped all the perfect fungi in the family Tuberculariaceae, macroconidia with the croissant shape
- 1935: 16 sections and 65 species (Wollenweber and Reinking)
- 1940: only 10 species (Snyder and Hansen)
- 1971: C. Booth recognize 44 species
- 1982 Gerlach and Nirenberg recognize 73 species and 23 varieties
- 1983: Nelson & et.al. only 30 species
- 2006: Leslie and Summerell recognize 70 species
- 2011: Watanabe more than 1000 species
- 2017: 1200 species

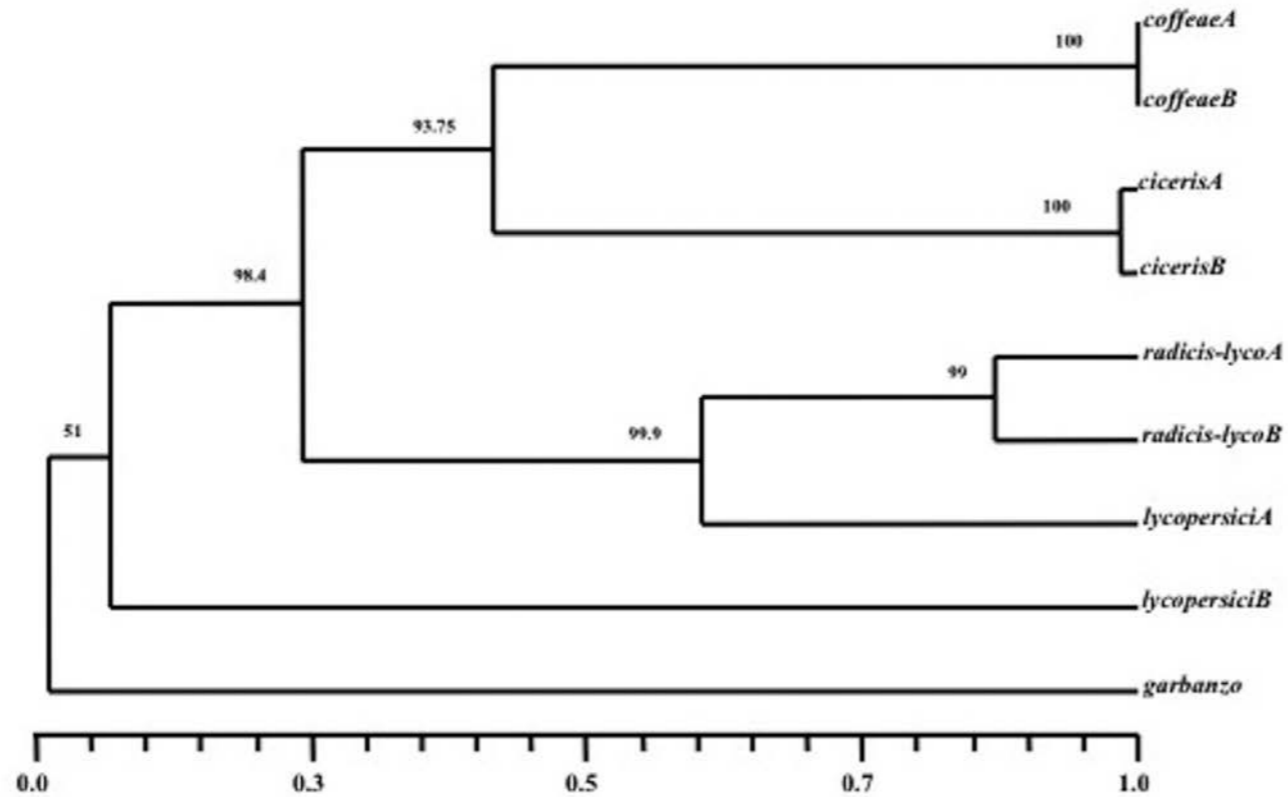
5 sections



13 sections

- *Arachnites*
- *Arthrosporiella*
- *Discolour*
- *Elegans*
- *Eupionnotes*
- *Gibbosum*
- *Lateritium*
- *Liseola*
- *Martiella*
- *Ventricosum*
- *Roseum*
- *Spicarioides*
- *Sporotrichiella*



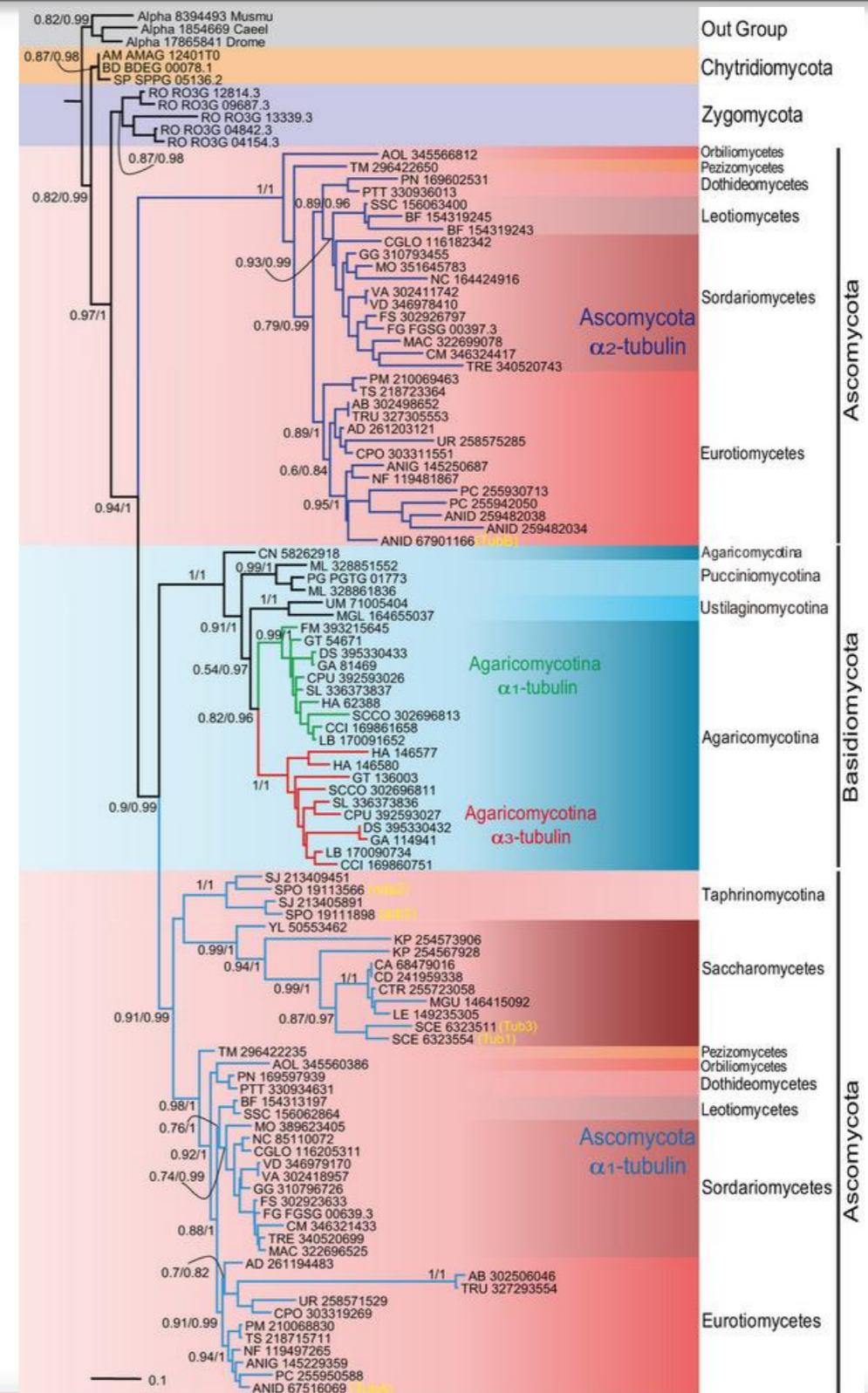


- Genus
- Subgenera
- Species
- f.sp.
- Races
- Isolates



Molecular evolution and functional divergence of tubulin superfamily in the fungal tree of life, 2012.

• [Zhongtao Zhao](#), [Huiquan Liu](#), [Yongping Luo](#), [Shanyue Zhou](#), [Lin An](#), [Chenfang Wang](#), [Qiaojun Jin](#), [Mingguo Zhou](#) & [Jin-Rong Xu](#)



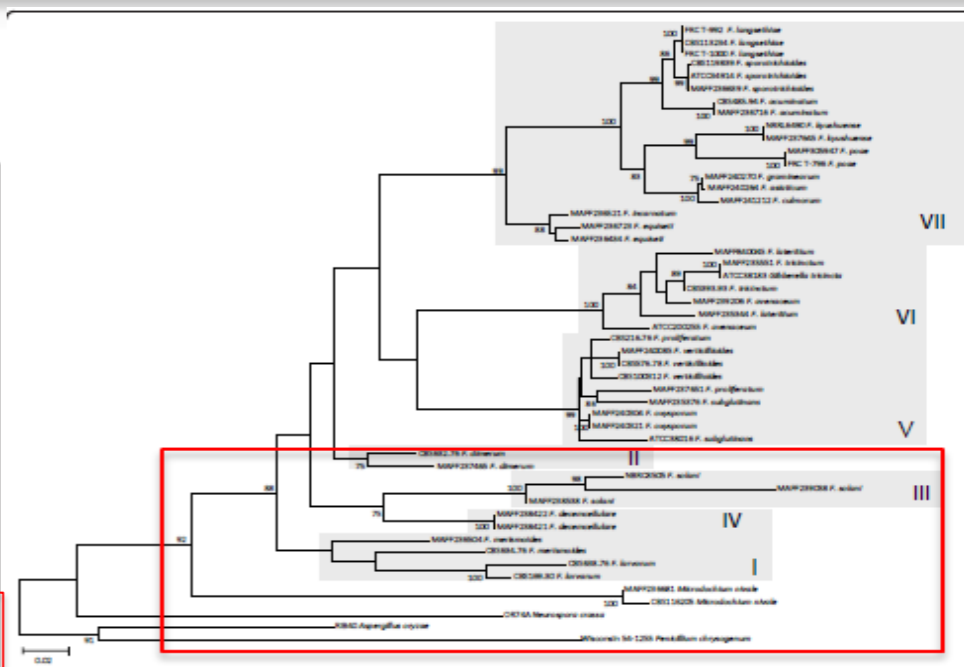
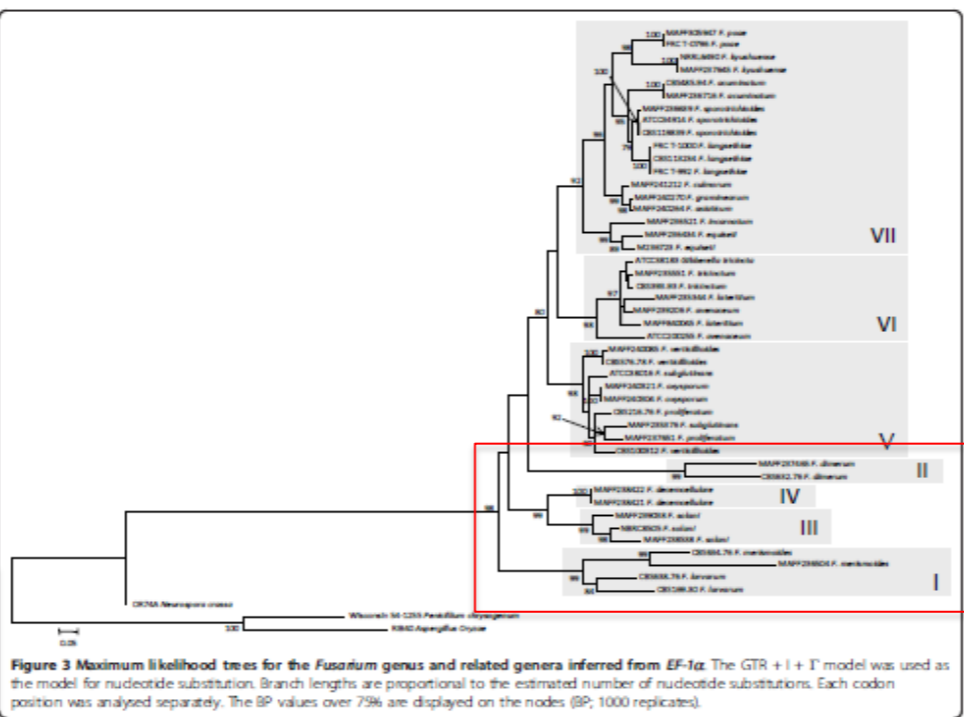
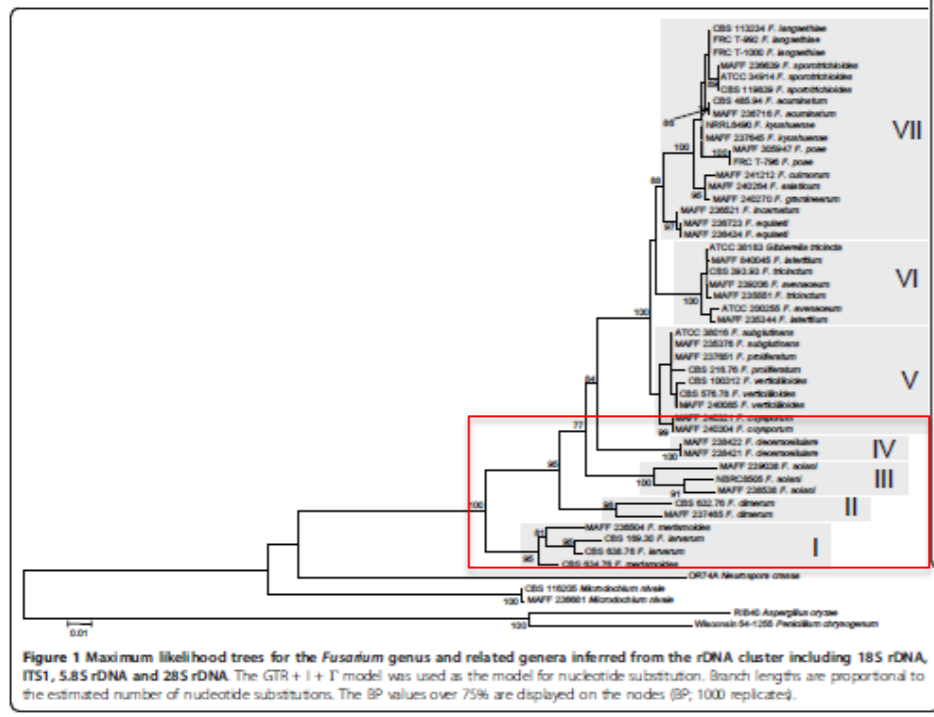


Figure 2 Maximum likelihood trees for the *Fusarium* genus and related genera inferred from β -*tub*. The GTR + I + Γ model was used as the model for nucleotide substitution. Branch lengths are proportional to the estimated number of nucleotide substitutions. Each codon position was analysed separately. The BP values over 75% are displayed on the nodes (BP, 1000 replicates).

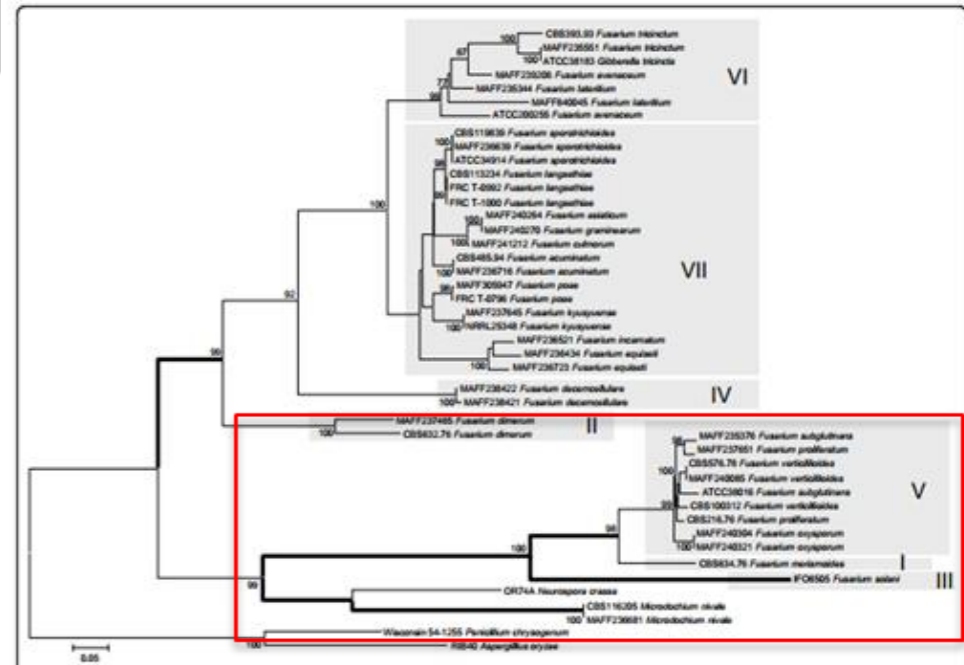
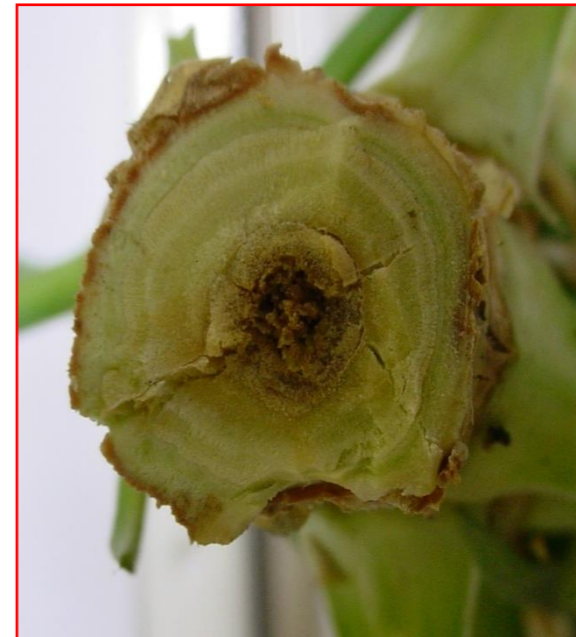


Figure 4 Maximum likelihood trees for the *Fusarium* genus and related genera inferred from *hys2*. The GTR + I + Γ model was used as the model for nucleotide substitution. Branch lengths are proportional to the estimated number of nucleotide substitutions. Each codon position was analysed separately. The BP values over 75% are displayed on the nodes (BP, 1000 replicates). The branches with bold lines indicate the lineages in which positive selection has occurred with the *p*-value under the null hypothesis that the ω (synonymous substitutions per synonymous site/non-synonymous substitutions per non-synonymous site) of the positively selected sites is equal to 1.0. ($p < 0.001$).

F. oxysporum

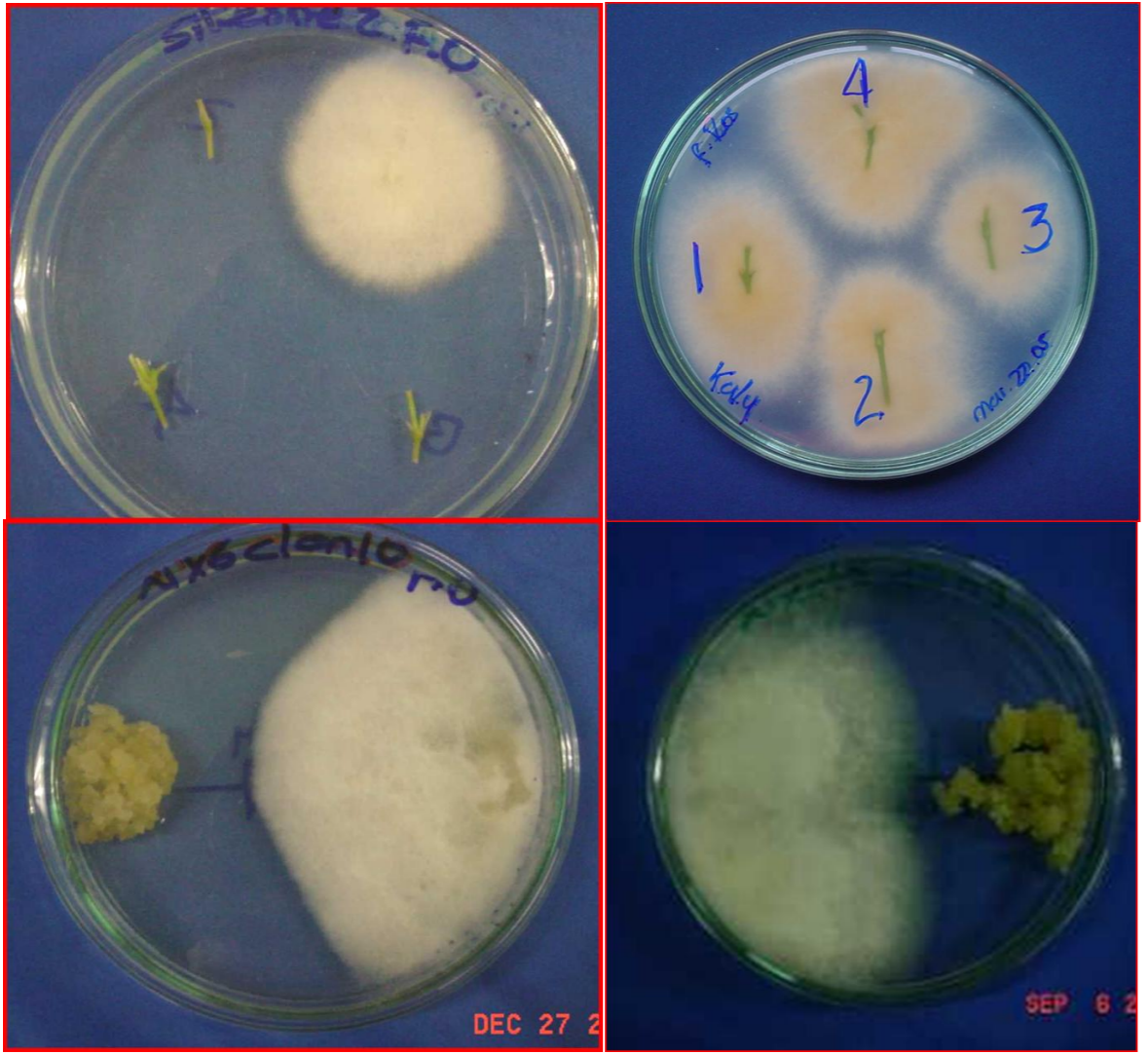


F. verticillioides

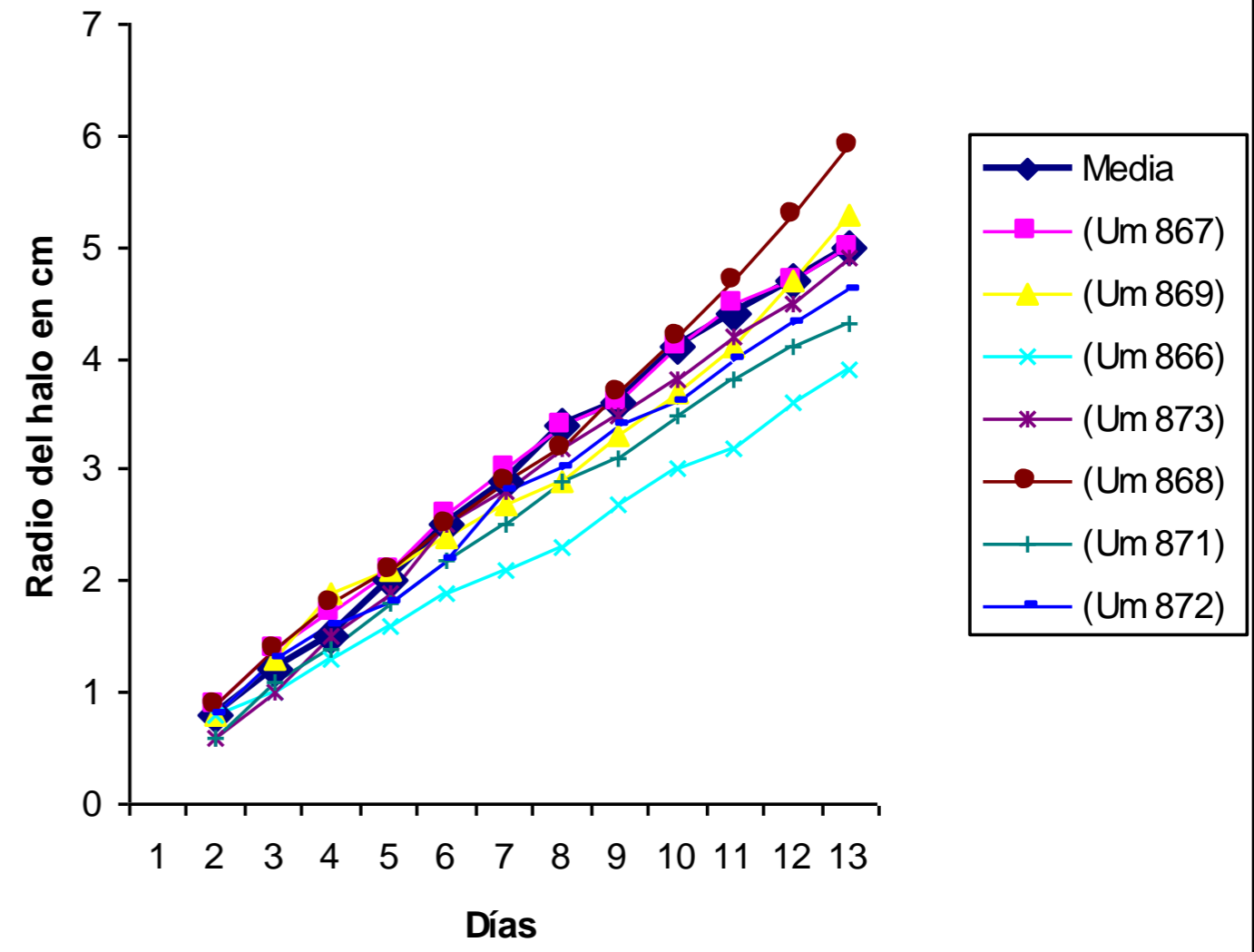


***F. verticillioides* - CARNATION INTERACTION IN COLOMBIA**

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 Department of Science, Biotechnology Laboratory, Military University "Nueva Granada",
 Bogotá - Colombia * jfilgdu@umng.edu.co



Resultado ensayo dual callos de los clones de la línea híbrida 8 frente a *Fusarium verticillioides*



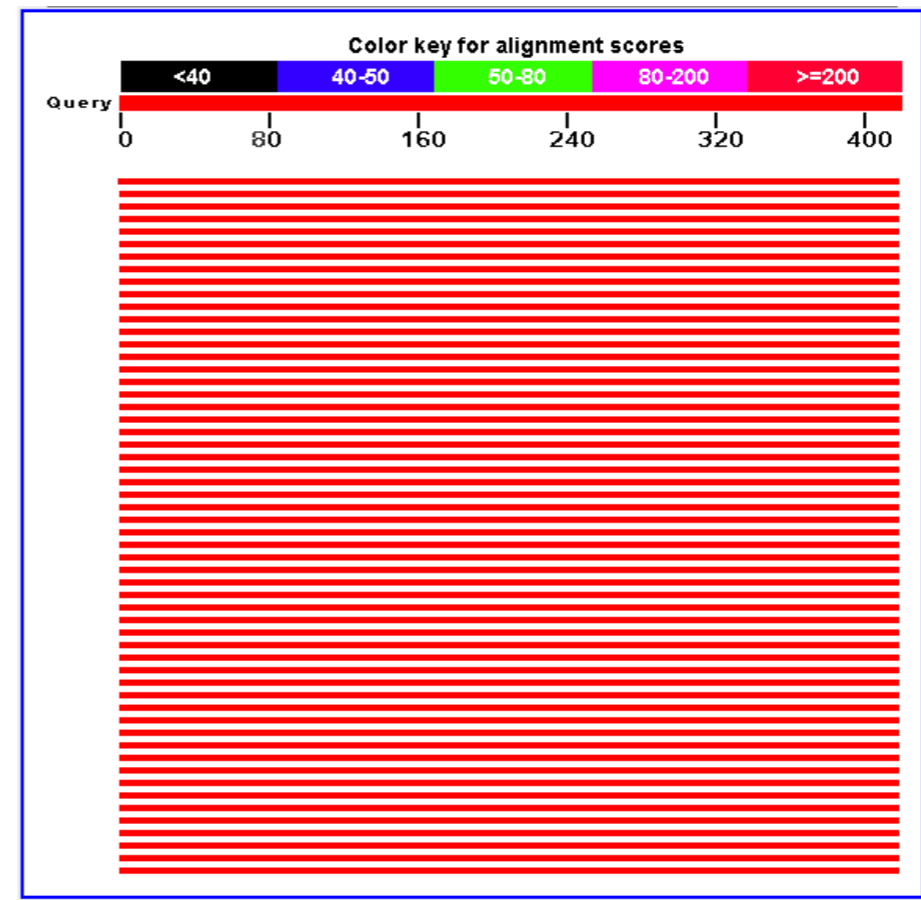
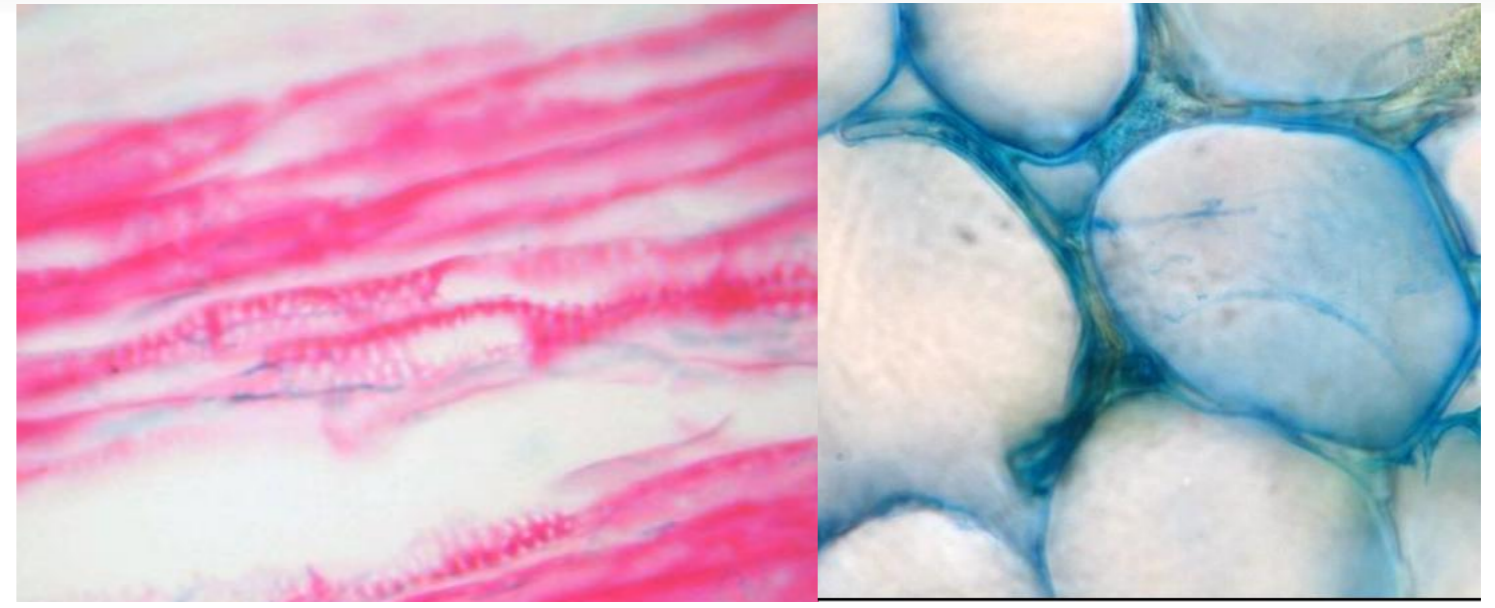
ESTUDIO DE *Fusarium foetens* ASOCIADO A LA PUDRICIÓN BASAL EN CLAVEL (*Dianthus caryophyllus* L.) VARIEDAD NELSON

BURAGLIA OSORIO, Guillermo Andrés- FILGUEIRA
DUARTE, Juan Jose

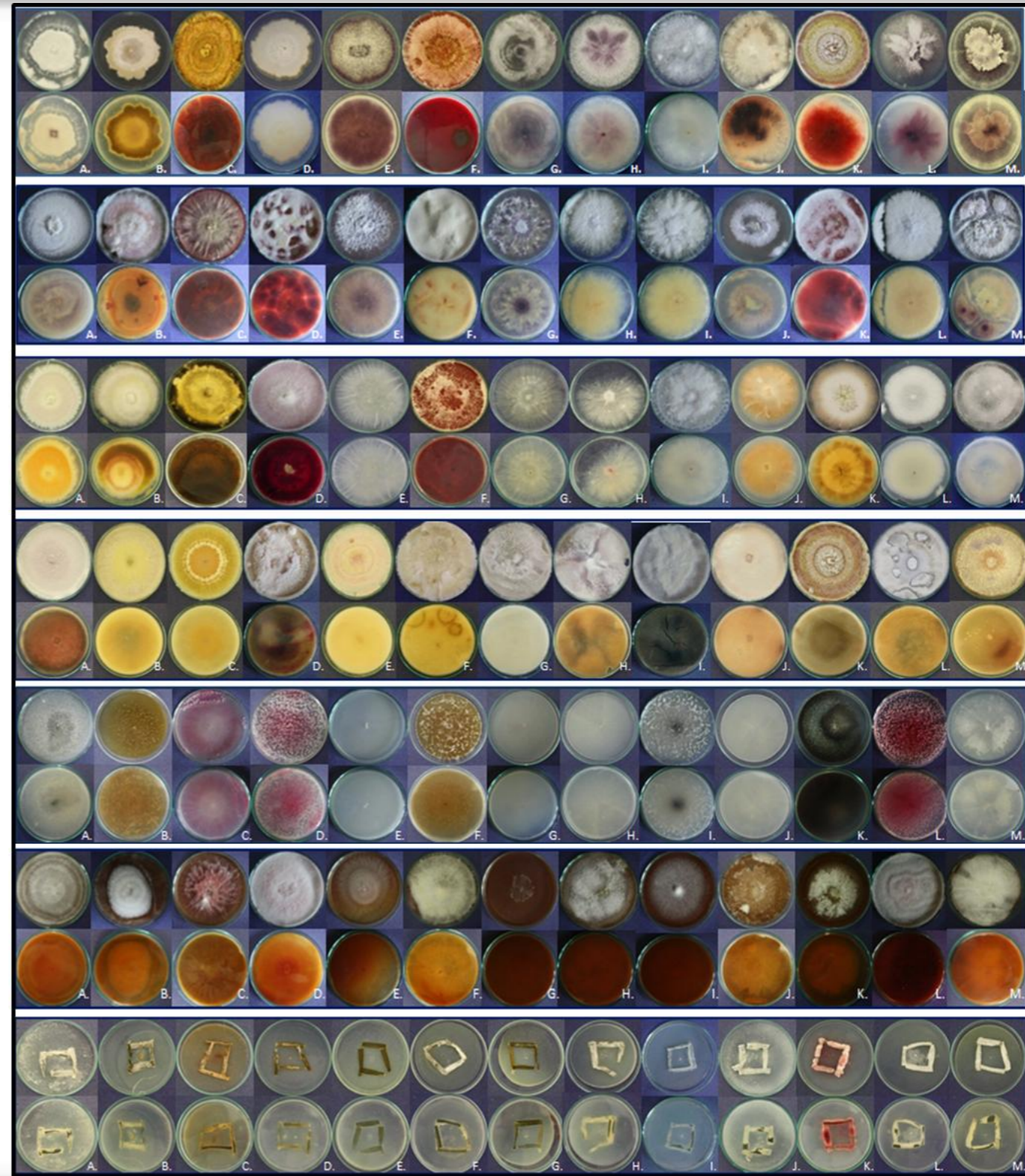
Grupo de Fitopatología Molecular, Universidad Militar Nueva
Granada – Colombia

muin24@g

du.co



13 Fusarium Species 7 different culture media



Uso de microsecuencias de genes útiles para hacer filogenia molecular para resolver problemas de clasificación taxonómica en el género *Fusarium*. Cindy Melissa Rincón. Universidad Nacional de Colombia. Maestría en Microbiología, Bogotá Colombia.



ID. No.	MACROCONIDIA			MICROCONIDIA		
	Forma	Apical	Pie	Forma	Fialide	Clamidosporas
<i>Fusarium anthophilum</i>	delgadas, poca curvatura, pared delgada	Curvada	Definido	piriforme, globosa, ovoide	si	no
<i>Fusarium avenaceum</i>	largas y delgadas	Larga	con una muesca	fusoide	si	no
<i>Fusarium culmorum</i>	Robusta	redondeada y roma	no definido	ausente	no	si
<i>Fusarium equiseti</i>	largas y delgadas	ahusada y elongada	Elongado	elipsoidal	si	si
<i>Fusarium foetens</i>	Falcada	Curvada	Redondeado	ovoide, elipsoidal	si	si
<i>Fusarium graminearum</i>	delgadas, pared gruesa, longitud media	Hoz	Definido	ausente	no	si
<i>Fusarium oxysporum</i>	recta o levemente curvada, relativamente delgada	ahusada y curvada	Definido	ovoide, elipsoidal, arriñonada	si	si
<i>Fusarium proliferatum</i>	delgadas, relativamente rectas, pared delgada	Curvada	poco definido	claviforme	si	no
<i>Fusarium solani</i>	relativamente anchas, rectas, gruesas y robustas	redondeada y roma	Redondeado	ovoide, elipsoidal, fusiforme	si	si
<i>Fusarium sp.</i>	largas y delgadas	Curvada	Definido	ovaladas	no	no
<i>Fusarium sporotrichioides</i>	Luna	curvada y ahusada	con una muesca	piriforme, elipsoidal, ovoide	si	si
<i>Fusarium subglutinans</i>	relativamente delgadas, pared delgada	Curvada	poco definido	ovoide	si	no
<i>Fusarium verticillioides</i>	largas y delgadas	Curvada	Definido	ovaladas	no	no

Uso de microsecuencias de genes útiles para hacer filogenia molecular para resolver problemas de clasificación taxonómica en el género *Fusarium*. Cindy Melissa Rincón. Universidad Nacional de Colombia. Maestría en Microbiología, Bogotá Colombia.



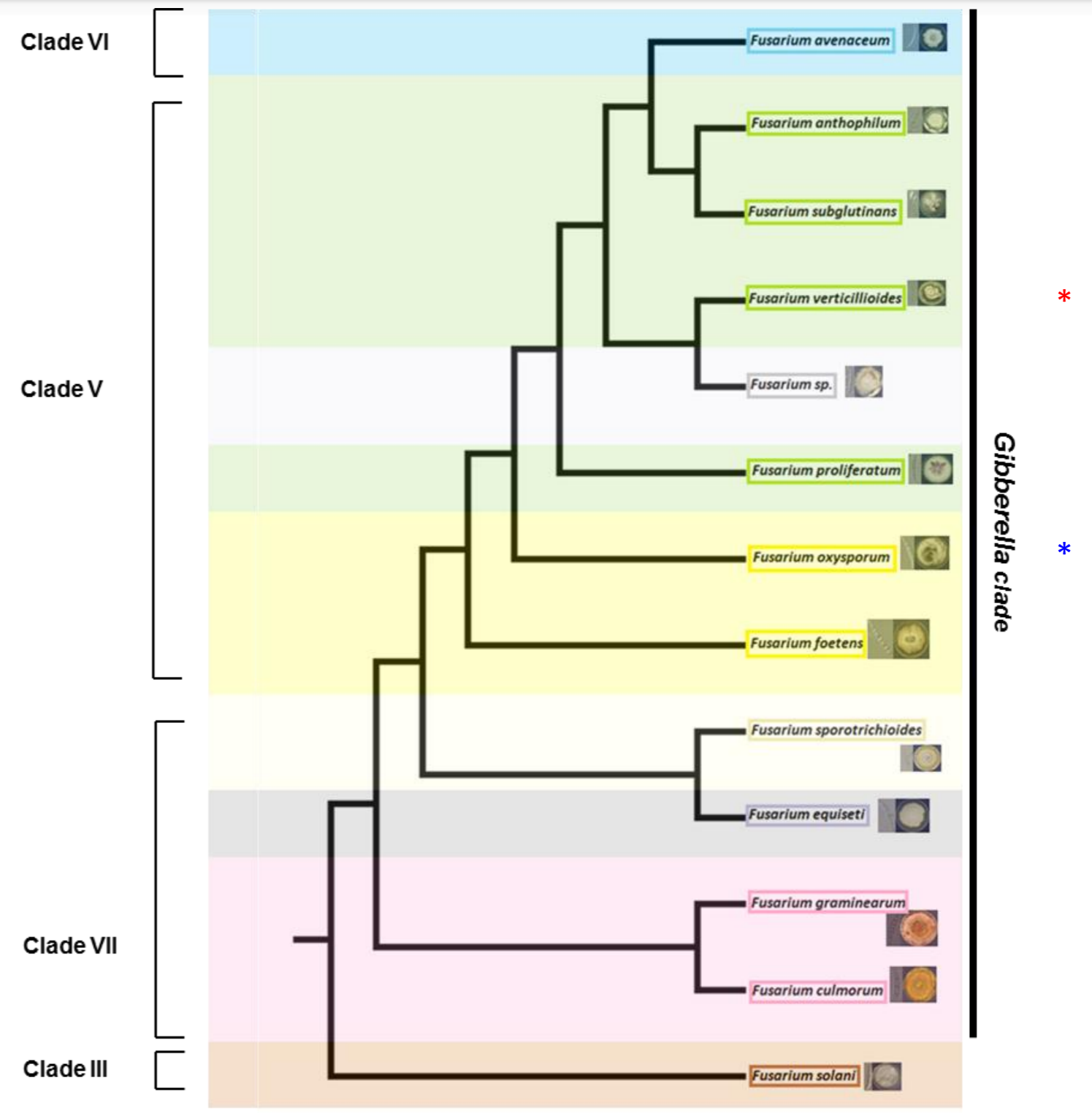
Especie	Área (μm)	Perímetro (μm)	Longitud eje mayor (μm)	Longitud eje menor (μm)
<i>Fusarium anthophilum</i>	59	524	216,5	34,5
<i>Fusarium avenaceum</i>	156	982	329	60
<i>Fusarium culmorum</i>	204	953	377	69
<i>Fusarium equiseti</i>	96	860	277	44
<i>Fusarium foetens</i>	163	1216	411	51
<i>Fusarium graminearum</i>	184,7	970	364,7	64,7
<i>Fusarium oxysporum</i>	123	779,5	290,5	53,75
<i>Fusarium proliferatum</i>	81,3	646	272	38
<i>Fusarium solani</i>	109	687,5	272,5	51
<i>Fusarium sp.</i>	104,5	807	324	41,5
<i>Fusarium sporotrichioides</i>	-	-	-	-
<i>Fusarium subglutinans</i>	53	462,5	217	31
<i>Fusarium verticillioides</i>	101	911	363,5	36

Uso de microsecuencias de genes útiles para hacer filogenia molecular para resolver problemas de clasificación taxonómica en el género *Fusarium*. Cindy Melissa Rincón. Universidad Nacional de Colombia. Maestría en Microbiología, Bogotá Colombia.

Character matrix built
with Mesquite software
V.2.5.

Taxon \ Character																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
	Macroconidia-abundant	Macroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present	Meroconidia-absent	Meroconidia-present
1 *Fusarium_anthophilum	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1	
2 *Fusarium_avenaceum	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	1	
3 *Fusarium_culmorum	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	1	0	
4 *Fusarium_equiseti	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	1	0	
5 *Fusarium_foetens	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	1	0	
6 *Fusarium_graminearum	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	1	0	
7 *Fusarium_oxysporum	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	1	0	
8 *Fusarium_proliferatum	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	
9 *Fusarium_solani	1	0	0	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	
10 *Fusarium_sp.	1	0	0	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	
11 *Fusarium_sporotrichioides	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	1	0	
12 *Fusarium_subglutinans	1	0	0	1	1	0	1	0	1	0	1	0	0	1	0	1	0	1	
13 *Fusarium_verticillioides	1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	1	0	1	

Dendrogram of the characters matrix using Mezquite

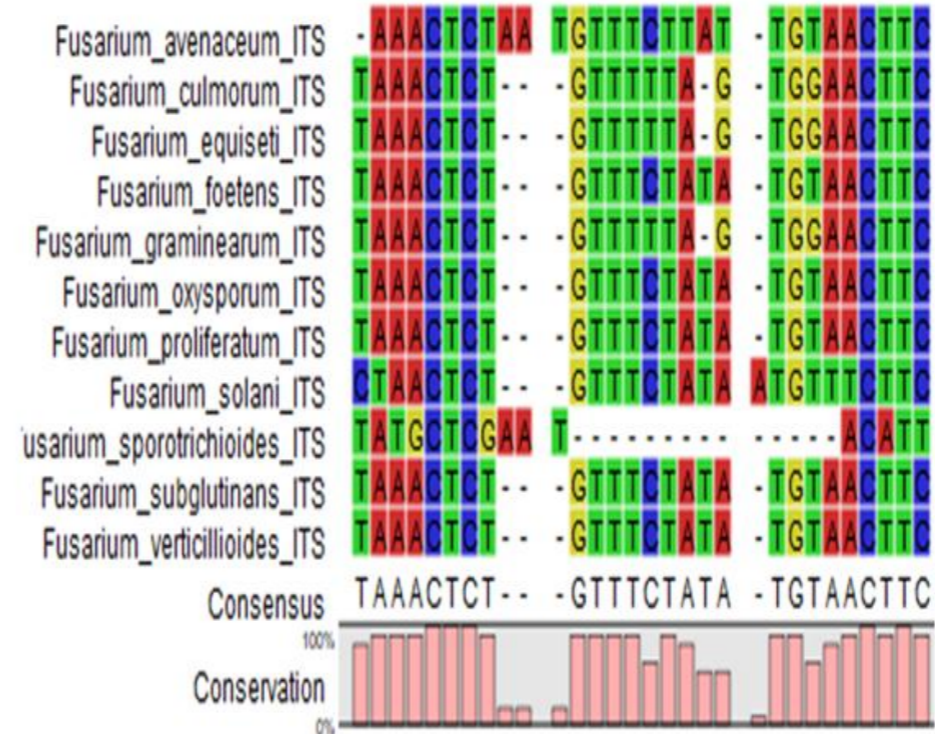
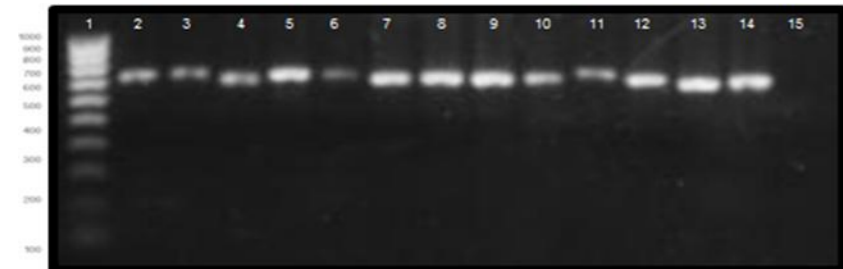
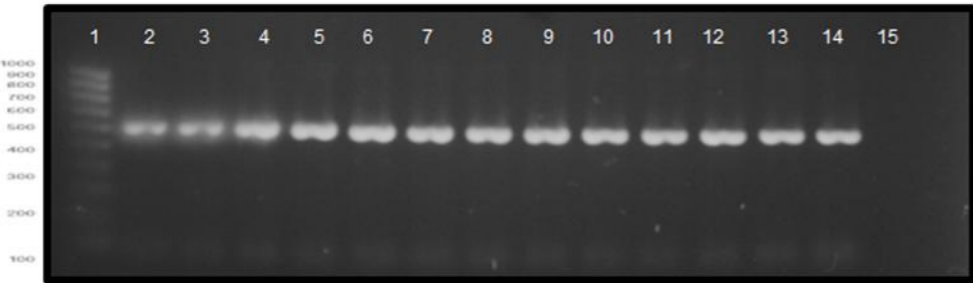
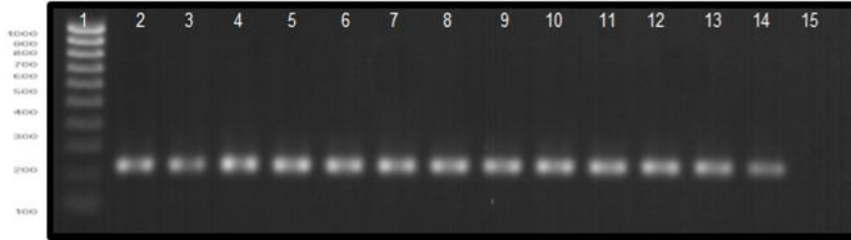
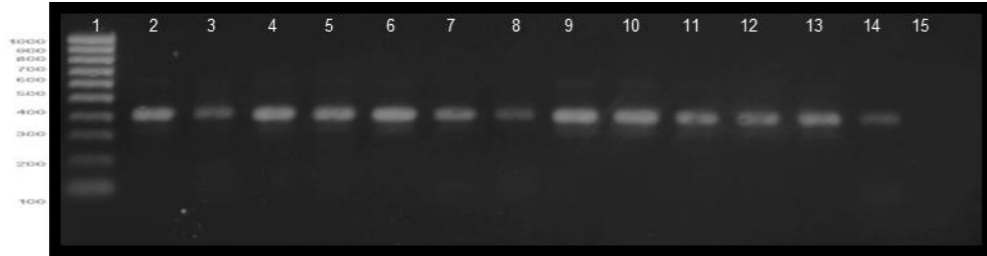


- What is Fusarium?
- Are Nectria, Gibberella, and Fusarium monophyletic?
- Are the sections natural?
- Can a phylogenetic species concept (PSC) be applied, and to what extent is it congruent with morphological and biological species concepts?
- Can sister group relationships of all taxa be resolved?
- How can the DNA database best be used to design molecular tools for the detection, and rapid and accurate identification of all species of Fusarium?

F. verticillioides – Aislamiento Sopo

Sequences producing significant alignments:

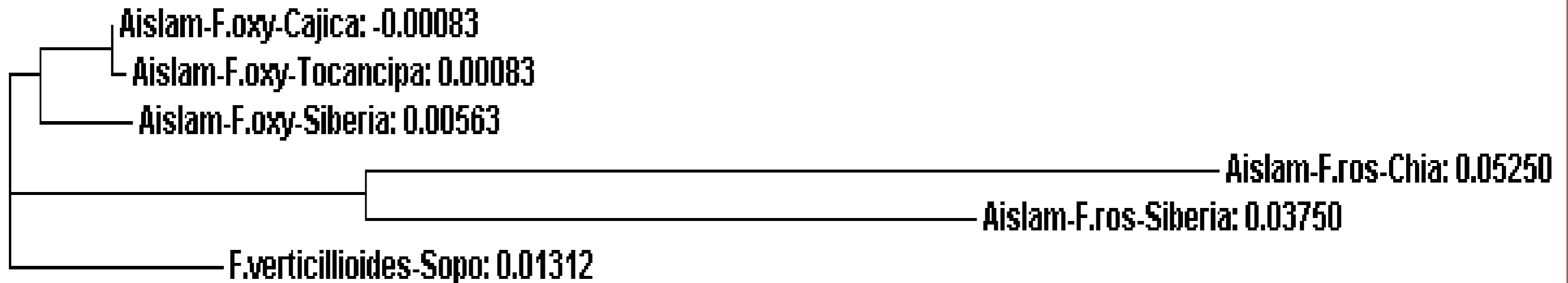
Sequence ID	Organism	Score (Bits)	E Value
gi 90855308 emb AM162679.1 	Uncultured Fusarium sp. 18S rRNA ...	696	0.0
gi 55793450 gb AY662326.1 	Gibberella moniliformis strain KSU...	696	0.0
gi 21666881 gb AF455450.1 	Gibberella sacchari isolate wb395 ...	696	0.0
gi 20531669 gb AF502862.1 	Leaf litter ascomycete strain its3...	696	0.0
gi 3320362 gb U61688.1 FSU61688	Fusarium sp. NRRL25195	696	0.0
gi 45479262 gb AY533376.1 	Gibberella moniliformis small subu...	696	0.0
gi 83272594 gb DQ297569.1 	Fusarium subglutinans isolate ZMS-...	696	0.0
gi 83272593 gb DQ297568.1 	Fusarium subglutinans isolate ZMS-...	696	0.0
gi 83272592 gb DQ297567.1 	Fusarium subglutinans isolate ZMS-...	696	0.0
gi 83272588 gb DQ297563.1 	Fusarium pseudonygamai isolate ZMS...	696	0.0



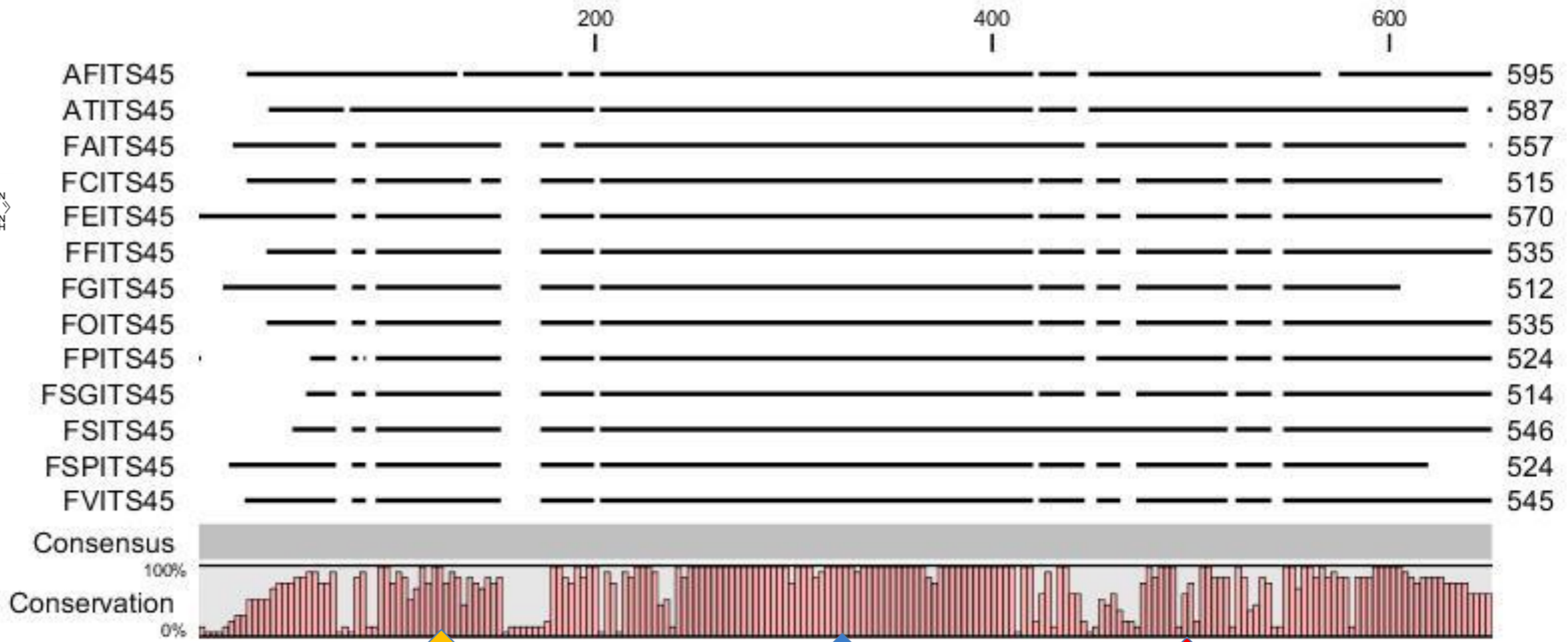
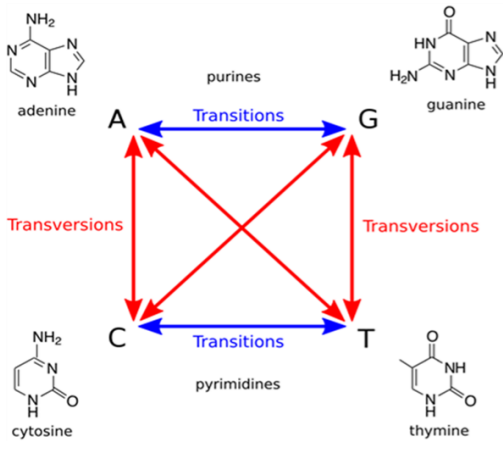
Genetic differences between regions of the same locus



Genetic variations between different FOX isolates in different farms



DNA Micro-Sequences Obtaining



Partially Conserved

Conserved

No Conserved

Calculate the Sequence variation (Sv)

$$Sv = \frac{GAPs (0.5) + Tv (0.375) + Ti (0.125)}{(GAPs + Tv + Ti)}$$

Tv = Transversions Ti = Transitions

MicSeq

Select file

Select Calculate

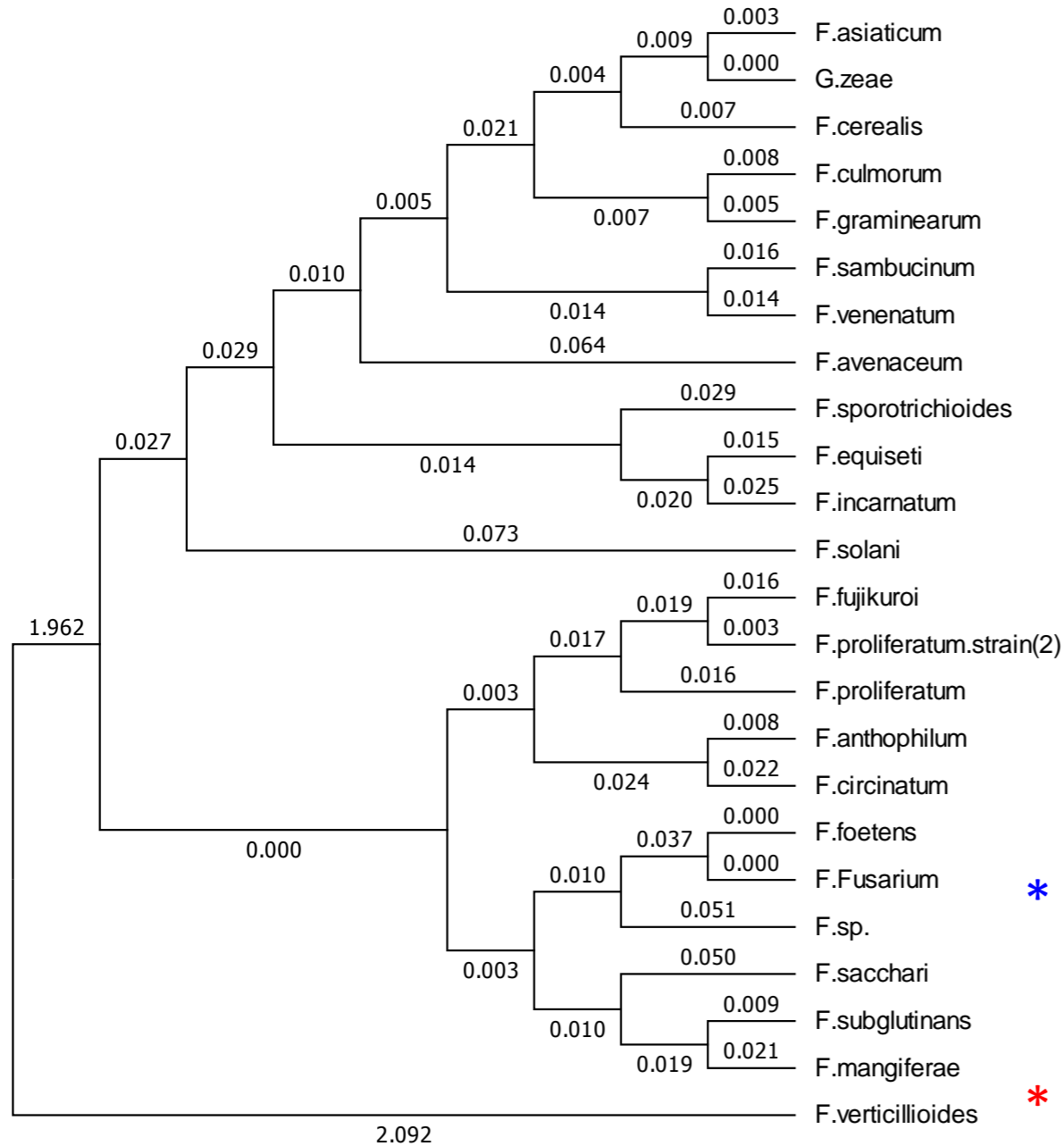
Result

Microsequence	Initial position	Final position	Transitions	Transversions	Indels	Ci index
1	31	100	45	8	0	0.01760204...
2	154	166	10	10	0	0.05494505...
3	217	229	10	2	0	0.02197802...
4	235	289	37	7	0	0.01883116...
5	337	349	8	3	0	0.02335164...
6	355	383	14	8	0	0.02339901...

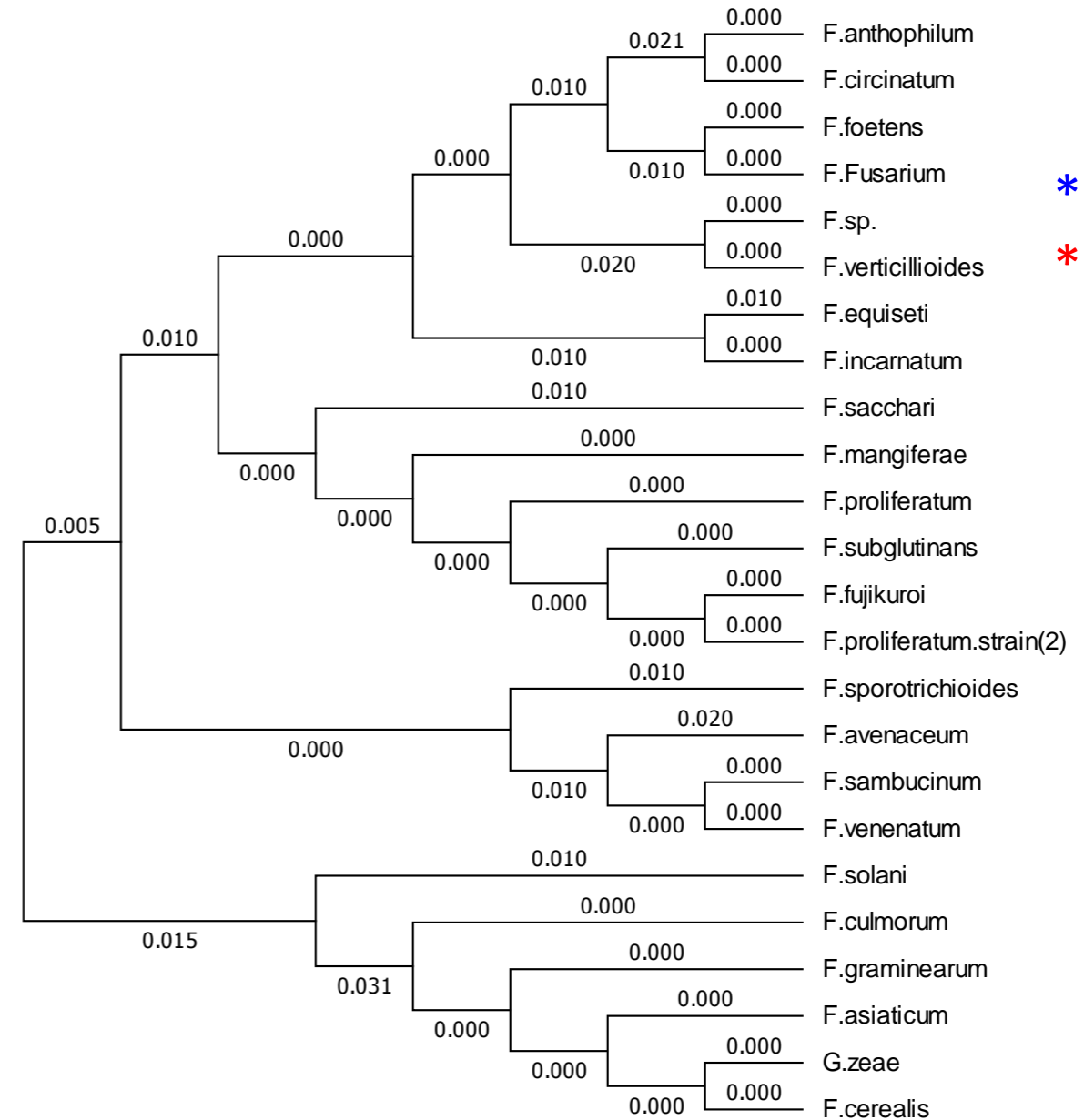
Export

Molecular Phylogenetic analysis by Maximum Likelihood method

H3 amplicon

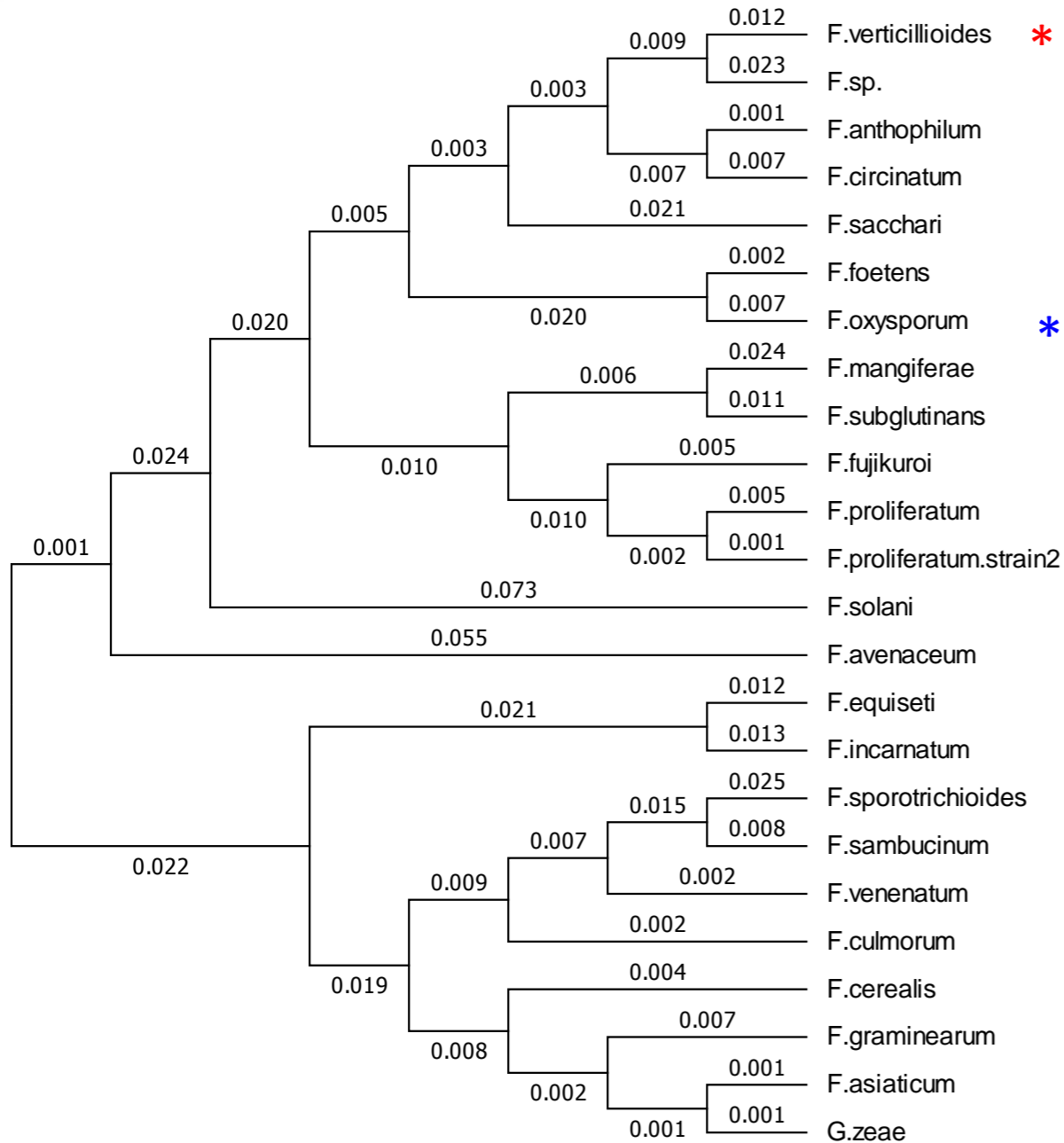


H3 MicroSeq

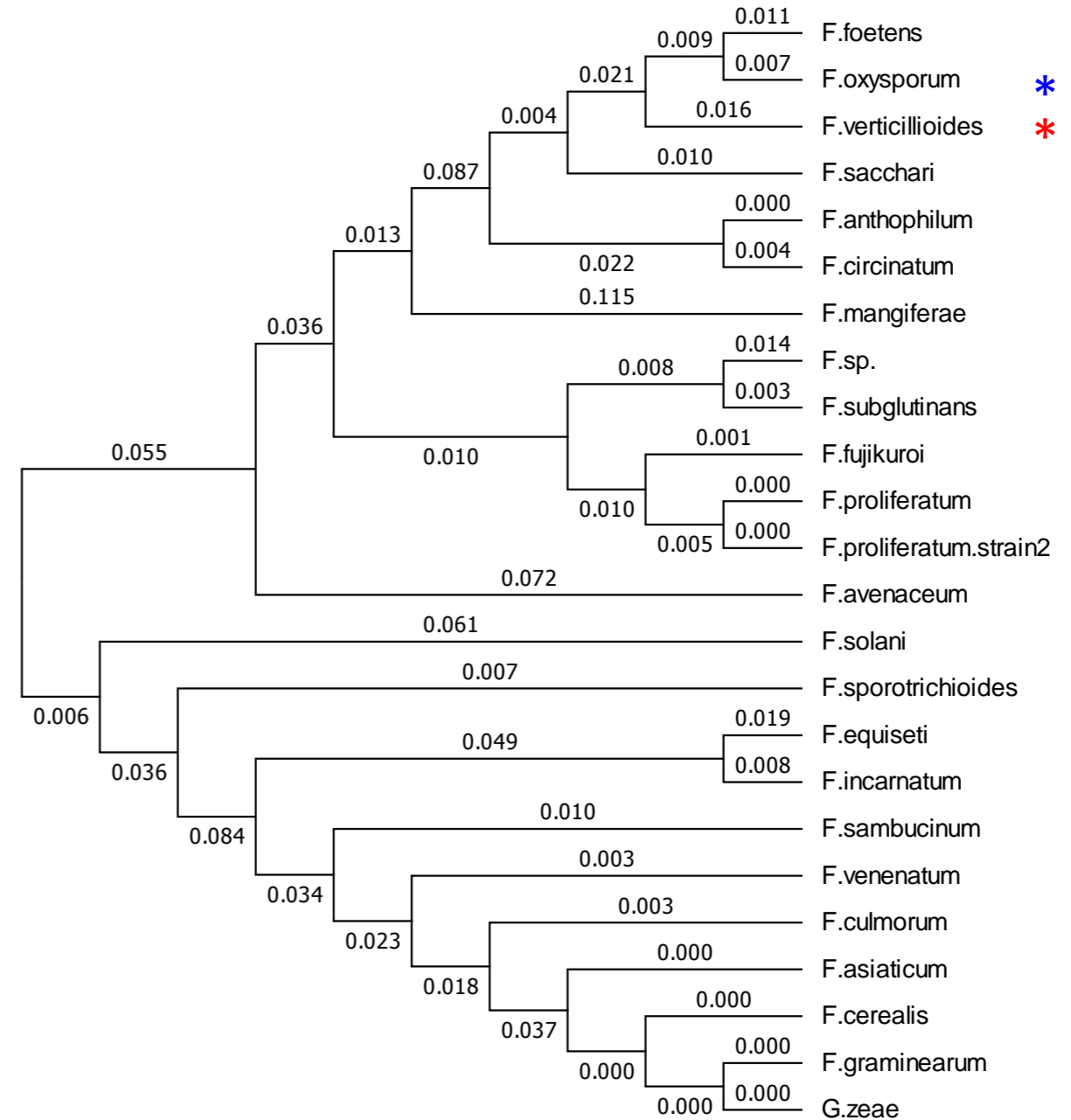


Molecular Phylogenetic analysis by Maximum Likelihood method

EF-1 H3 mtSSU ITS 2-4 amplicon



EF-1 H3 mtSSU ITS 2-4 MicroSeq



Bt1a/Bt1b

Bt2a/Bt2b

Ahy-FuF/AHyFuR

EF-1H/EF-2T

H3-1a/H3-1b

H4-1a/H4-1b

ITS FuR/ITS FuF

ITS-1/ITS-2

ITS-1/ITS-4

ITS-4/ITS-5



Bt2a/Bt2b

Bt1a/Bt1b

Ahy-FuF/AHyFuR

EF-1H/EF-2T

H3-1a/H3-1b

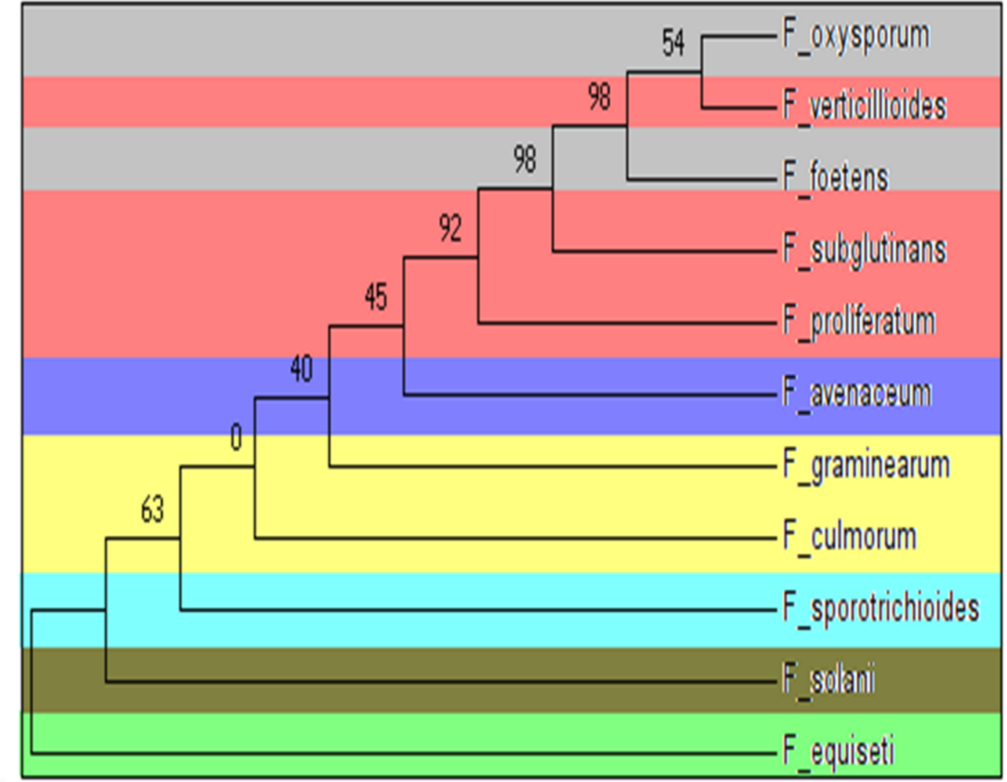
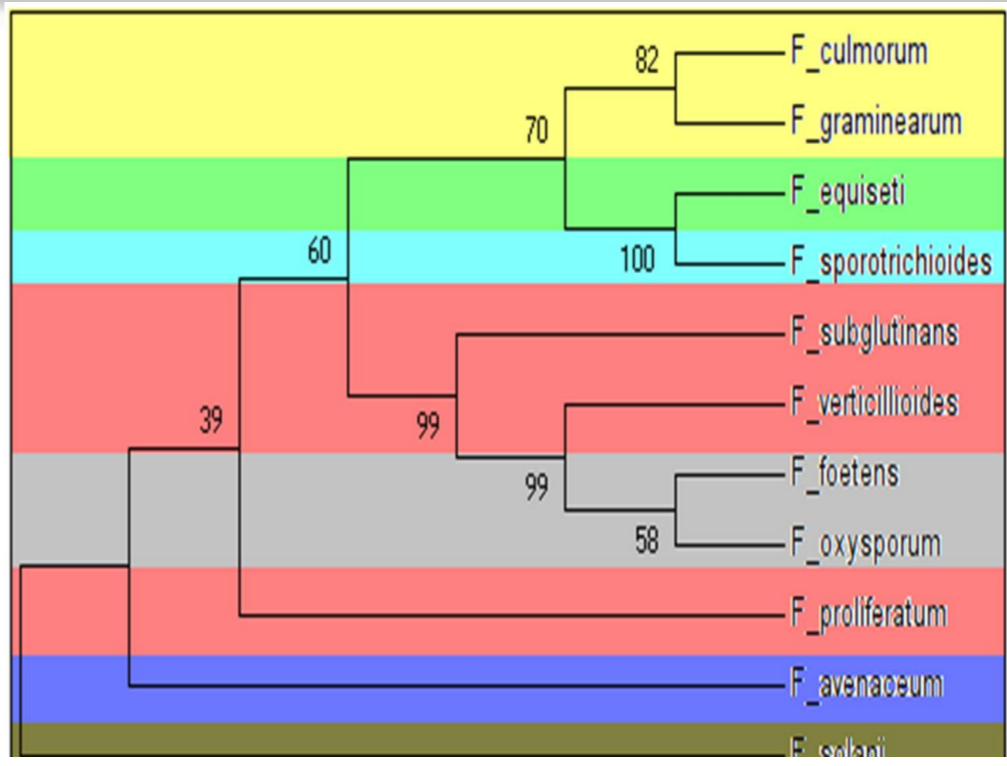
H4-1a/H4-1b

ITS FuR/ITS FuF

ITS-1/ITS-2

ITS-1/ITS-4

ITS-4/ITS-5



Neighbour-joining Distance Matrices

Monogen

Genes	Especies	EF-1	H3	ITS	mtSSU	Promedio
	Ver/Foe	0,026	3,838	0,000	0,006	0,967
	Ver/Oxy	0,032	3,838	0,004	0,006	0,970
	Foe/Oxy	0,032	0,000	0,004	0,000	0,009
	Pro/Strain	0,005	0,035	0,000	0,006	0,012
	Pro/Fuji	0,018	0,049	0,000	0,008	0,019
	Strain/Fuji	0,018	0,019	0,000	0,002	0,010
	Sacha/Mang	0,026	0,069	0,312	0,002	0,102

Genes	Especies	EF-1	H3	ITS	mtSSU	Promedio
	Ver/Foe	0,018	0,040	0,000	0,021	0,020
	Ver/Oxy	0,018	0,040	0,011	0,021	0,023
	Foe/Oxy	0,018	0,000	0,011	0,000	0,007
	Pro/Strain	0,000	0,000	0,000	0,021	0,005
	Pro/Fuji	0,009	0,000	0,000	0,029	0,009
	Strain/Fuji	0,009	0,000	0,000	0,007	0,004
	Sacha/Mang	0,037	0,010	1,144	0,014	0,301

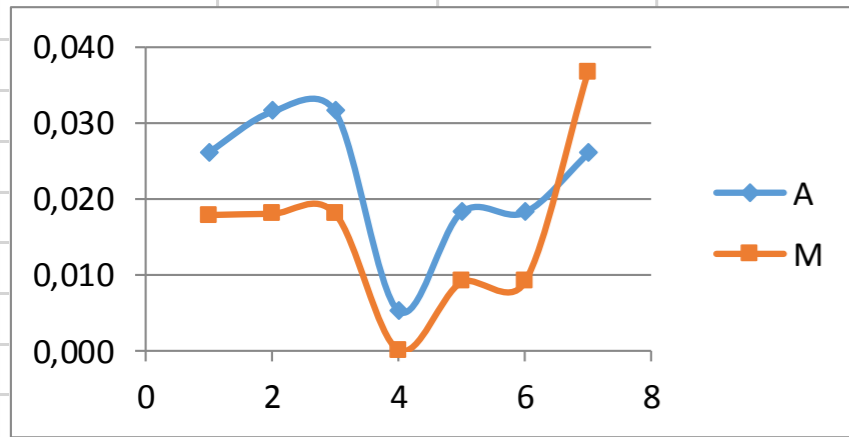
Poligen

Genes	Especies	EF-1+H3	EF+H3+mtSSU	EF+H3+mtSSU+ITS	Promedios
	Ver/Foe	0,018	0,045	0,036	0,033
	Ver/Oxy	0,073	0,047	0,040	0,053
	Foe/Oxy	0,014	0,009	0,009	0,011
	Pro/Strain	0,010	0,009	0,006	0,009
	Pro/Fuji	0,022	0,019	0,012	0,017
	Strain/Fuji	0,016	0,012	0,009	0,013
	Sacha/Mang	0,050	0,032	0,051	0,044

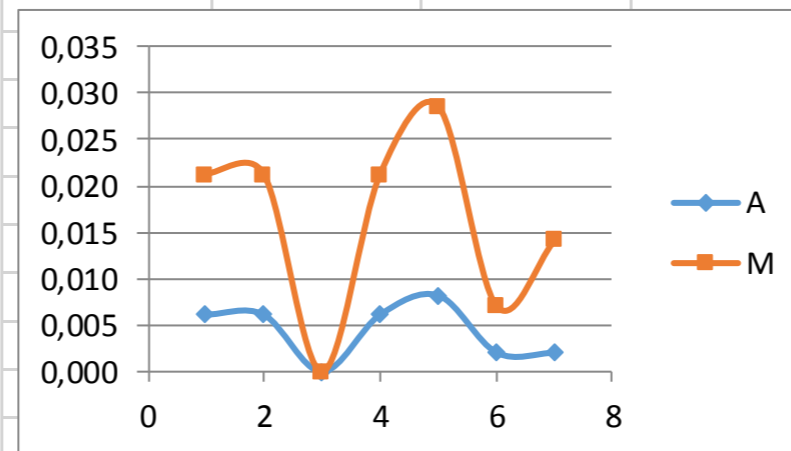
Genes	Especies	EF-1+H3	EF+H3+mtSSU	EF+H3+mtSSU+ITS	Promedios
	Ver/Foe	0,018	0,043	0,032	0,031
	Ver/Oxy	0,014	0,043	0,032	0,030
	Foe/Oxy	0,043	0,014	0,018	0,025
	Pro/Strain	0,000	0,000	0,000	0,000
	Pro/Fuji	0,007	0,007	0,004	0,006
	Strain/Fuji	0,007	0,007	0,004	0,005
	Sacha/Mang	0,043	0,029	0,208	0,093

Differences in gene variability and microsequence

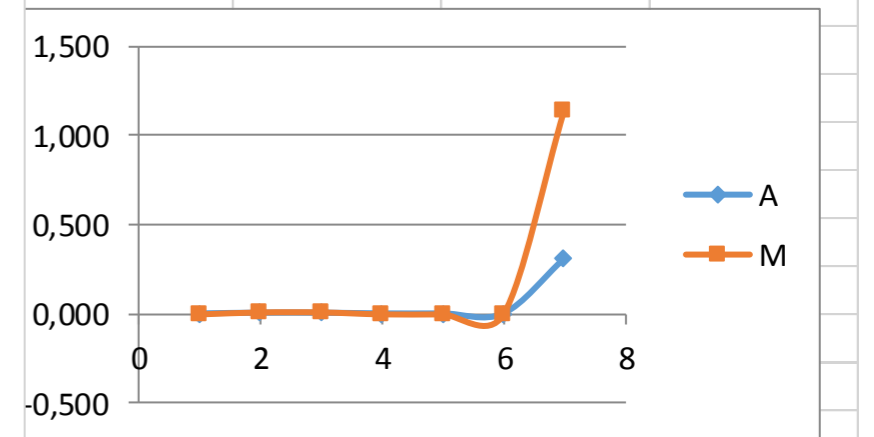
EF-1	A	M
VF	0,026	0,018
VO	0,032	0,018
FO	0,032	0,018
PS	0,005	0,000
PF	0,018	0,009
SF	0,018	0,009
SM	0,026	0,037



mtSSU	A	M
VF	0,006	0,021
VO	0,006	0,021
FO	0,000	0,000
PS	0,006	0,021
PF	0,008	0,029
SF	0,002	0,007
SM	0,002	0,014

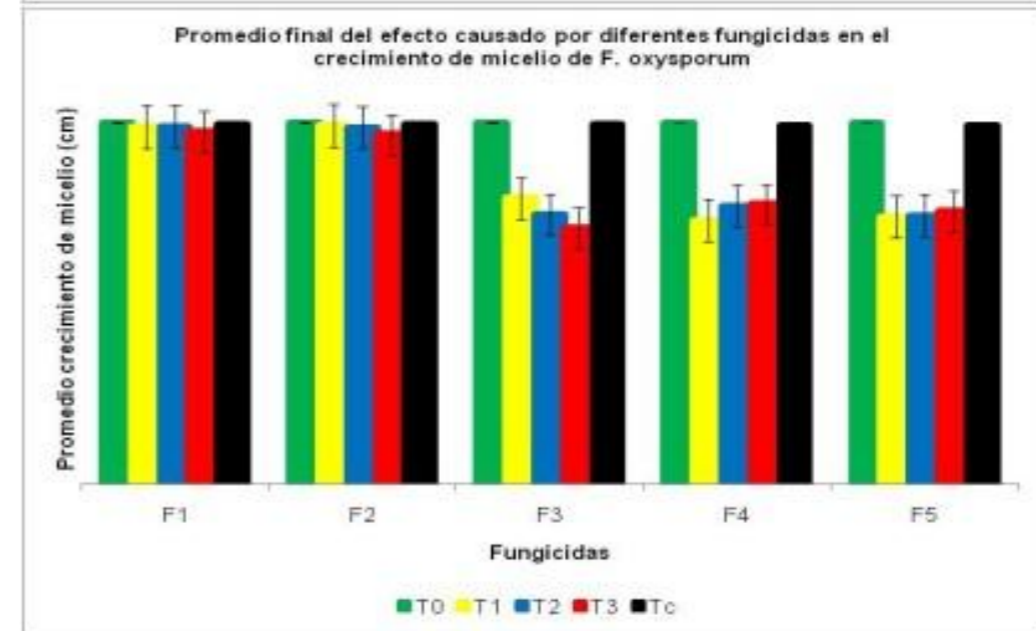
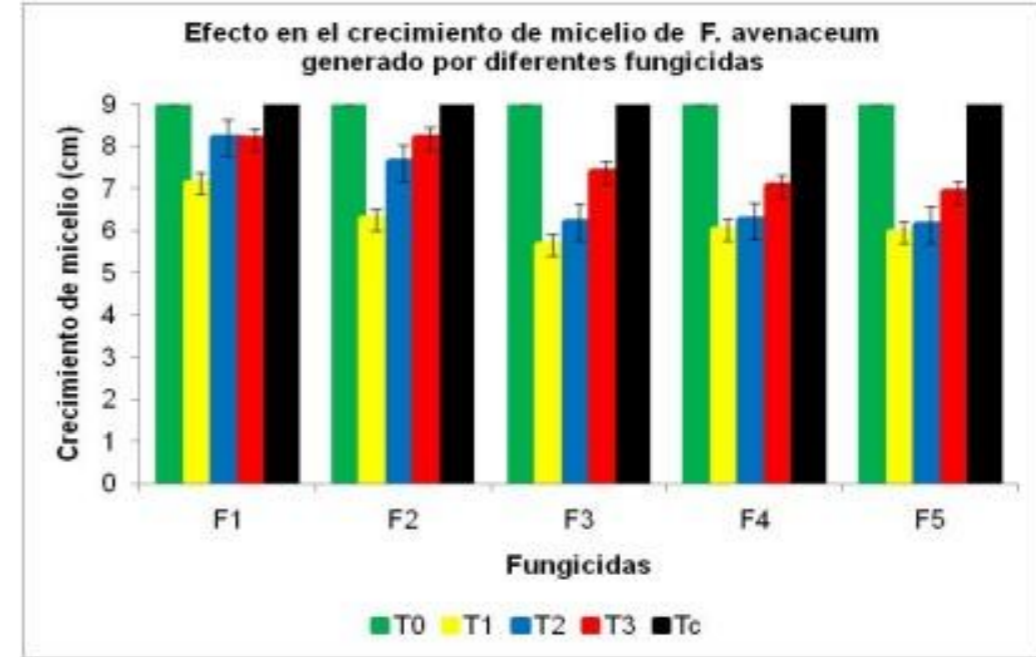
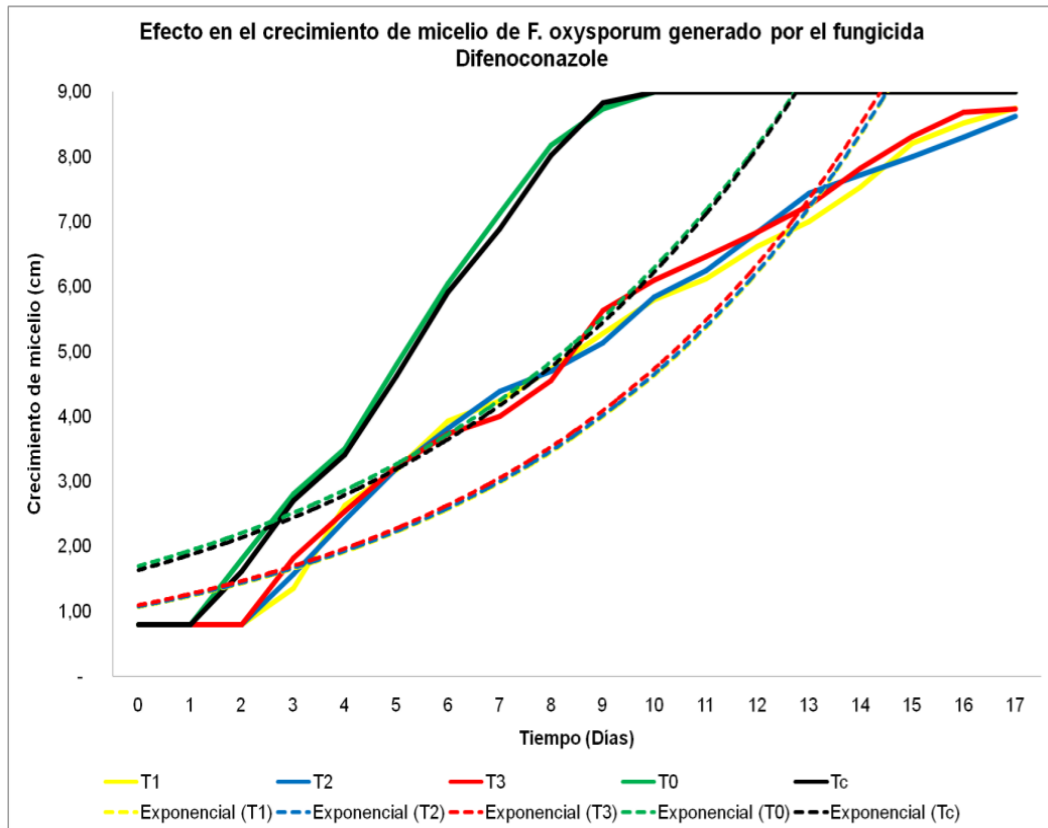
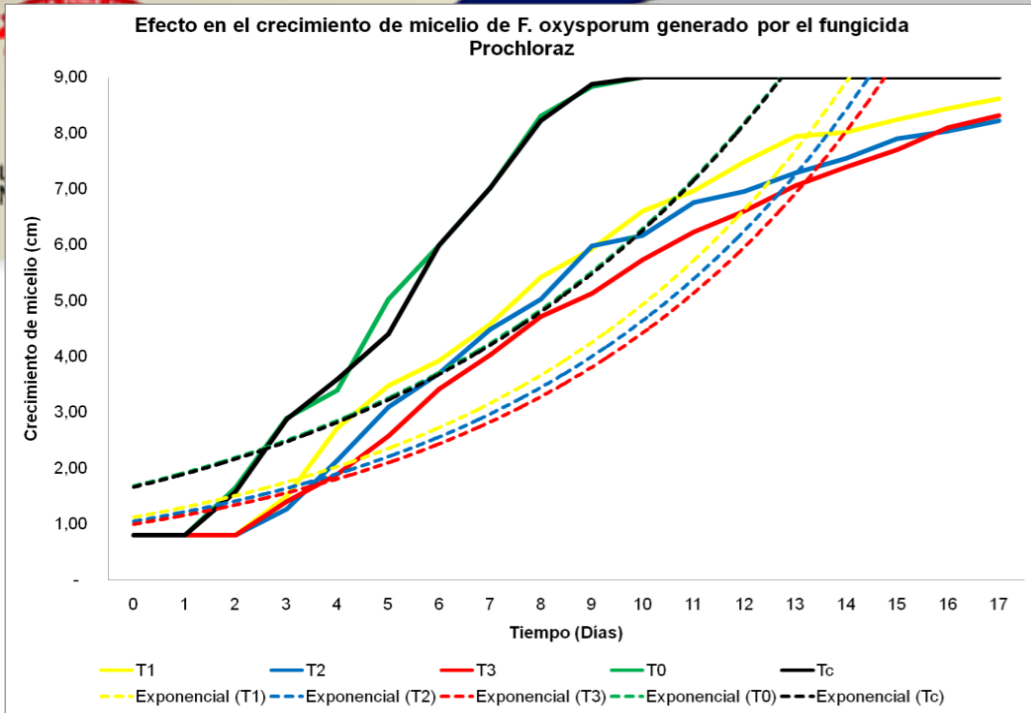


ITS	A	M
VF	0,000	0,000
VO	0,004	0,011
FO	0,004	0,011
PS	0,000	0,000
PF	0,000	0,000
SF	0,000	0,000
SM	0,312	1,144

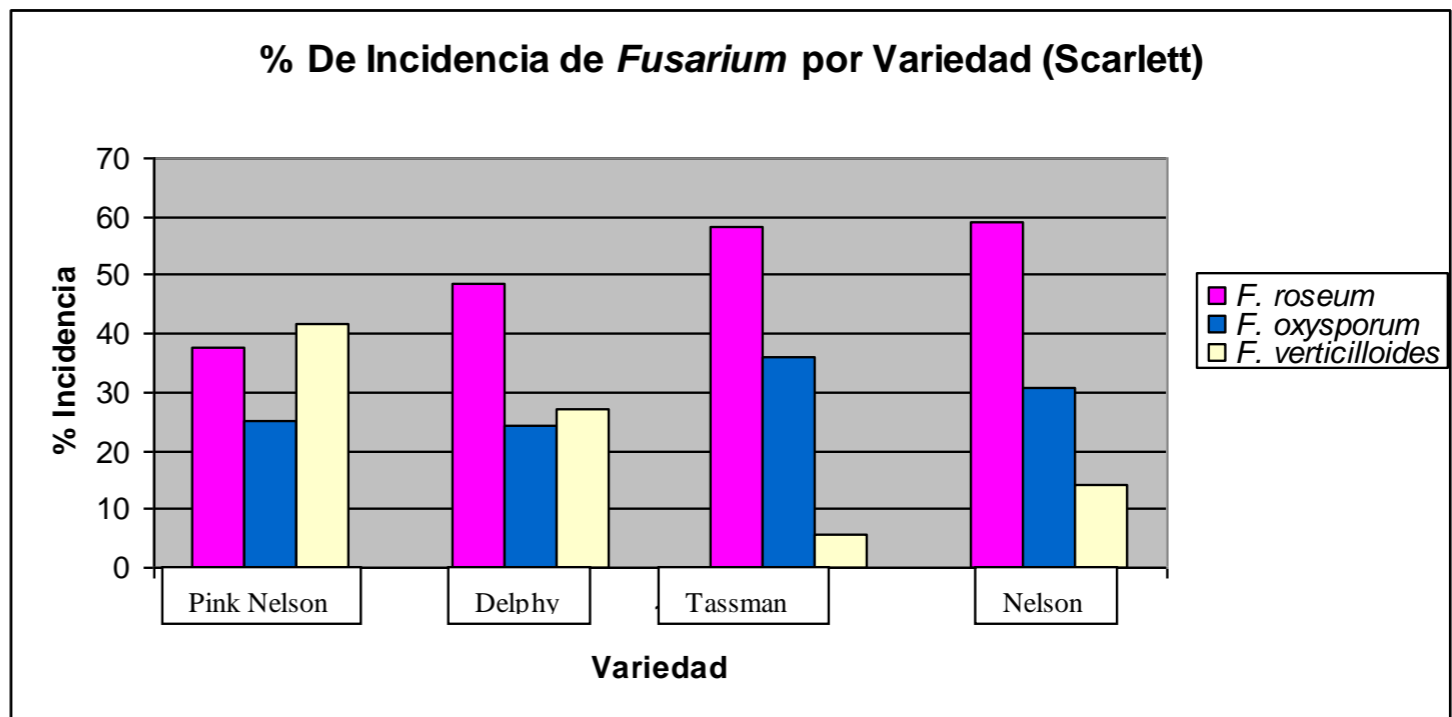
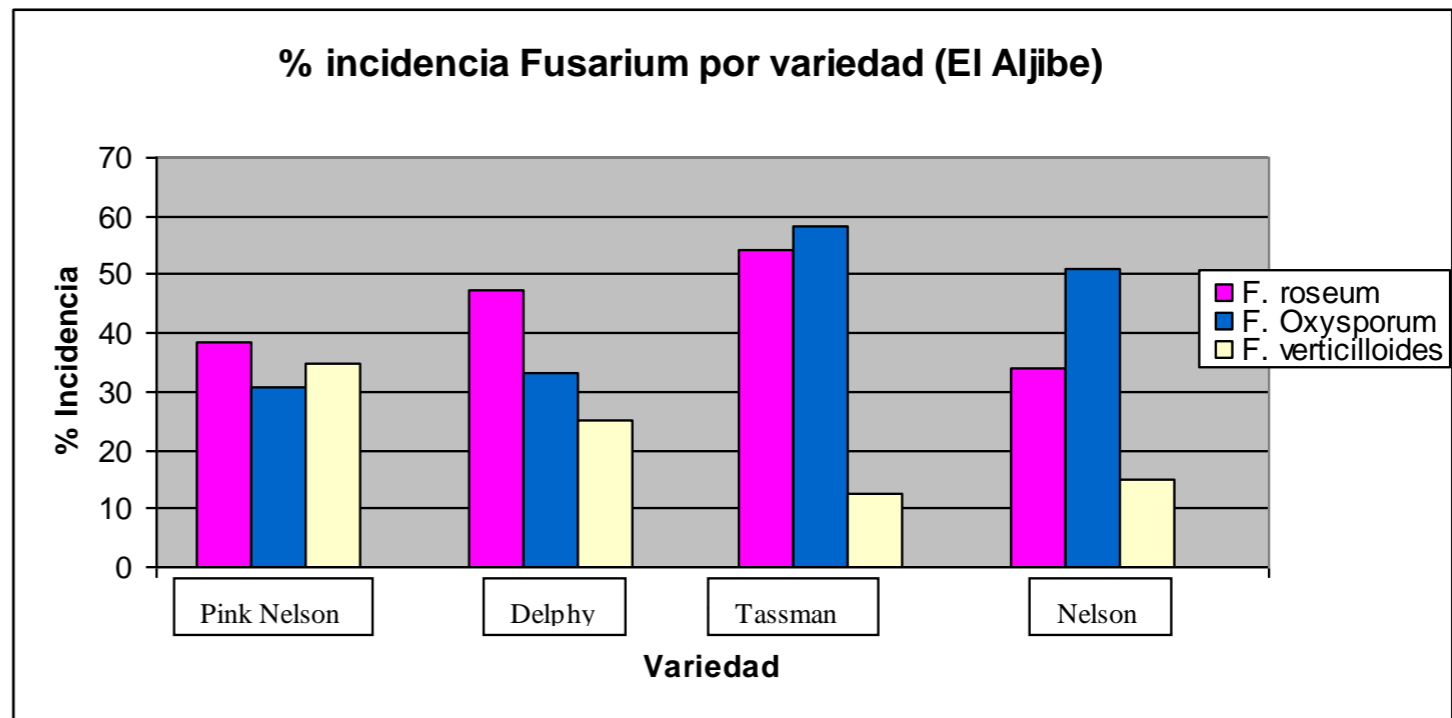


EVALUACIÓN DEL EFECTO DE FUNGICIDAS EN SEIS ESPECIES DE Fusarium PATÓGENOS DEL CLAVEL (*Dianthus caryophyllus*)

TRIANA BARROTE, Diana Lorena ; ARBELAEZ TORRES, Germán; FERNANDEZ DIAZ, Gina Paola;
 FILGUEIRA DUARTE, Juan José
 Programa Biología Aplicada, Universidad Militar Nueva Granada – Colombia
 juan.filgueira@unimilitar.edu.co

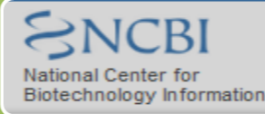


Variation of the incidence of different Fusarium species in different varieties of carnation and different farms.

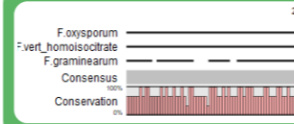


Búsqueda de microsecuencias de ADN útiles para hacer taxonomía y filogenia molecular en genes del género fusarium en bases de datos biológicas.

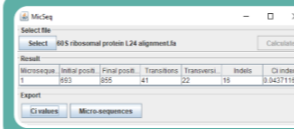
Ángela María Niño Nova., Juan, D. Henao., Daniela Londoño.,
Adrian R. Gómez P., Juan J. Filgueira D.,



Búsqueda de genes ortólogos



Alineamientos de los genes ortólogos



Análisis Cuantitativo de las microsecuencias



Análisis de taxonomía molecular



Análisis de filogenia molecular

Especie	Total genes	Orthologous genes
<i>Fusarium oxysporum</i> f.sp <i>Lycopersisi</i>	27.348	402
<i>Fusarium graminearum</i>	14.144	
<i>Fusarium verticilloides</i>	20.575	

Microsequences in orthologos genes

Nombre	Total Microsecuencias	Promedio de Transiciones	Promedio de Transversiones	Promedio de Indels	Promedio de índice Mi	Promedio de Longitud Microsecuencias (pb)
30S ribosomal protein S12	2	20,5	14,5	5	0,033	100,5
50S ribosomal protein L14	1	20	19	86	0,124	141
Atp-dependent RNA helicase DBP4	6	15,2	9,8	0	0,025	72,8
Chorismate synthase	4	13,5	11,3	3	0,032	73,8
COP9 signalosome complex subuni	3	11	7,7	0	0,025	56
Diphosphomevalonate decarboxyla	1	15	6	0	0,020	69
Diphthamide biosynthesis protei	6	17,7	15	0	0,031	84,5
DNA primase small subunit	2	29,5	13	3	0,021	139,5
Ferrochelataze	2	16	4	0	0,015	73,5
Formyltetrahydrofolate deformyl	1	13	3	0	0,014	66
Histone acetyltransferase type	5	27,2	17,4	0	0,027	114
Homoisocitrate dehydrogenase	2	158,5	144,5	5	0,035	723,5
Ornithine decarboxylase	3	17,7	14	22,7	0,074	88,7
Palmitoyltransferase PFA3	4	14,3	8	1,5	0,028	65,75
Quinate dehydrogenase	4	12,5	13	0	0,030	70,25
Rhomboid protein 2	4	33,8	21,3	19	0,052	141,5
RNA exonuclease 4	4	18,5	10,3	1,5	0,029	82,25
Swr1-complex protein 5	5	20	14,8	8,4	0,045	90,8
Transcription elongation factor	6	14	4,8	2	0,022	66,5
Transcription factor IWS1	3	35,3	30,3	10	0,048	141,3
Transcription initiation factor	1	13	8,0	6	0,042	60

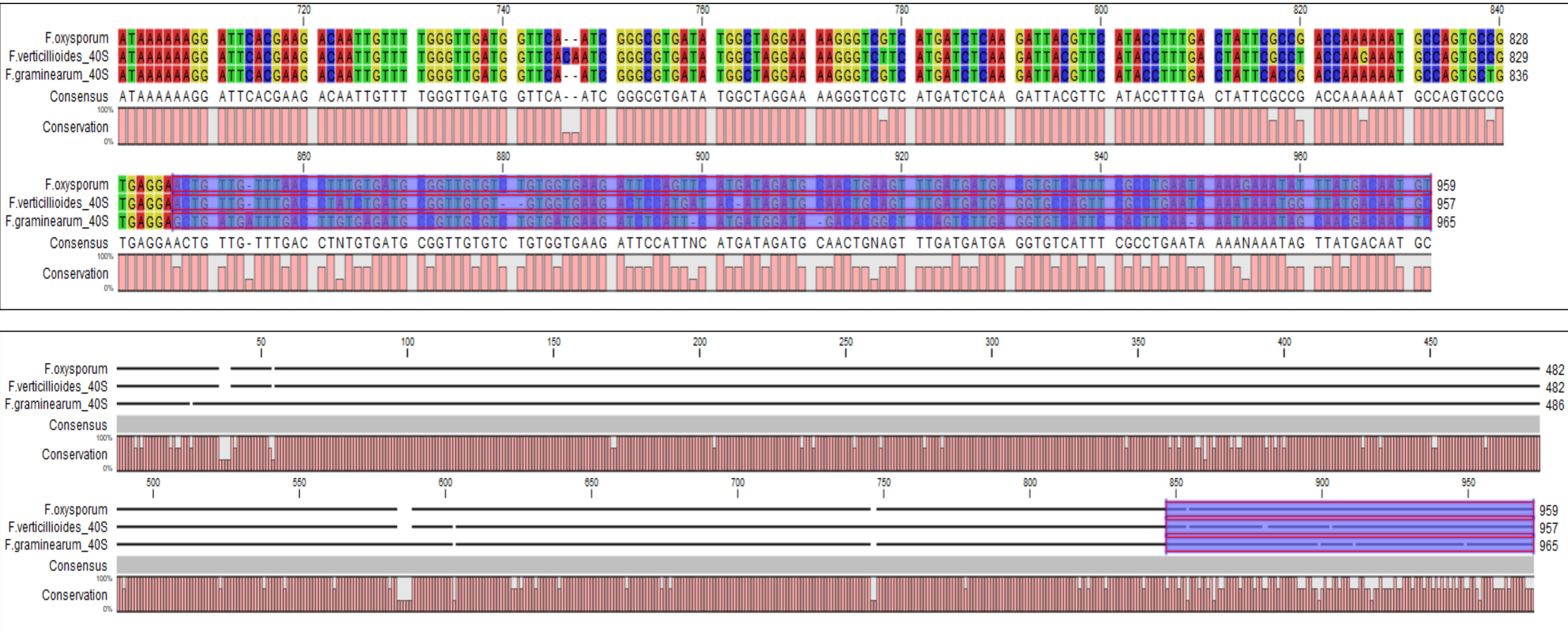
Matrices of distance N-J Microsequences of orthologous genes

	Ferrochelataase	Quinate dehydrogenase	Transcription elongation factor	Diphthamide biosynthesis protein	COP9 signalosome complex subunit 5	Atp-dependent RNA helicase	Histone acetyltransferase type B
F.oxy/F.gram	0,255	0,339	0,449	0,500	0,422	0,457	0,532
F.ver/F.gram	0,300	0,364	0,457	0,565	0,530	0,530	0,604
F.ver/F.oxy	0,064	0,245	0,087	0,155	0,200	0,200	0,155

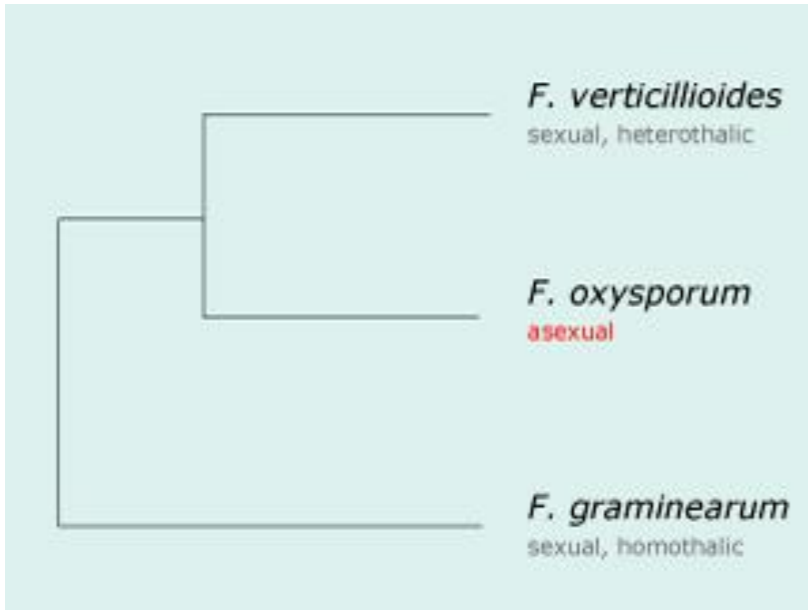
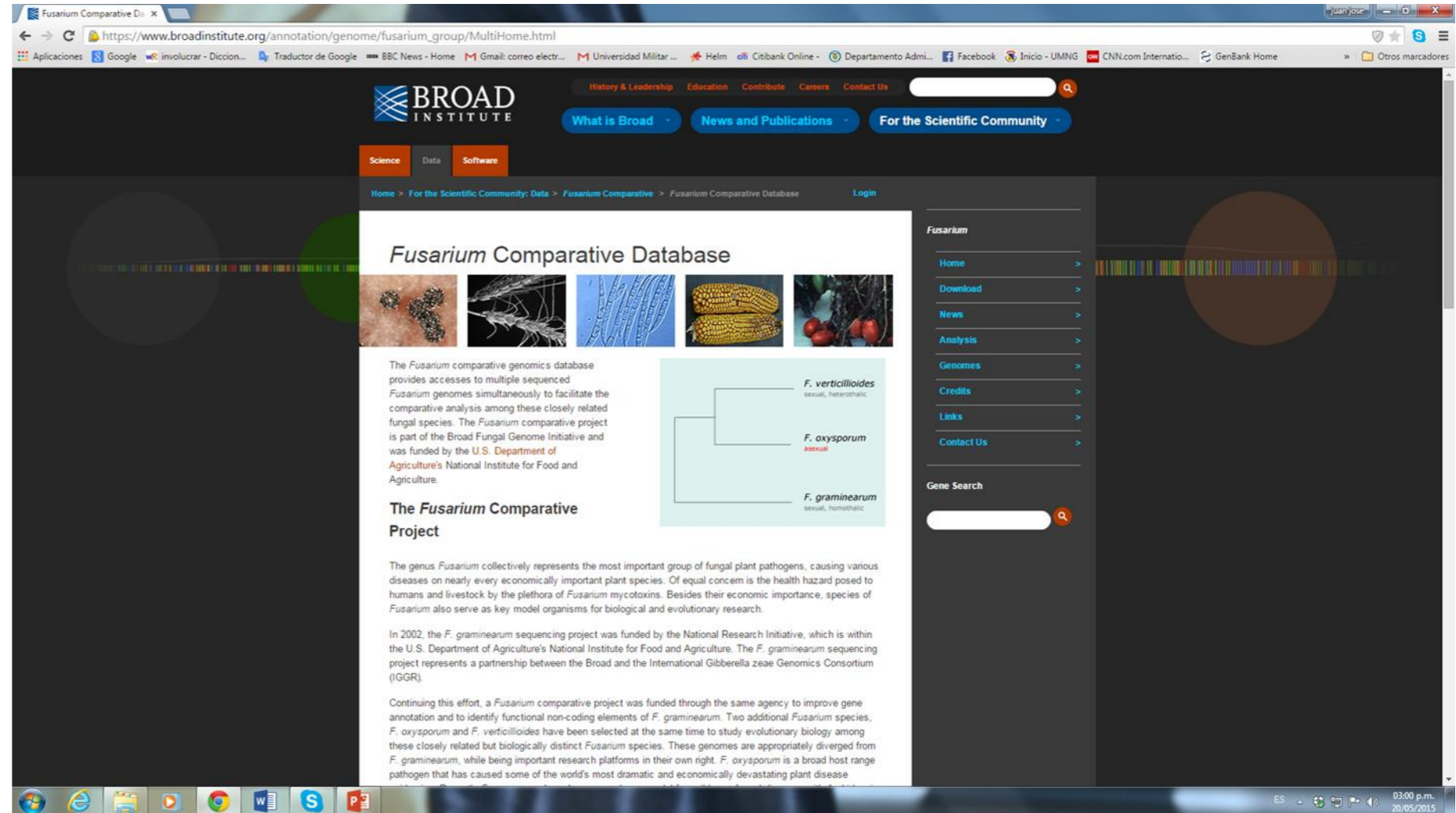
Orthologous genes, grouped in the same way to the species

They present similar distances despite being different genes

Conservation map obtained for the Ribosomal 40S protein gene for 3 species of the genus *Fusarium*.

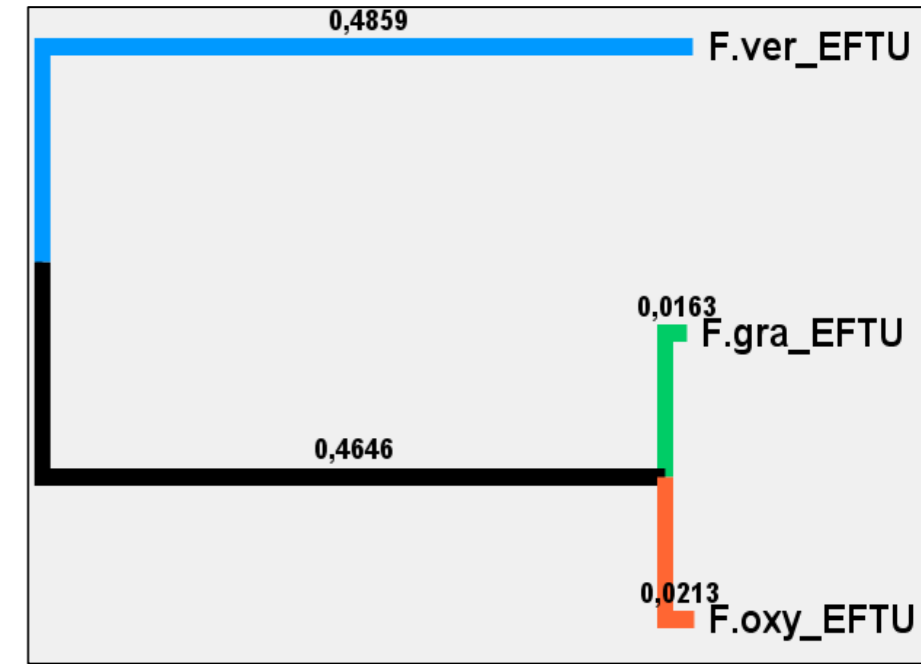
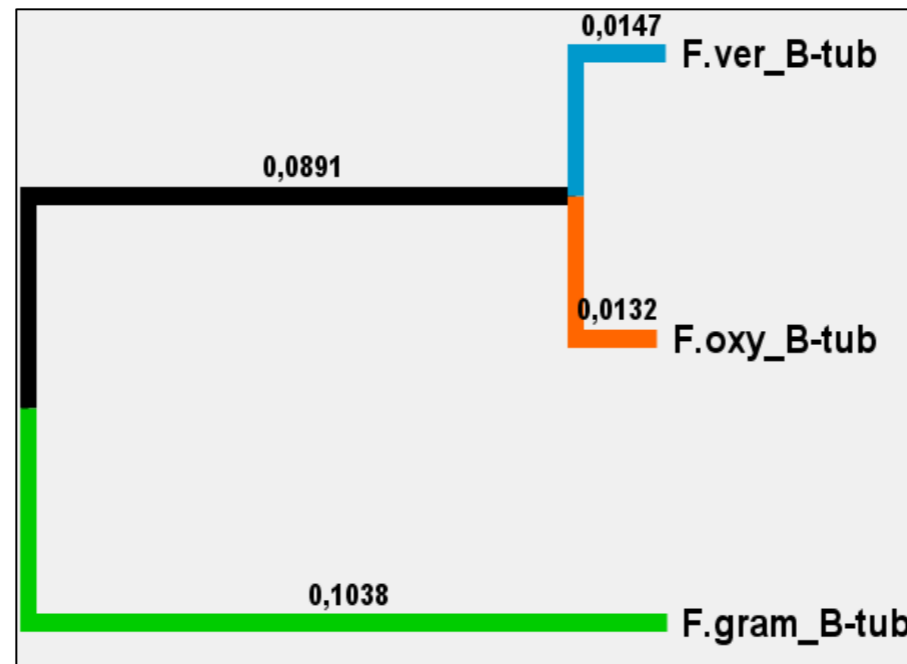
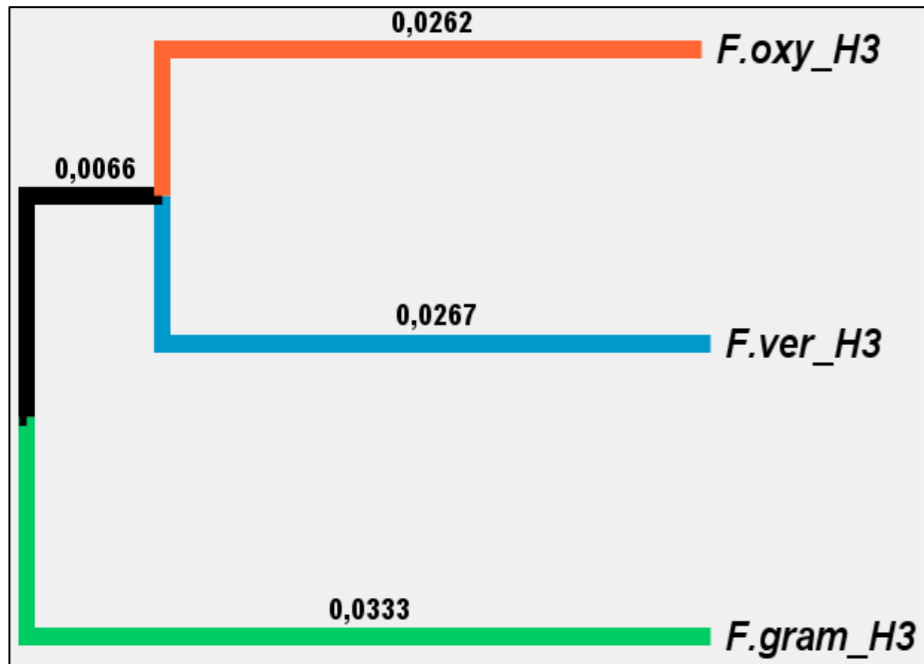


Fusarium Comparative Database

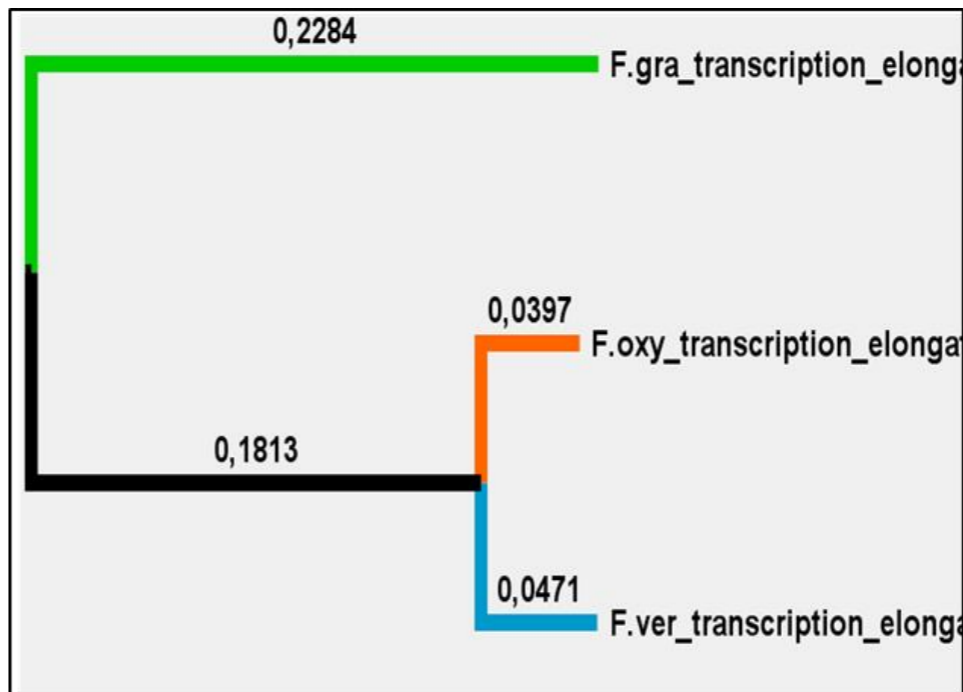
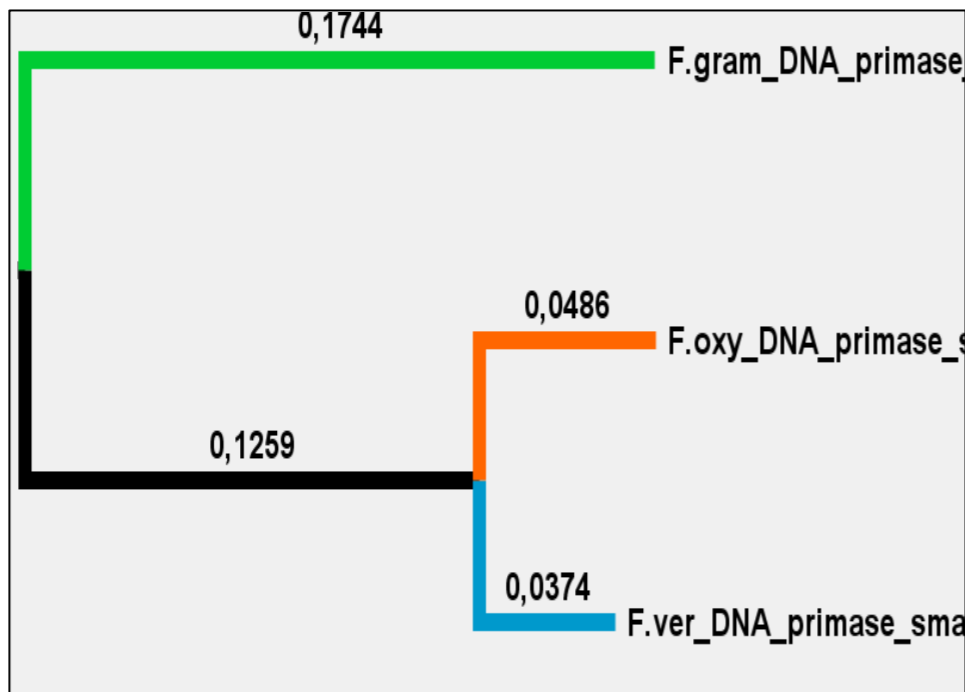
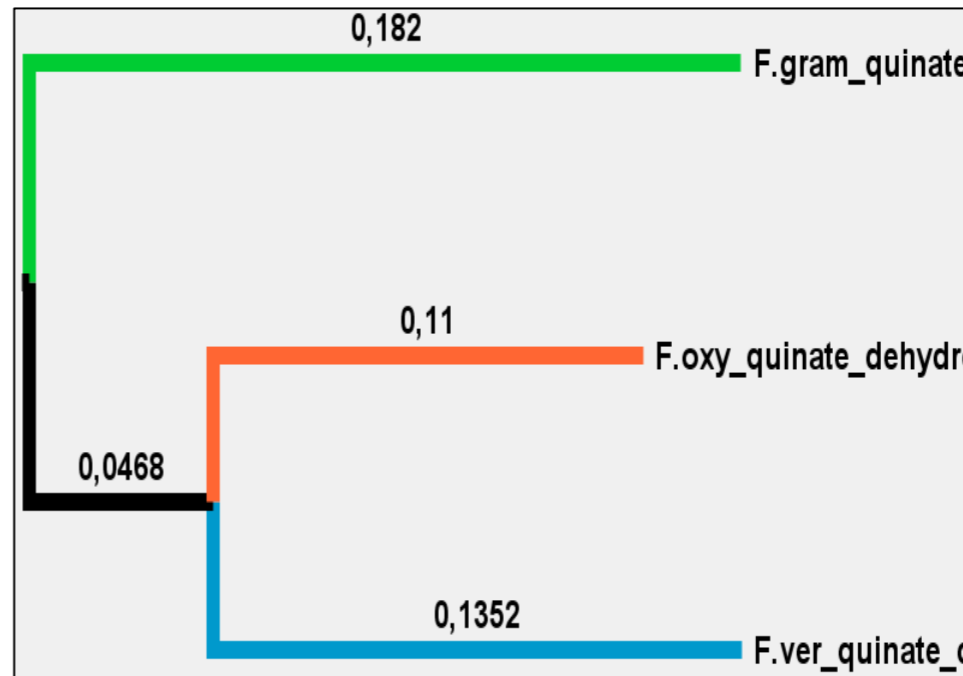
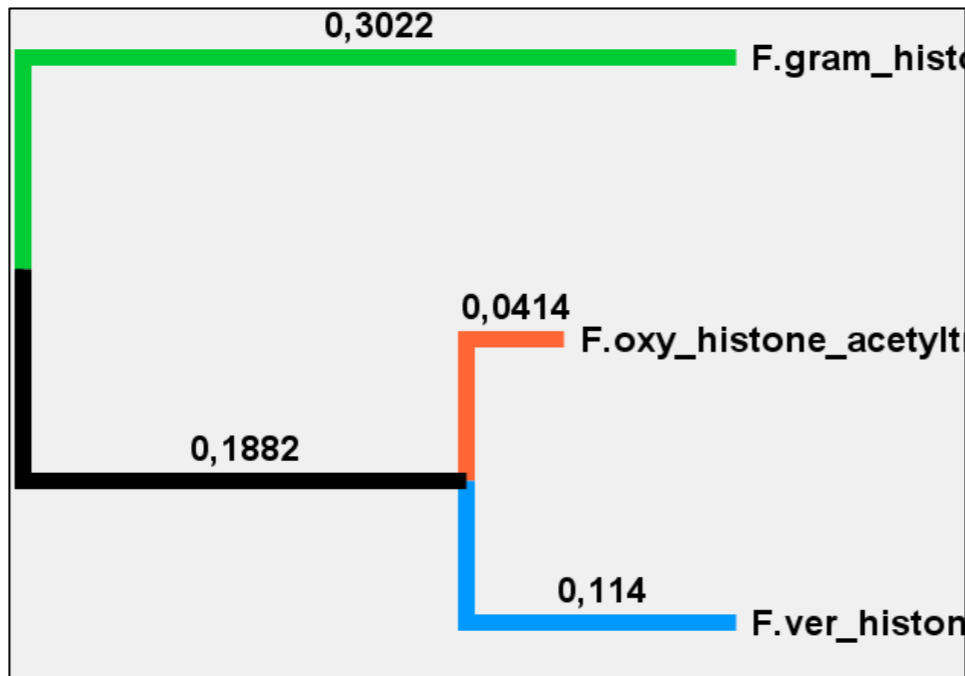
The screenshot shows the website interface for the Fusarium Comparative Database. The header includes the Broad Institute logo and navigation menus for 'What is Broad', 'News and Publications', and 'For the Scientific Community'. The main content area features a title 'Fusarium Comparative Database' and a descriptive paragraph: 'The Fusarium comparative genomics database provides access to multiple sequenced Fusarium genomes simultaneously to facilitate the comparative analysis among these closely related fungal species. The Fusarium comparative project is part of the Broad Fungal Genome Initiative and was funded by the U.S. Department of Agriculture's National Institute for Food and Agriculture.' Below this is a section titled 'The Fusarium Comparative Project' with further text. A small phylogenetic tree is also visible on the right side of the main content area. The right sidebar contains a 'Fusarium' menu with links to Home, Download, News, Analysis, Genomes, Credits, Links, and Contact Us, along with a 'Gene Search' field. The browser's address bar shows the URL: https://www.broadinstitute.org/annotation/genome/fusarium_group/MultiHome.html. The system tray at the bottom indicates the time is 03:00 p.m. on 20/05/2015.

Dendrograms with complete genes





Phylogeny using orthologous genes





Thanks

