

Mycored 2<sup>nd</sup> Mediterranean Workshop on Mycotoxins and Toxigenic Fungi

Istanbul, October 14th 2011

## *Fusarium mycotoxins, an emerging problem in dried figs.*

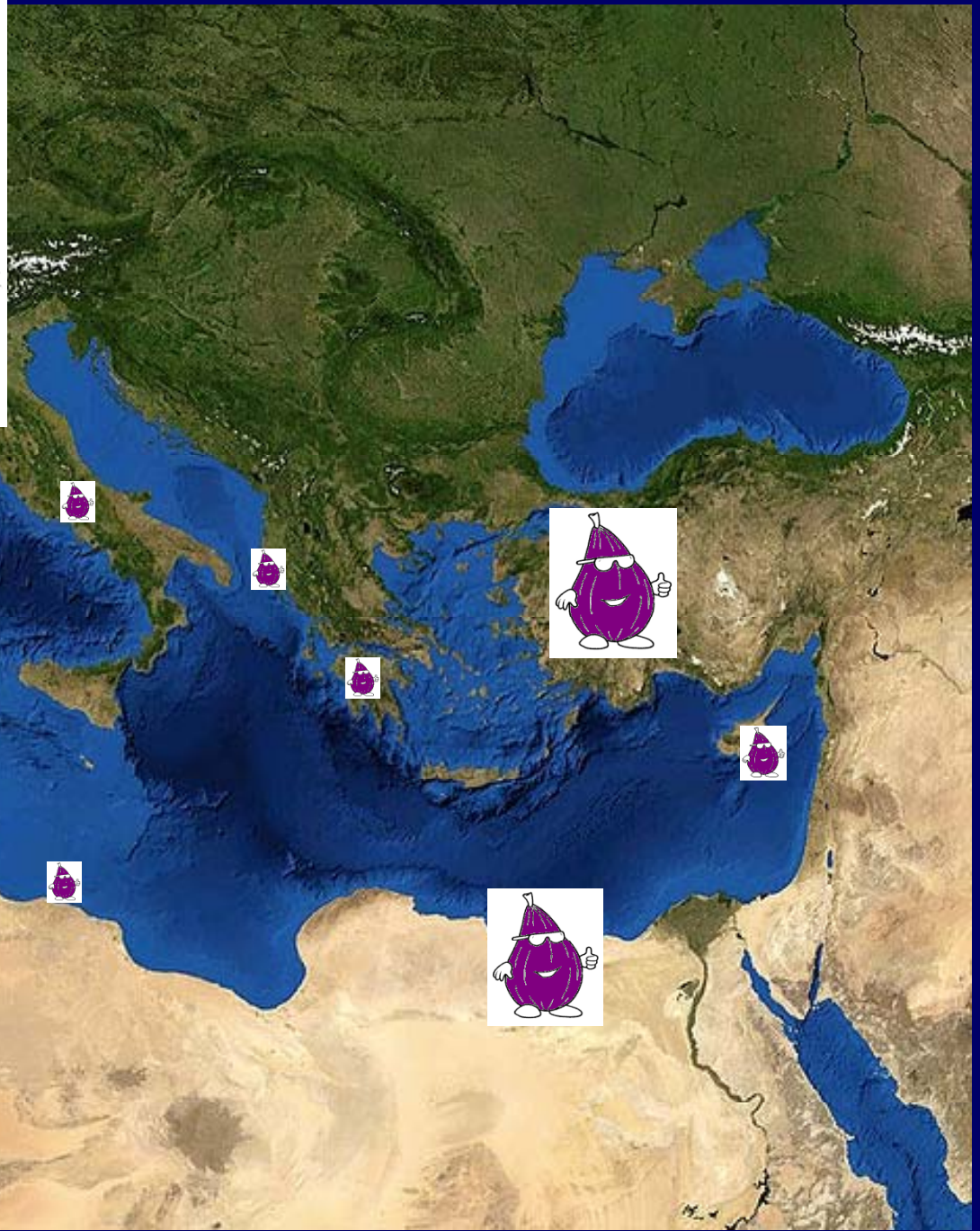
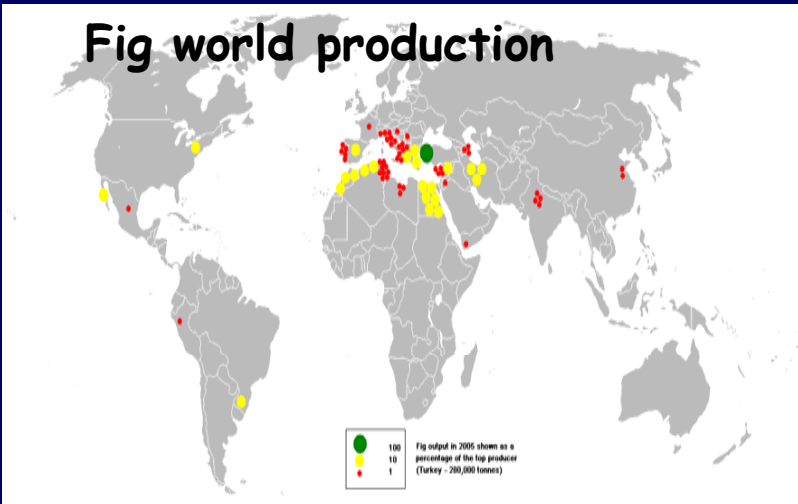
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# Fig world production





17 localities/ 5 fields each  
32% of fruits contaminated  
by *Fusarium* species

4-26  $\mu\text{g}/\text{kg}$  of  $\text{FB}_1$

43/85 samples of fresh  
fig fruits from the field

115 samples collected from  
orchards during drying stage

85/115 samples of dried  
figs from orchards

46-3,200  $\mu\text{g}/\text{kg}$  of  $\text{FB}_1$



# *Fusarium* species associated with fig endosepsis

**Caldis**

*F. moniliforme* var. *fici*

**Subbarao and Michailides**

*F. moniliforme*

**Subbarao and Michailides**

*F. moniliforme*

*F. solani*

*F. dimerum*

**Marasas**

*F. proliferatum*

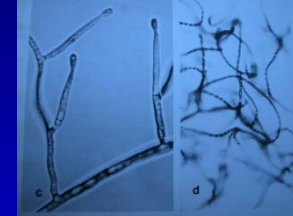
*F. subglutinans*

**Nirenberg and O'Donnell**

*F. ramigenum*

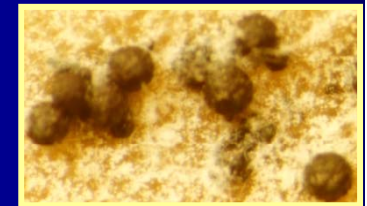
*F. lactis*

# *Gibberella fujikuroi* species complex

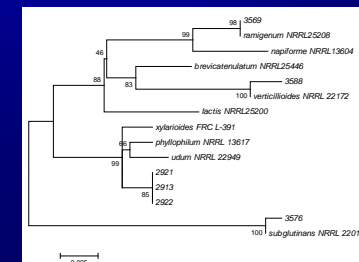


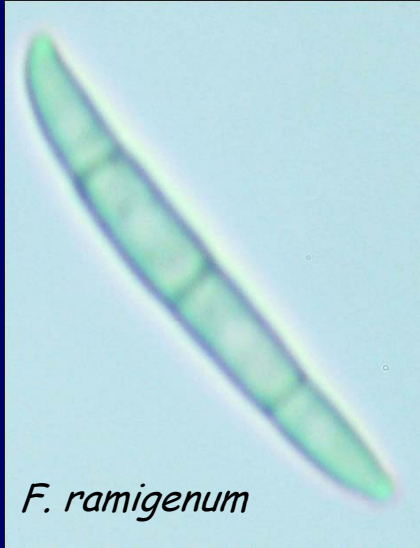
Morphological species concept based on conidiogenesis; **4-10 species**

Biological species concept based on fertility tests: **at least 11 species**



Phylogenetical species concept based on gene sequences: **46 species**

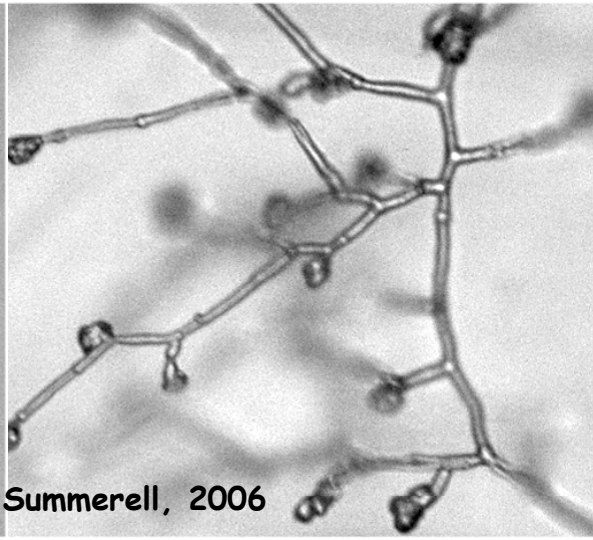




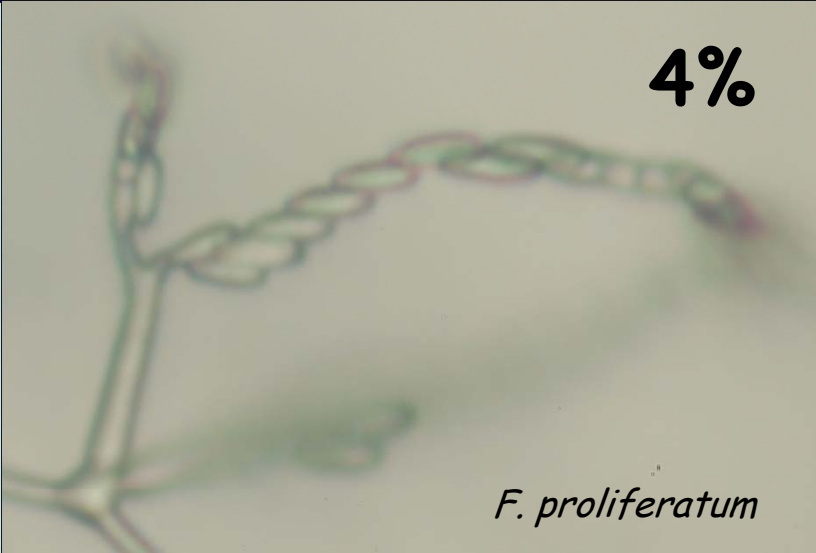
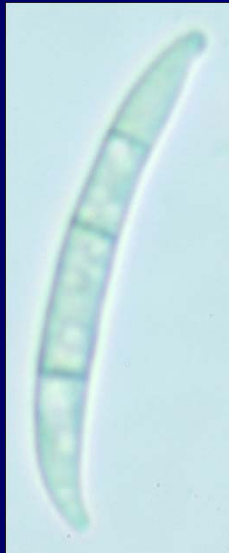
*F. ramigenum*



From Leslie and Summerell, 2006

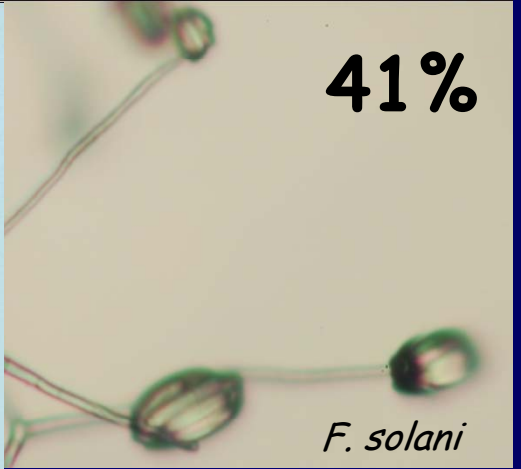
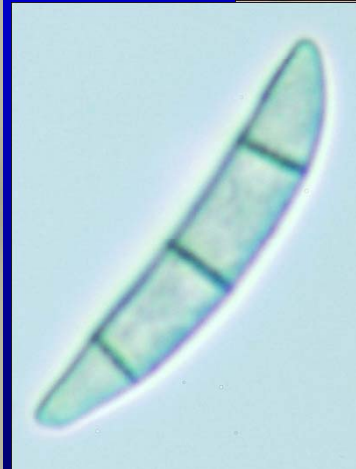


**55%**



**4%**

*F. proliferatum*

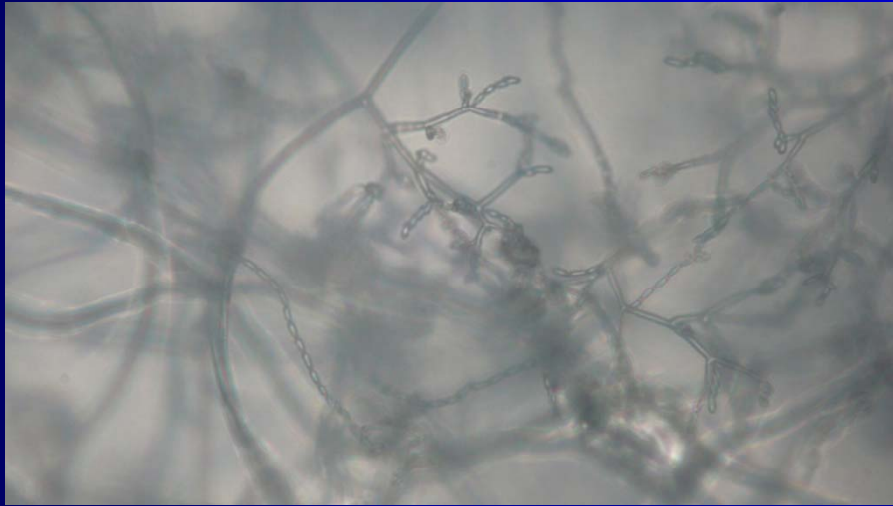


**41%**

*F. solani*



*F. ramigenum*

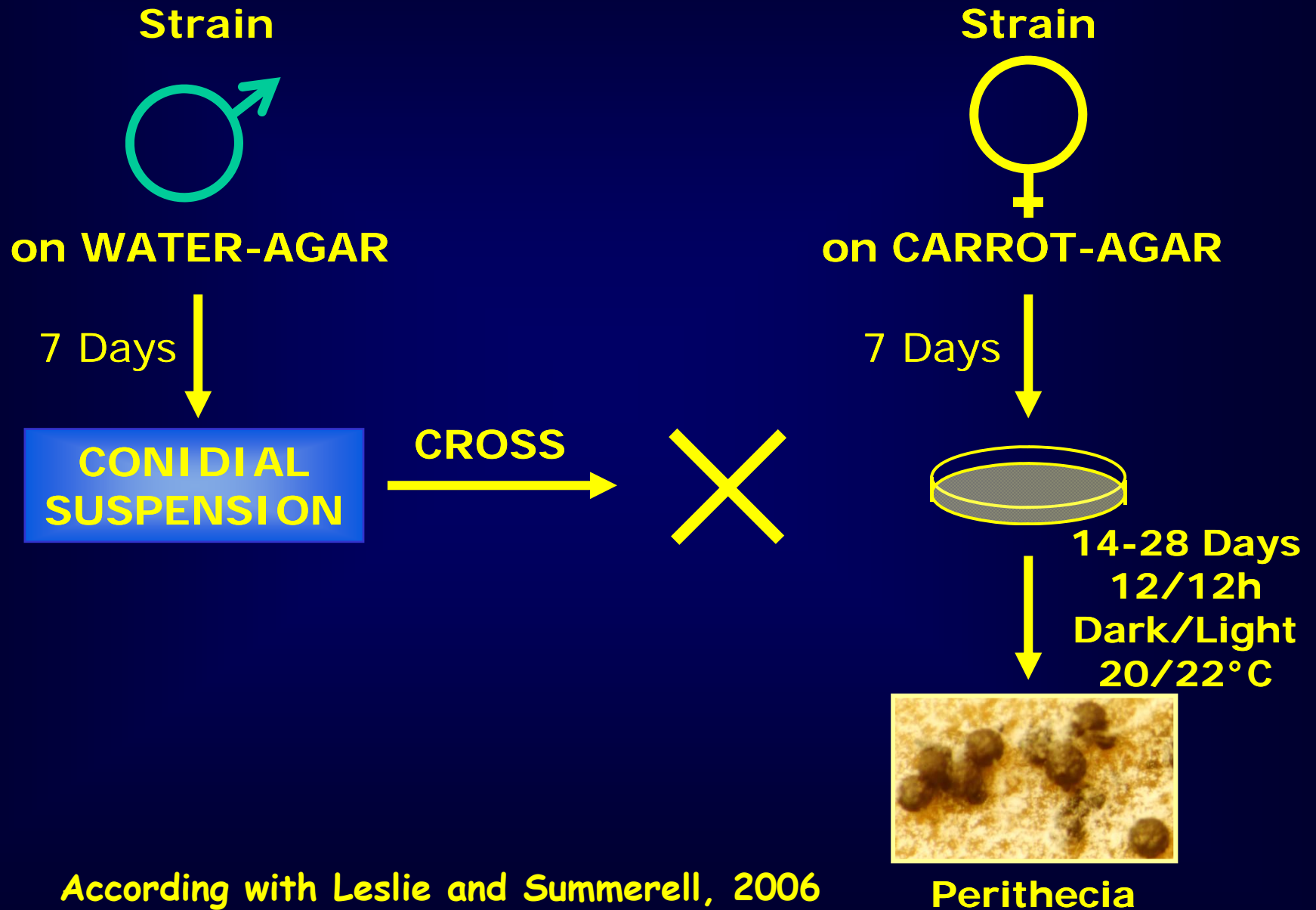


*F. proliferatum*

Strains from Turkey



# Mating test in *Gibberella fujikuroi* complex





## "Sexual Stage", preferential stage and toxin profile in *Fusarium* species of the *Gibberella fujikuroi* complex

Sexual stage (Syn. Mating population)	Anamorphic stage	Preferential host	Mycotoxins					
			FBs	BEA	FUP	GA	FA	MON
<i>G. moniliformis</i> (A)	<i>F. verticillioides</i>	Maize	+	-	-	-	+	low
<i>G. sacchari</i> (B)	<i>F. sacchari</i>	Sugar cane	-	+	-	-	+	?
<i>G. fujikuroi</i> (C)	<i>F. fujikuroi</i>	Rice	low	+	-	+	+	+
<i>G. intermedia</i> (D)	<i>F. proliferatum</i>	Various	+	+	+	-	+	+
<i>G. subglutinans</i> (E)	<i>F. subglutinans</i>	Maize	-	+	+	-	+	+
<i>G. thapsina</i> (F)	<i>F. thapsinum</i>	Sorghum	-	-	-	-	+	+
<i>G. nygamai</i> (G)	<i>F. nygamai</i>	Soil	+	+	-	-	+	+
<i>G. circinata</i> (H)	<i>F. circinatum</i>	Pine	-	+	-	-	+	?
<i>G. konza</i> (I)	<i>F. konzum</i>	Talgrass	+	+	+	-	+	?
<i>G. gaditjirii</i> (J)	<i>F. gaditjirii</i>		?	?	?	?	?	?
<i>G. xylarioides</i> (K)	<i>F. xylarioides</i>	Coffe	?	?	?	?	?	?

FB<sub>s</sub> = fumonisines; BEA = beauvericin; FUP = fusaproliferin; MON = moniliformin; GA = gibberellic acid; FA = fusaric acid.

# Toxin-producing strains and their range of production (mg/Kg) (Italian set)

*F. ramigenum*

*F. proliferatum*

N. of isolates

71

5

Np/Na

Range

>10mg/Kg

Np/Na

Range

>10mg/Kg

FUP

2/71

155-345

2

1/5

820

1

BEA

30/71

1-120

24

2/5

10-205

0

FA

39/71

0.3-525

5

5/5

0.1-3.5

0

FB<sub>1</sub>

43/71

0.1-1080

22

1/5

1100

1

FB<sub>2</sub>

47/71

0.1-655

12

1/5

470

1

# Toxin-producing strains and their range of production (mg/Kg) (Turkish set)

*F. ramigenum*

*F. proliferatum*

N. of isolates

**10**

**31**

Np/Na

Range

>10mg/Kg

Np/Na

Range

>10mg/Kg

FB<sub>1</sub>

10/10

1.20-4010

6

0

-

0/31

FB<sub>2</sub>

10/10

0.2-1280

7

0

-

0/31

FB<sub>3</sub>

10/10

0.3-445

6

0

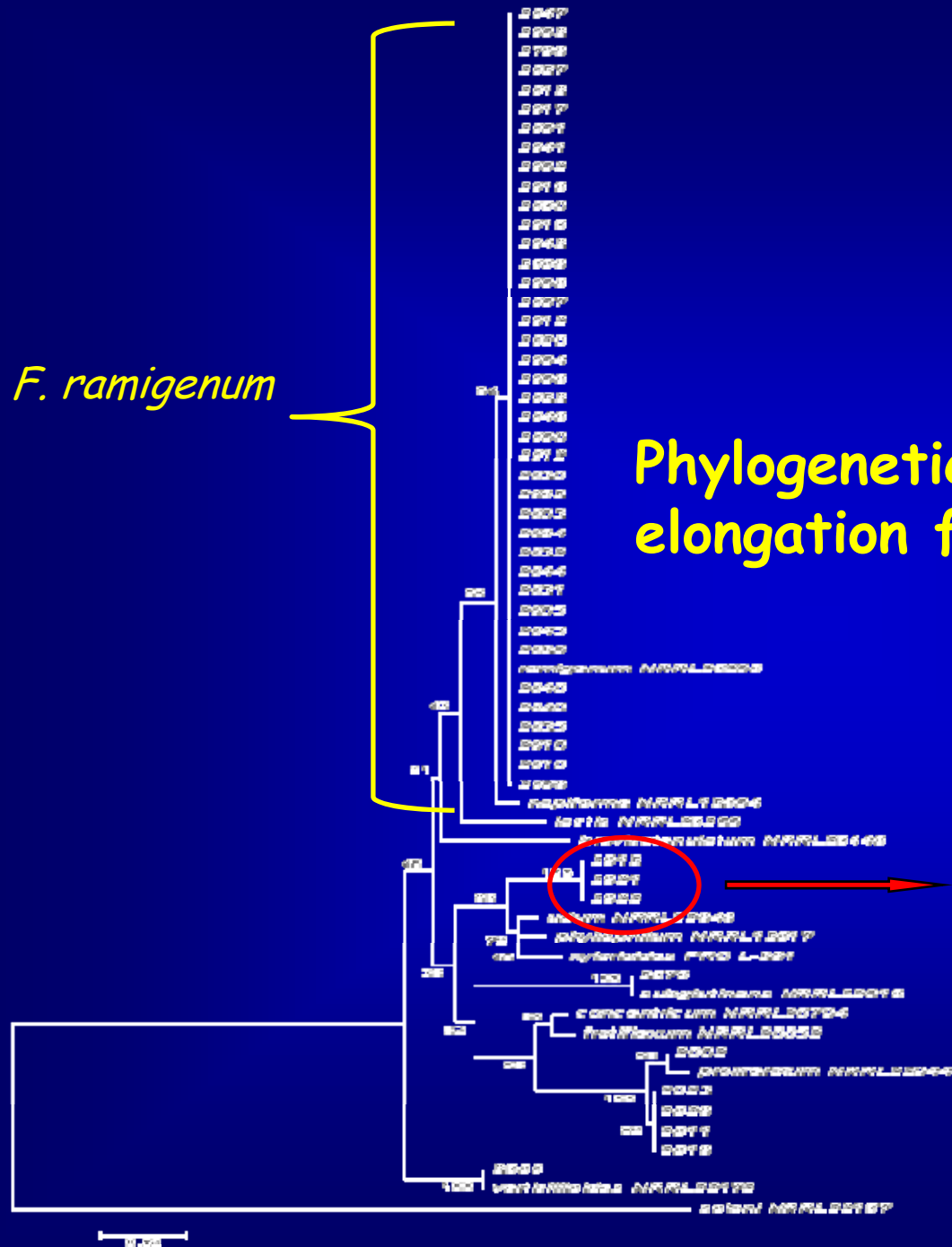
-

0/31

*F. ramigenum*

# Phylogenetic analysis of translation elongation factor-1 $\alpha$ sequences

Evolutionary relationships of 63 taxa  
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.51572582 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.



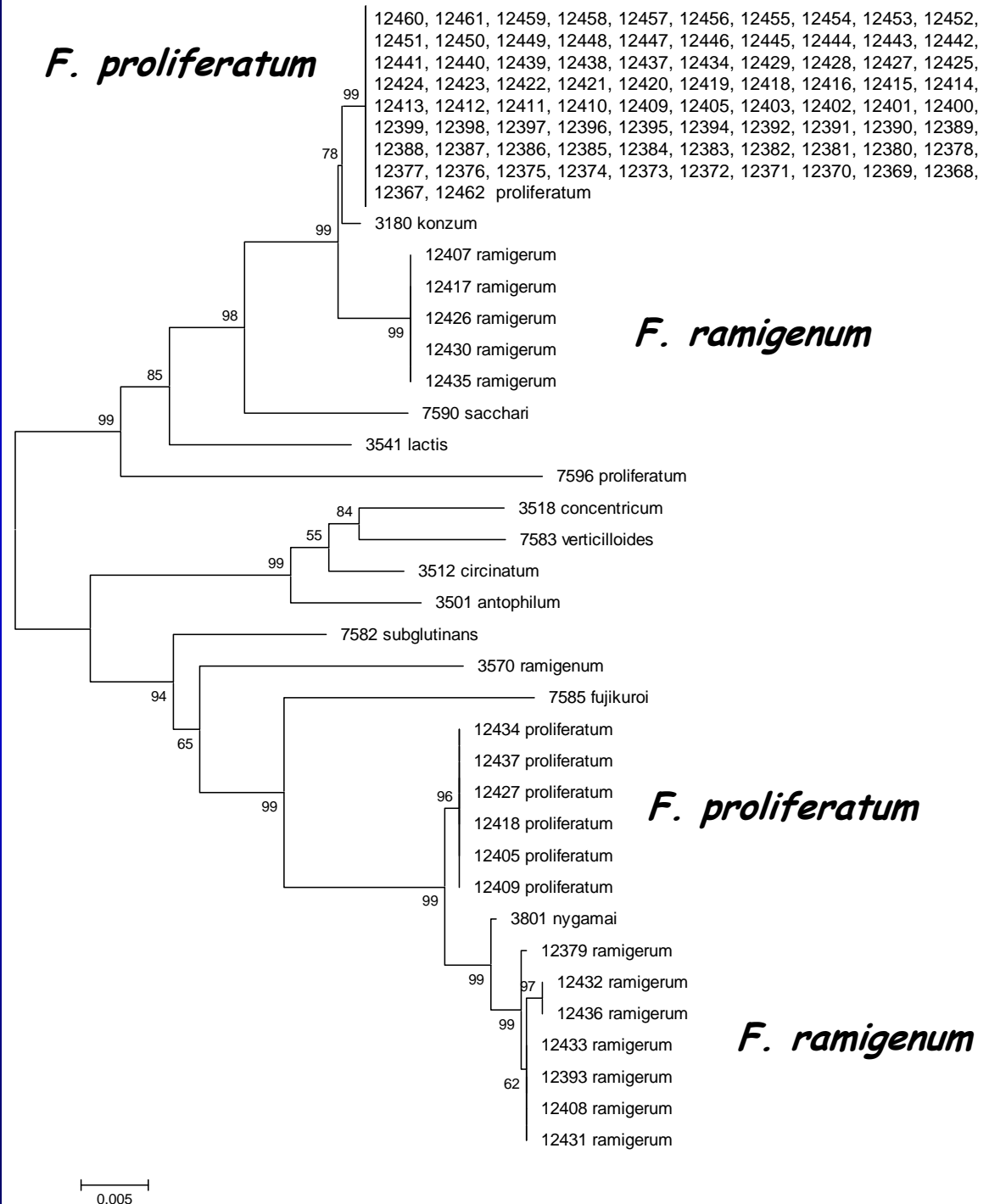
Unknown species

*F. proliferatum*

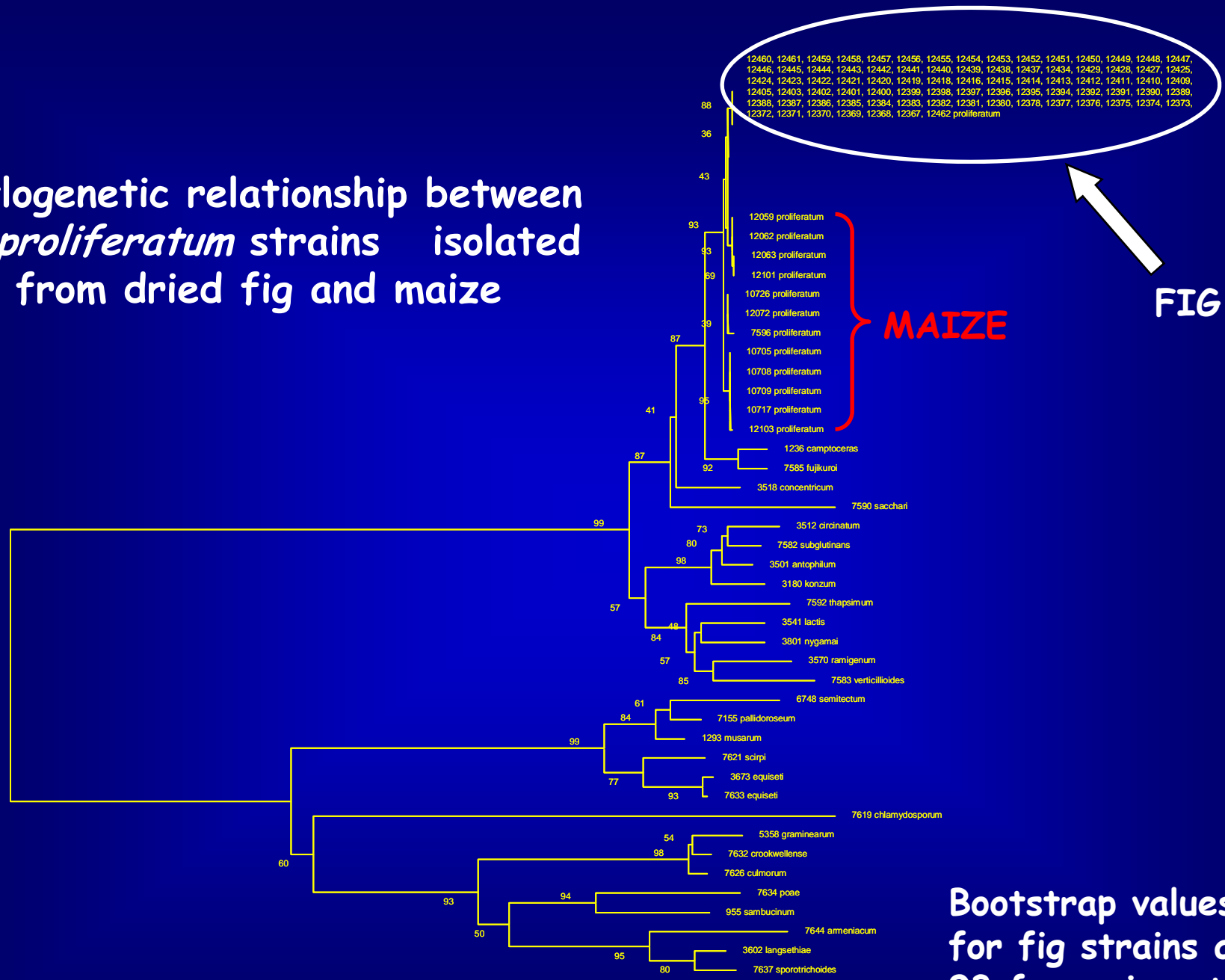
0.02

# Phylogenetic relationship of strains isolated from dried fig in Turkey

Evolutionary relationships of *F. proliferatum* (88 strains) and *F. ramigenum* (12 strains) obtained from the combined gene sequences analyses of RPB2,  $\beta t$ , caM e TEF genes. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.51572582 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutive distance is expressed in number of substituions of bases for each site. by using Maximum Composite Likelihood method

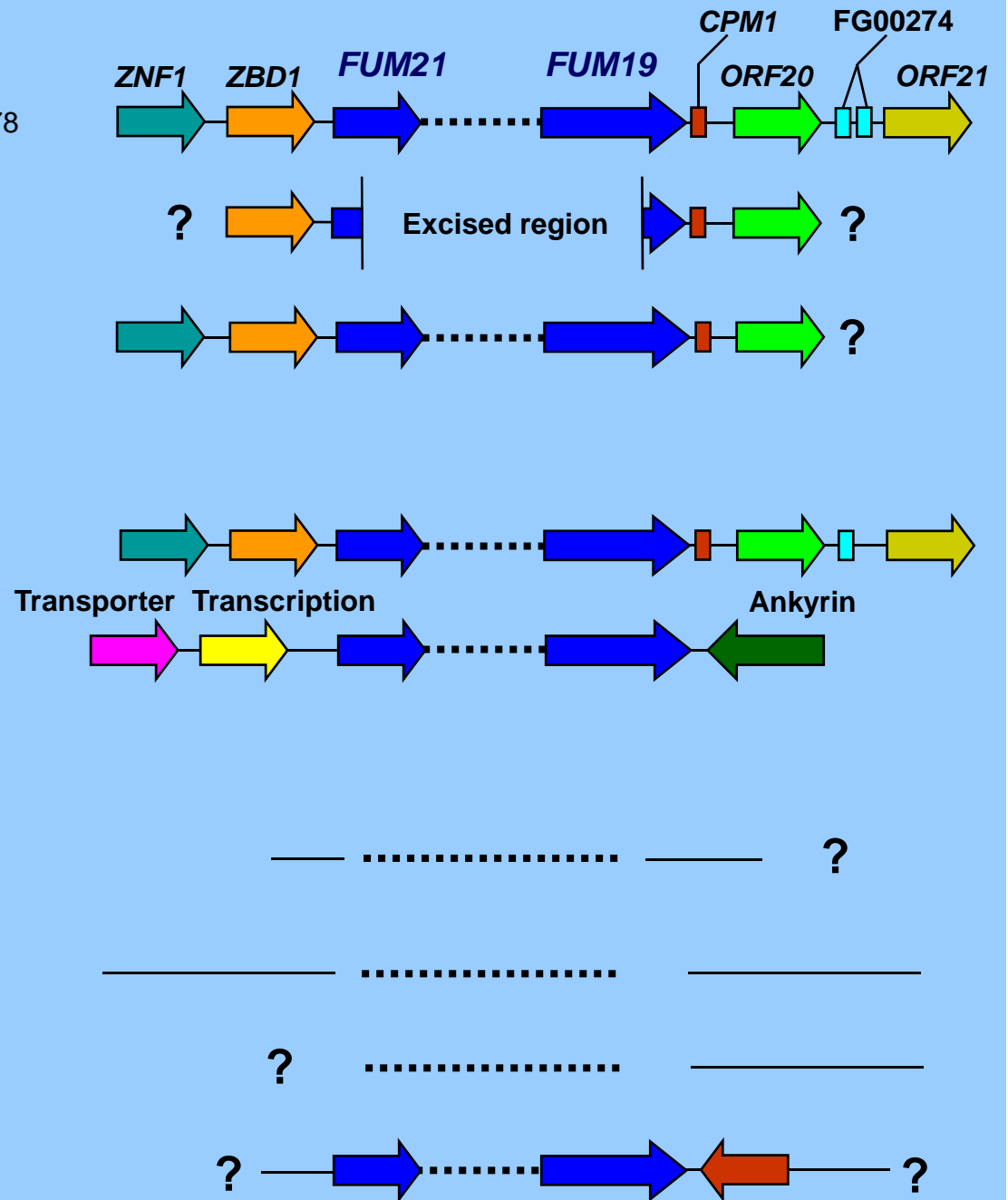
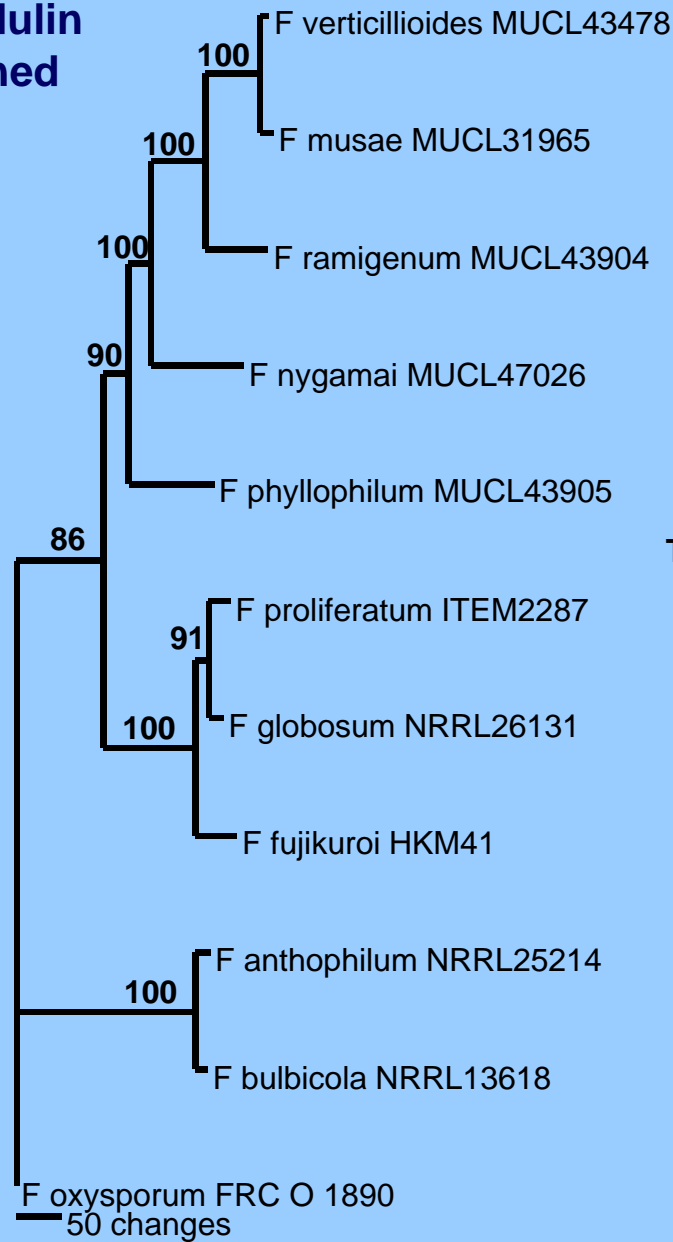


# Phylogenetic relationship between *F. proliferatum* strains isolated from dried fig and maize

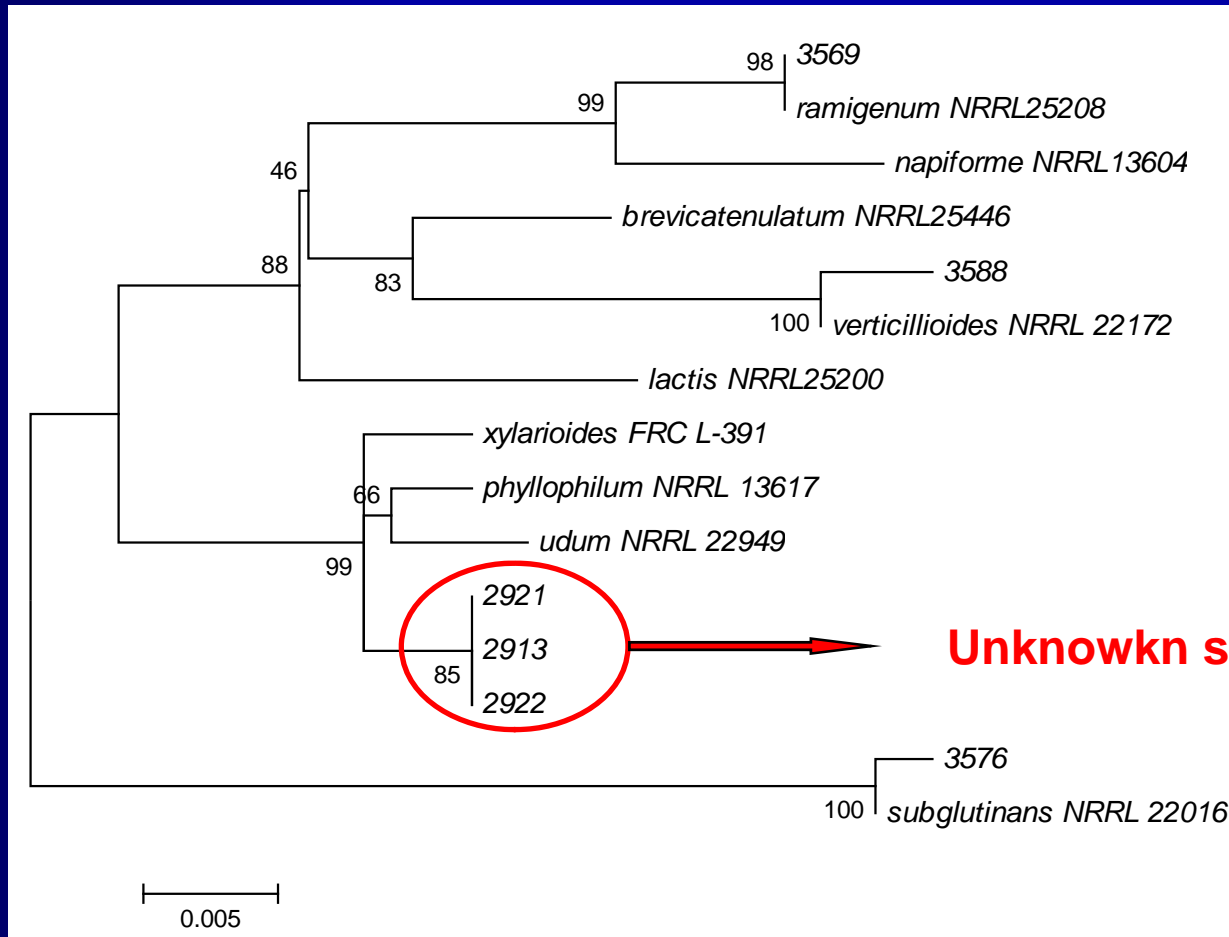


Bootstrap values: 88 for fig strains and 93 for maize strains.

**RPB2, EF-1a,  
beta-tubulin,  
calmodulin  
combined  
MPT**



# Phylogenetic analysis of beta-tubulin sequences



Evolutionary relationships of 15 taxa

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.14034532 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

**Unknown species**



## Toxin production (mg/Kg) and fertility of unknown strains from fig

<b>isolate</b>	<b>MP</b>	<b>FUP</b>	<b>BEA</b>	<b>FA</b>	<b>FB1</b>	<b>FB2</b>
<b>2913</b>	<b>MAT?</b>	<b>nd</b>	<b>nd</b>	<b>0.1</b>	<b>nd</b>	<b>0.1</b>
<b>2921</b>	<b>MAT?</b>	<b>nd</b>	<b>39</b>	<b>0.1</b>	<b>nd</b>	<b>0.1</b>
<b>2922</b>	<b>MAT?</b>	<b>nd</b>	<b>20</b>	<b>nd</b>	<b>nd</b>	<b>20</b>

## Toxicity in brine shrimp assay of no or low toxin-producing *Fusarium ramigenum* strains isolated from fig fruits

Strain ITEM	FUP mg/kg	BEA mg/kg	FA mg/kg	FB <sub>1</sub> mg/Kg	FB <sub>2</sub> mg/Kg	% of Toxicity
2805	nd	3	nd	nd	nd	100
2806	nd	2	0.6	0.4	1	100
2812	nd	nd	1	nd	nd	100
2821	nd	nd	0.4	0.3	0.1	93
2917	nd	nd	1	0.1	0.1	100
2928	nd	15	nd	nd	nd	100
2946	nd	nd	0.1	nd	nd	100

# Conclusions

- There is a high risk of fig contamination by *Fusarium* species and related mycotoxins (mainly fumonisins)
- The main species associated with fig endosepsis in Apulia are *F. ramigenum* and *F. proliferatum*
- Although the fumonisin production by *F. ramigenum* strains was highly variable, this species can be considered the main responsible for fumonisin contamination of figs

To be continued..

- *F. ramigenum* strains could not be assigned to any mating population of *G. fujikuroi* complex since the strains here investigated were unfertile

- The *F. proliferatum* strains from fig could represent a distinct species. The fumonisin pathway gene occurrence is under investigations in order to provide a genetic confirmation of the lack of fumonisin production. Together with subtle genetic divergence with *F. proliferatum* from maize and their infertility, this could be a further evidence for a new species.

- Molecular analyses showed that further species not identified yet could occur on fig plants and need to be better characterized by using further molecular tools. This work is in progress



Which *Fusarium* species  
at that time on figs?

Thank you for your attention