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New species and populations in *Fusarium*: examples from the tropics

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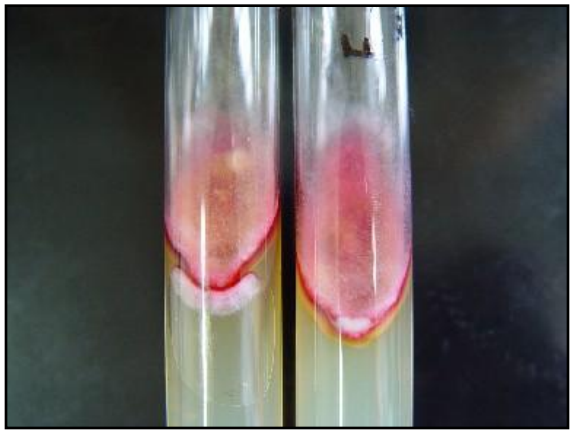
Problems and constraints with regard to information on tropical species, populations and plant diseases

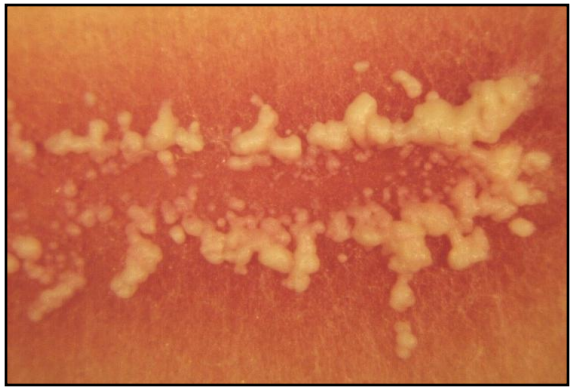
1. **Data on geographical distribution of species and diversity in the tropics are more related to research activities than to facts;**
2. **Many regions are absolutely under-investigated. This is true also for the genus *Fusarium*;**

Problems and constraints with regard to information on tropical species, populations and plant diseases

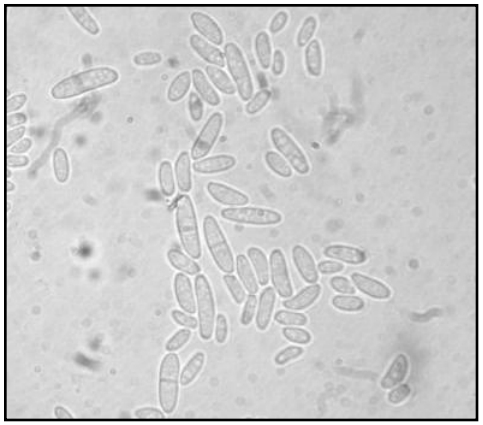
3. **Causal agents of diseases are not always the same in every country, region or continent;**
4. **Names used in older reports, like *Fusarium moniliforme* or *Fusarium roseum*, are no longer in use, and stand for a large diversity of different forms and populations.**

Fusarium is a typical anamorph form-genus, with species sharing morphological markers



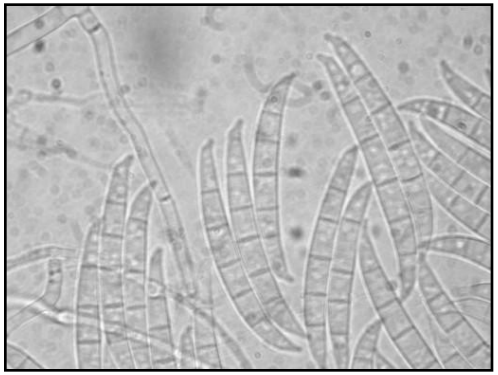


The culture

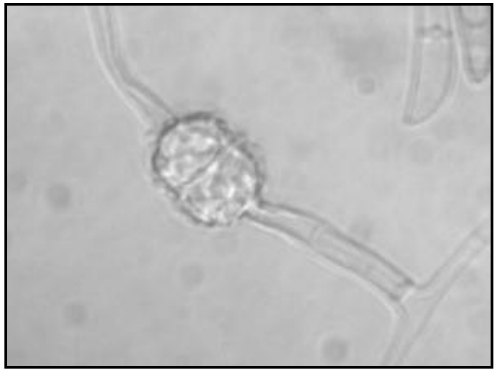


Microconidia

Mono- and polyphialides

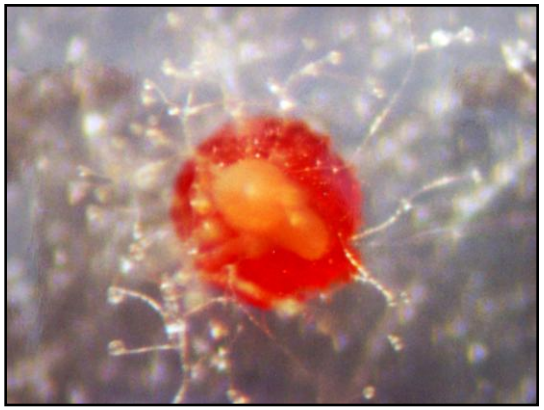


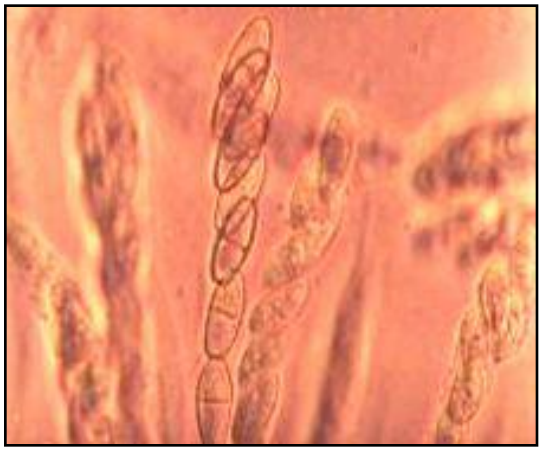
Macroconídia

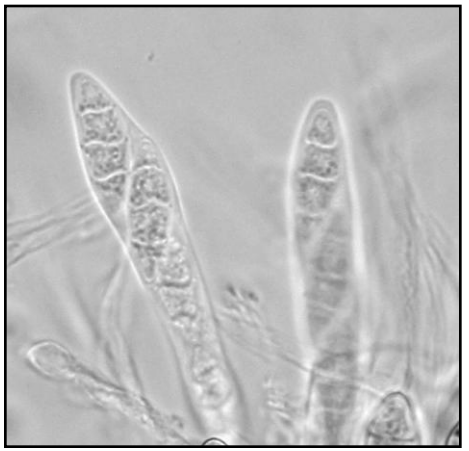


Chlamydospores

Telomorphs of *Fusarium* species



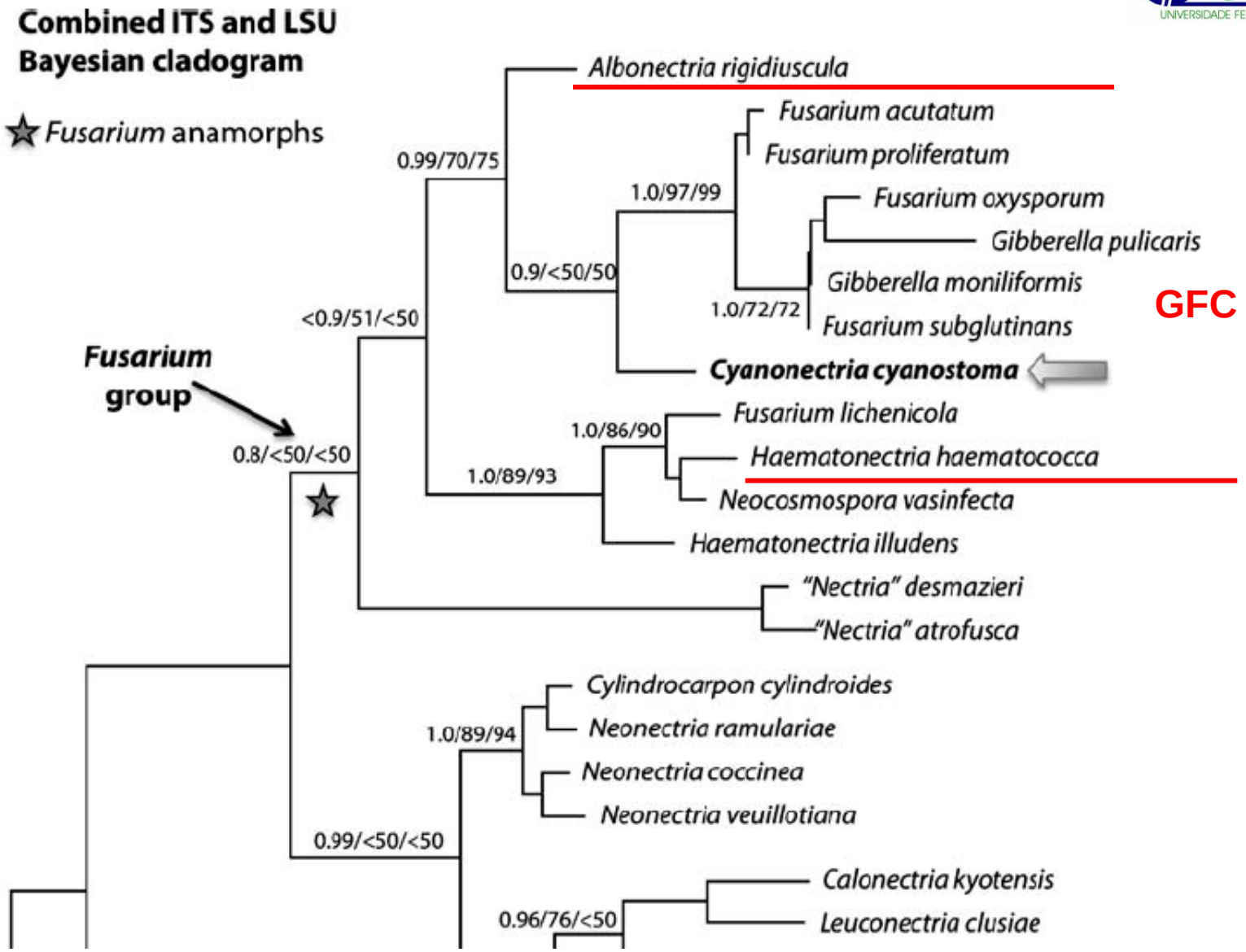




Gibberella

Haematonectria

Albonectria



Samuels et al. 2009. Mycol. Progress

Why is Fusarium important ?

- Biology and ecology
- Economical importance
- Model for species concepts and molecular characterization

Plant pathogen

- **Soil fungus** → soil and rhizosphere, root rots
- **Vascular fungus** → wilts, dieback

Endophyte → plants, seeds, etc.

Toxin producer → contamination of plant products

Human pathogen → nosocomial infection

Examples of species with mostly tropical and subtropical distribution :

- ***Fusarium lateritium, Fusarium stilboides***
- ***Fusarium guttiforme, Fusarium ananatum* - GFC**
- ***Fusarium mangiferae*, *F. sterilihyphosum* - GFC**
- ***Fusarium decemcellulare – Albonectria rigidiuscula***

Form-species, a high diversity of typically tropical populations, many phylogenetic or biological species :

- ***Fusarium oxysporum***
- ***Fusarium solani – Haematonectria / Neocosmospora***

Species Concepts

- **Morphological Species Concept – MSC**
  Population shares morphological characters

- **Biological Species Concept - BSC**
  Population delimited by reproductive barrier –
  mating population

- **Phylogenetic Species Concept - PSC**
  Population delimited by concordance in groupings generated based on DNA sequences - clades

Morphological Species

Fusarium solani

Morphological markers :
macro- and microconidia frequent
chlamydospores frequent
monophialides in the aerial mycelium are long

Many host plants
some *formae speciales*
Also clinical

→ **Species Complex**

- **Biological Species**
  ***Biological Species Concept* - BSC**

Population of individuals that breed and form fertil descendents

Population is delimited by a reproductive barrier
mating population* or *breeding population

Ex. Gibberella fujikuroi Complex

- **Phylogenetic Species**

  ***Phylogenetic Species Concept* - PSC**

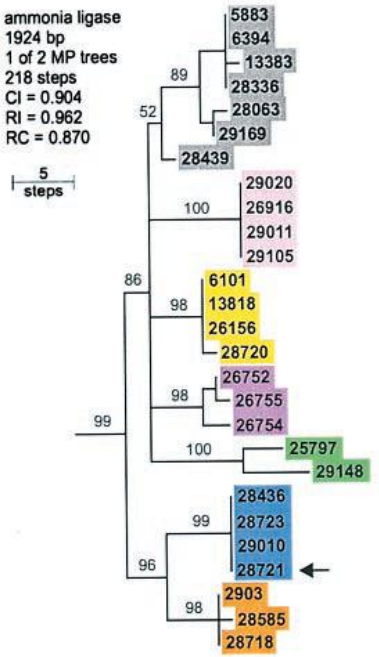
  Population delimited by concordance in groupings generated based on DNA sequences - clades

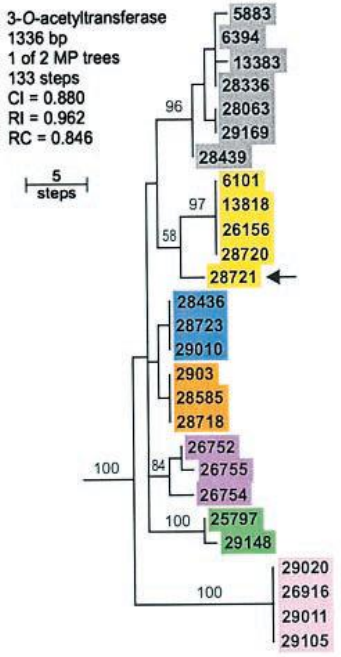
  Population of individuals that share apomorphic characters

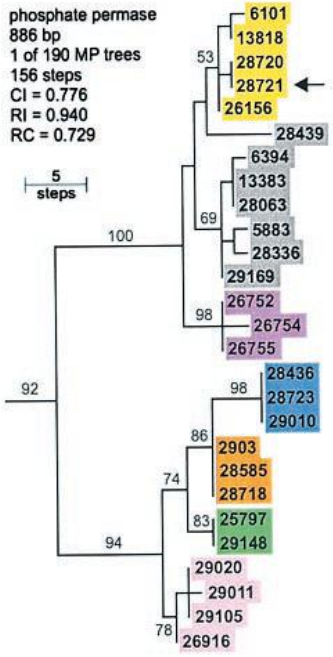
  p.ex. substitution of nucleotides etc.

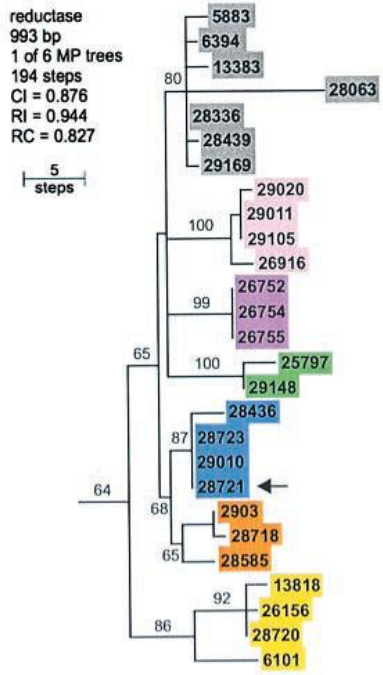
  Geneological Concordance Phylogenetic Species Recognition

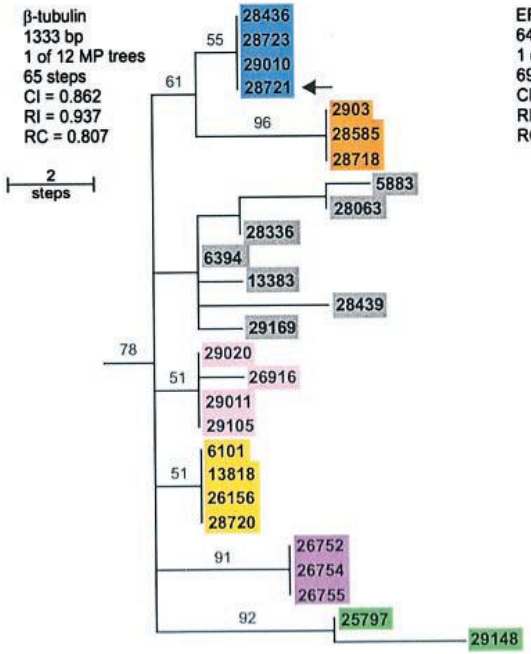
  Taylor et al. 2000. Fungal Genetics Biology

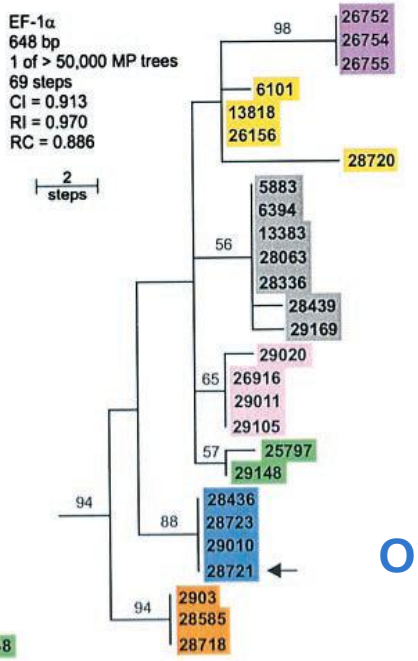












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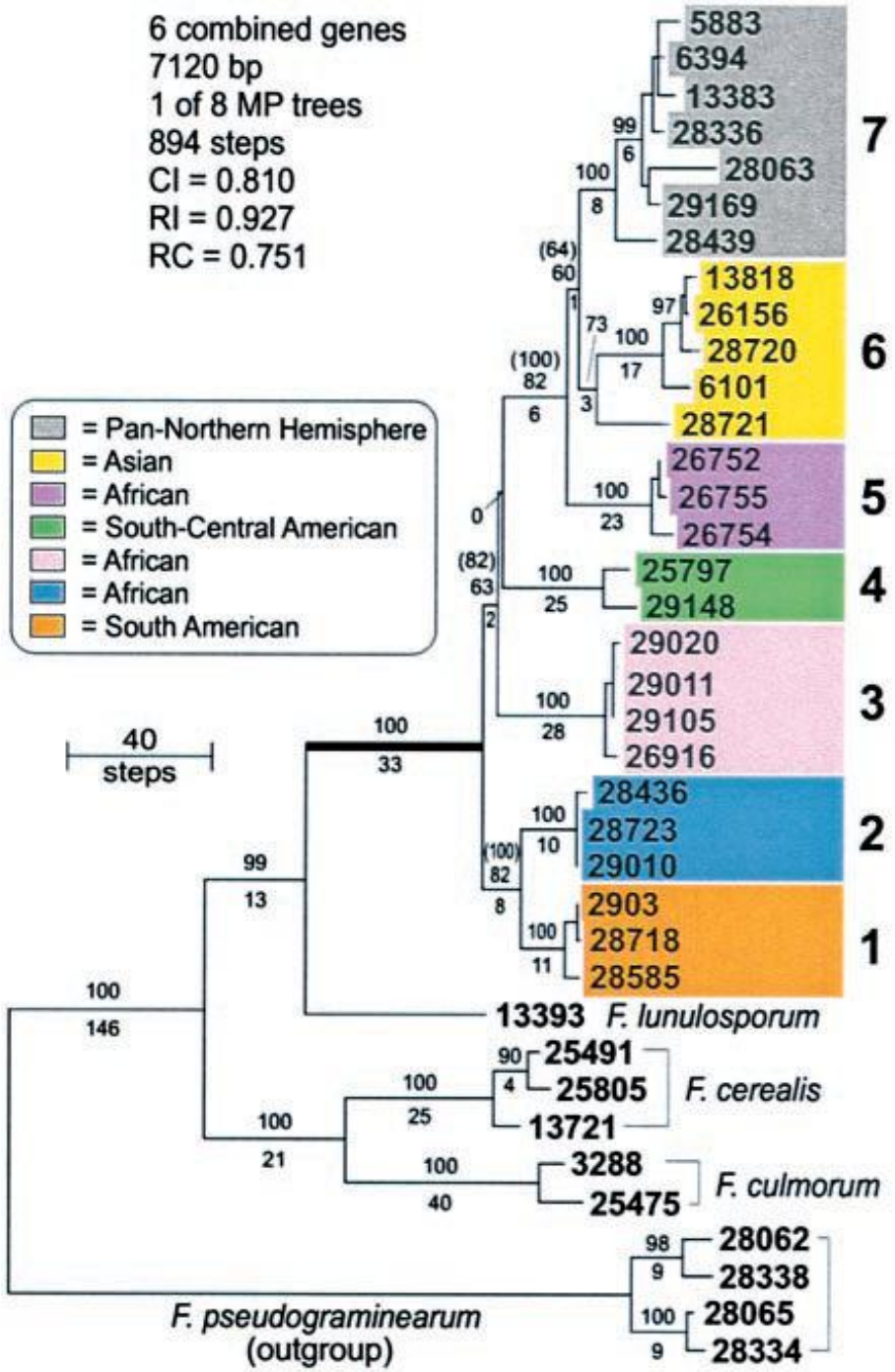
Fusarium graminearum

Wheat pathogen

O´Donnell et al. 2000. PNAS



O´Donnell et al. 2000. PNAS

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Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade☆

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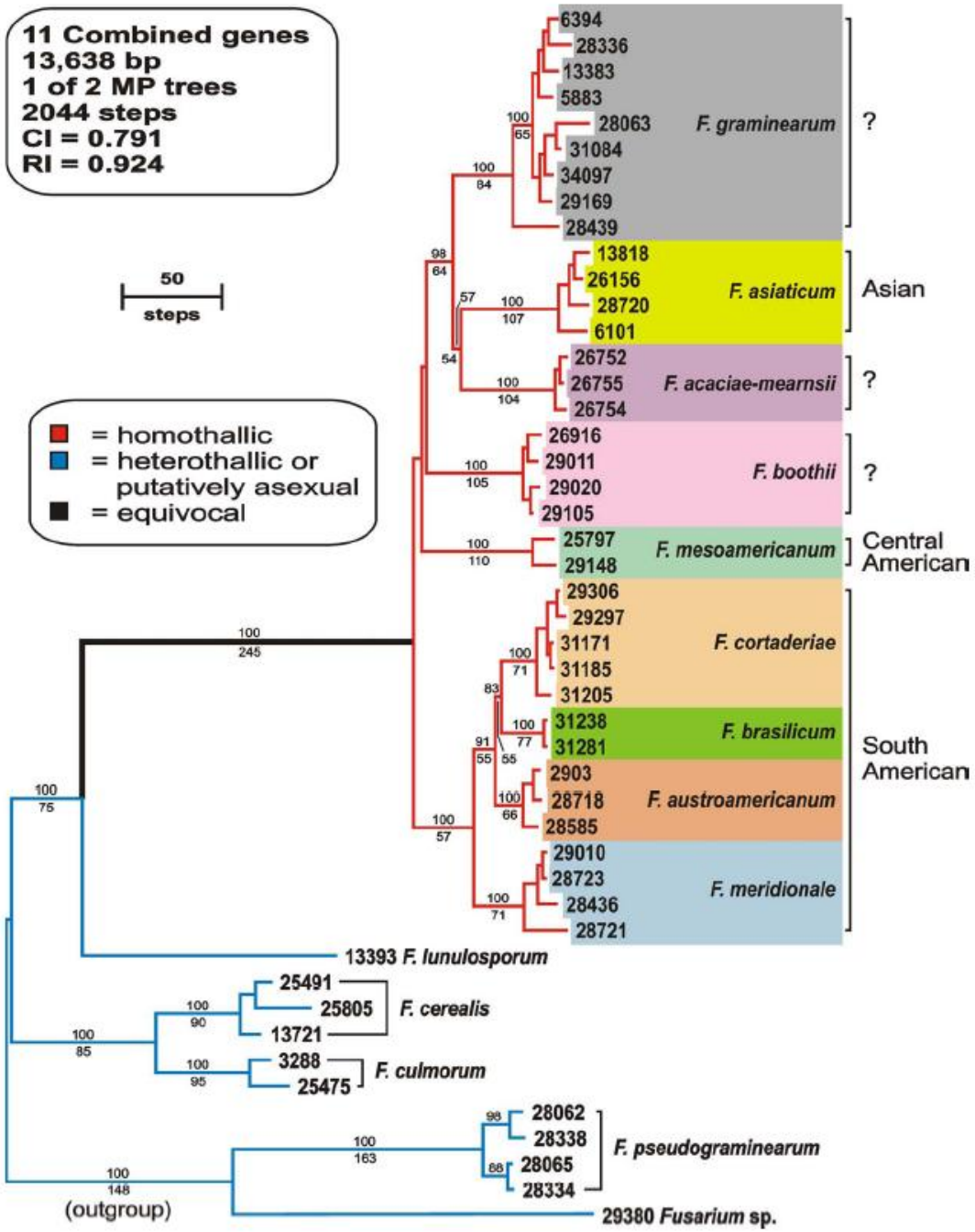
[d] *Genetic Diversity Department, National Institute of Agrobiological Sciences (NIAS), 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan*



O´Donnell et al. 2004. FGB

Examples of tropical crops which develop diseases, caused by *Fusarium* species :

i. **Fruit crops:**
   Mango, pineapple, banana, passionfruit

ii. **Palm trees:**
   oil palm, date palm, canary palm

iii. **Coffee and Cocoa**

iv. **Spices, others:**
   Black pepper, tobacco, vanilla, cotton

v. **Monocots: Sugarcane, rice, sorghum, maize**

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Etiology of Mango Malformation in Brazil

Mangifera indica L.

Healthy tree

Symptoms of malformation

Floral - inflorescense

Vegetative - shoot

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Objectives

- **To identify the causal agents of mango malformation disease in Brazil**

  Methods AFLP analysis, Gene Sequencing, Morphological Characterization, Pathogenicity Test

- **To induce the sexual stage for the causal agents**

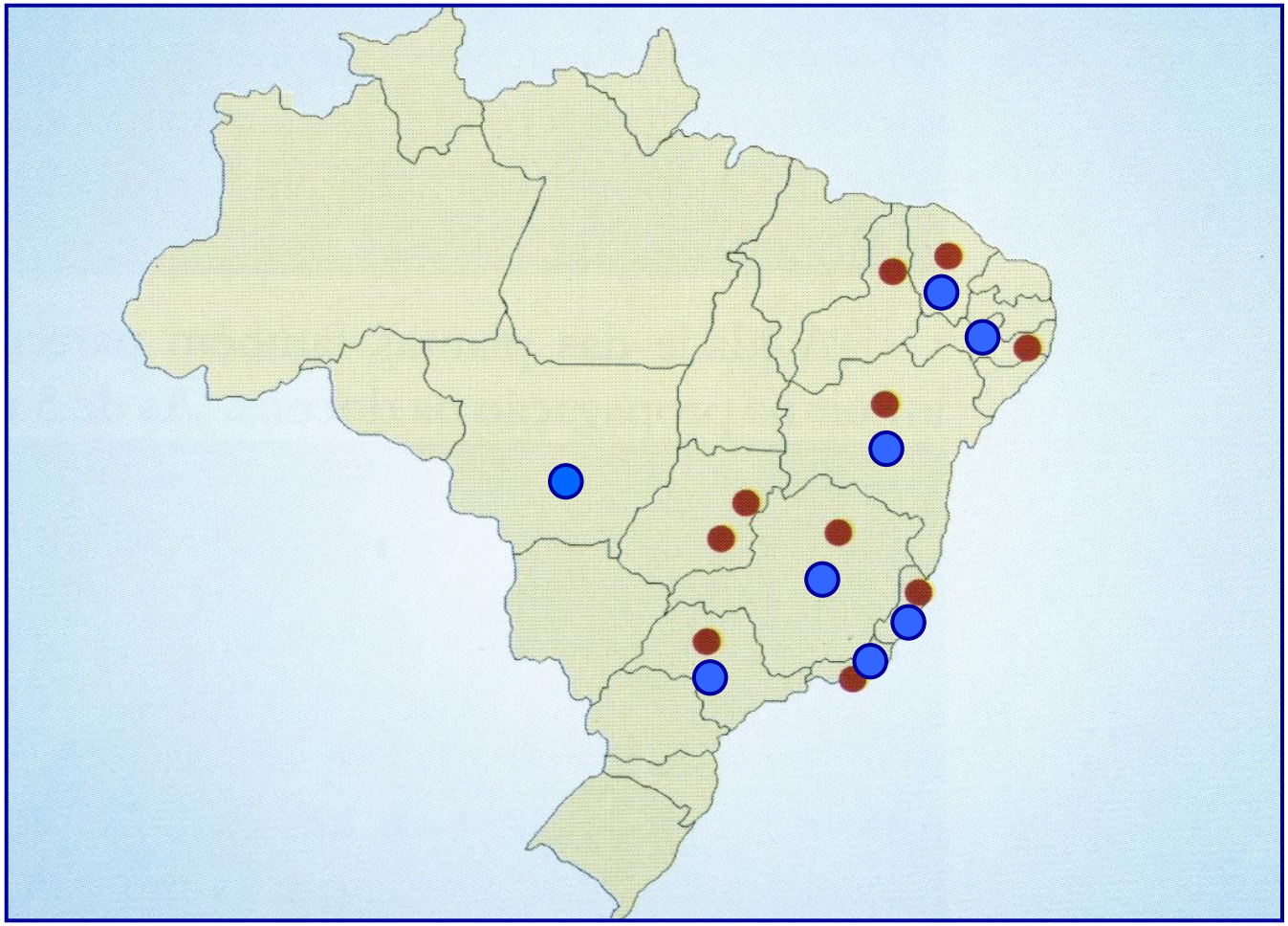
  Methods Identification of mating type (PCR) and crossing

- **To develop a PCR-based method for the detection and identification of the causal agents**

  Methods primer design, tests for specificity and sensitivity

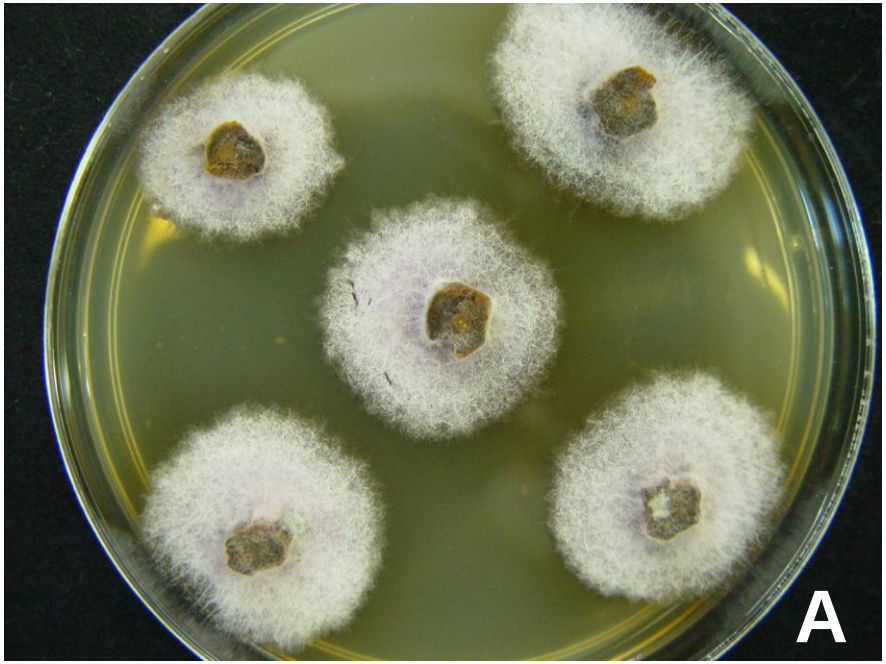
Disease Distribution in Brazil

- Lima 2006, 59 isolates, 8 states
- Cunha et al 2000



Pathogen x Endophyte's isolation

Malformed tissue
Fusarium only

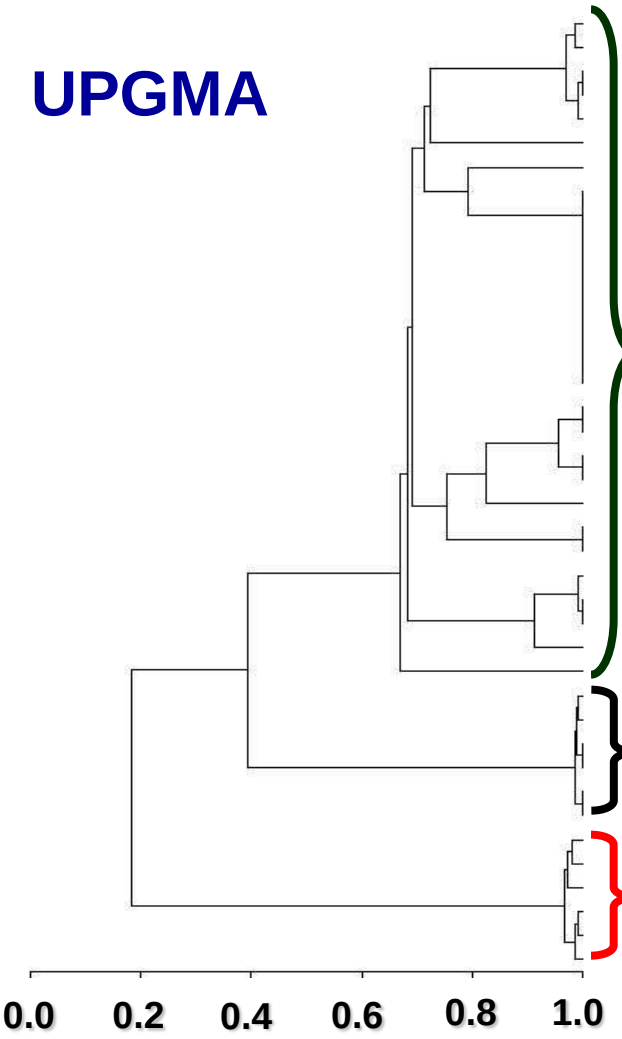


Asymptomatic tissue
about 15 species

AFLP analysis of *Fusarium* from mango

UPGMA

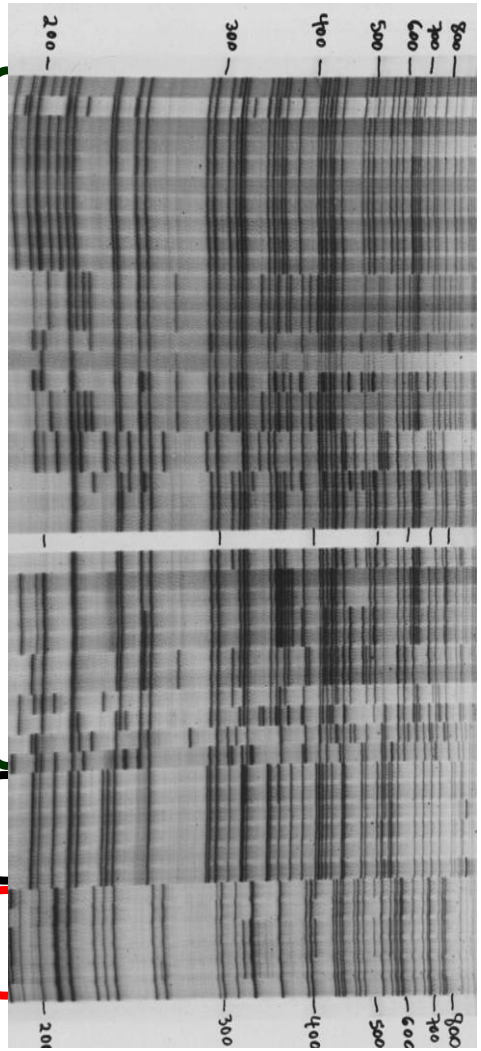
AFLP bands



Fusarium sp. nov.

F. sterilihyphosum

F. mangiferae



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Sequence analysis of genes encoding β-tubulin and translation elongation factor 1-α

Primers tub2 – T1 and T2 - O'Donnell et al., 1998, Mycologia

Primers tef1 – EF1 and EF2 - O'Donnell et al., 1998, PNAS

Sequencing – MegaBACE 500, both directions

Analysis – Maximum Parsimony

Program – PAUP 4 version beta 10

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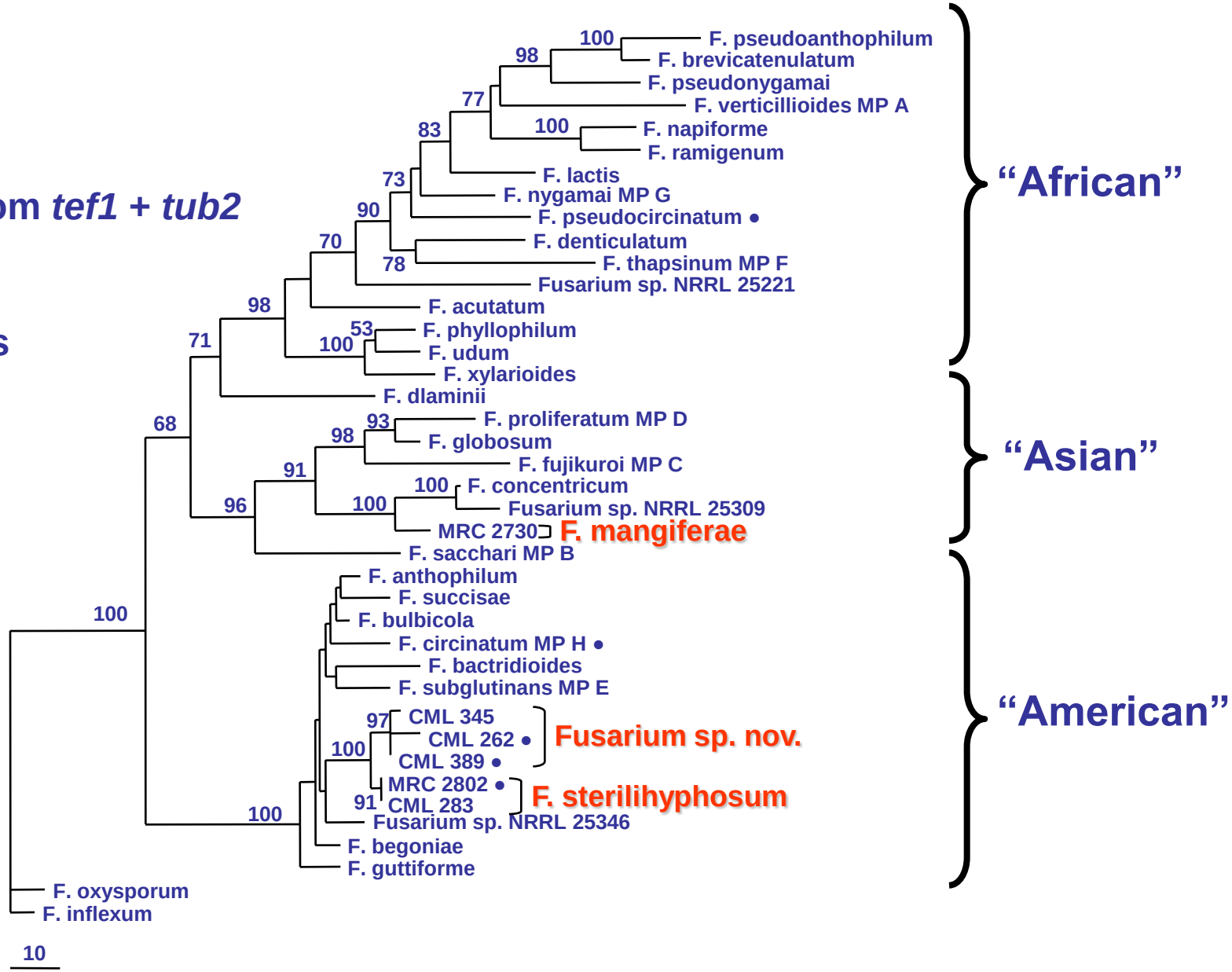
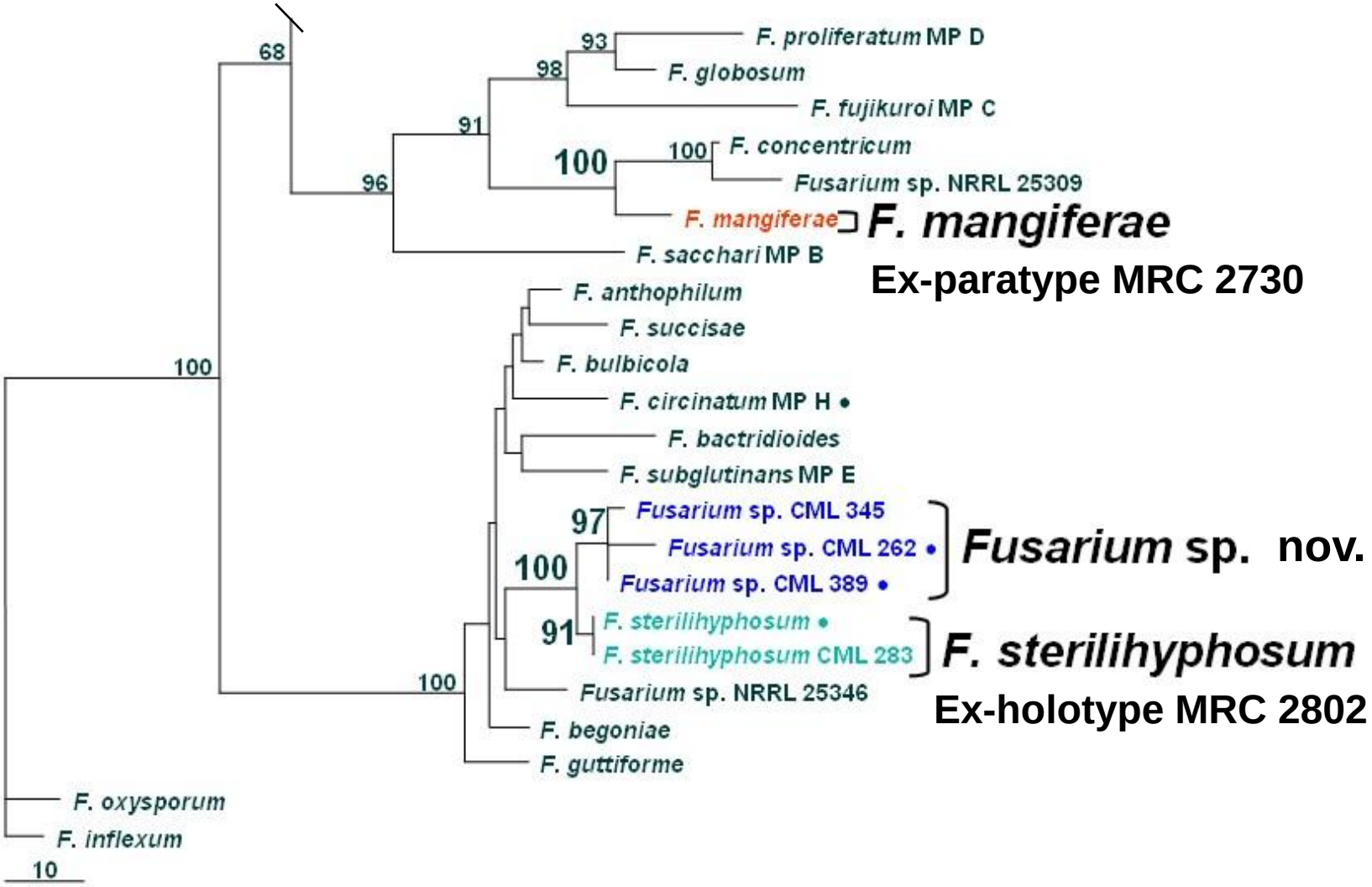
MP tree from *tef1* + *tub2*

1252 bp
1 of 2 trees
816 steps
CI 0.659
RI 0.804
HI 0.341





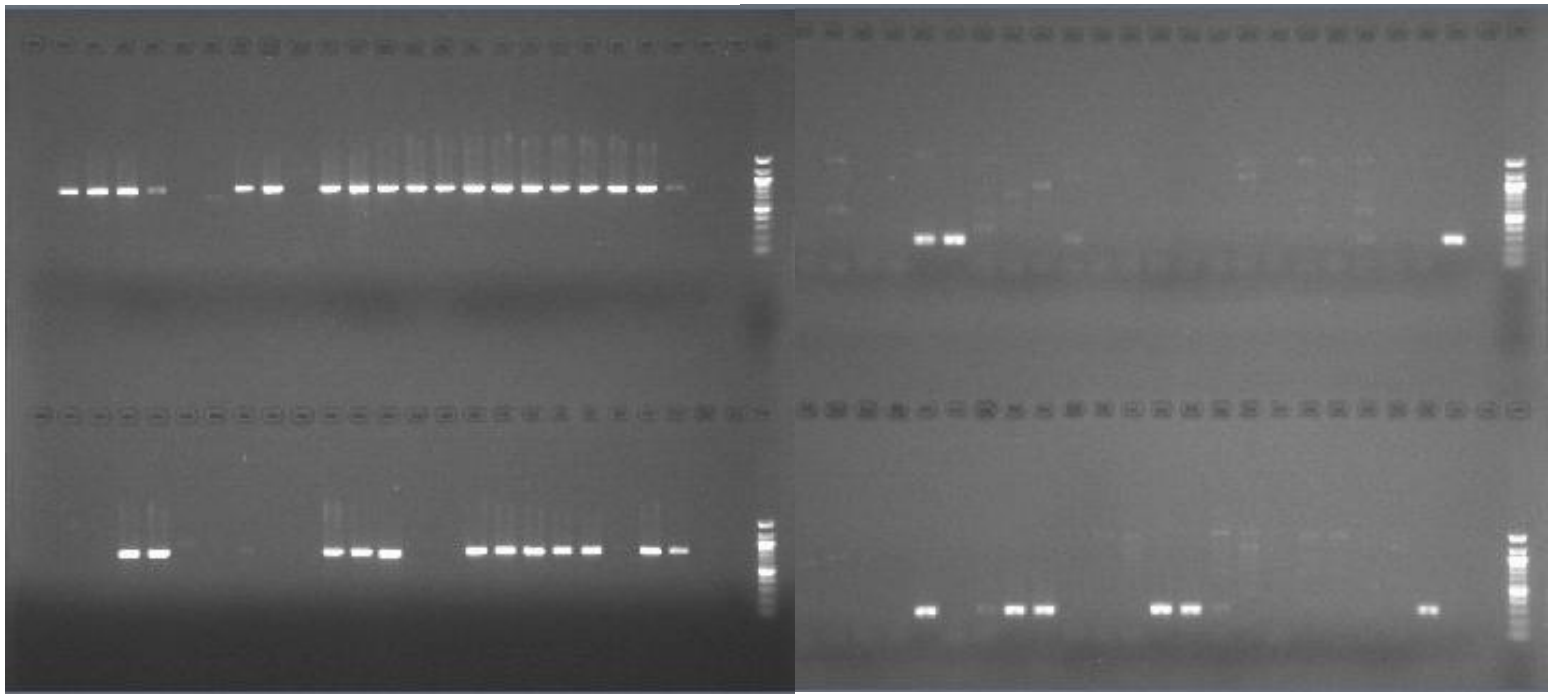
Maximum Parsimony Tree of Combined tub2 and tef1

Crossing

Identification of mating type

primers according to Steenkamp et al. (2000)

MAT-2 ***MAT-1***



Fertile crosses only to *Fusarium* sp. nov.

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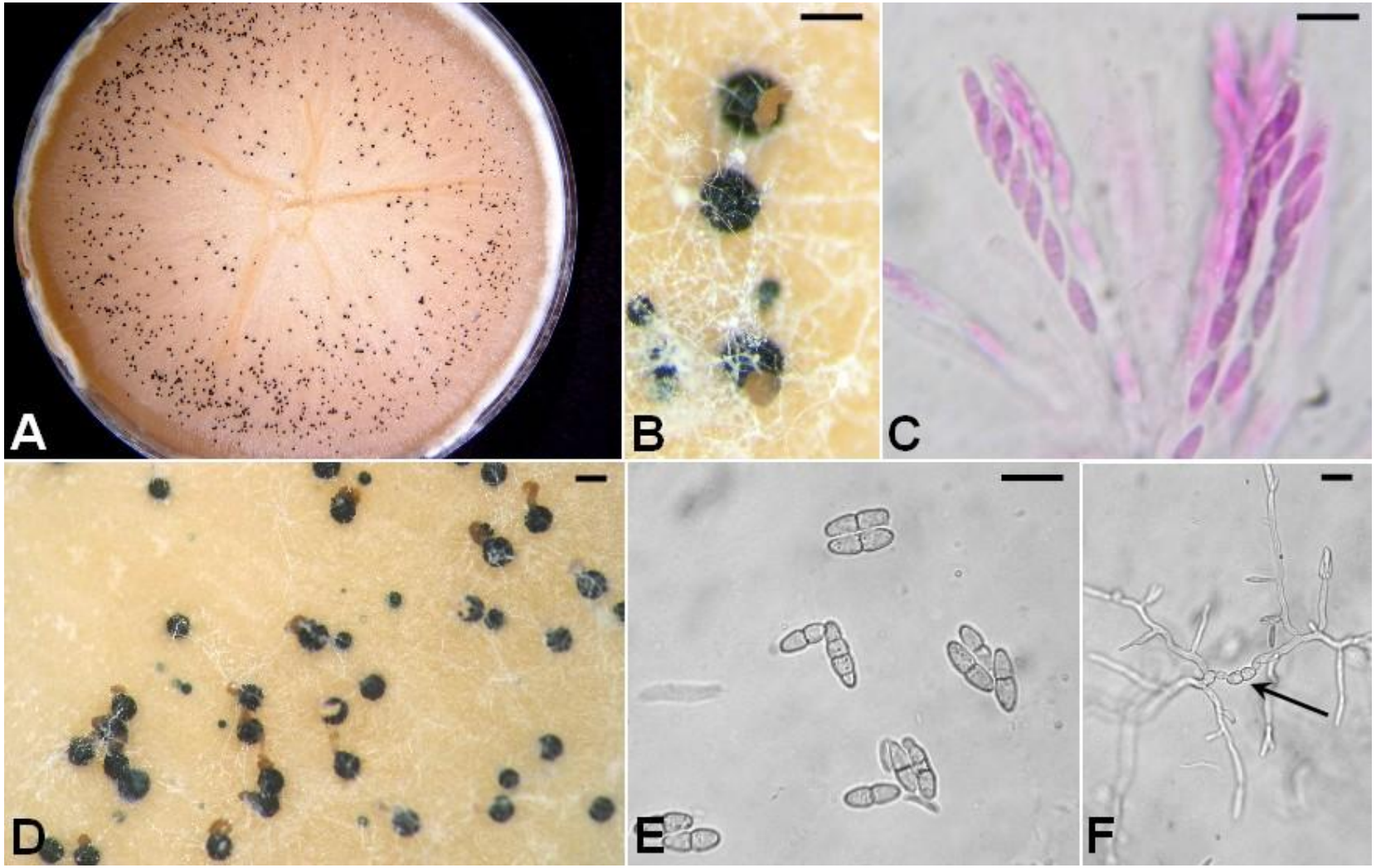
Evidence from morphology, AFLP fingerprint, phylogeny and crossing

F. mangiferae – **cosmopolitan, but not found in Brazil**

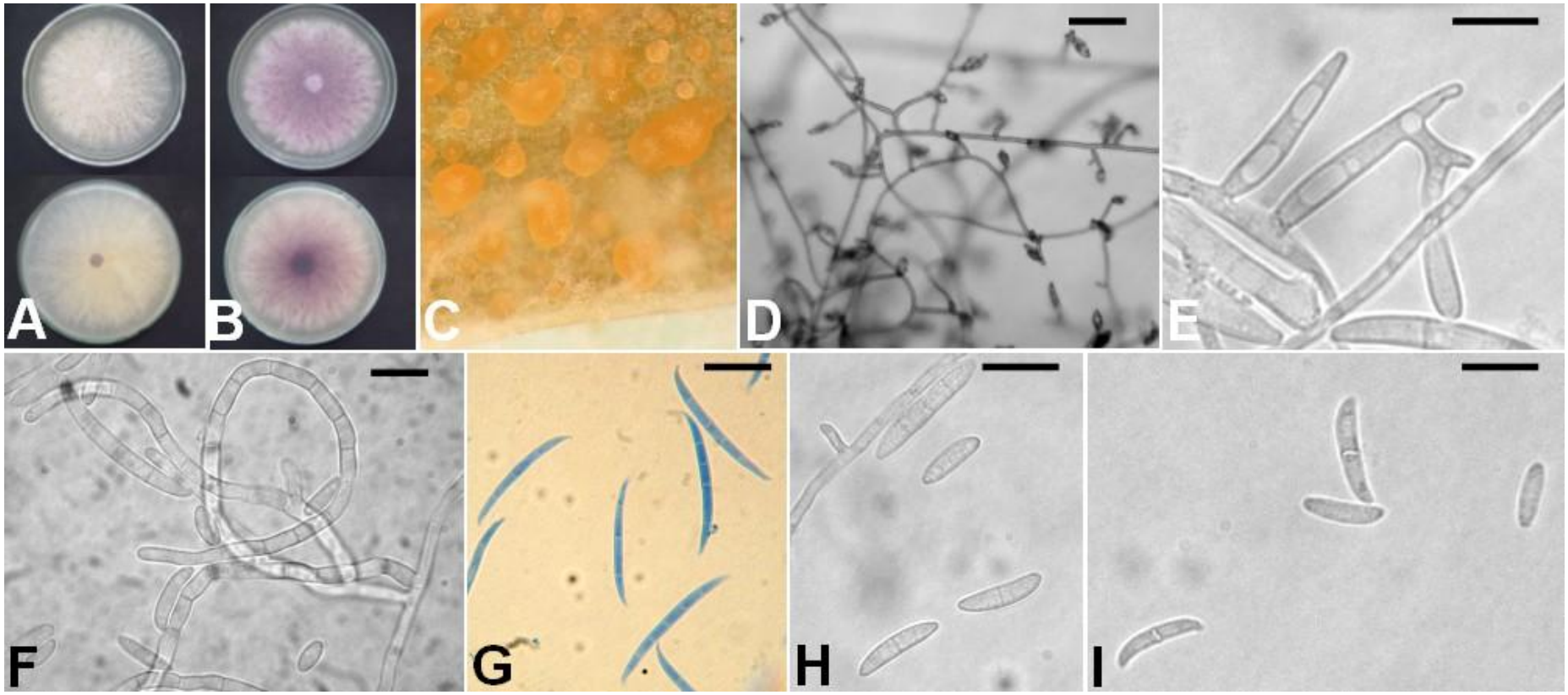
F. sterilihyphosum – **South Africa and Brazil**

Gibberella **sp. nov. - Brazil**

Gibberella sp. nov. Lima, Pfenning and Leslie



Gibberella anam. nov. Lima, Pfenning and Leslie



Pathogenicity

Control

Fusarium sp. nov.

F. sterilihyphosum

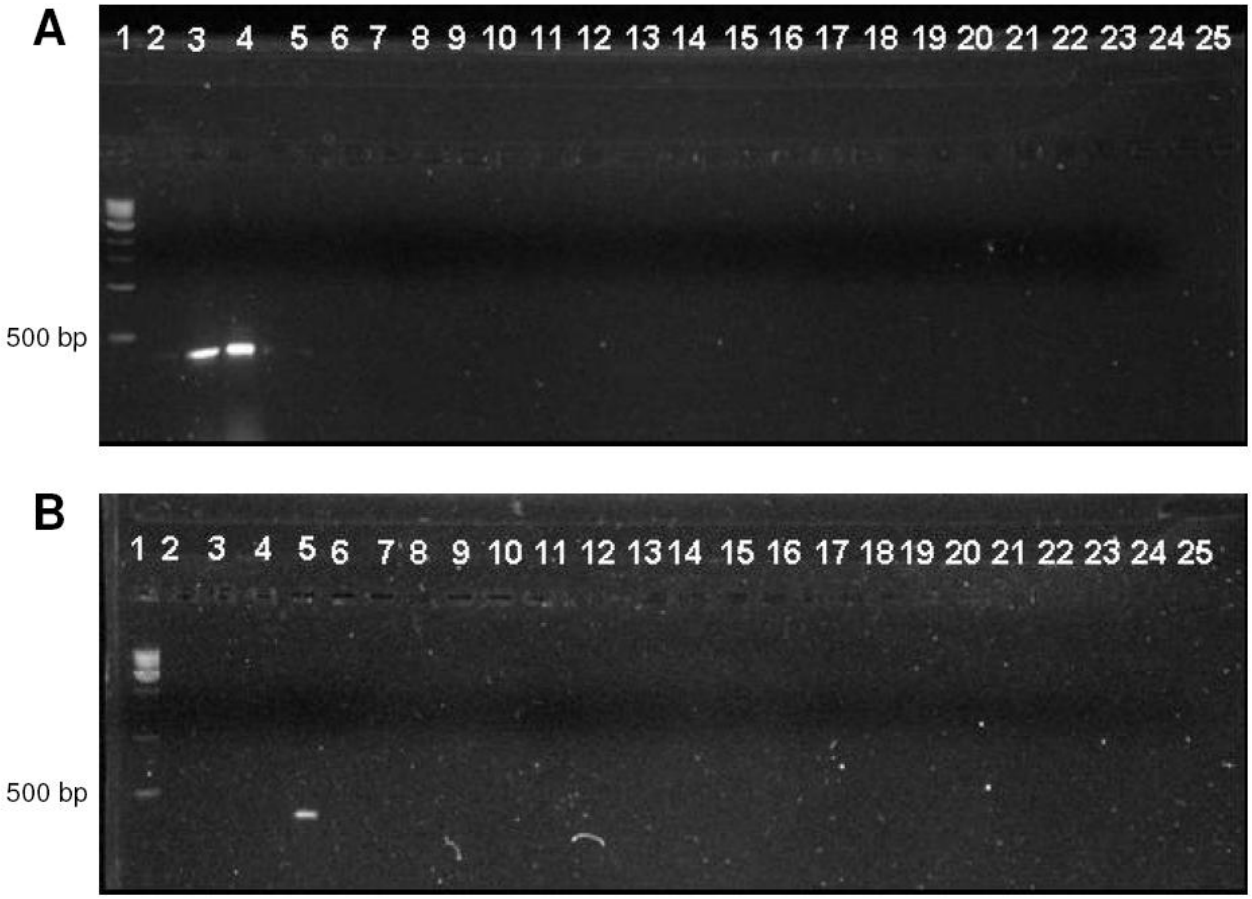
PCR detection of the causal agents of mango malformation

- Primer Sets designed from the tef1 sequences

  Fbra – Fusarium sp. nov. and F. sterilihyphosum (~380 bp)

  Fman – F. mangiferae (~217 bp)

- Specificity – pure DNA from mango pathogens and endophytes
- Sensitivity – DNA dilutions from 20 ng to 1 fg
- Pathogens' detection in shoot, leaf, bud, flower, fruit peel mango tissue
- Inoculation – according to Freeman et al. (1999)

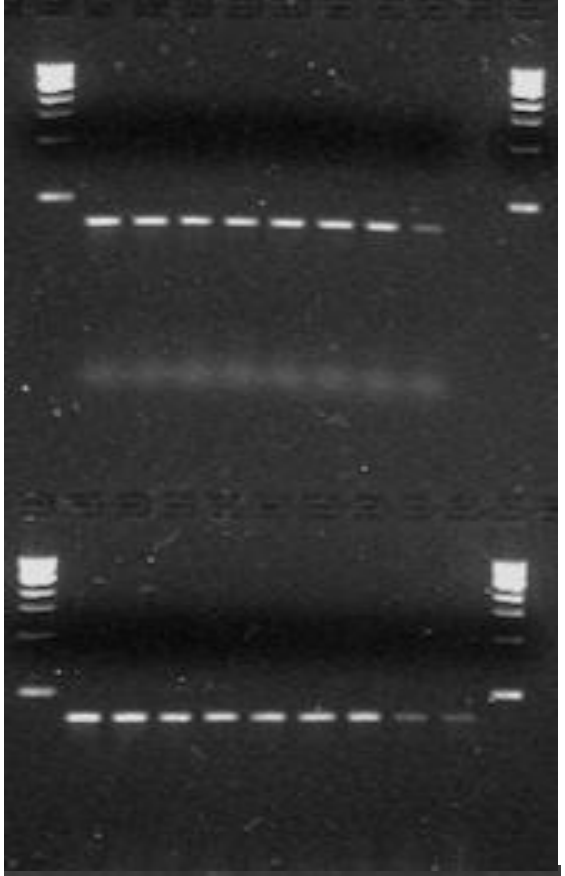


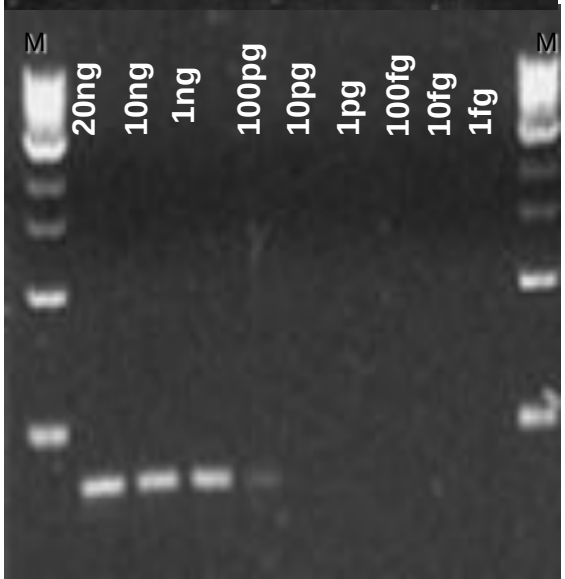
Specificity

A Fbra primer set (~380 bp)

B Fman primer set (~217 bp)

1. Marker
2. Control - water
3. Fusarium sp. nov. (DNA)
4. F. sterilihyphosum (DNA)
5. F. mangiferae (DNA)
6. F. proliferatum
7. F. sacchari
8. F. subglutinans
9. F. pseudocircinatum
10. F. oxysporum
11. F. semitectum
12. F. decemcellulare
13. Neocosmospora sp.
14. Phomopsis mangifera
15. Cladosp. cladosporioides
16. Epiccocum purpurascens
17. Fusiccocum mangiferum
18. Alternaria alternata
19. Coll. gloeosporioides
20. Aspergillus niger
21. Penicillium sp.
22. Pestalotiopsis sp.
23. Chalara fimbriata
24. Lasiodiplodia sp.
25. Phoma sp.





A

B

C

Sensitivity

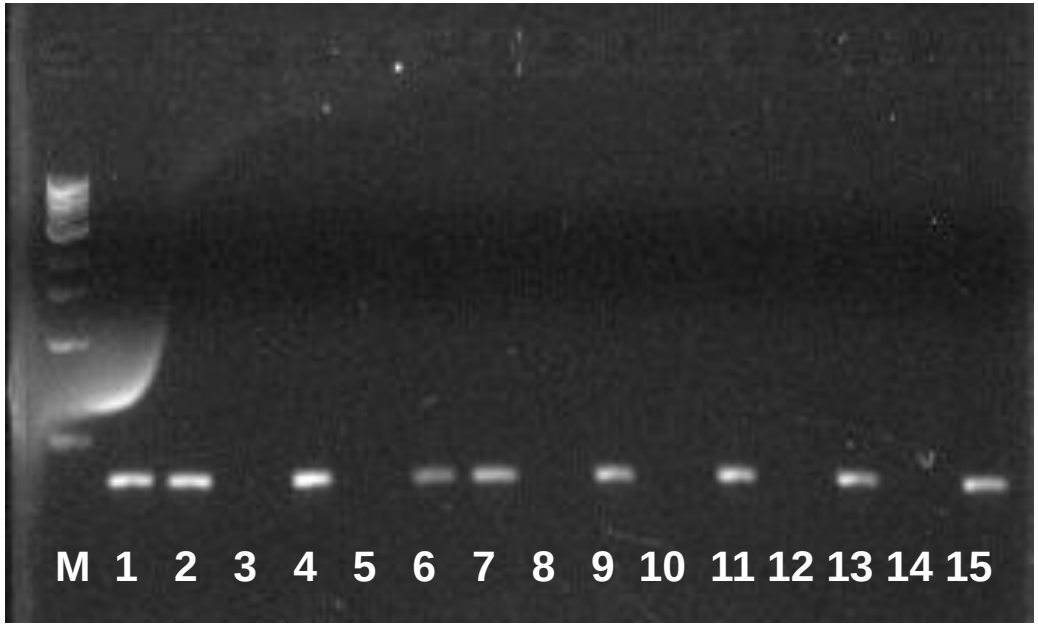
A. Fbra - Fusarium sp. nov.

B. Fbra - F. sterilihyphosum

C. Fman - F. mangiferae

Detection of Fusarium sp. nov. and F. sterilihyphosum

Fbra primer set



1. Fusarium sp. nov. (DNA)
2. F. sterilihyphosum (DNA)
3. F. mangiferae (DNA)
4. Naturally infected panicle
5. Asymptomatic inflorescence
6. Malformed plant inoculated with Fusarium sp. nov.
7. Malformed plant inoculated with F. sterilihyphosum.

8 and 9. Asymptomatic plant

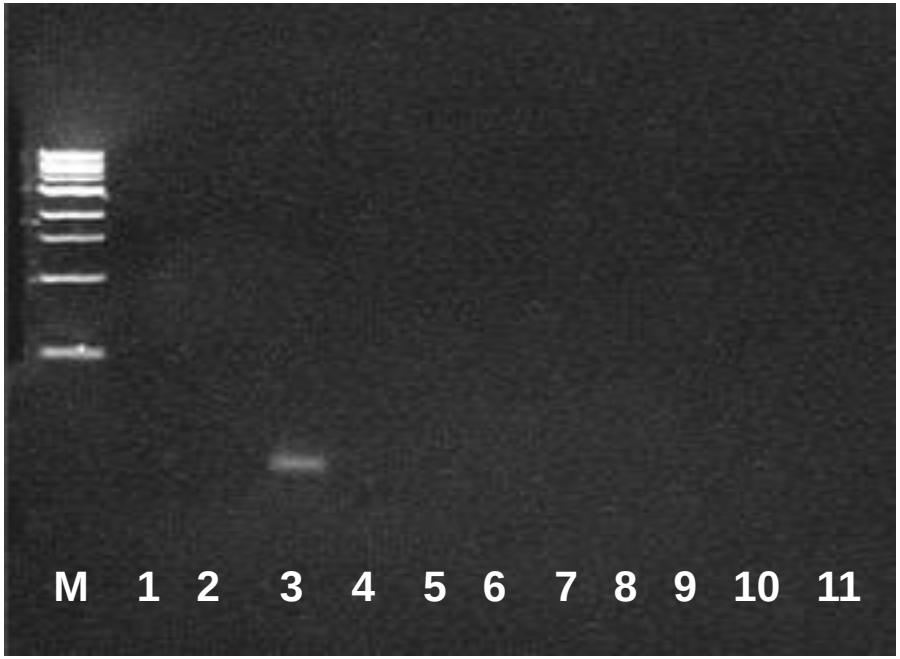
10 and 11. Fruit peel

12 and 13. Fruit peduncle

14 and 15. Mango leaf

Detection of F. mangiferae

Fman primer set



1. Fusarium sp. nov. - DNA
2. F. sterilihyphosum - DNA
3. F. mangiferae - DNA
4. Naturally infected panicle
5. Asymptomatic inflorescence
6. Malformed plant inoculated with Fusarium sp. nov.
7. Malformed plant inoculated with F. sterilihyphosum
8. Asymptomatic plant
9. Fruit peel
10. Fruit peduncle
11. Mango leaf

Conclusions

- The main causal agent of mango malformation disease in Brazil corresponds to a new Fusarium species within the Gibberella fujikuroi species complex
- The teleomorphic stage of Fusarium sp. nov. can be induced in the laboratory
- Fusarium sp. nov. and F. sterilihyphosum are the only known causal agents of the disease in Brazil and can be detected through PCR

Fusariosis of Pineapple - *Ananas comosus*

Most important disease of this crop, restricted to South America ?

Fusarium subglutinans

Fusarium subglutinans* f.sp. *ananas

Fusarium guttiforme

Morphology, Specificity, tub and tef genes

Limited number of isolates

Nirenberg & O'Donnell 1998

***Gibberella fujikuroi* complex – GFC, but no teleomorph known so far**

Fusariosis of Pineapple - *Ananas comosus*

Fusarium associated with pineapple

Symptomatology



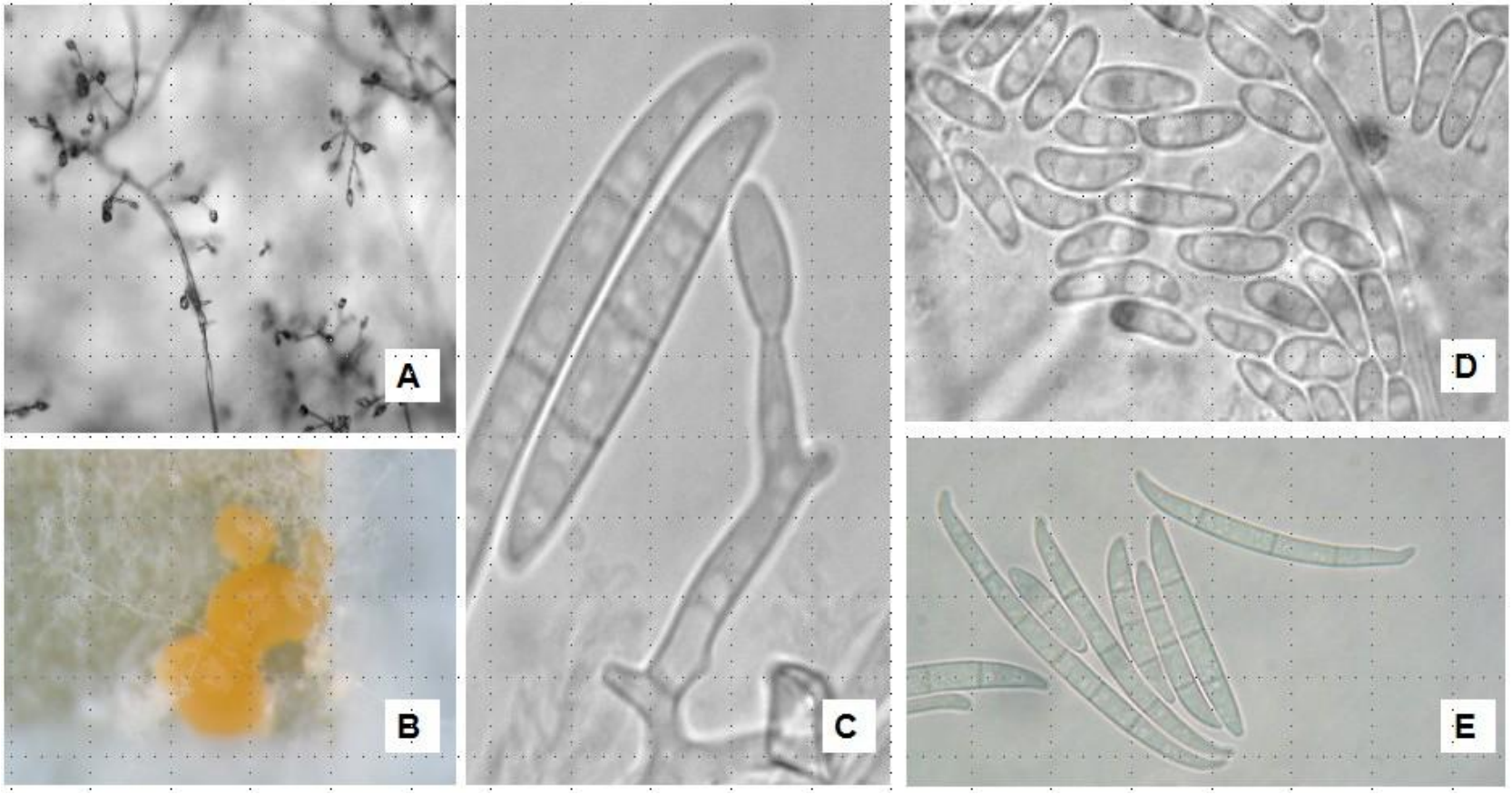


Fusariosis of Pineapple - *Ananas comosus*

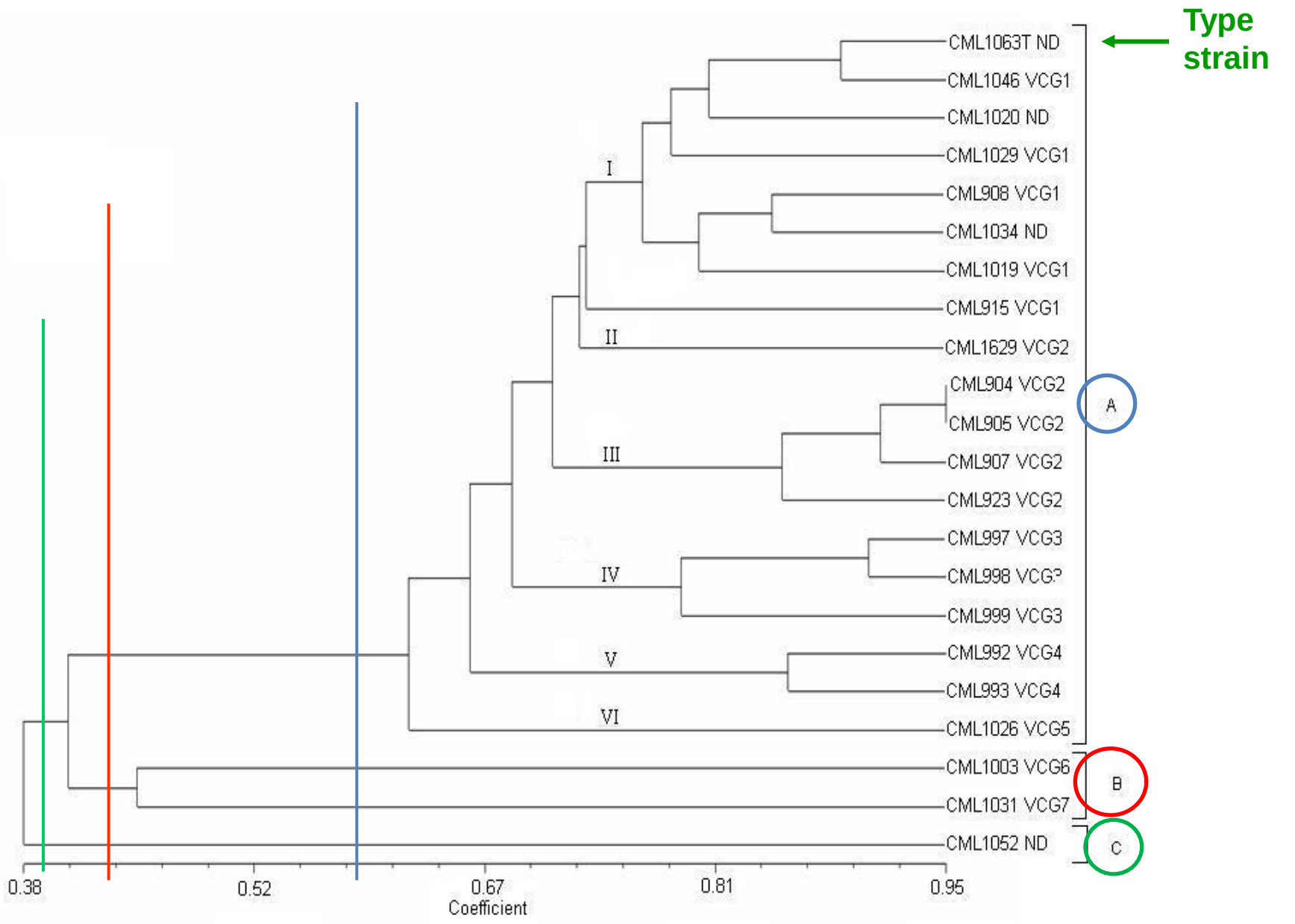
- Collection of isolates from all over Brazil
- Morphological markers
- Pathogenicity tests
- VCG analysis
- AFLP fingerprint
- Phylogenetic analysis of *tub* and *tef* genes

Fusariosis of Pineapple - *Ananas comosus*

Morphology of *Fusarium guttiforme*



Groups evidenced by VCG and AFLP fingerprint analysis



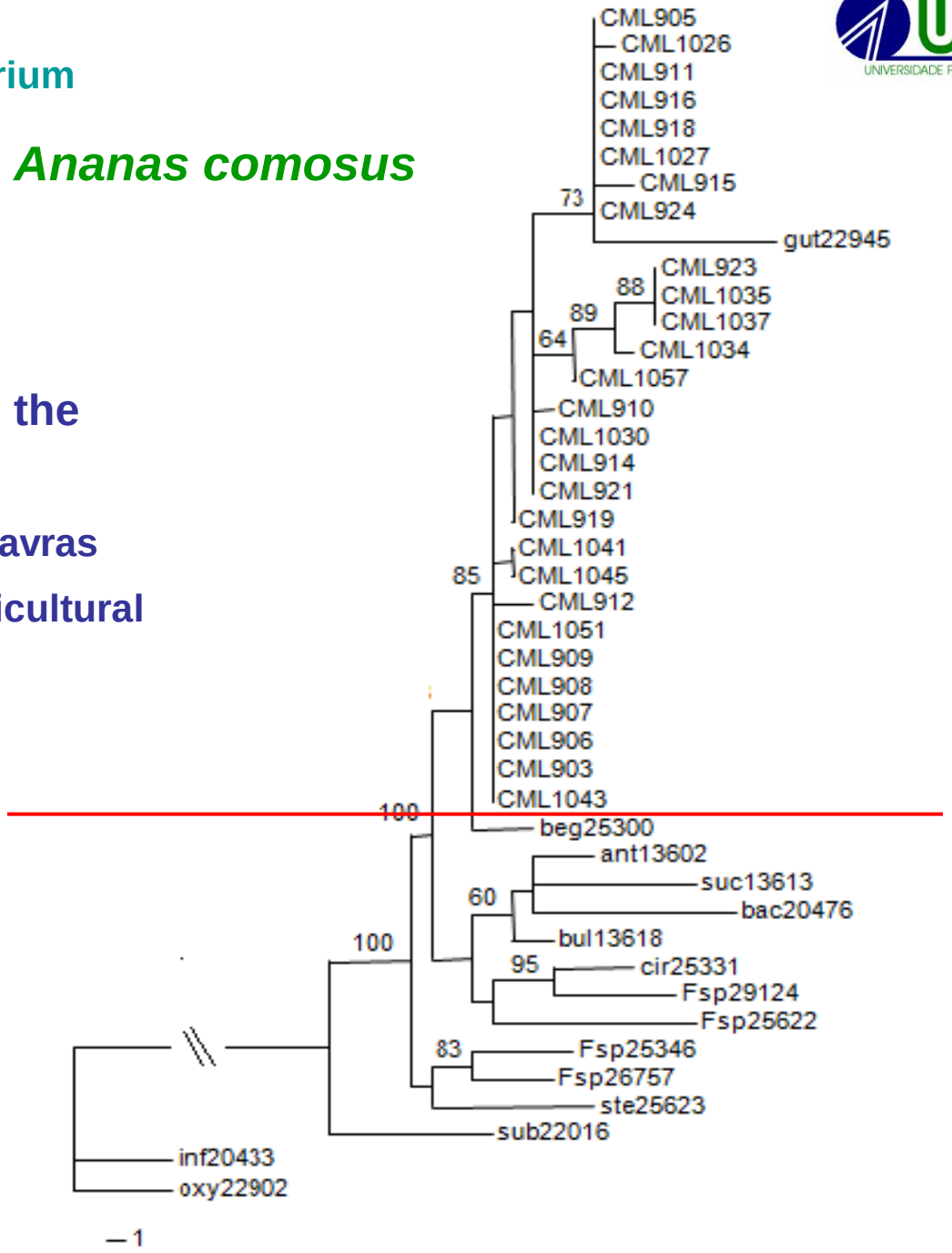
Fusariosis of Pineapple - *Ananas comosus*

tub2* and *tef1

***F. guttiforme* and species in the American clade of GFC**

CML - Coleção Micológica de Lavras

NRRL - National Center for Agricultural Utilization Research



Fusariosis of Pineapple - *Ananas comosus*

Conclusions

- ***F. guttiforme*** **confirmed as the main pathogen of *Fusarium* wilt and resinosis of pineapple in Brazil.**
- **High genetic diversity observed in the population is consistent with the hypothesis that it is near the center of origin, and the possibility of the occurrence of sexual reproduction in species cannot be excluded.**
- **The observation of two divergent AFLP groups with similarity below 40% in comparison with the main group suggests that there may be distinct populations causing wilt in pineapple in Brazil.**

Perspectives and future work

- **Further collection, including native species of *Ananas***
- **Crossing experiments**
- **Metabolite profiling**
- **And ……**

Fusariosis of Pineapple - *Ananas comosus*

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Fusarium ananatum sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa

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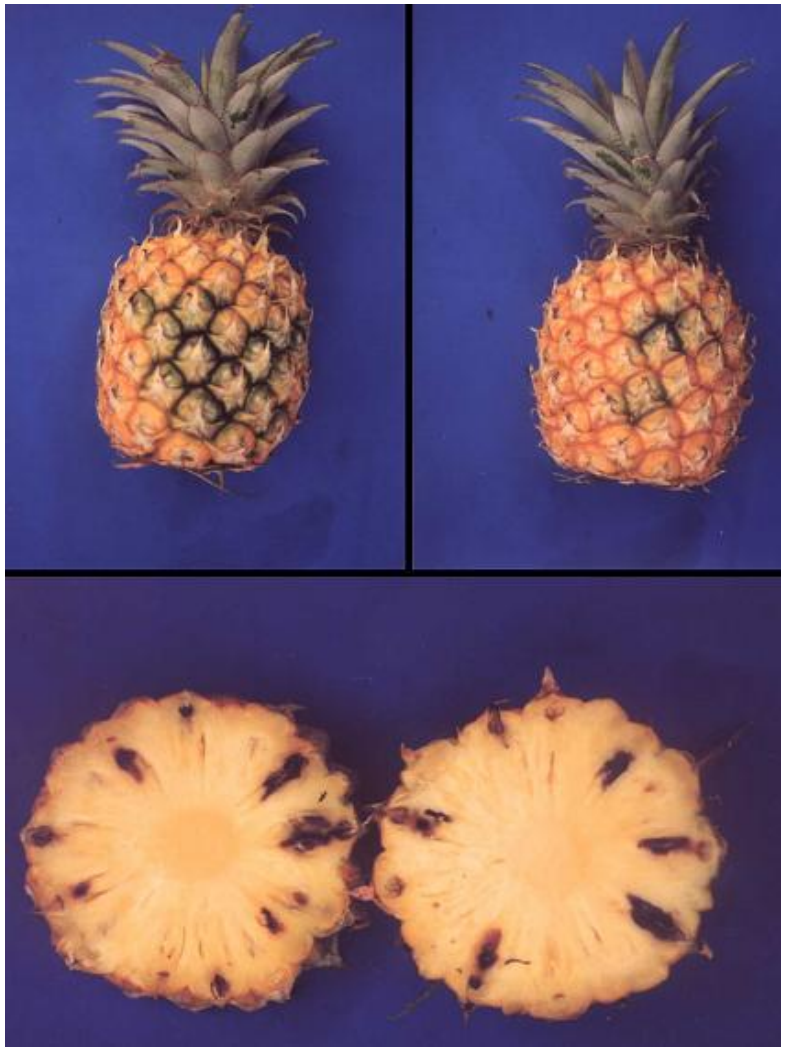
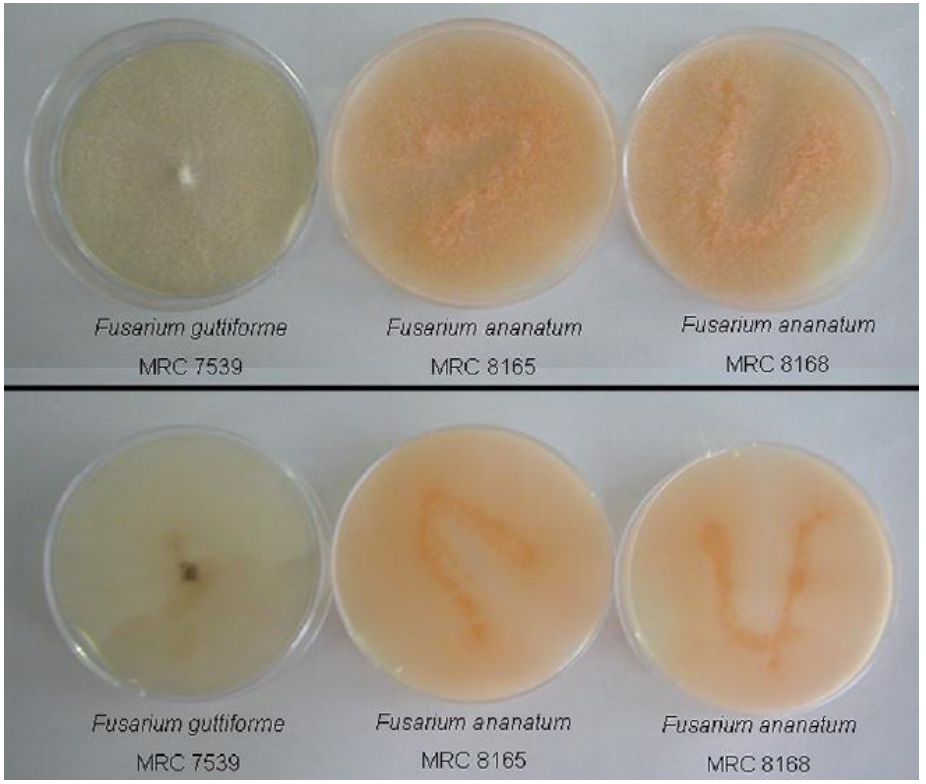
Fusariosis of Pineapple - *Ananas comosus*



Symptoms

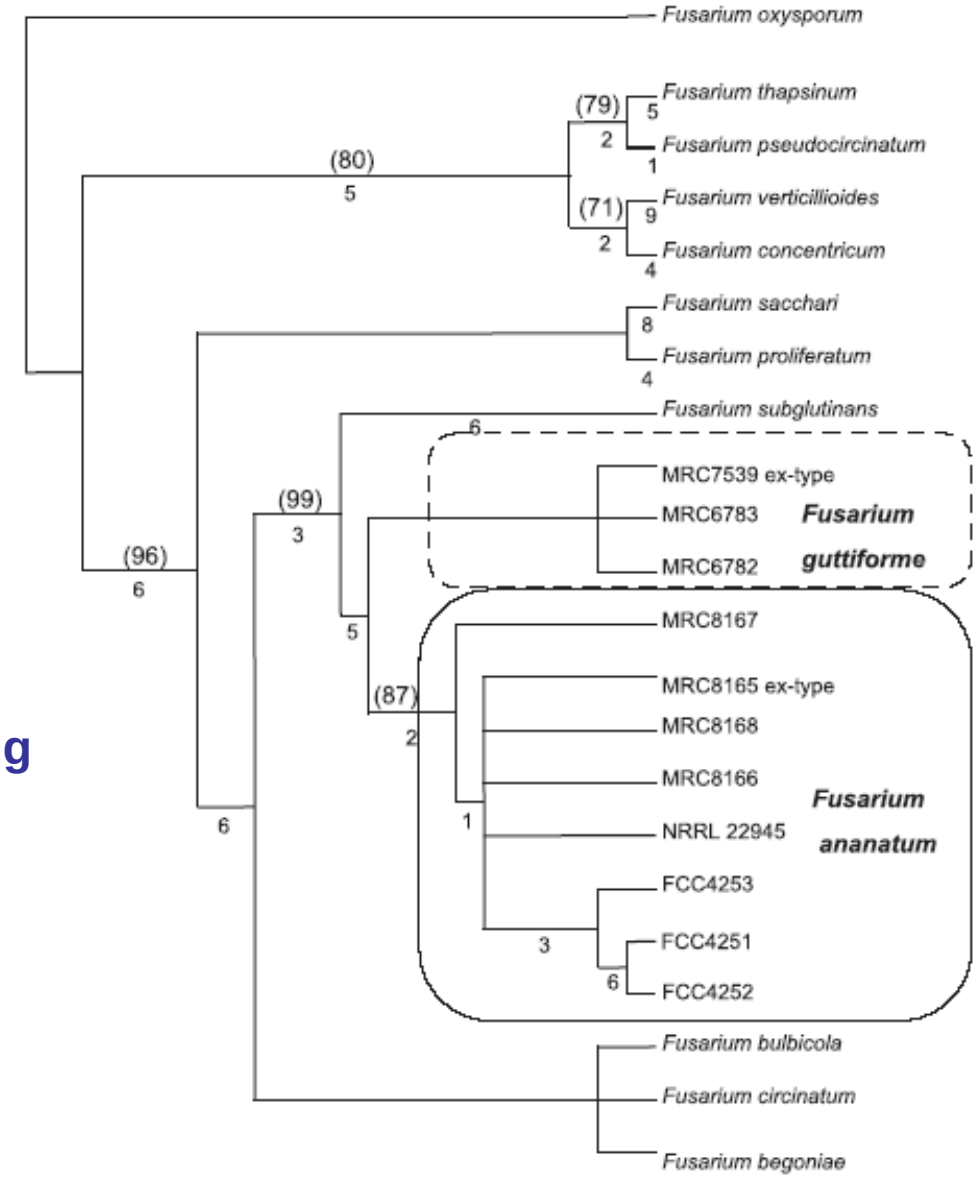
Fusarium ananatum

Black spot



Culture morphology

Fusariosis of Pineapple - *Ananas comosus*



Phylogenetic tree of F. ananatum and related species produced using parsimony of tef-1a

Informative characters 53

CI = 0.6737

RI = 0.8510

g1 = -0.476216

Panama Disease, Fusariosis, wilt disease

Banana - *Musa* spp.

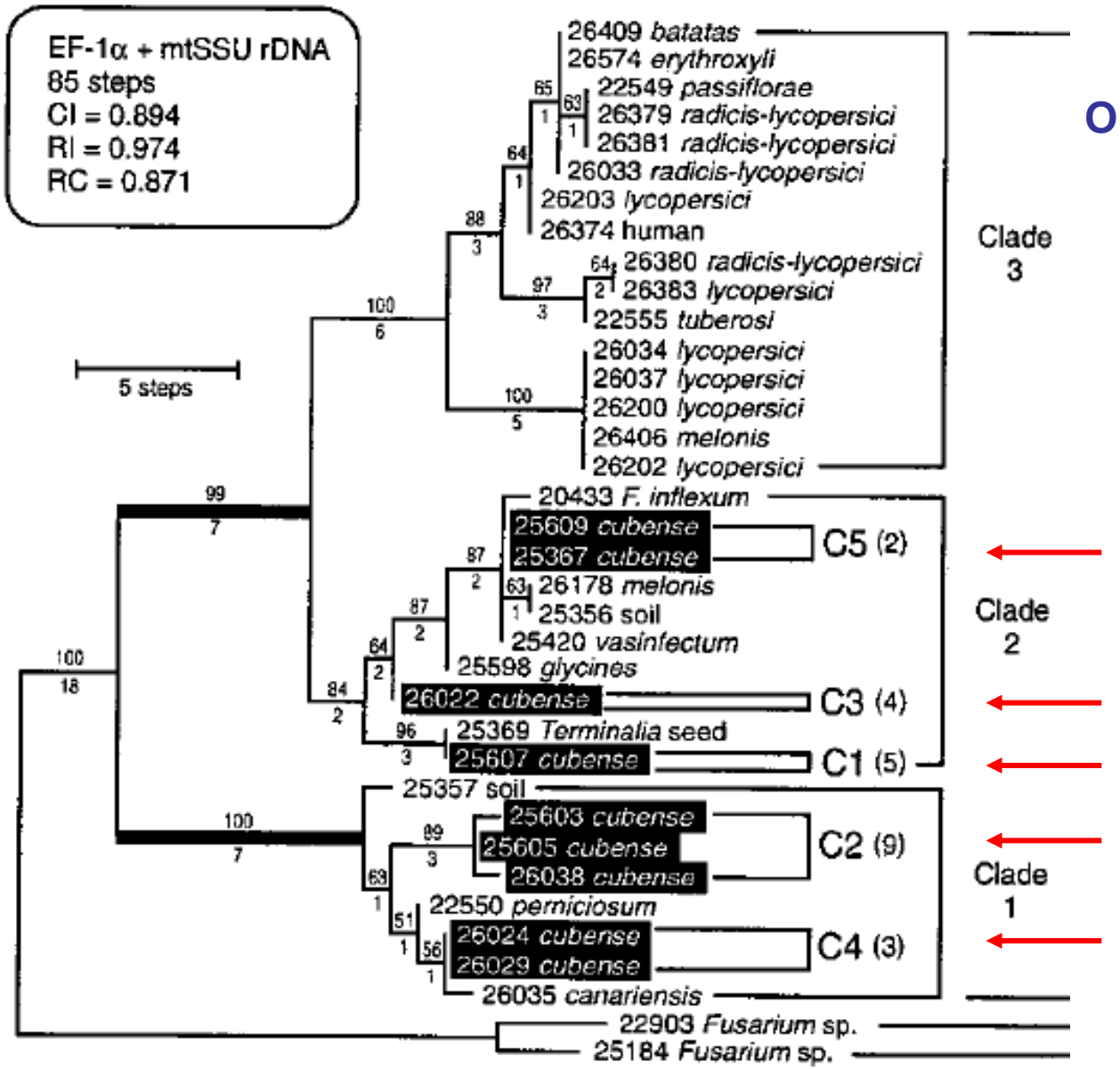
Fusarium oxysporum* f. sp. *cubense

Teleomorph unknown

Four races ??

- **Polyphyletic**
- **Distinct evolutionary origin**
- **... Five species (?), maybe more**

Panama disease of banana



O'Donnell et al. 1998

Panama disease of banana

Applied and Environmental Microbiology, July 2009, p. 4770–4781 Vol. 75, No. 14
0099-2240/09/$08.00+0 doi:10.1128/AEM.00370-09

Evolutionary Relationships among the *Fusarium oxysporum* f. sp. *cubense* Vegetative Compatibility Groups▽

Gerda Fourie,[1]* E. T. Steenkamp,[1] T. R. Gordon,[2] and A. Viljoen[1,3]

Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa[1]; Department of Plant Pathology, University of California, Davis, California 95616[2]; and Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa[3]

Received 16 February 2009/Accepted 21 May 2009

…. clearly showed that ability of *F. oxy. cub*

1 to cause disease on banana has emerged multiple times, independently

2 to cause disease to a specific banana cultivar is also a polyphyletic trait

Panama disease of banana

Disease caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*

Ploetz 2006

- ***Fusarium oxysporum* f. sp. *cubense* represents at least five distinct clonal lineages**
- **Races of *F. oxysporum* f. sp. *cubense* may belong to different species**
- **Concept of races is not consistent**
- **The *formae speciales* concept is not phylogenetically informative**

Fusariosis of Passion fruit

Passiflora spp. - Disease complex

Causal agents:

1 ***Fusarium oxysporum* f. sp. *passiflorae* - Wilt**

2 ***Fusarium solani***

root rot, stem rot and canker

develop to wilting and dieback

present also as an endophyte

Ploetz 2003

Nunes & Albuquerque 1995, Pará, Brazil
Fusarium solani - Nectria haematococca

Nirenberg & Brielmeyer-Liebetanz 1996, Germany
Fusarium striatum – Haematonectria ipomoeae

UNIVERSIDADE FEDERAL DE LAVRAS

Fusariosis of Passion fruit

Wilt syndrom in the field

Photos: Jeferson M. Dariva

Fusariosis of Passion fruit

F. oxysporum f.sp. passiflorae

Fusarium solani

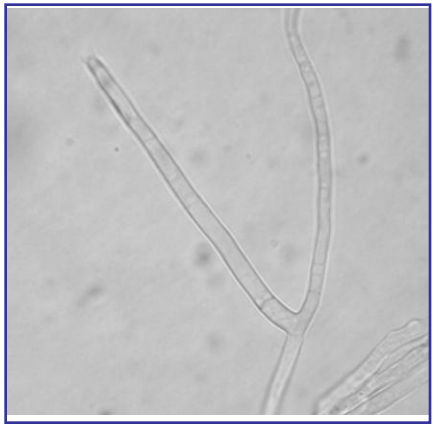
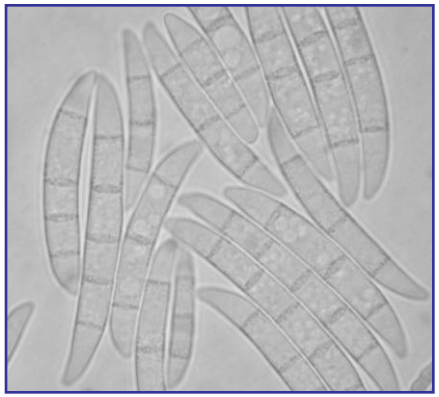
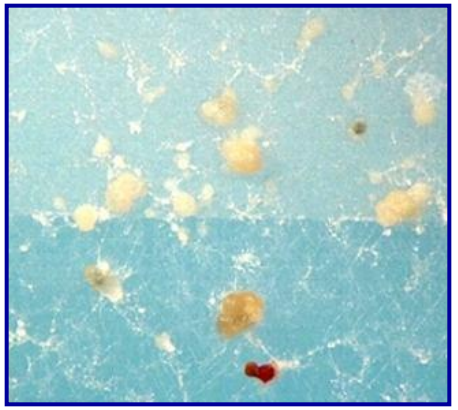
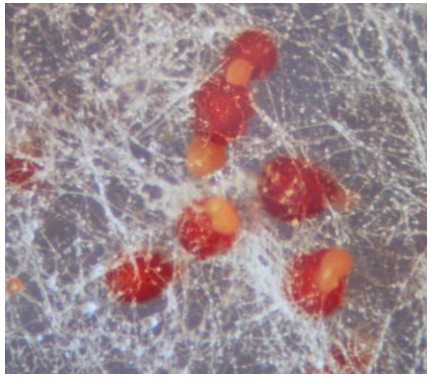
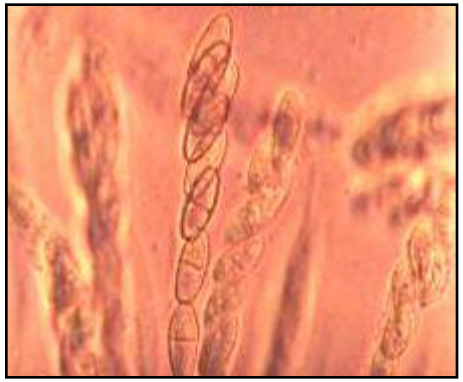
Photos: Jeferson M. Dariva

Morphological markers within the form species *Fusarium solani*

- Growth rate of colony, pigmentation
- Ramification of the conidiophore
- Format, septation and size of micro- and macroconidia
- Specificity, Others ?

Telomorph – *Haematonectria*

- Homothallic + heterothallic spp.
- Ascospores

Form species *Fusarium solani* - Section Martiella

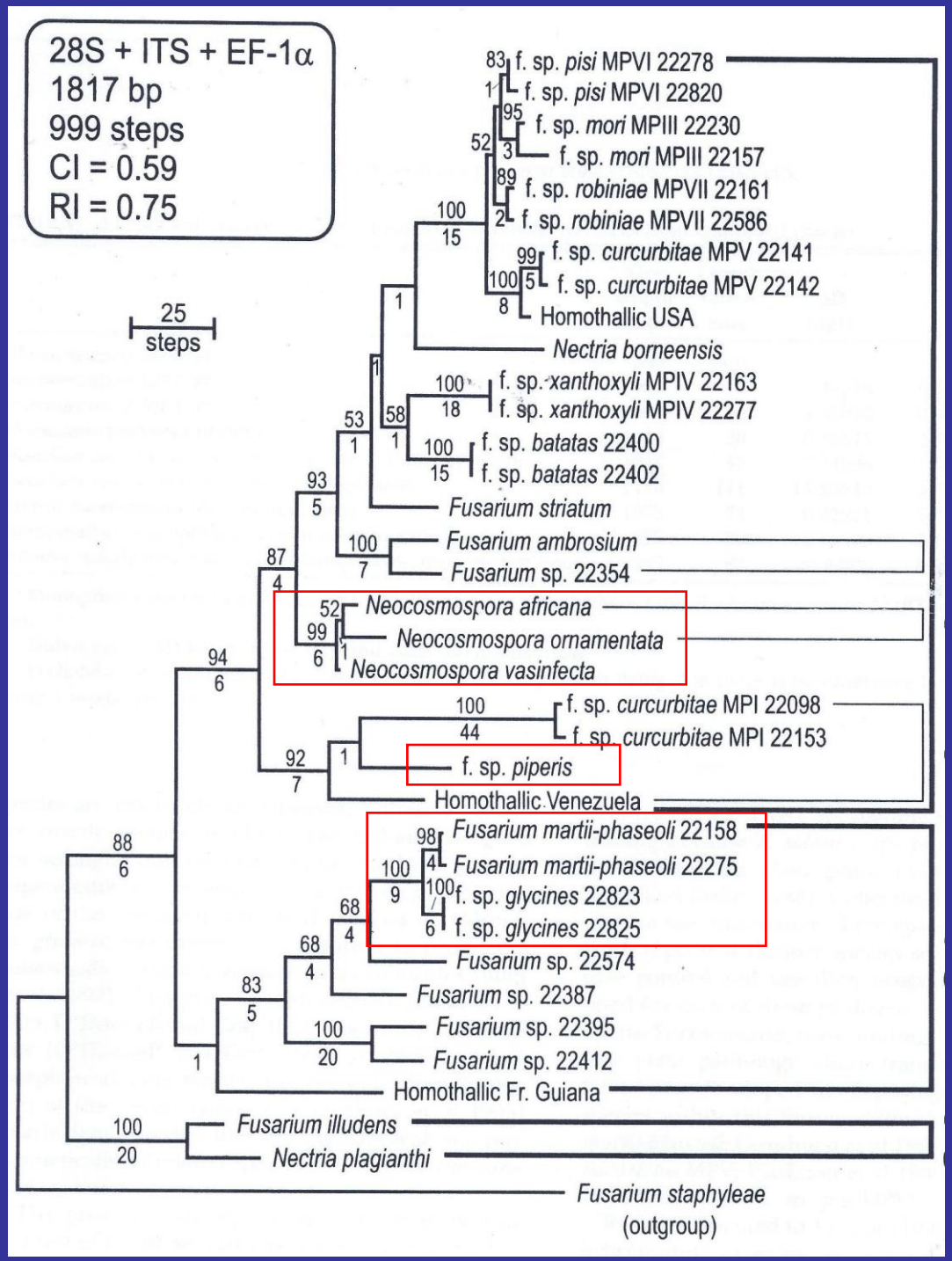
Named species, populations, strains etc. :

Fusarium ambrosium	India
Fusarium illudens	Nova Zelandia – tel *Nectria illudens*
Fusarium martii-phaseoli	USA, *Phaseolus vulgaris*
Fusarium striatum (homothallic)	Panama – tel *Haematonectria ipomoeae*
Fusarium sp.	Guiania
Fusarium sp.	Venezuela
Nectria borneensis	Indonesia
Nectria plagianthi	Nova Zelandia
Neocosmospora africana	
N. vasinfecta	
N. ornamentata (homothallic)	

(O'Donnell 2000)

Named *formae speciales* and *mating populations* in the Fusarium solani species complex - FSSC

1	*F sol* f. sp. *batatas*	MP-II	*Ipomoea batatas*
2	*F sol* f. sp. *cucurbitae* race 1	MP-I	*Cucurbita* spp.
3	*F sol* f. sp. *cucurbitae* race 2	MP-V	*Cucurbita* spp.
4	*F sol* f. sp. *glycines*		*Glycine max*
5	*F sol* f. sp. *mori*	MP-III	*Morus alba*
6	*F sol* f. sp. *piperis*		*Piper nigrum*
7	*F sol* f. sp. *pisi*	MP-VI	*Pisum sativum*
8	*F sol* f. sp. *robiniae*	MP-VII	*Robinia* spp.
9	*F sol* f. sp. *xanthoxyli*	MP-IV	*Xanthoxylum* sp.



Strict consensus cladogram

Clade 3

Asian

Africa

S-American

Clade 2 - SDS pathogens soy beans

S-American

O'Donnell 2000

Clade 1

New Zealand

SDS - Sudden death syndrome of soybean

Causal agent: ***Fusarium solani*** **f.sp.** ***glycinis***

Mycoscience (2005) 46:162–183
DOI 10.1007/s10267-005-0235-y

FULL PAPER

Takayuki Aoki · Kerry O'Donnell
María Mercedes Scandiani

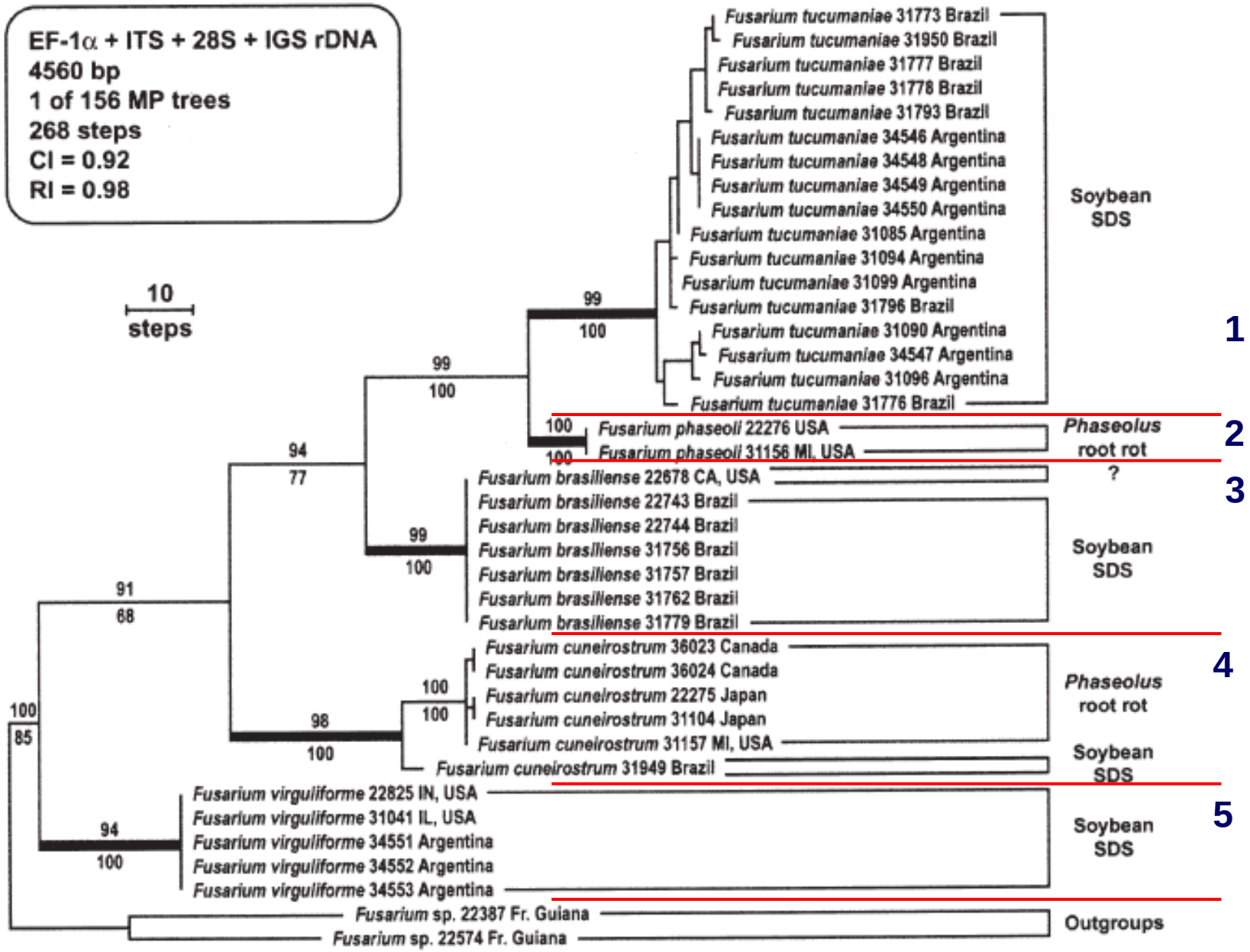
Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*

Fusarium solani **on soybean and common bean**

Morphology; pathogenicity; ITS, 28S, IGS, EF-1α

5 distinct species, only a few isolates from Brazil were included

SDS - Sudden death syndrome of soybean



Aoki et al. 2005

Etiology of Sudden death syndrome of soybean in Brazil

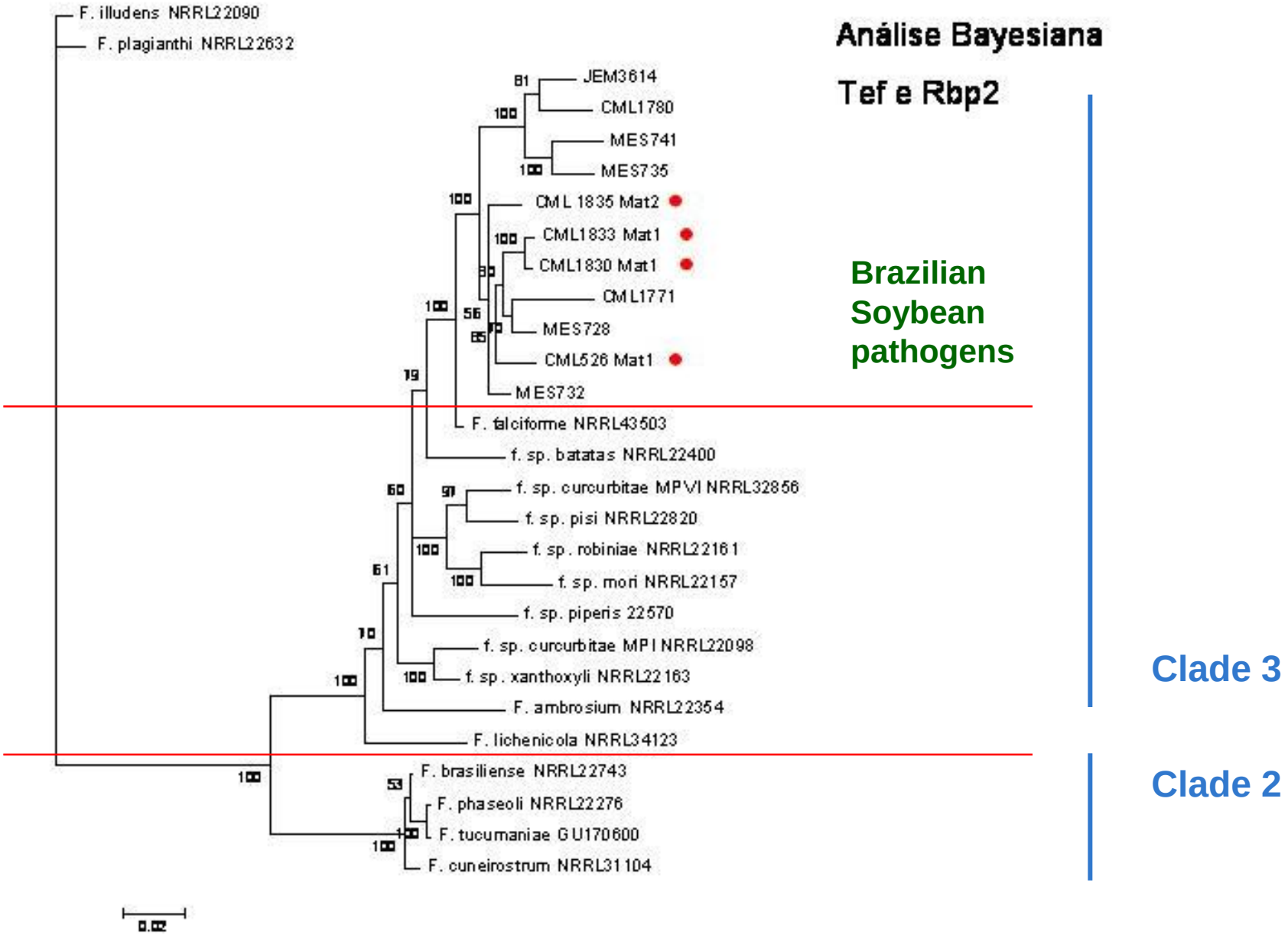
Objectives

- **To identify the causal agents of Sudden death syndrome of soybean in Brazil**

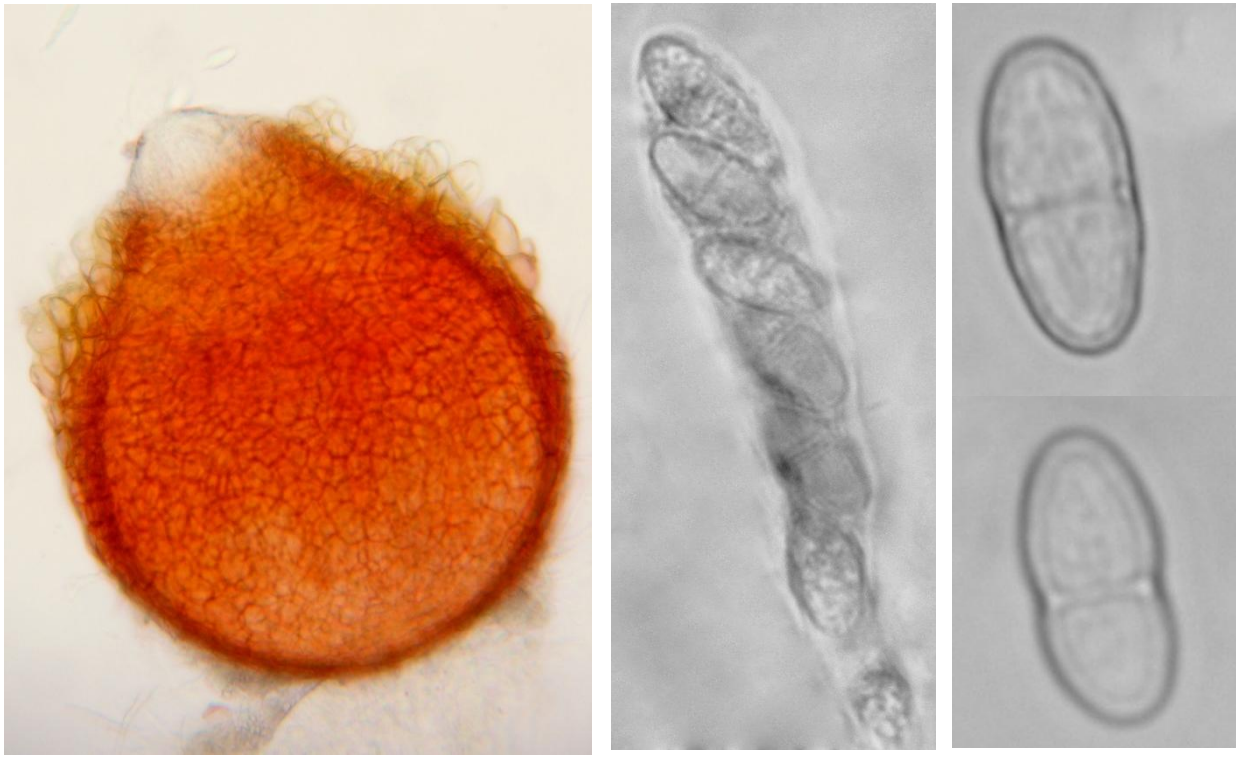
  Methods Gene Sequencing, Morphological Characterization, Pathogenicity Test

- **To identify biological species - mating populations**

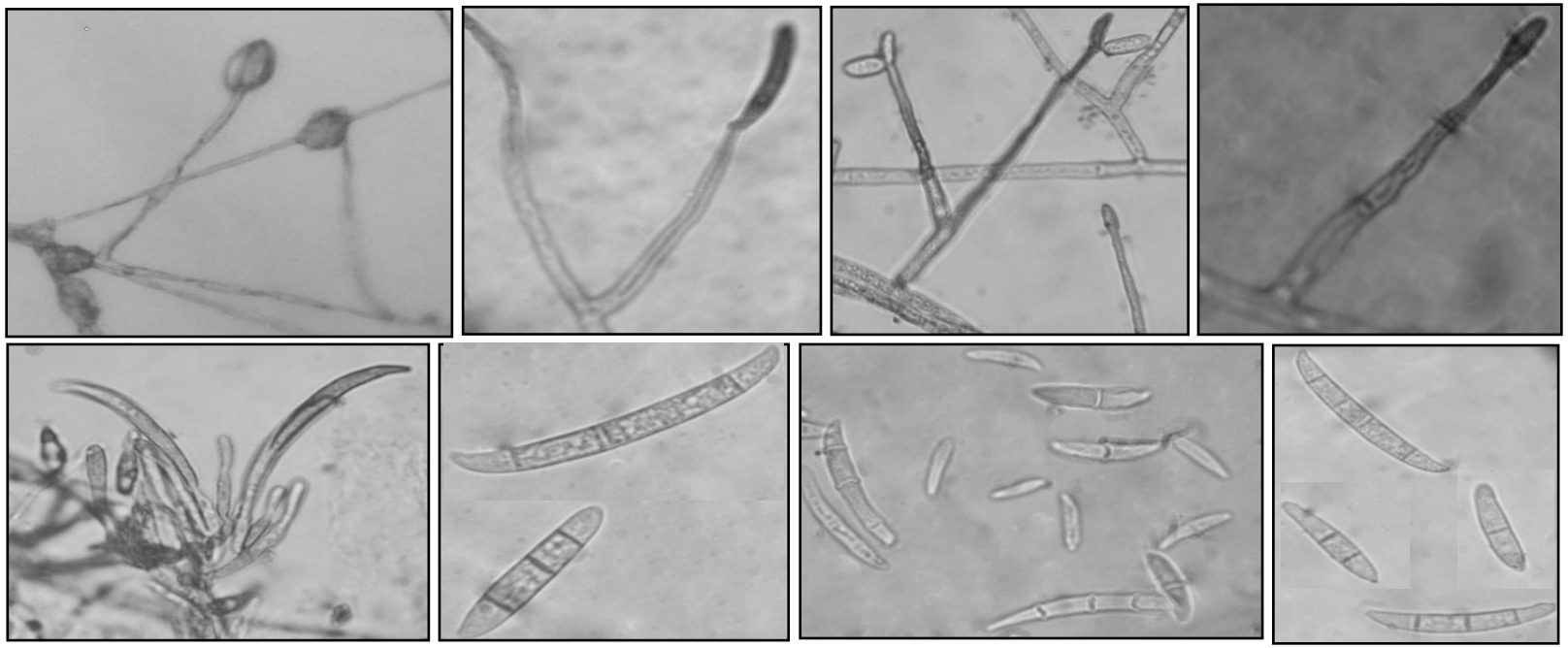
  Methods Identification of mating type (PCR) and crossing


Análise Bayesiana
Tef e Rbp2
F. illudens NRRL22090
F. plagianthi NRRL22632
JEM3614
CML1780
MES741
MES735
CML1835 Mat2
CML1833 Mat1
CML1830 Mat1
CML1771
MES728
CML526 Mat1
MES732
F. falciforme NRRL43503
f. sp. batatas NRRL22400
f. sp. curcurbitae MPVI NRRL32856
f. sp. pisi NRRL22820
f. sp. robiniae NRRL22161
f. sp. mori NRRL22157
f. sp. piperis 22570
f. sp. curcurbitae MPI NRRL22098
f. sp. xanthoxyli NRRL22163
F. ambrosium NRRL22354
F. lichenicola NRRL34123
F. brasiliense NRRL22743
F. phaseoli NRRL22276
F. tucumaniae GU170600
F. cuneirostrum NRRL31104
Brazilian Soybean pathogens
Clade 3
Clade 2

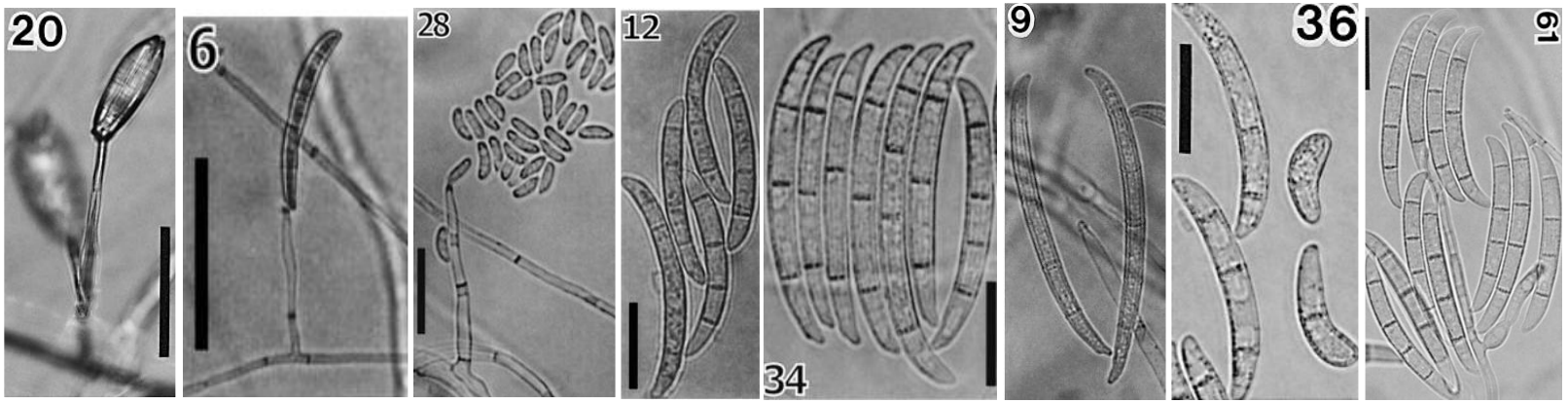
Sexual reproduction in the soybean sudden death syndrome pathogen



Morphological characters



Brazilian isolates



Aoki et al; 2003, 2005

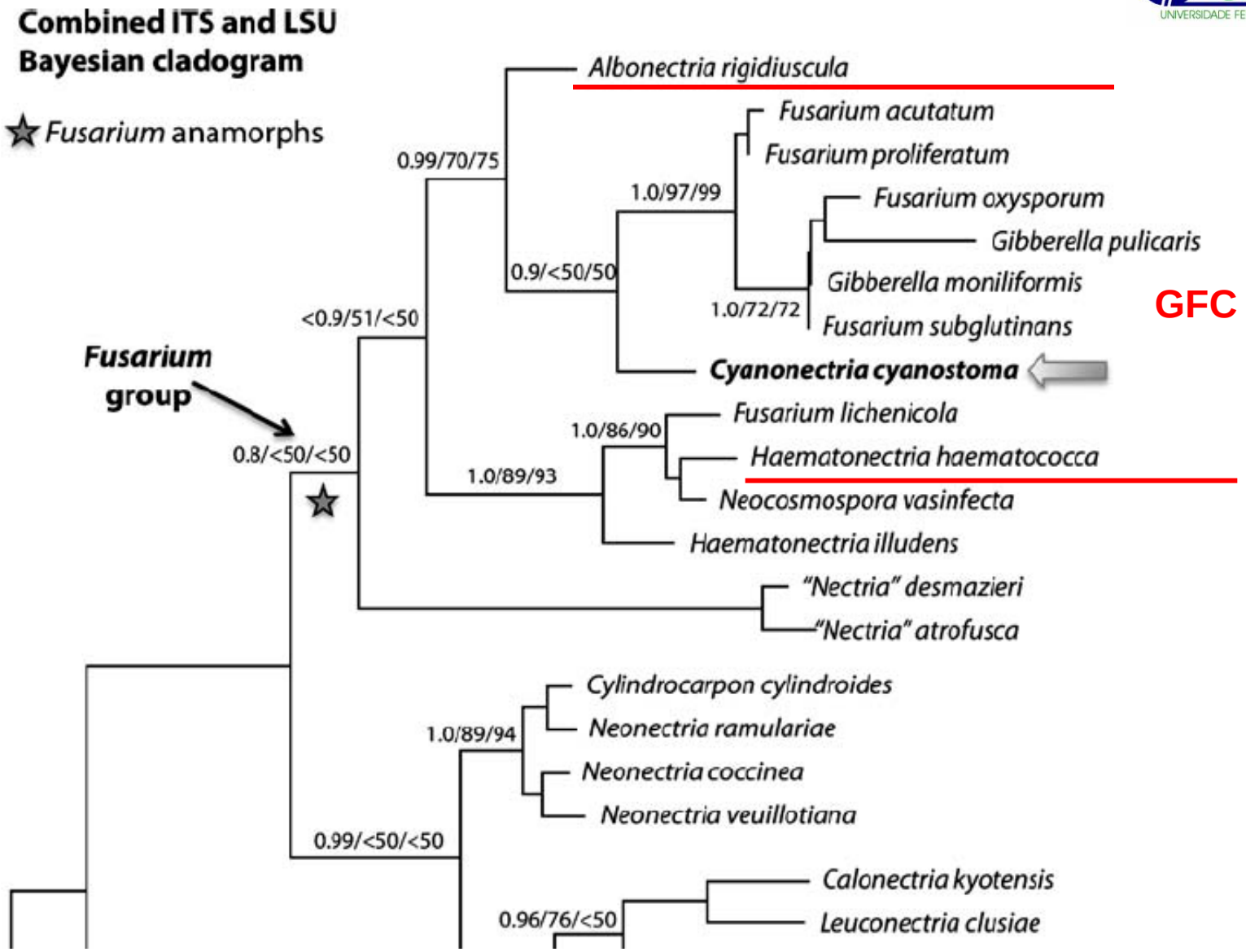
Piper nigrum - Black Pepper

Fusarium solani f. sp. *piperis*

Most important disease of black pepper in Brazil

Is supposed to be a distinct species within FSSC

Photos: Lahyre I. Gomes



Samuels et al. 2009. Mycol. Progress

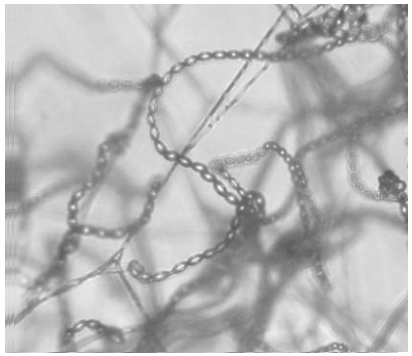
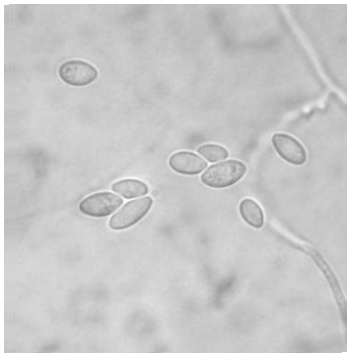
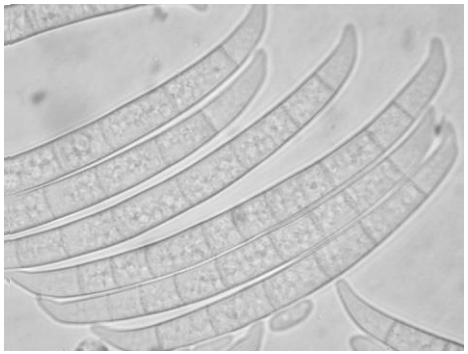
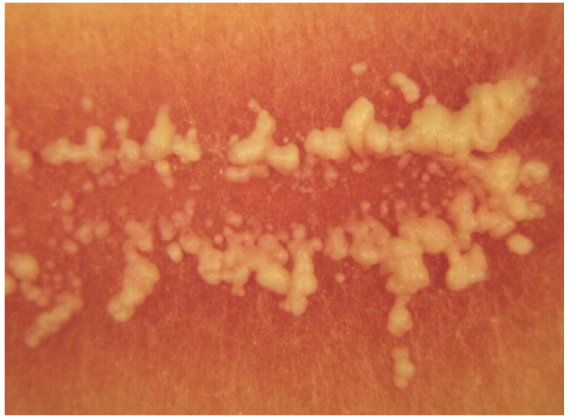
Cocoa - *Theobroma cacau*

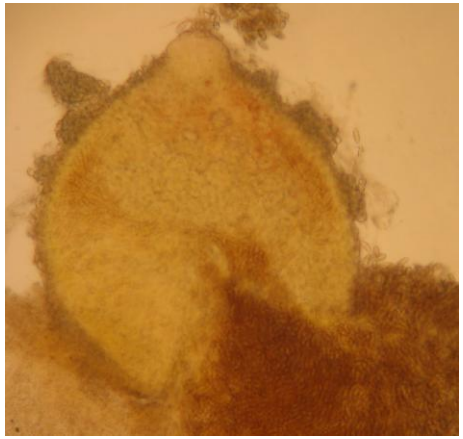
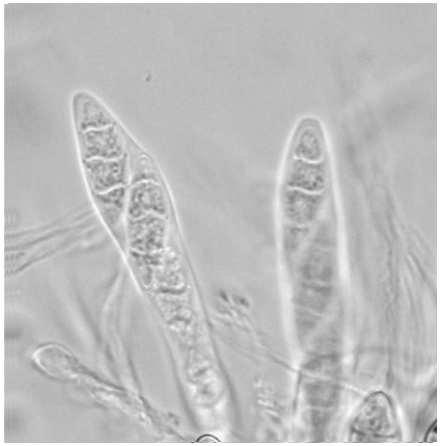
Fusarium decemcellulare

Albonectria rigidiuscula

Disease: cushion gall

Fusarium decemcellulare
Teleomorph *Albonectria rigidiuscula*


Characterization of populations - approaches

- **Morphology** MSC
- **AFLP *fingerprint***
- **Sequencing and Phylogenetic analysis** PSC
- **Sexual Compatibility, *mating populations*** BSC
- **Vegetative Compatibility, *vegetative compatibility groups***

Phylogenetic Classification System -

only monophyletic taxa are accepted

Characterization of populations and species

The Ideal World – El Mundo Ideal – O Mundo Ideal

MSC = BSC = PSC

Biological Resource Centers:

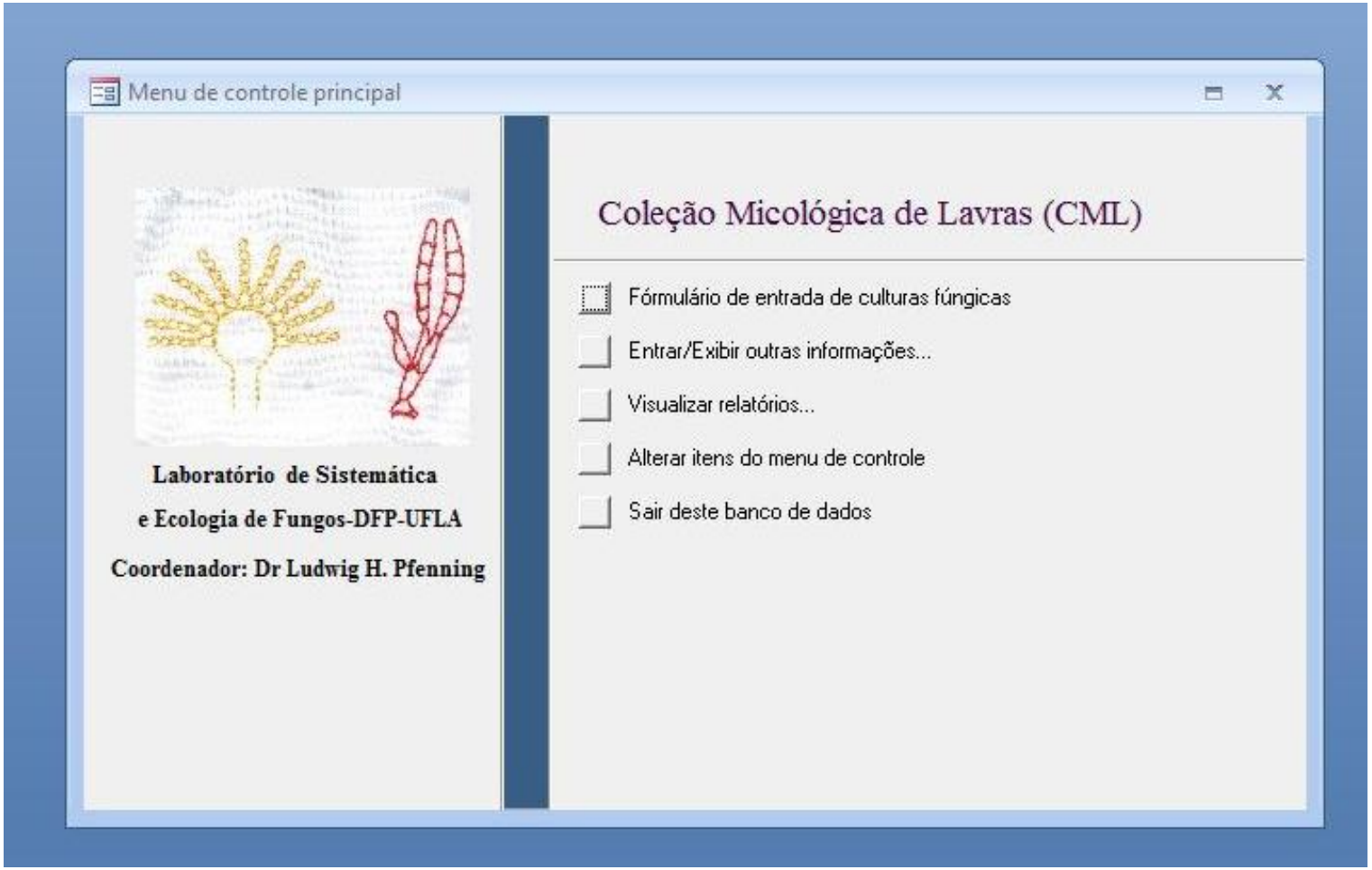
Gateway to biodiversity and services to innovation in biotechnology

Our research activities :

- Impact of agriculture on soil fungi diversity
- Diversity and specificity of fungal endophytes in crops and plants of natural vegetation
- Metabolite profiling for chemotaxonomy and bioprospection
- Species concepts and characterization of populations of plant pathogens, for the development of PCR-based protocols for diagnostics and quarantine porposes

Biological Resource Centers:

Gateway to biodiversity and services to innovation in biotechnology



COLEÇÃO MICOLÓGICA DE LAVRAS (CML)

FORMULÁRIO DE REGISTRO DE CULTURAS FÚNGICAS

IDENTIFICAÇÃO

Número de acesso	CML 0001
Gênero	Fusarium
Espécie	verticillioides
Autoridade científica	J. Sheld
Sinônimos	F. moniliforme
Estado alternativo	
Família	Nectriaceae
Ordem	Hypocreales
Depositante	Ludwig Pfenning
Data de acesso	01/12/1998
Histórico	CCT 4776

ORIGEM

Substrato / hospedeiro	maracujá (Passiflora edulis)
Cultivar	nd
Especificação	fruto
Habitat	campo cultivado

Acknowledgements

Sarah S. Costa and Virginia G. Elizei, PhD students

Kedma S. Matos and Erica Sfalsin, Master students

Edson L. Rezende, Ana Karla F. Machado, Culture Collection CML

Lucas M. Abreu, Pos-Doc

Cristiano S. Lima - UFRPE, Recife PE, Brazil

José Aires Ventura - INCAPER, Vitória ES, Brazil

Jeferson M. Dariva - Unimontes, Janaúba MG, Brazil

John F. Leslie - KSU, Manhattan, Kansas, USA