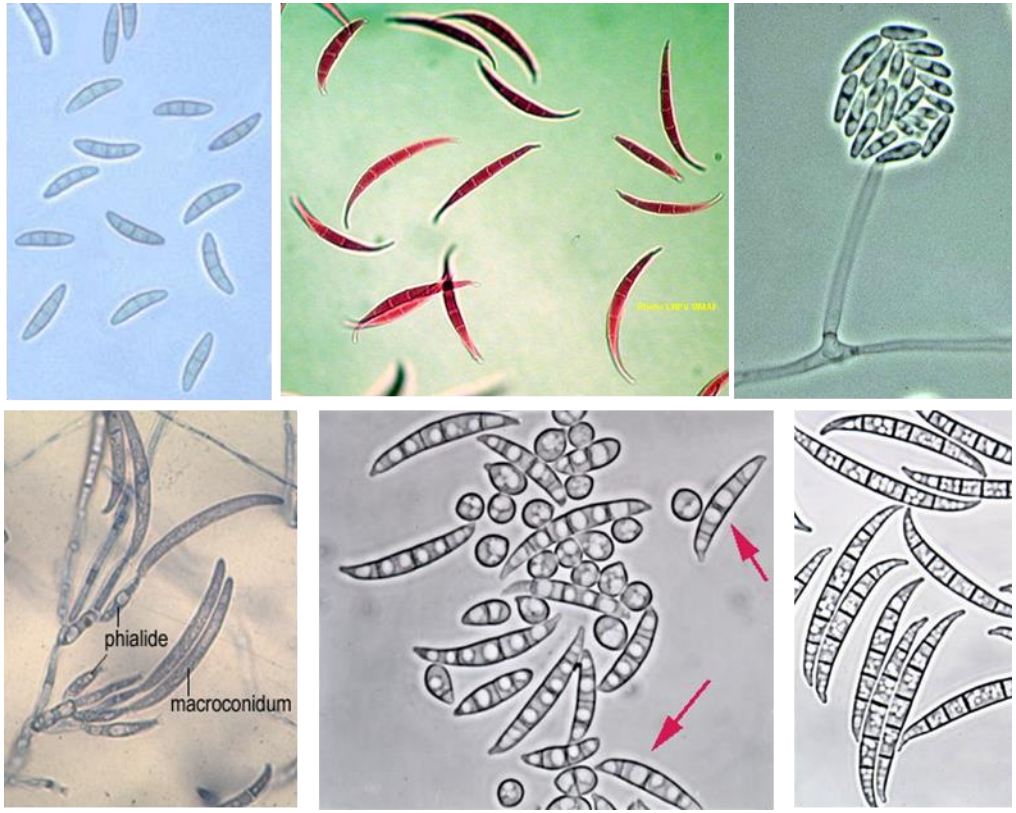
Comparative genomic analyses of mycotoxin production in *Fusarium*

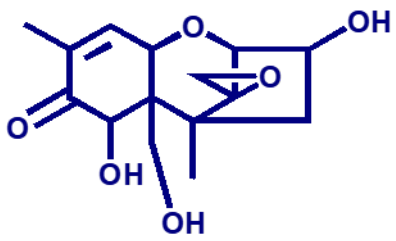


Robert Proctor
National Center for Agricultural Utilization Research
Mycotoxin Prevention and Applied Microbiology

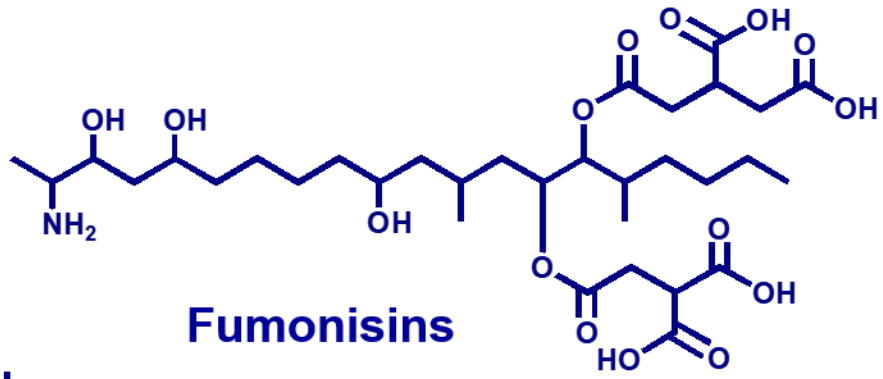
USDA

ars

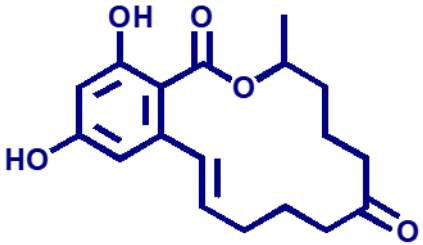
Fusarium produces multiple mycotoxins and many other secondary metabolites



Trichothecenes

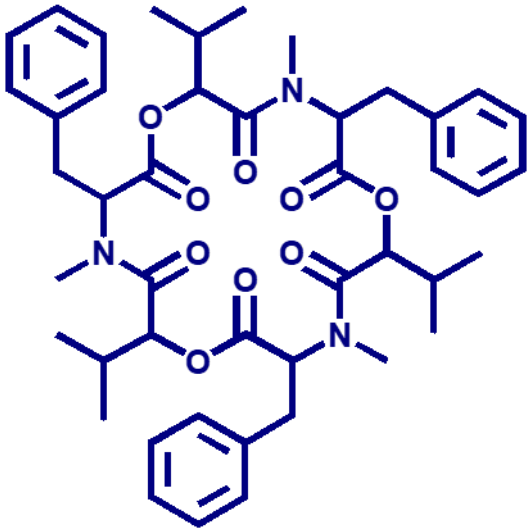


Fumonisins

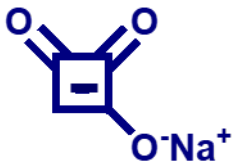


Zearalenone

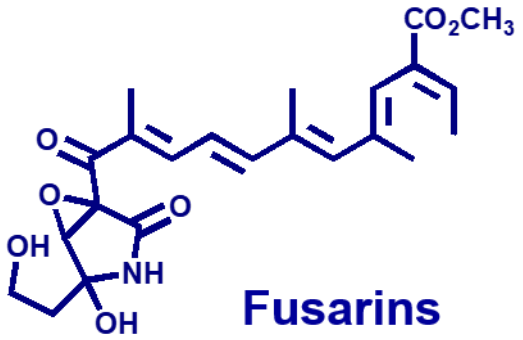
Fusaric Acid



Beauvericin

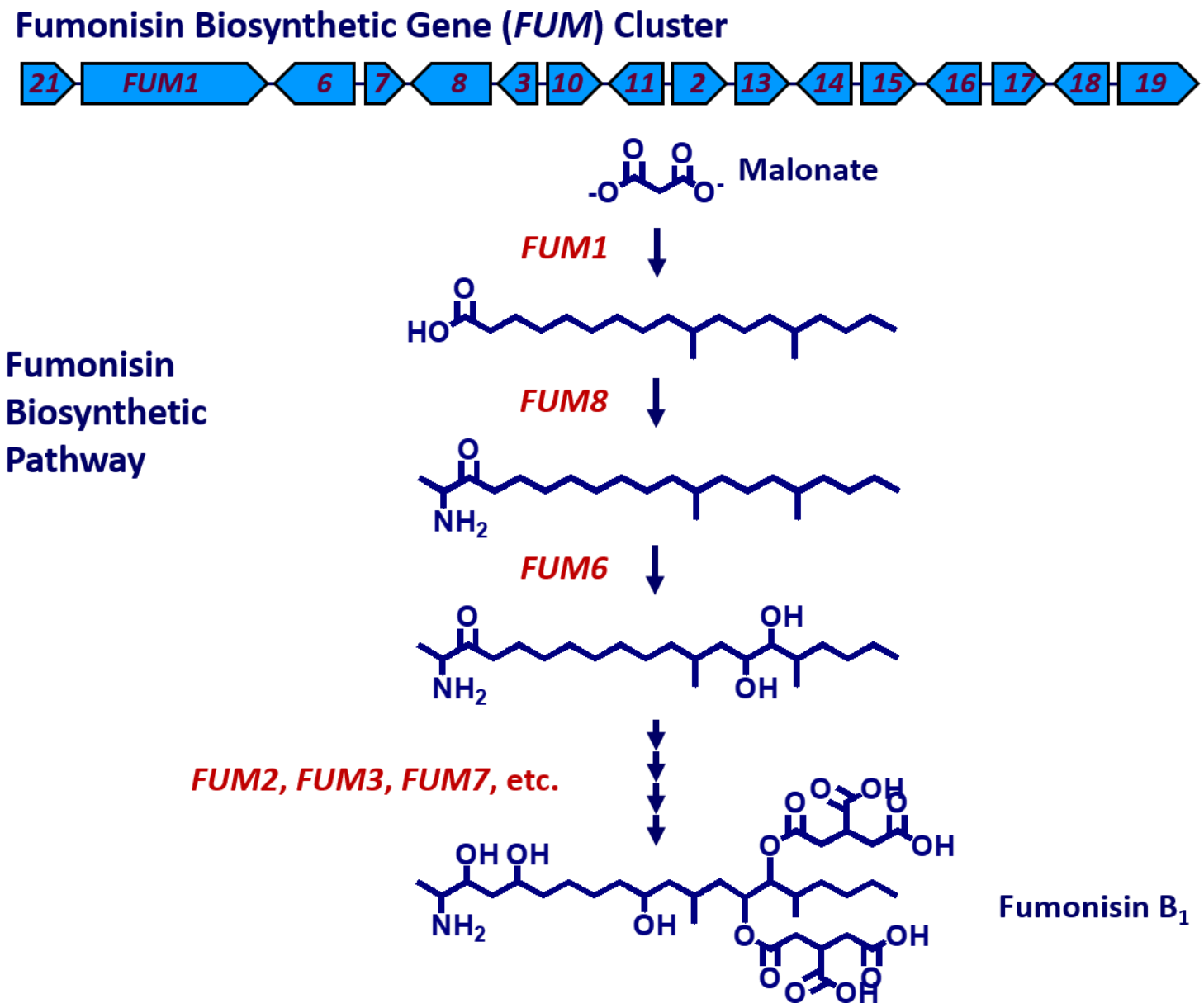


Moniliformin



Fusarins

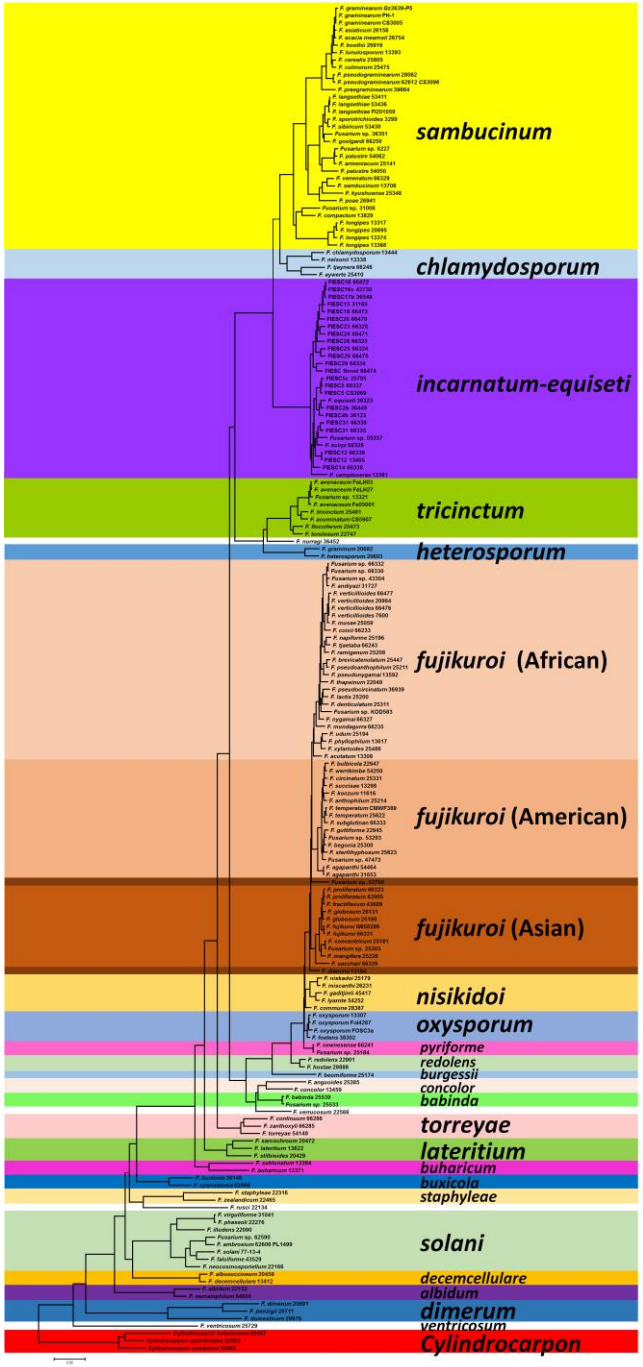
Genes responsible for biosynthesis of the same mycotoxin are typically located in a cluster



Fusarium Genome Sequence Database

- **305 *Fusarium* genome sequences**
- **288 genome sequences generated at/for NCAUR**
- **250 *Fusarium* species**

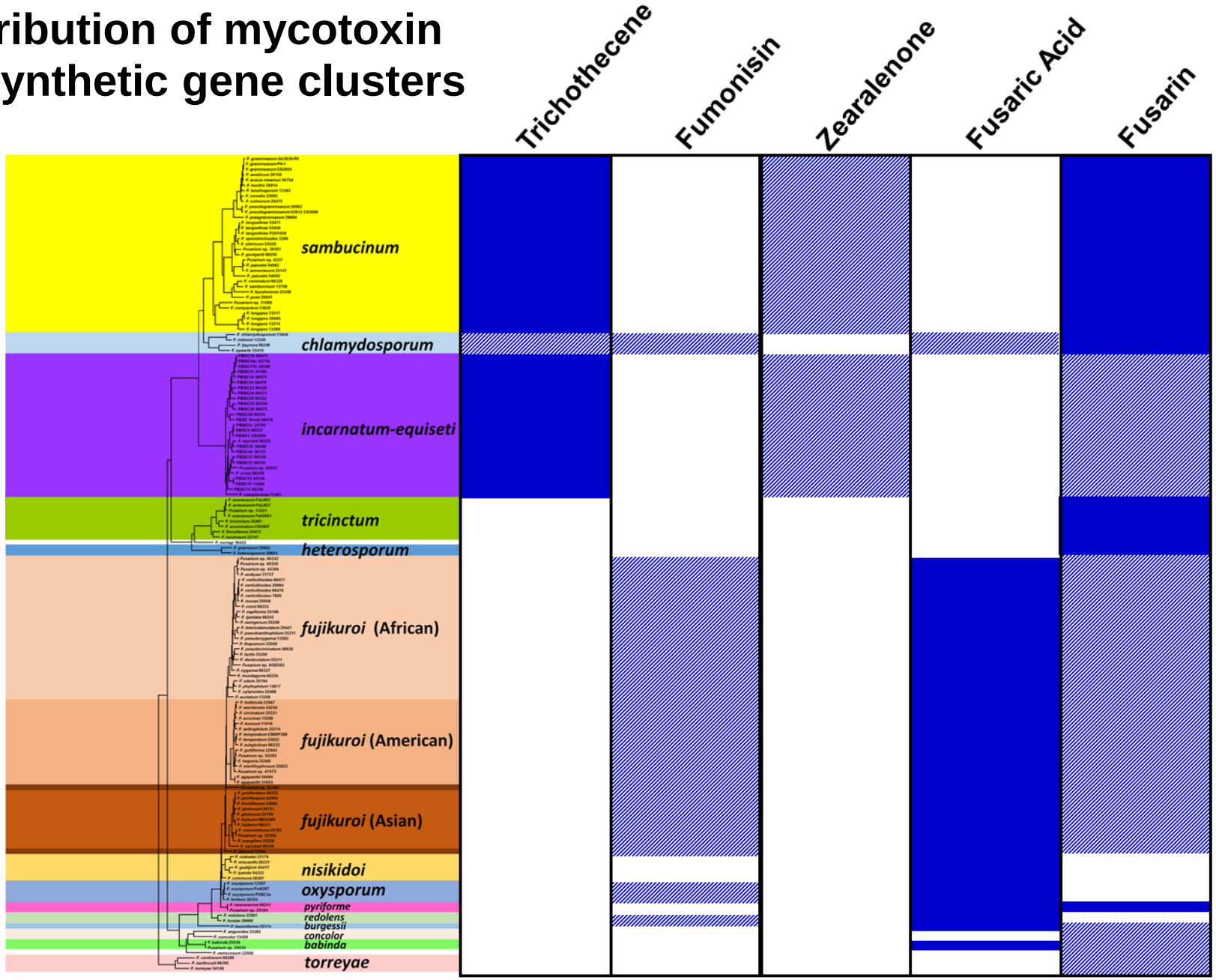
Species Complex



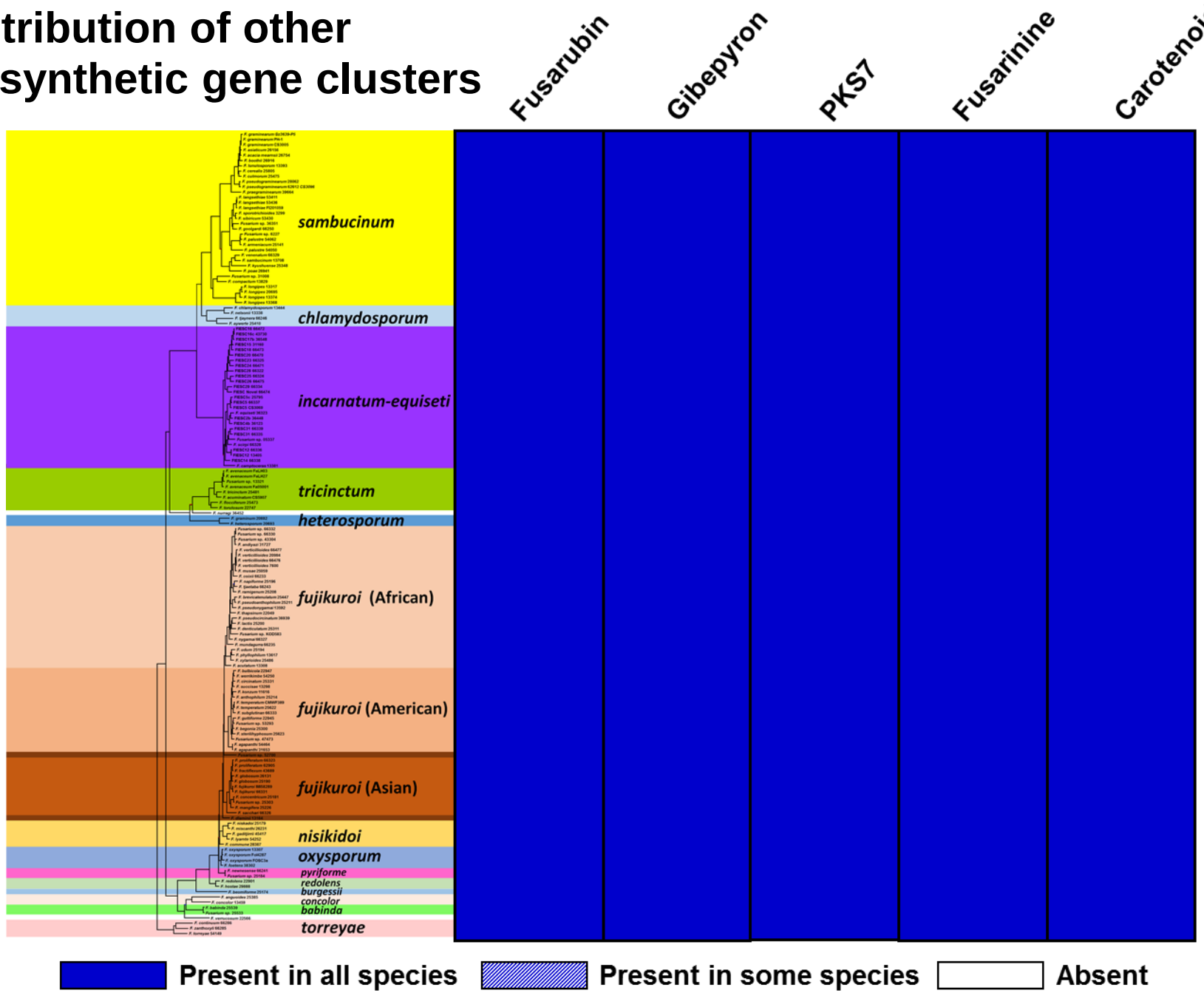
***Fusarium* species phylogeny**
Based on 19 housekeeping genes

22 multi-species lineages (species complexes)

Distribution of mycotoxin biosynthetic gene clusters



Distribution of other biosynthetic gene clusters

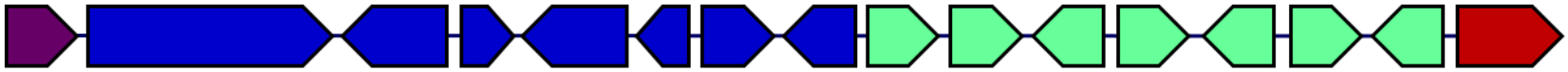


Processes that affect distribution of *Fusarium* mycotoxin biosynthetic genes

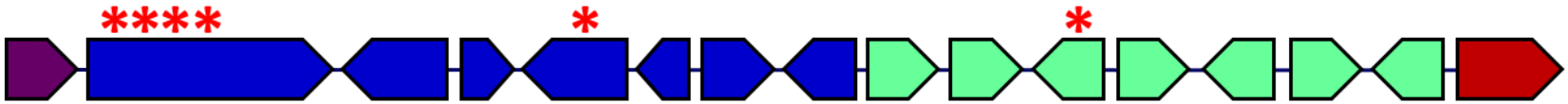
- Vertical inheritance
- Loss
- Horizontal transfer
- Duplication

Evidence for gene cluster loss: degeneration of fumonisin cluster in the *F. fujikuroi* species complex

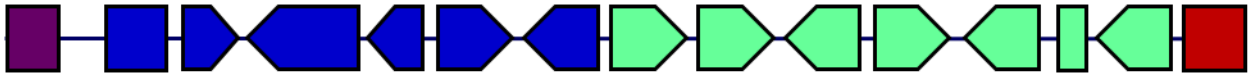
Functional cluster



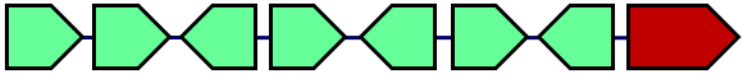
Mutation(s) in one or more essential genes



Mutation(s) in multiple essential genes

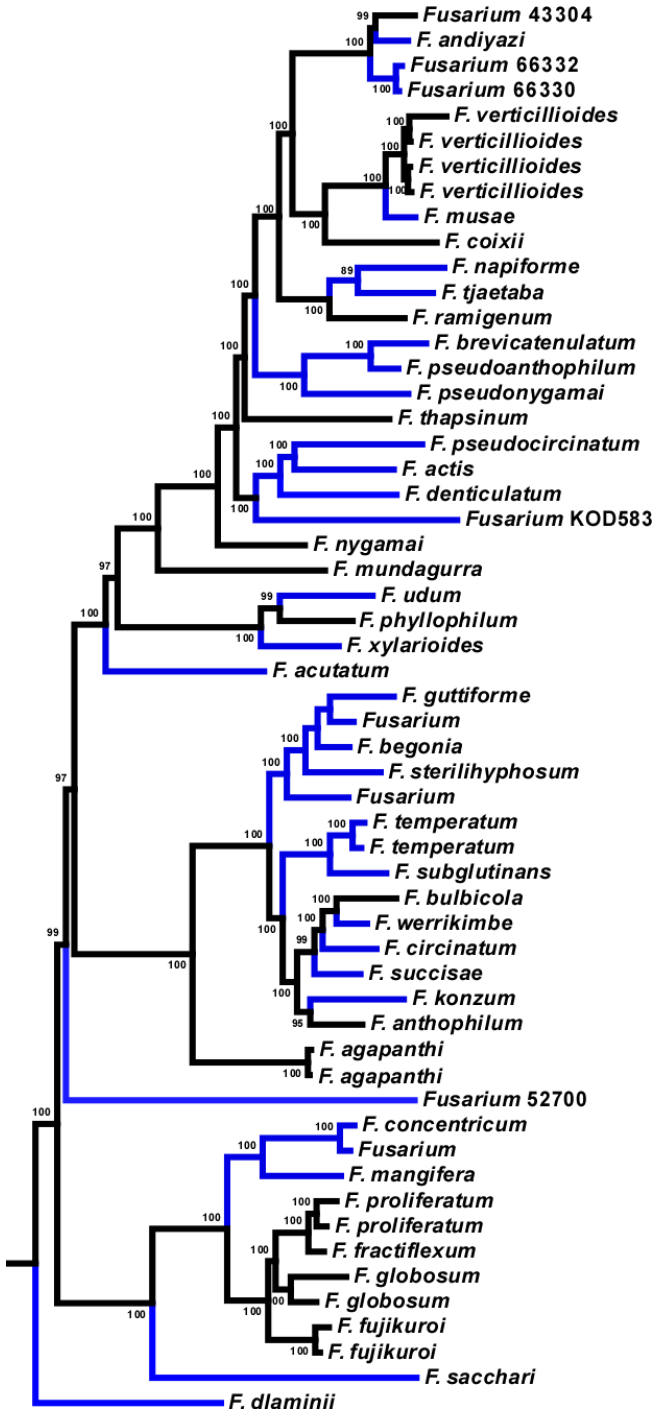


Partial deletion of *FUM* cluster



Almost complete deletion of cluster

* = premature stop codon

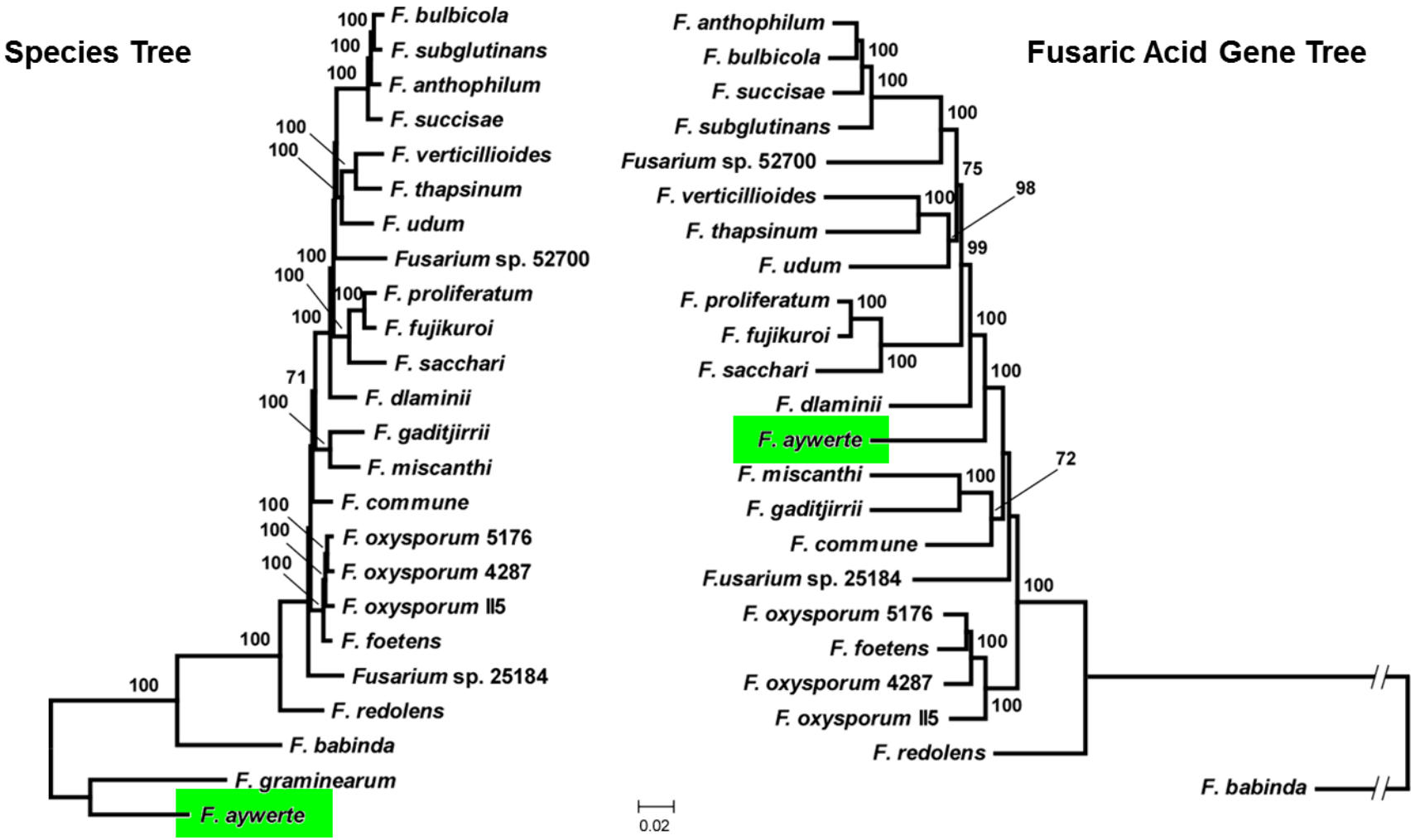


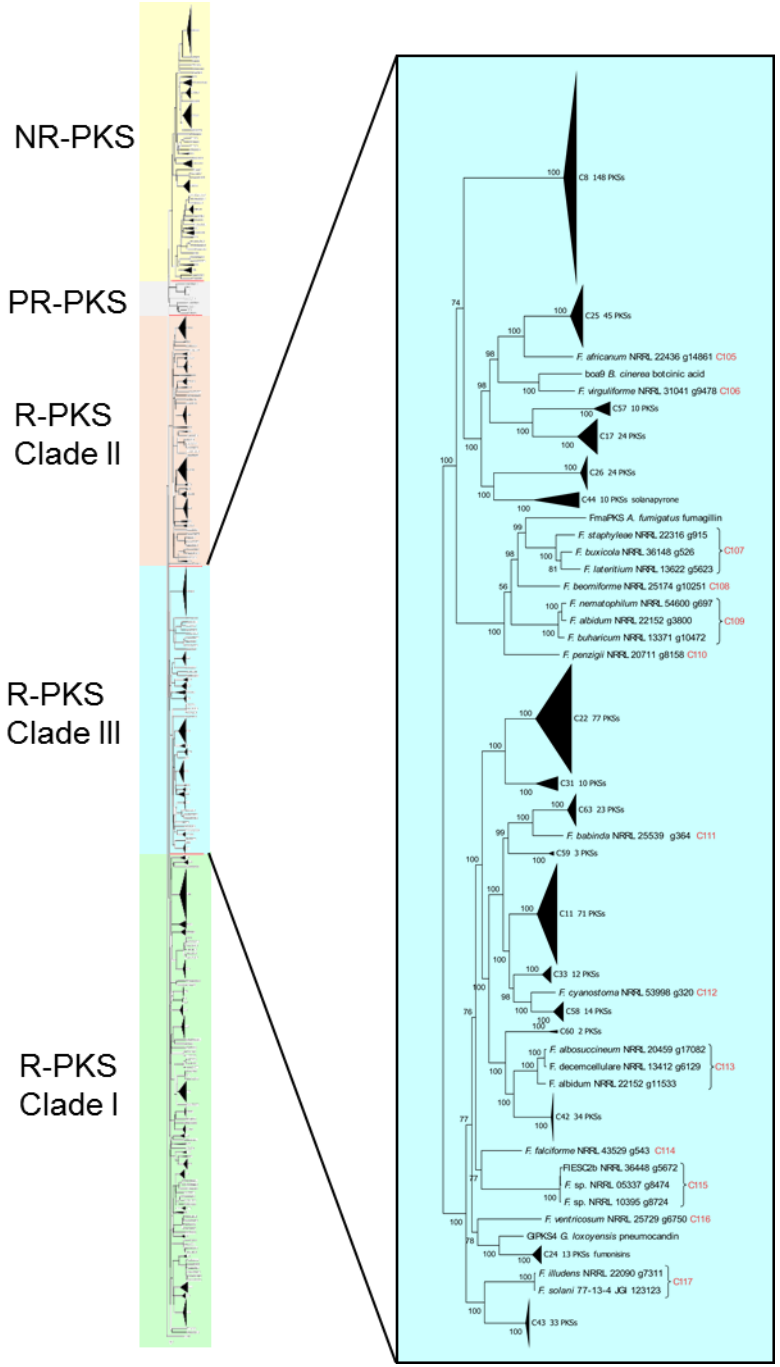
18 loss events of fumonisin cluster in *F. fujikuroi* species complex

cluster present

cluster absent

Evidence for horizontal transfer of a mycotoxin gene cluster: distantly related species have closely related mycotoxin genes





Fusarium polyketide synthase (PKS) genes

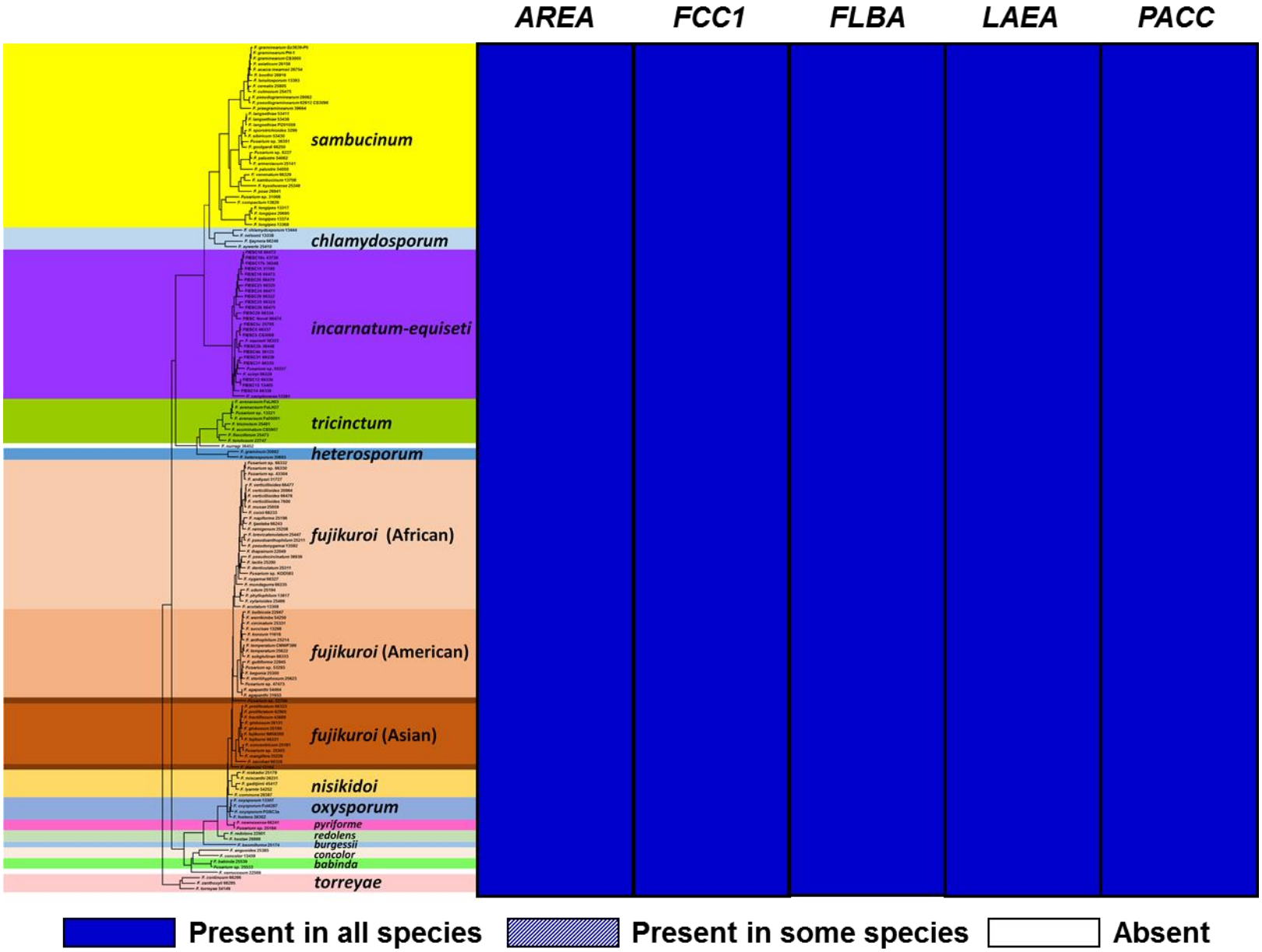
- 2236 PKS genes
- grouped into 116 clades
- Horizontal gene transfer in 47% of clades

Brown *et al.*

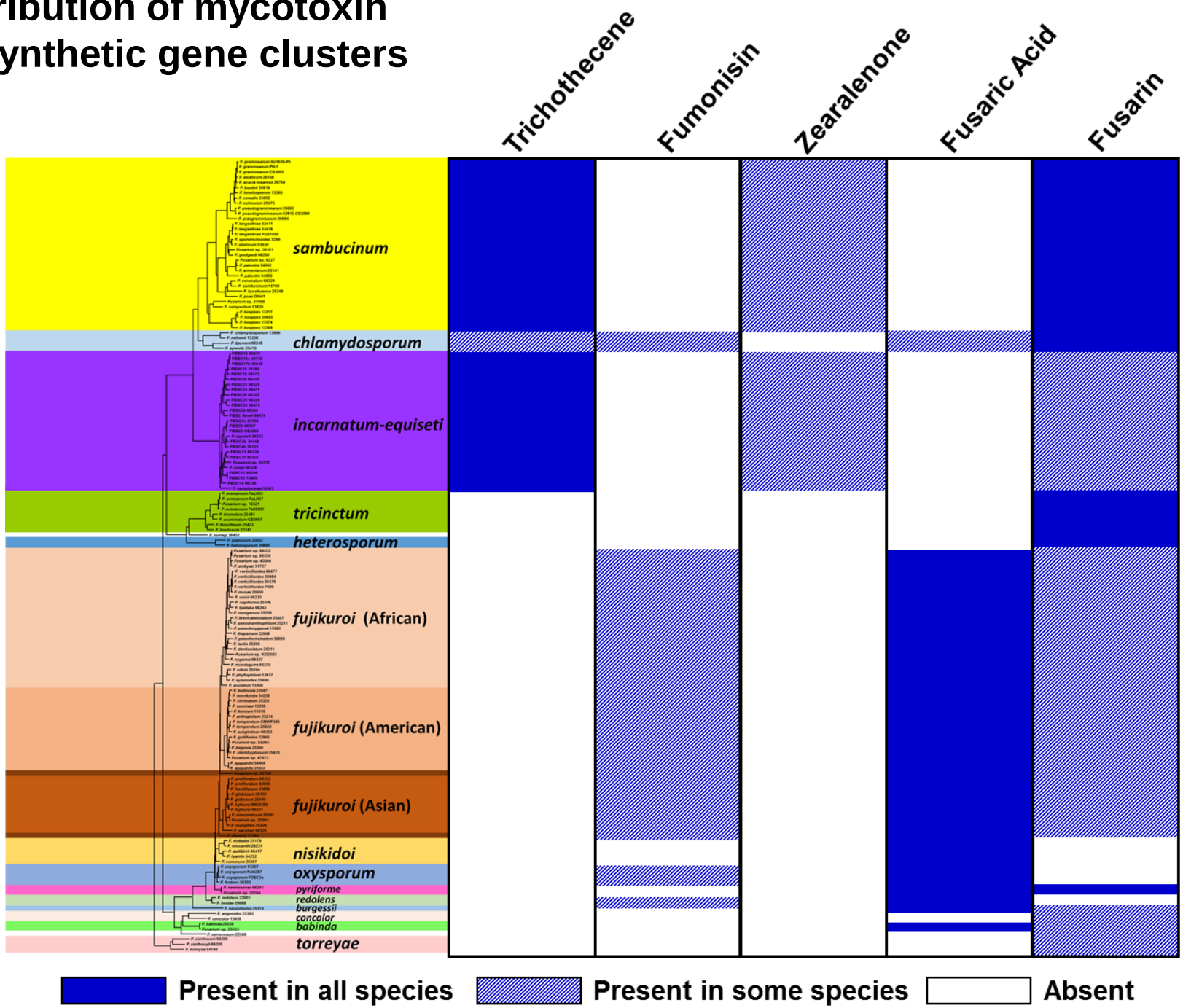
Horizontal transfer of mycotoxin biosynthetic gene clusters between *Fusarium* species complexes

Gene Cluster	Donor Complex	→	Recipient Complex
Fumonisin	fujikuroi		oxysporum
Fumonisin	fujikuroi		redolens
Fusaric acid	fujikuroi		chlamydosporum
Fusarin	tricinctum		incarnatum-equiseti
Enniatin	fujikuroi		incarnatum-equiseti
Butenolide	sambucinum		incarnatum-equiseti
Butenolide	incarnatum-equiseti		sambucinum

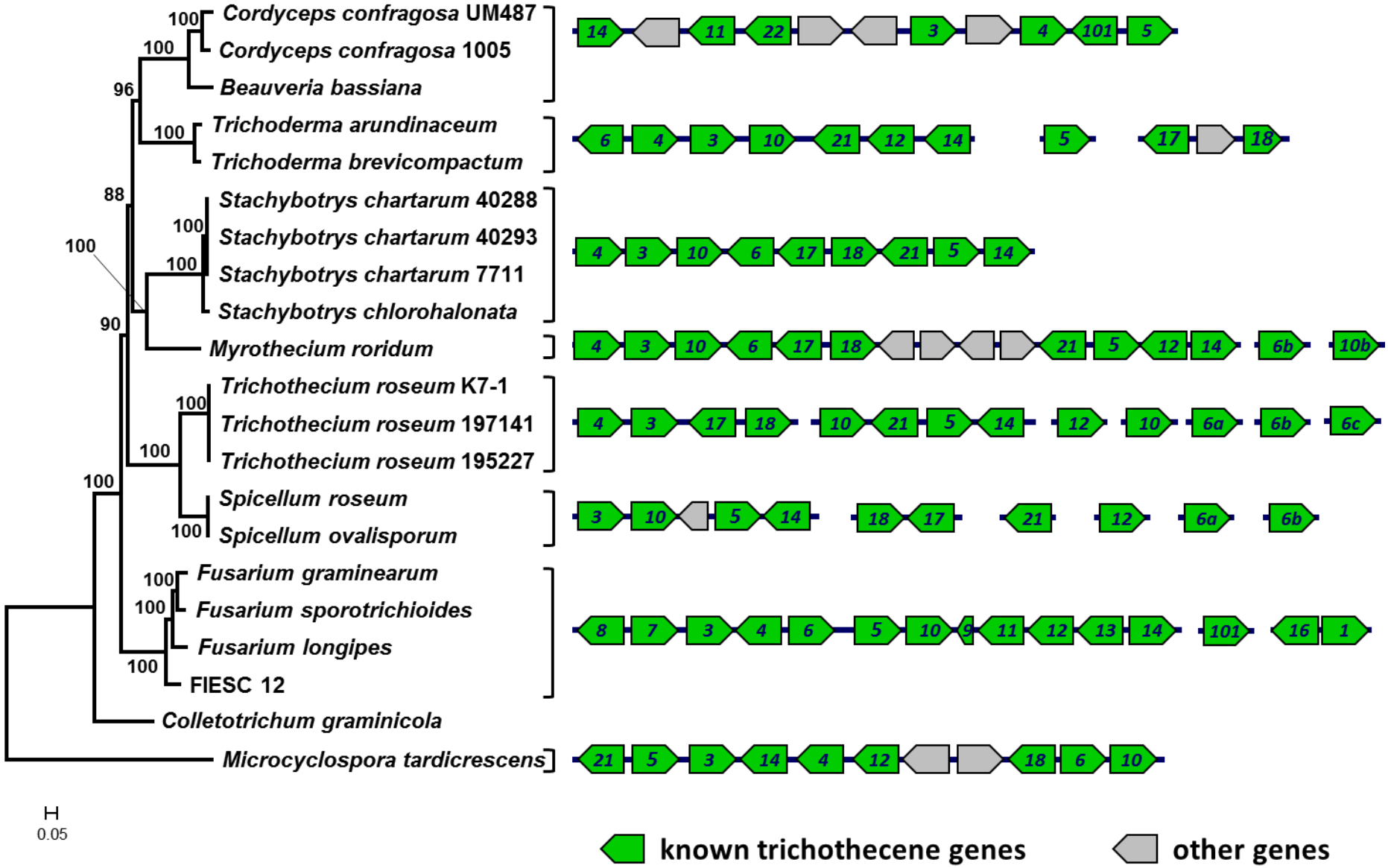
Distribution of genes that regulate mycotoxin biosynthesis



Distribution of mycotoxin biosynthetic gene clusters



Trichothecene biosynthetic gene cluster occurs in nine fungal genera



Conclusions

- Presence of mycotoxin biosynthetic gene clusters varies among *Fusarium* species and species complexes; distribution of clusters tends to be limited
- Vertical inheritance, loss and horizontal transfer have contributed to current distribution of mycotoxin biosynthetic genes
- Non-cluster regulatory genes that affect mycotoxin production are conserved
- Conservation of regulatory genes likely facilitates expression of mycotoxin biosynthetic genes following horizontal transfer

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Thank you

CRIS Project Outline

Genomic and Metabolomic Approaches for Detection and Control of *Fusarium*, Fumonisins and Other Mycotoxins on Corn

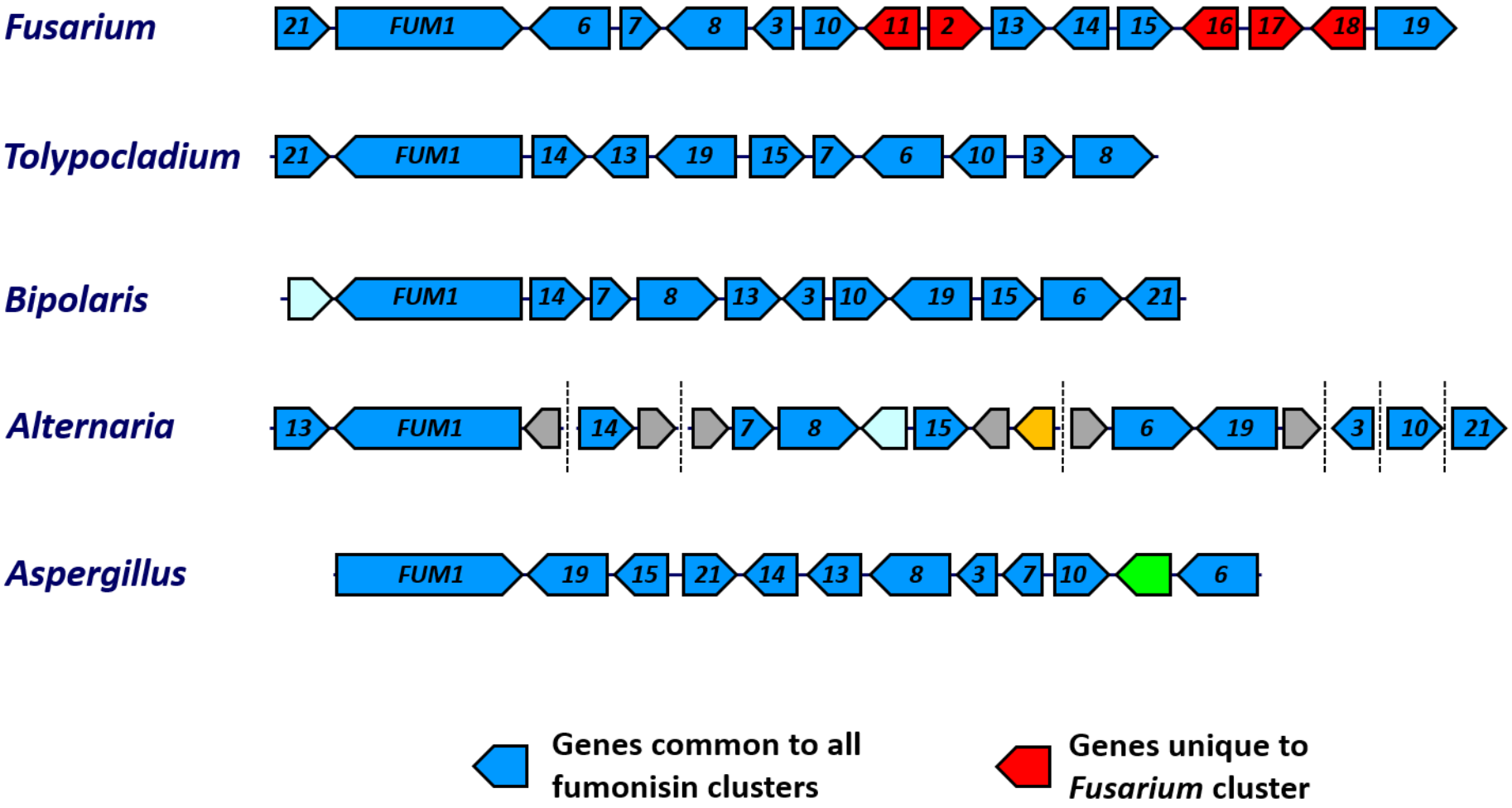
Objective 1: comparative genomics for detection of *Fusarium* and to assess distribution and evolution of genes responsible for mycotoxin biosynthesis **(Brown, O'Donnell, Proctor)**

Objective 2: metabolomics: mass-spectrometry-based analysis of *Fusarium verticillioides* and maize **(Busman)**

Objective 3: protein chemistry: identify and characterize plant and fungal factors that impact mycotoxin contamination **(Naumann)**

Objective 4: Identify and characterize components of fungus-fungus interactions that contribute to or inhibit mycotoxin contamination of crops **(Peterson, Brown, Proctor)**

Fumonisin biosynthetic gene cluster occurs in five fungal genera



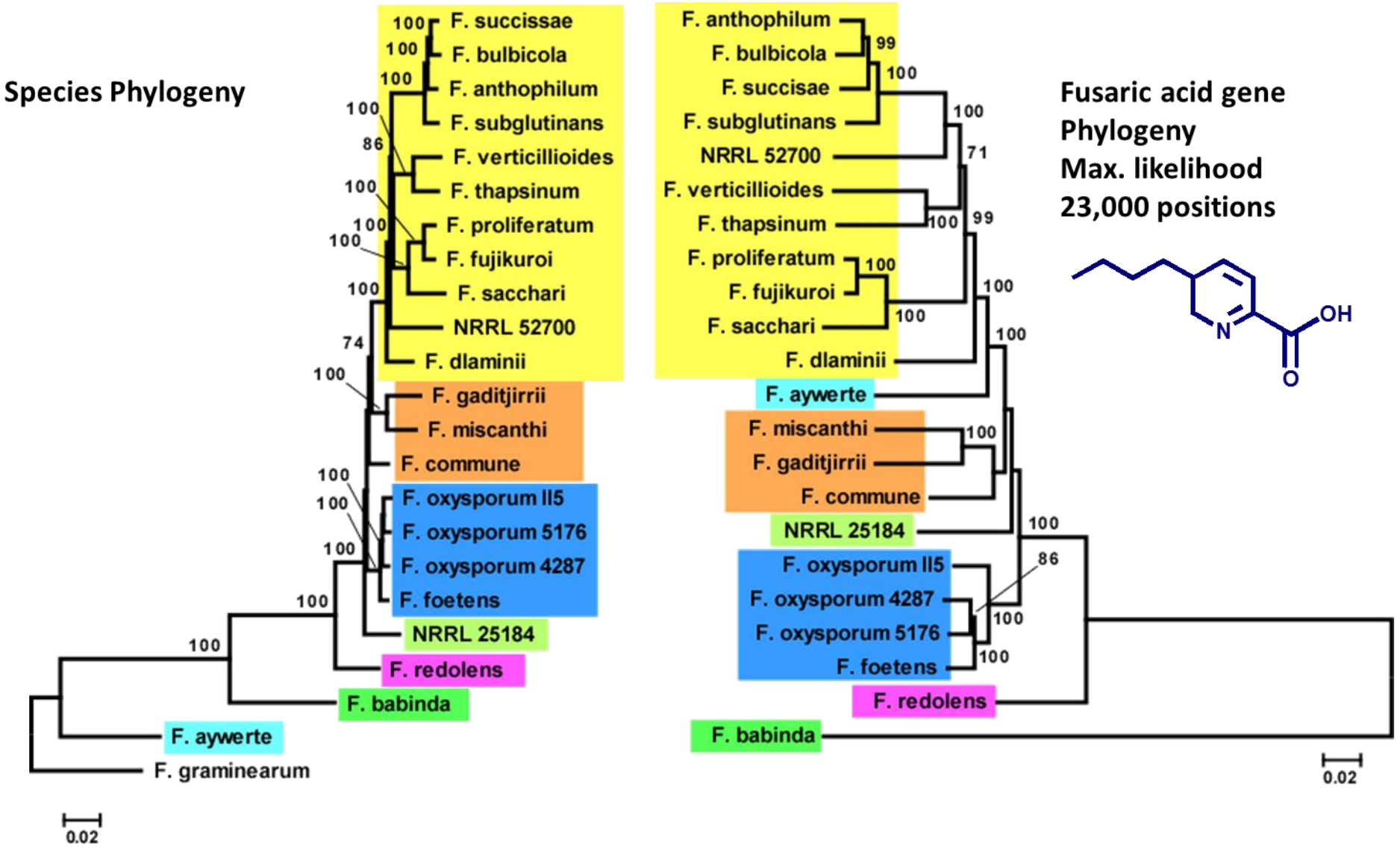
Data

Chemical analyses indicate that Fusarium can produce >50 families of secondary metabolites

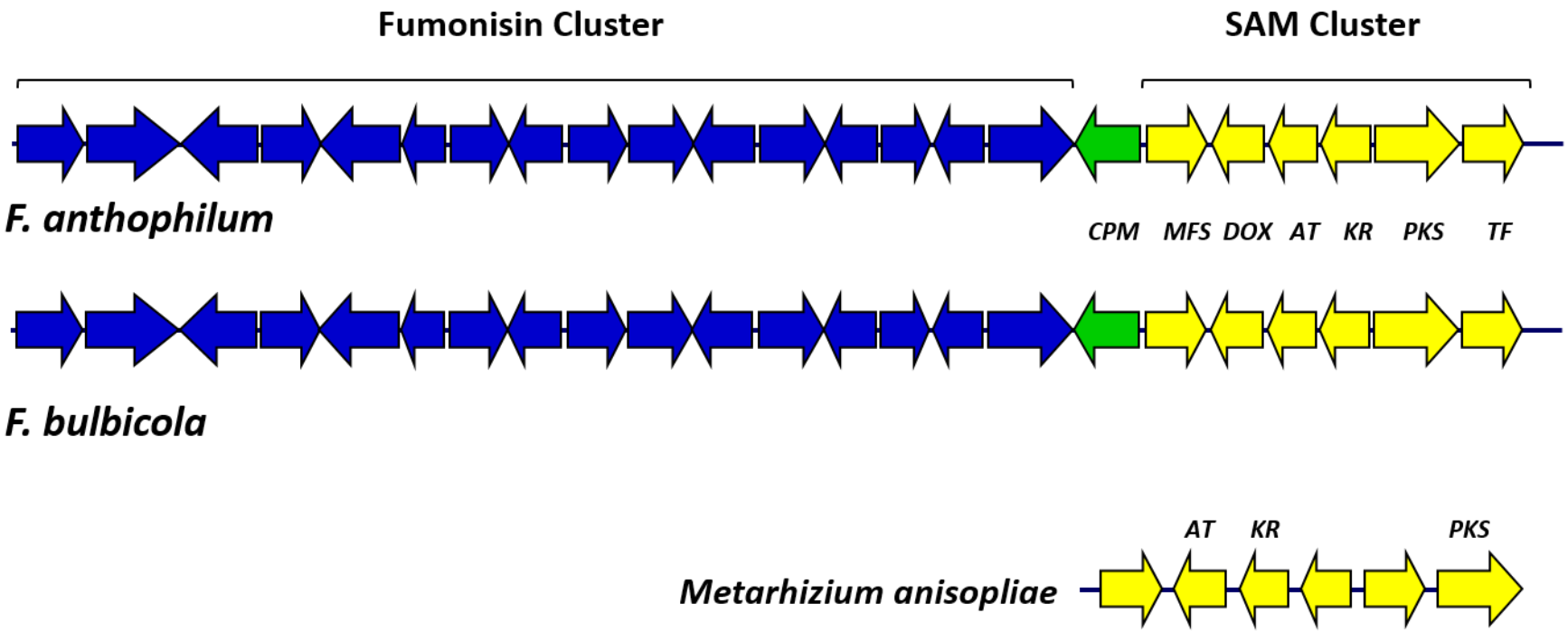
Daren's PKS analysis: 2236 Fusarium PKSs (this includes 2 PKSs from a Fusarium identified as "F. heterosporum (ATCC 74349)".

Phylogenetic analysis grouped the 2236 PKSs into 116 monophyletic clades, and each clade contains homologs that are responsible for the same polyketide; at least 108 different chemicals (i.e. 8 pairs of PKSs)

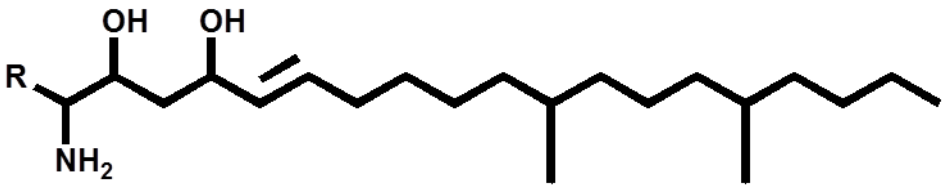
Evidence for vertical inheritance: mycotoxin biosynthetic gene tree mirrors housekeeping gene tree (species phylogeny)



Putative Sphinganine Analog Metabolite (SAM) cluster next to the fumonisin cluster

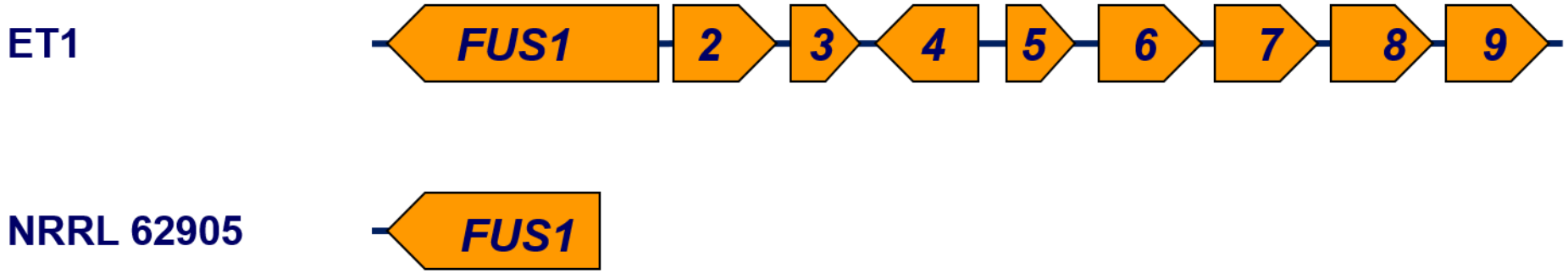


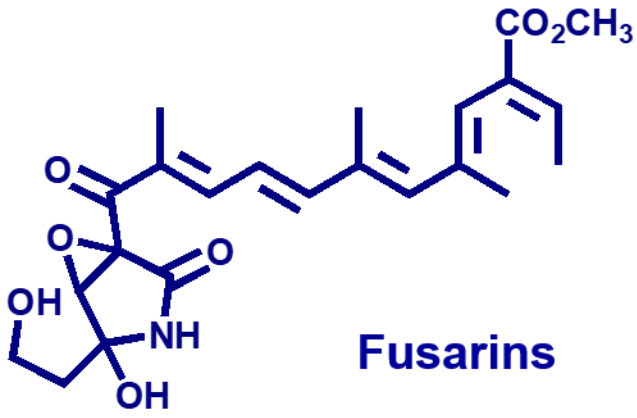
Educated guess at structure of metabolite product



Intra-species variation in the presence and absence of mycotoxin biosynthetic genes

F. proliferatum – fusarin biosynthetic gene cluster

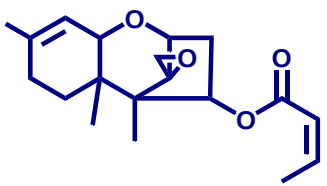




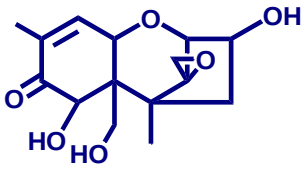
Fusarins

Trichothecenes produced by fungi in multiple genera

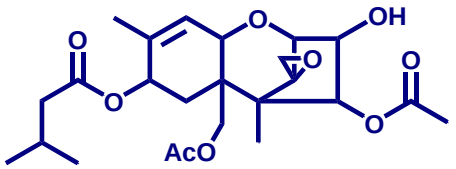
Spicellum roseum



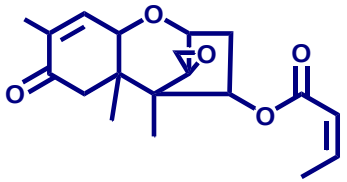
Fusarium graminearum



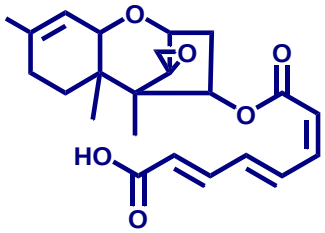
Fusarium sporotrichioides



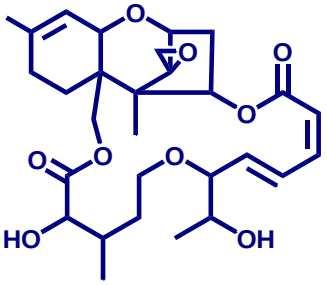
Trichothecium roseum



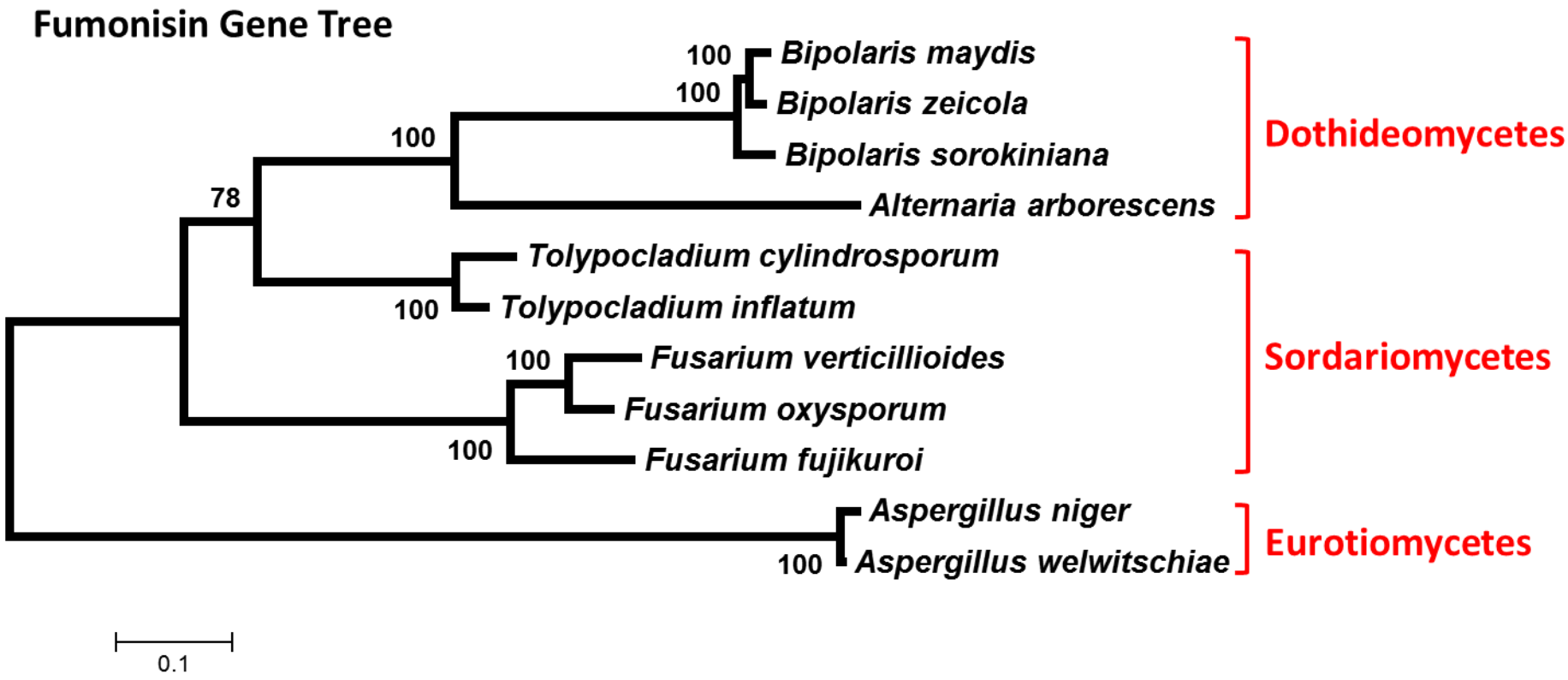
Trichoderma



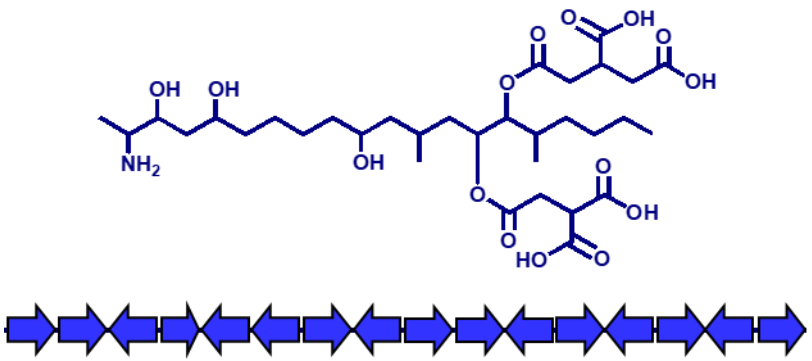
Myrothecium & Stachybotrys



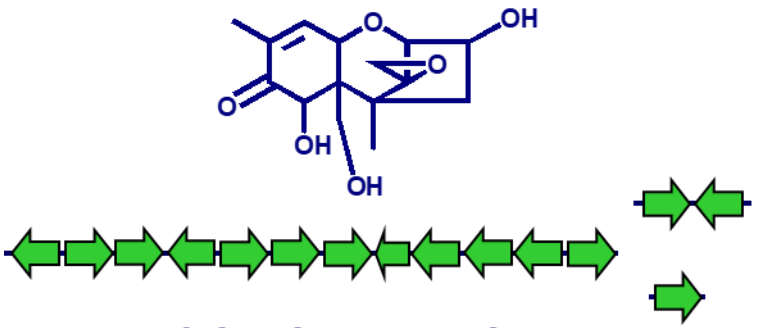
The Fumonisin cluster is present in multiple fungal genera



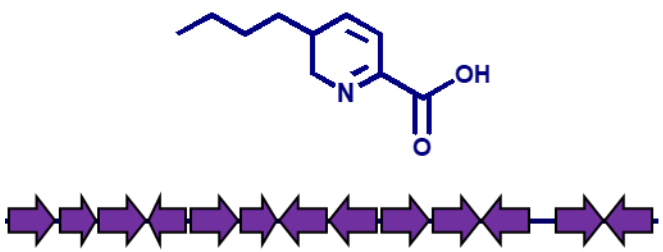
Mycotoxin biosynthetic gene clusters



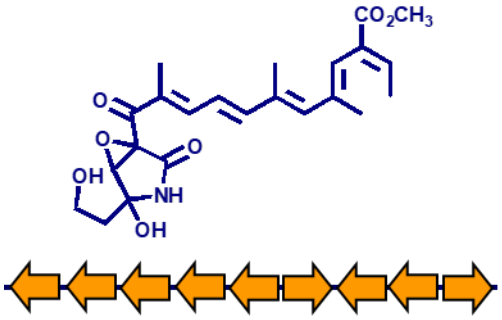
Fumonisin cluster



Trichothecene cluster



Fusaric acid cluster



Fusarin cluster