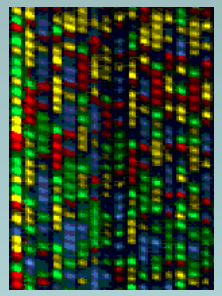
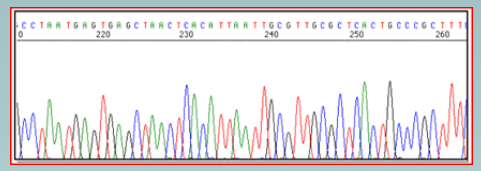
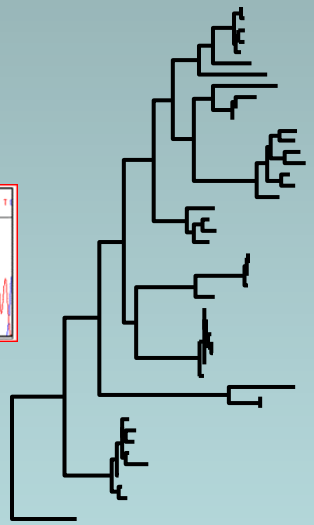
A hierarchical genetic framework for risk assessment of the microbial agent *Beauveria bassiana*







Stephen A. Rehner
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USDA, ARS
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Molecular markers enable identification and genotyping of agronomically important microbes

- Pathogen diagnosis, epidemiology
- Commerce: import (APHIS) and export
- Biocontrol organisms
  - Ecology
  - Risk Assessment
  - Environmental Persistence

Selecting genetic markers

- Variability (Resolution)
- Type
- Number

Constraints to developing genetic markers

- Available genetic resources
- Time
- Expense

Level of Genetic Resolution

1) Deep Phylogeny

2) Species-level phylogeny

3) Intra-specific: phylogeny
   - Phylogeography
   - Demographic history

4) Intraspecific- population genetics
   - strain genotyping

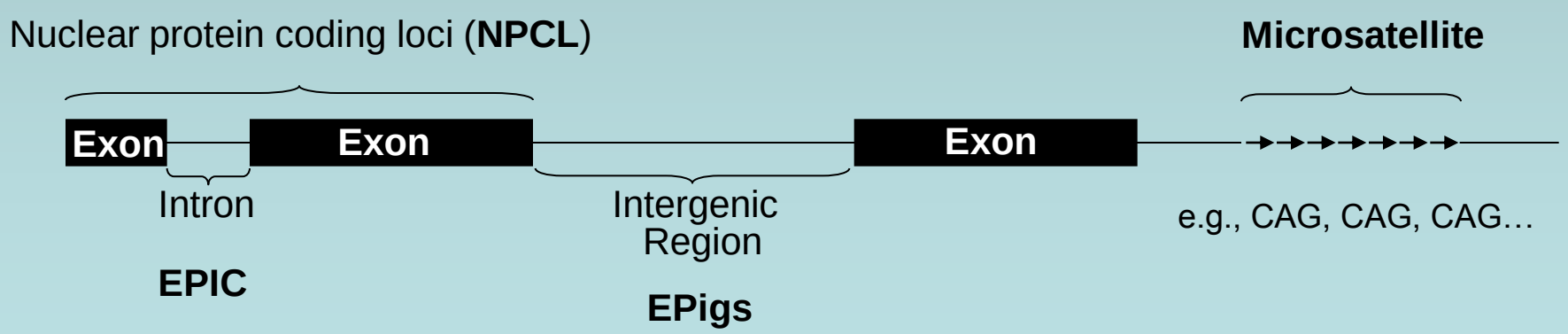
Genetic Marker Categories

1) Nuclear protein coding loci (NPCL)

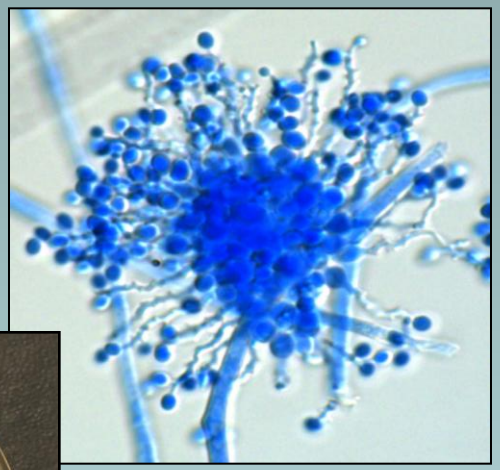
2) NPCL or EPIC/"Epigs"
   (Exon-primed intron/intergenic crossing)

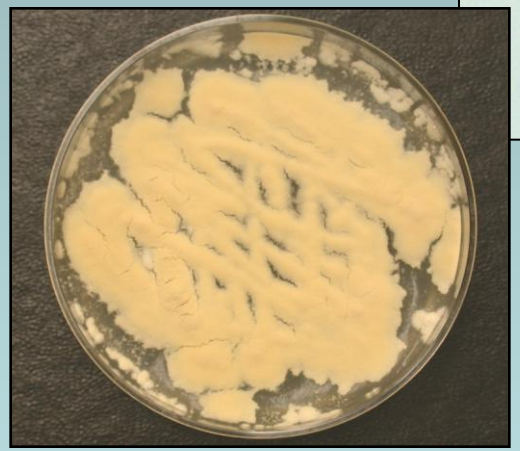
3) EPIC

4) Microsatellites (SSR), SNPs



Beauveria bassiana





Generalist entomopathogen

Biocontrol formulations

Hierarchical genetic markers for Beauveria

1) Deep phylogeny: phylogenetic systematics (NPCL)

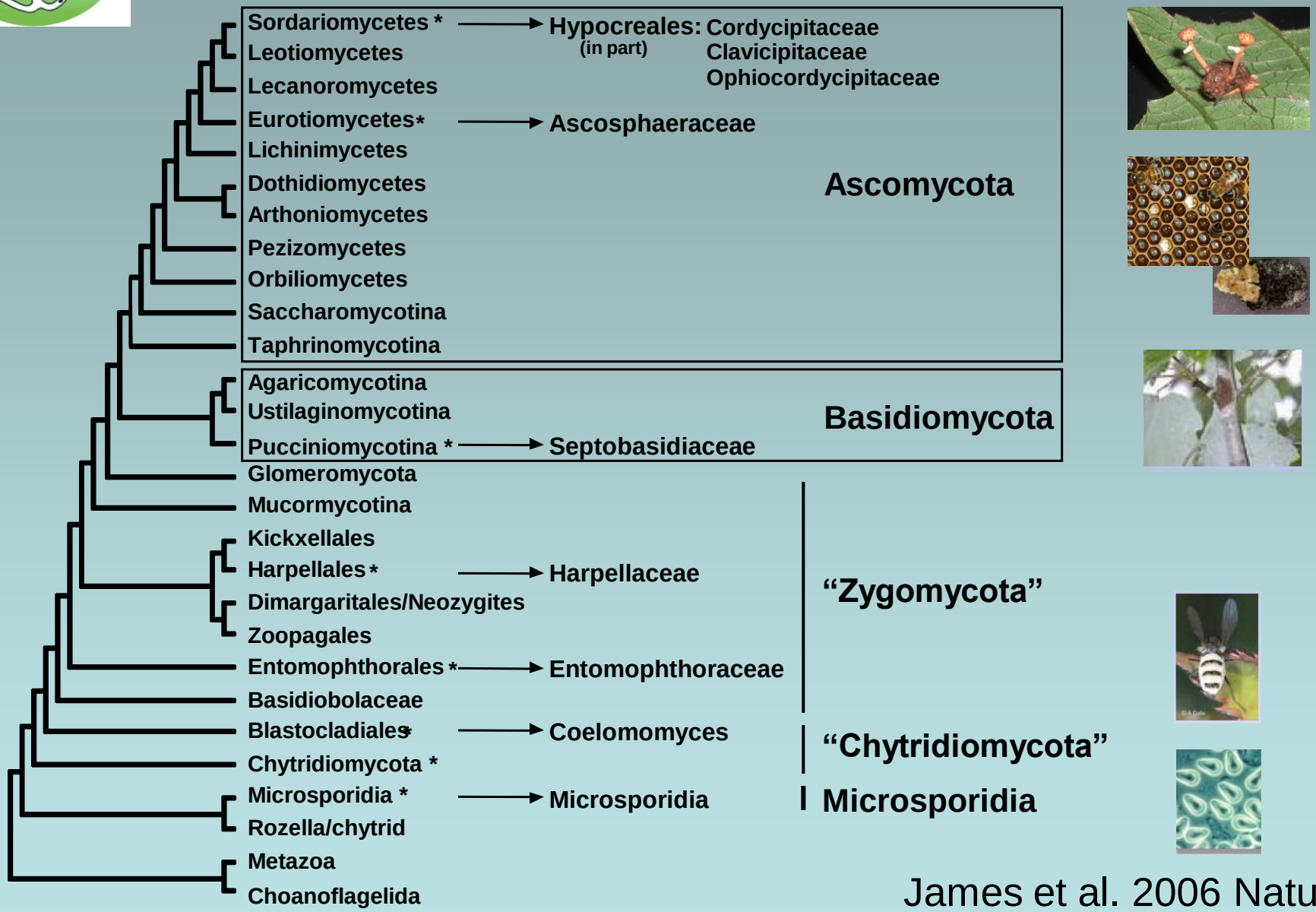
2) Intraspecific phylogeny (EPigs):

***B. bassiana* phylogeny, biogeography**

3) *B. bassiana* reproductive mode (Mating type loci)

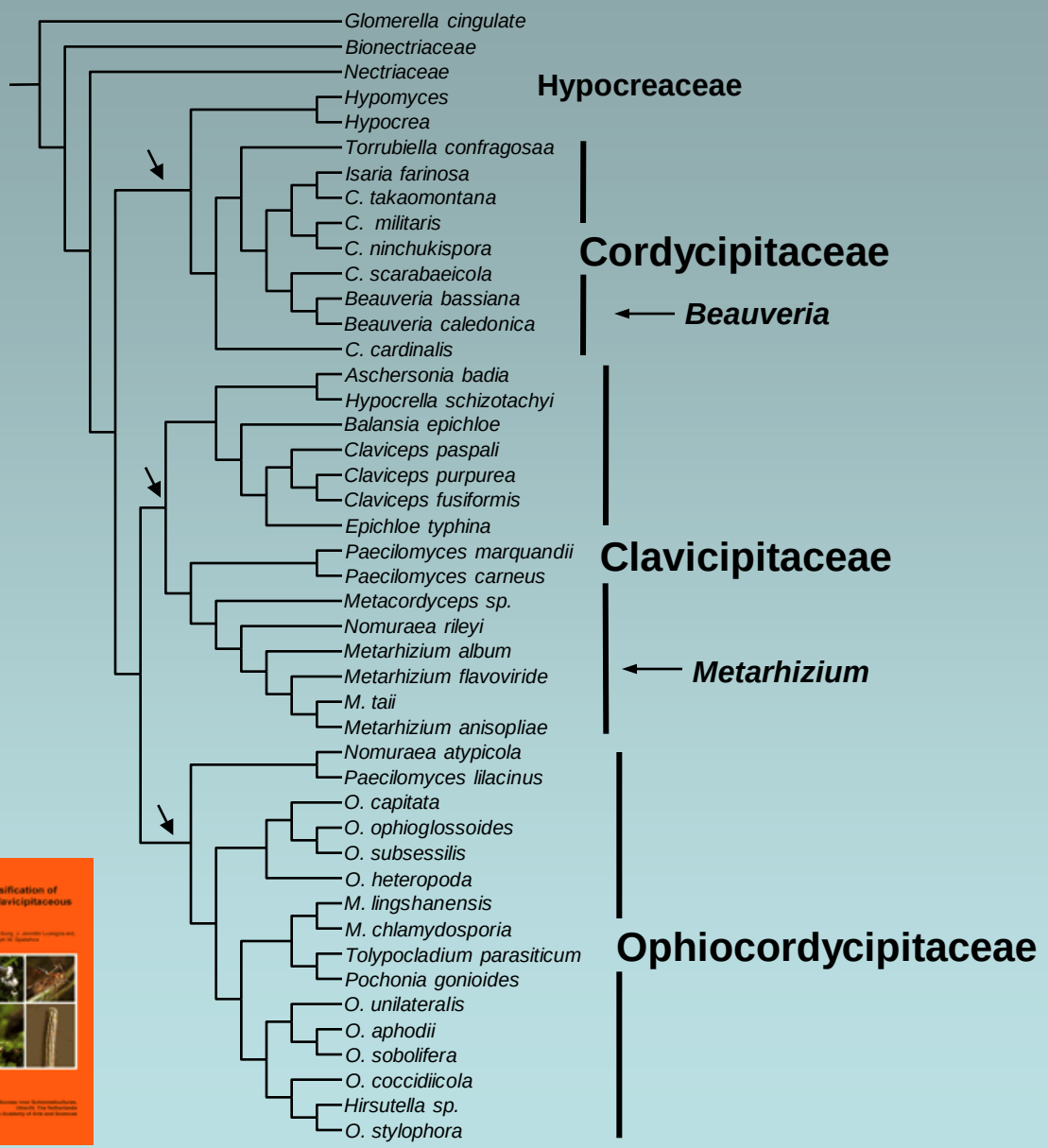
4) *B. bassiana* population genetics (Microsatellites)

Fungal Tree of Life: AFTOL (http://aftol.org)



James et al. 2006 Nature

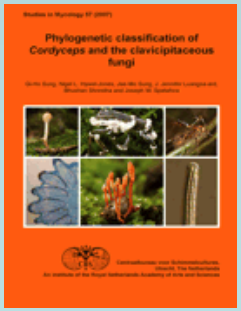
Clavicipitalean Multi-locus Phylogeny



Cordyceps

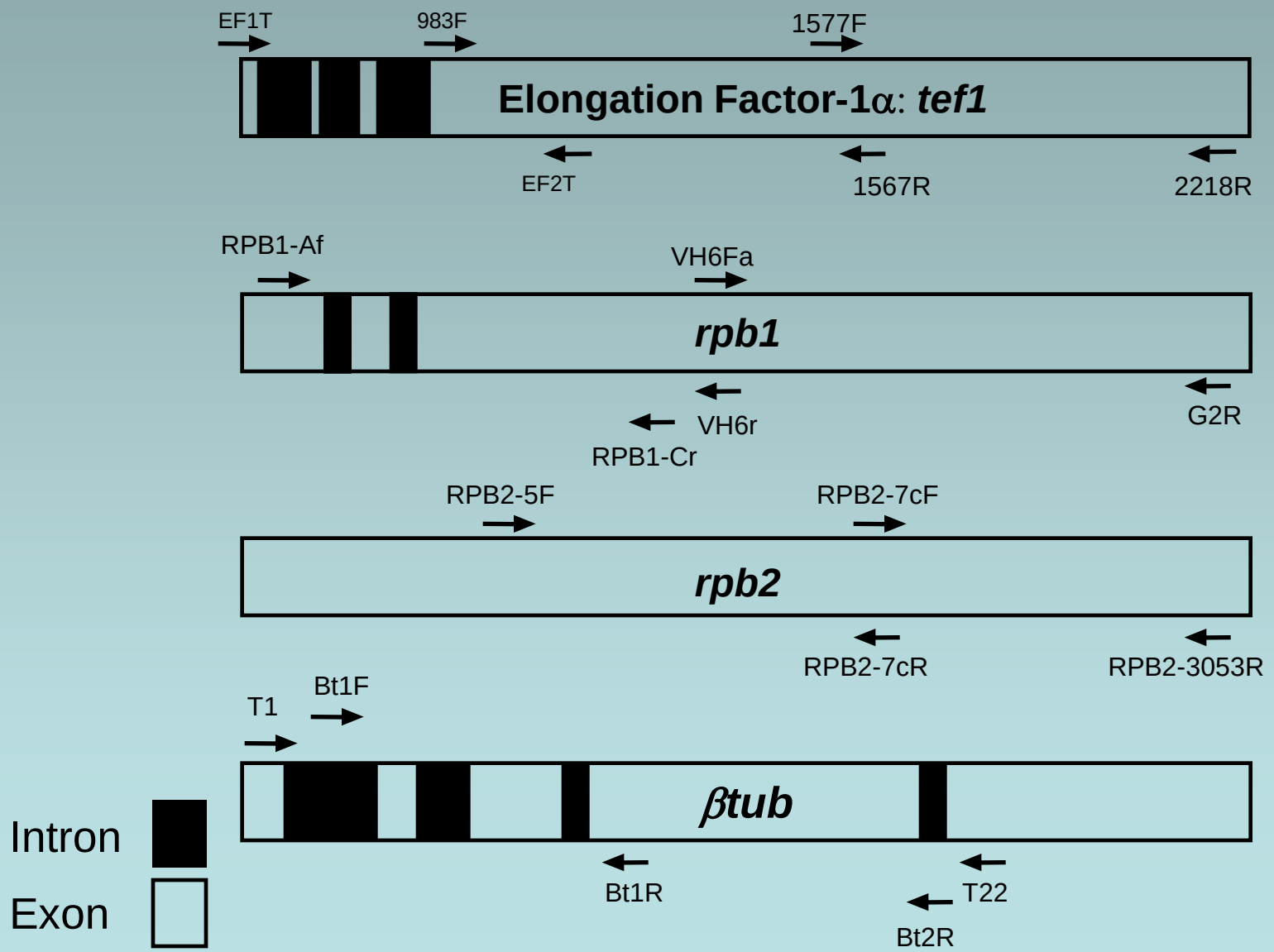
Metacordyceps

Ophiocordyceps



Sung et al. 2007 Studies in Mycology 57:1-63

AFTOL-1 NCPL for *Beauveria*

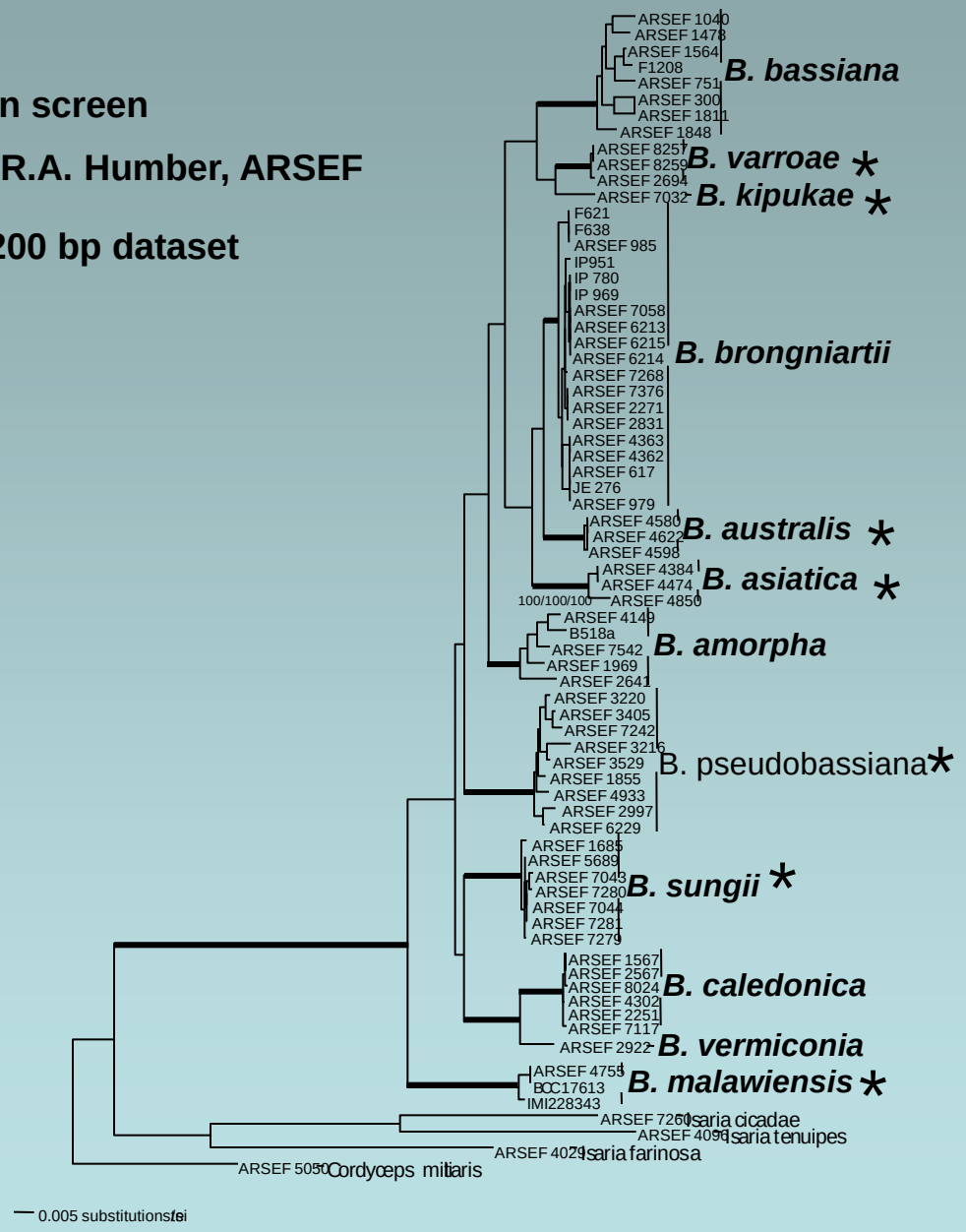


Beauveria Species Boundaries and Relationships

- 1200 strain screen

  Ackno: R.A. Humber, ARSEF

- 5 gene, 7200 bp dataset



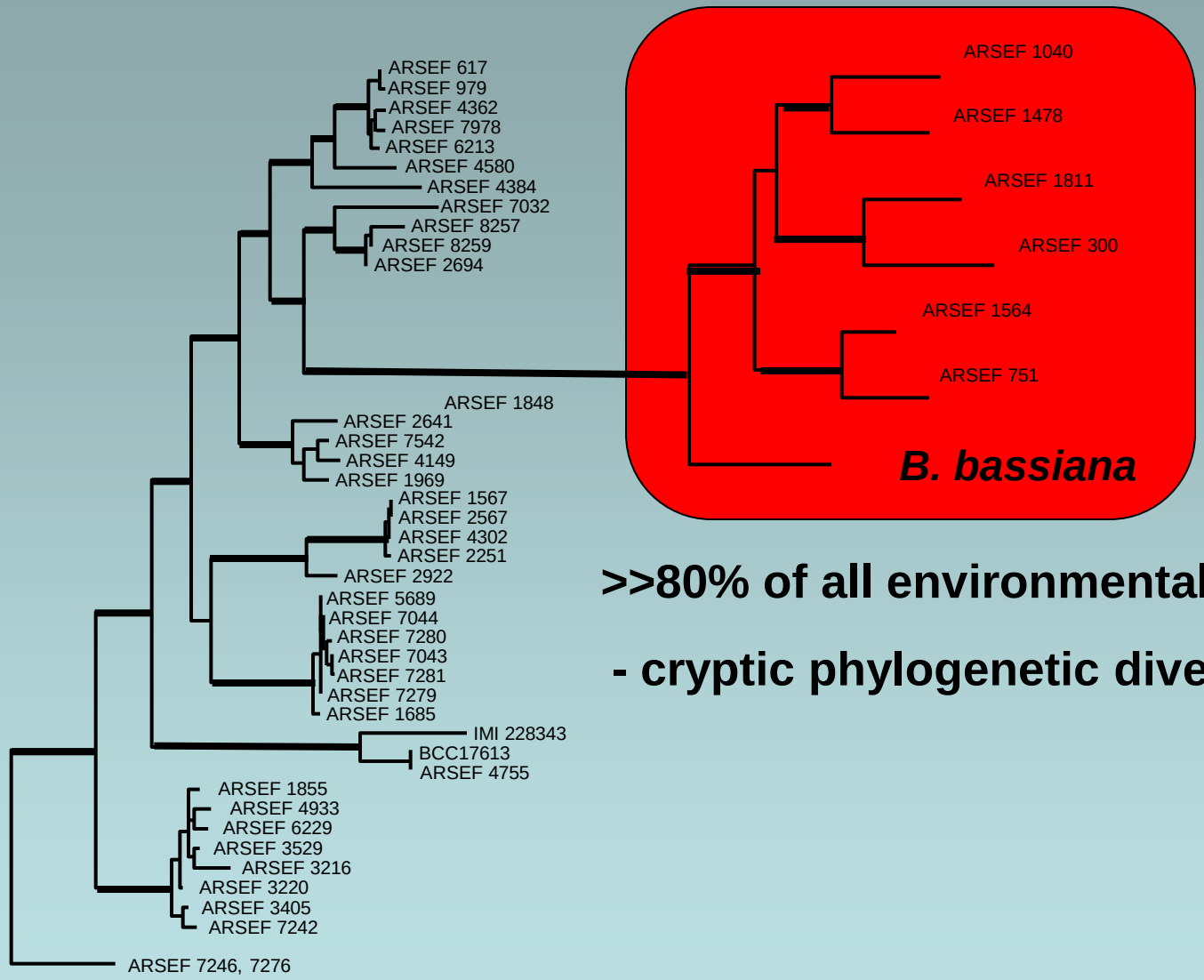
- species boundaries defined

- 7 new species *

- database for species identification

Rehner et al. Mycologia, accepted

Beauveria



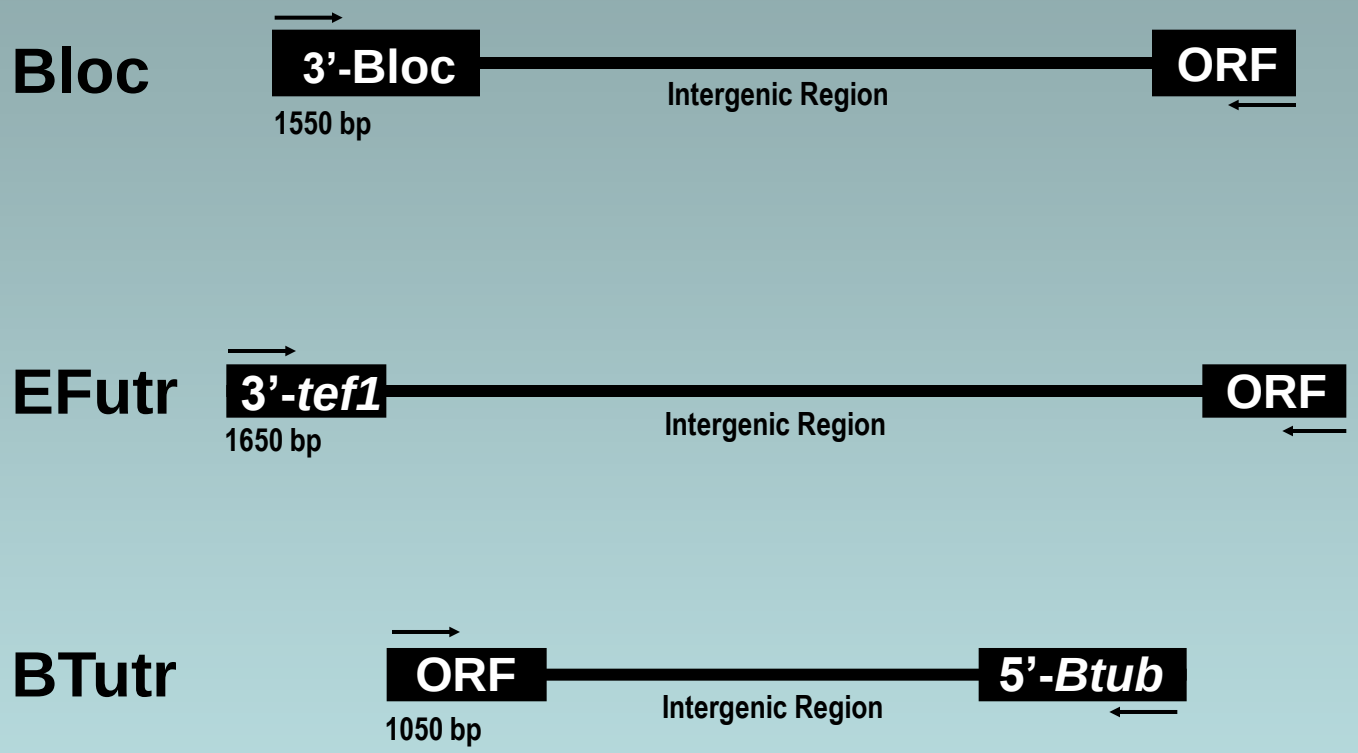
>>80% of all environmental isolates

- cryptic phylogenetic diversity

- 10 changes

EPigs Markers for *B. bassiana*

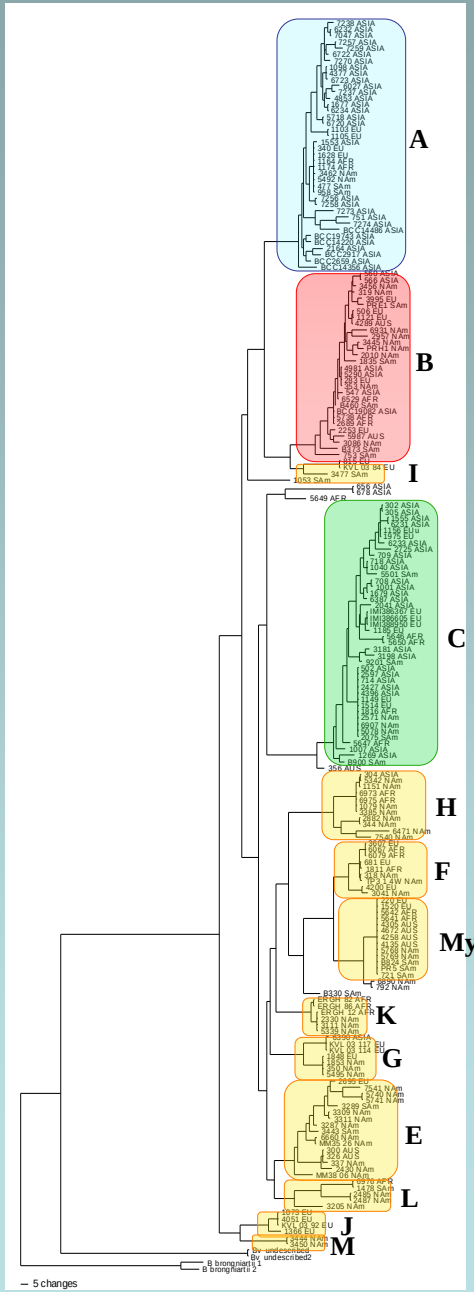
(Exon-primed intergenic-crossing)

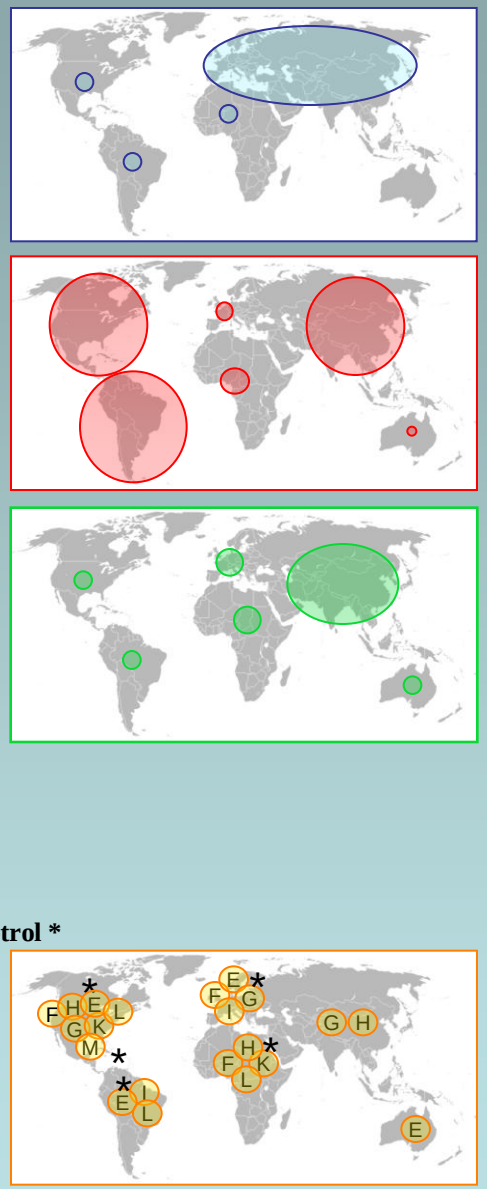


more…..

Rehner et al. JIP 2006, unpubl.

Global Phylogeography of the *B. bassiana*-group with EPigs markers



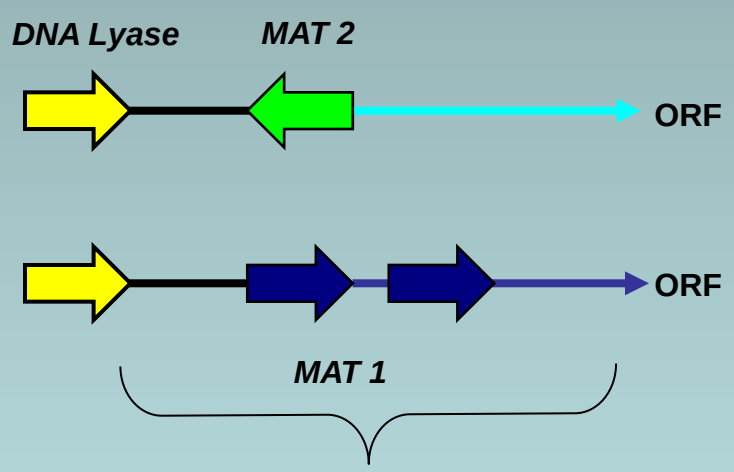


- EPigs resolve cryptic diversity
- 3 principal species: A, B, C
- numerous rare lineages
- Mycotrol, “rare lineage”
  - detected on 4 continents

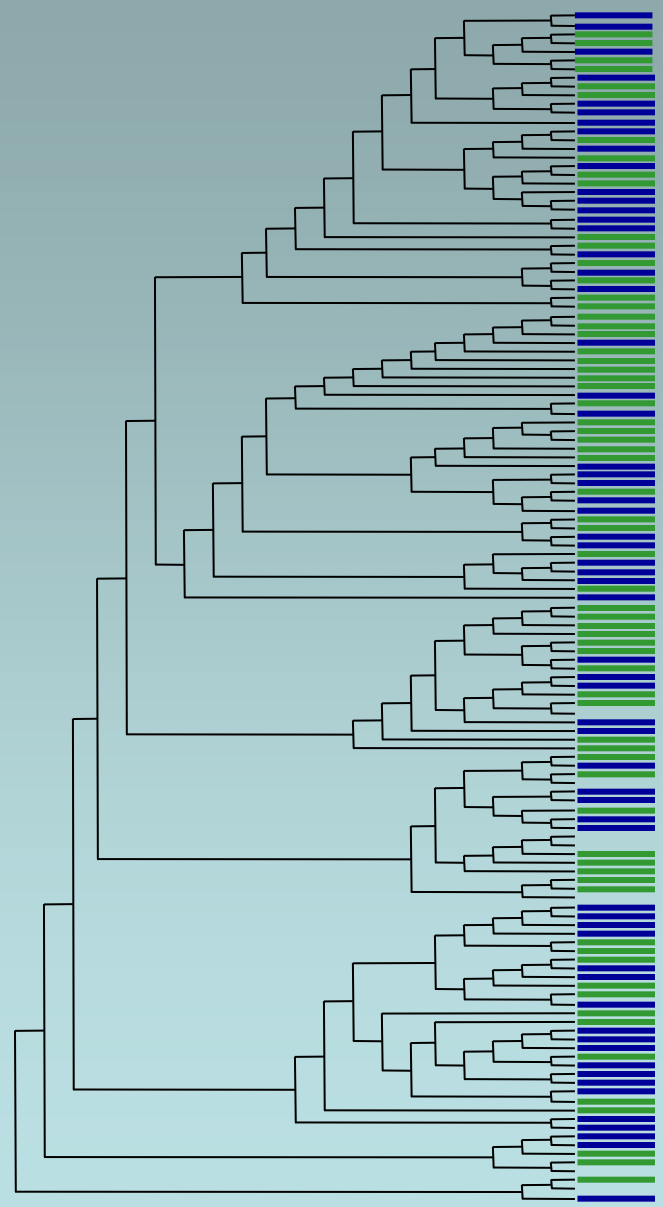
Mating type genes in the *B. bassiana*-group

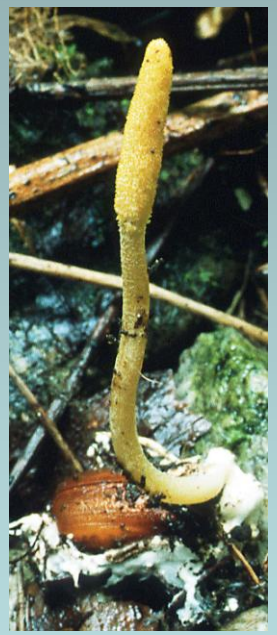
MAT-1

MAT-2



Mating Type Locus

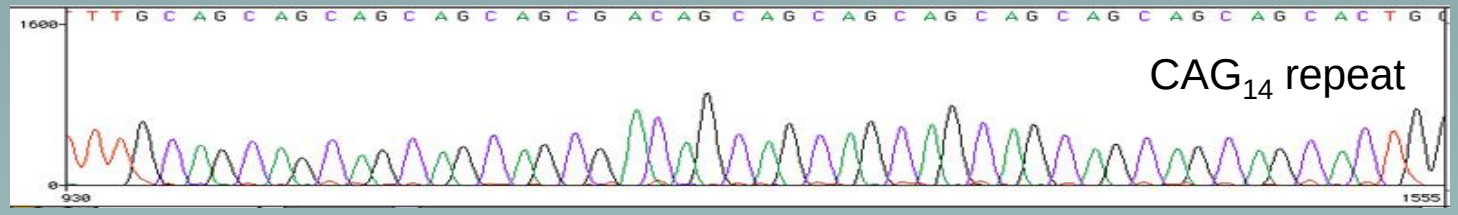


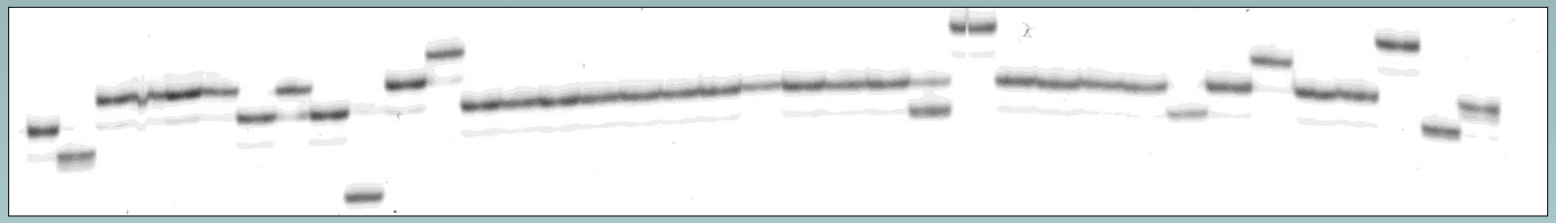


!

Population genetic markers for *B. bassiana*

Microsatellite (SSR)





- 18 polymorphic trinucleotide microsatellite markers
- amplify across *B. bassiana* s.s. complex
    - strain-specific genotyping
    - population genetic analysis

Rehner & Buckley, MEN 2003

Meyling et al., Mol. Ecol. 2009

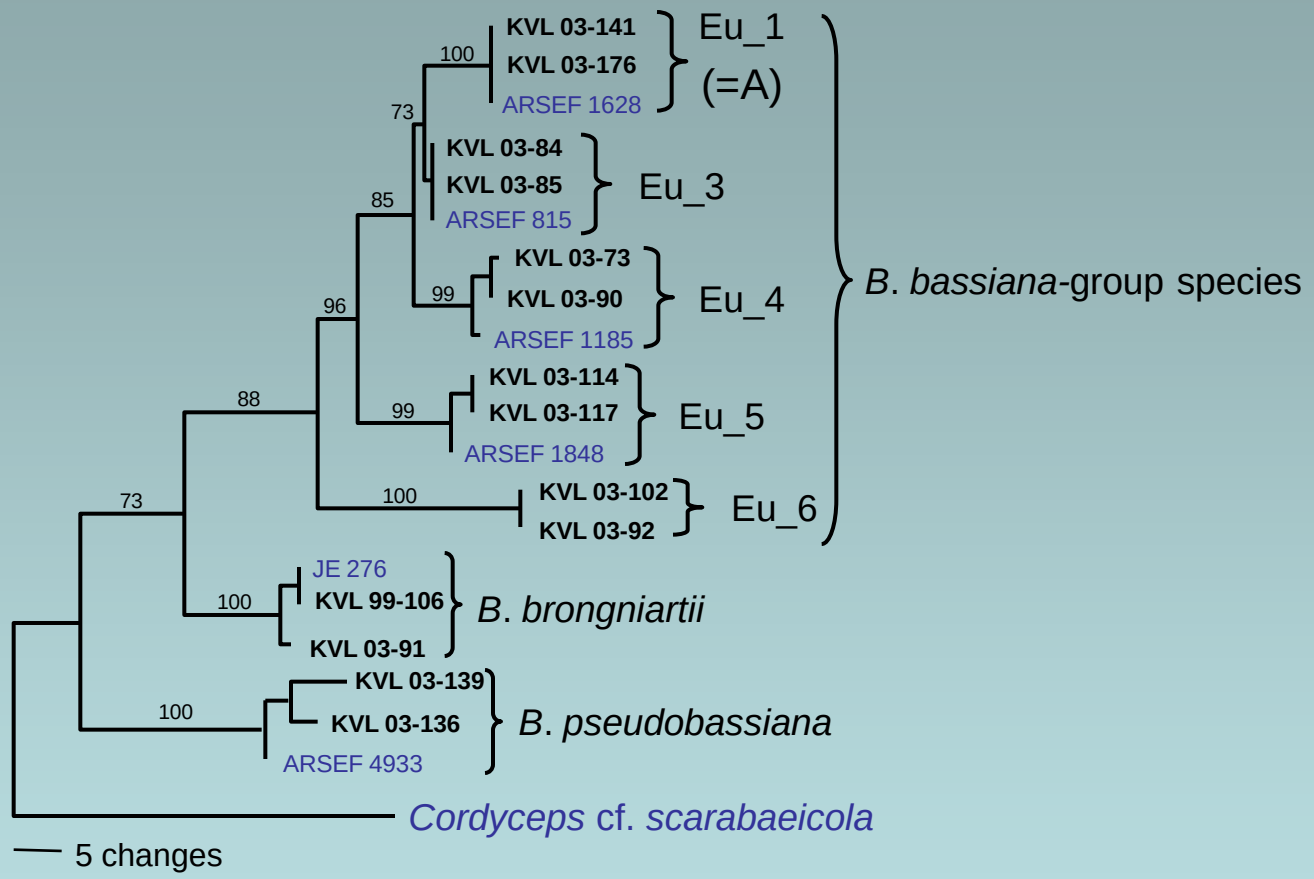
Genetic Diversity of *Beauveria* in Denmark: Cultivated vs. Adjacent Hedgerow Habitats

Meyling et al. Mol. Ecol. 2009

N. V. Meyling

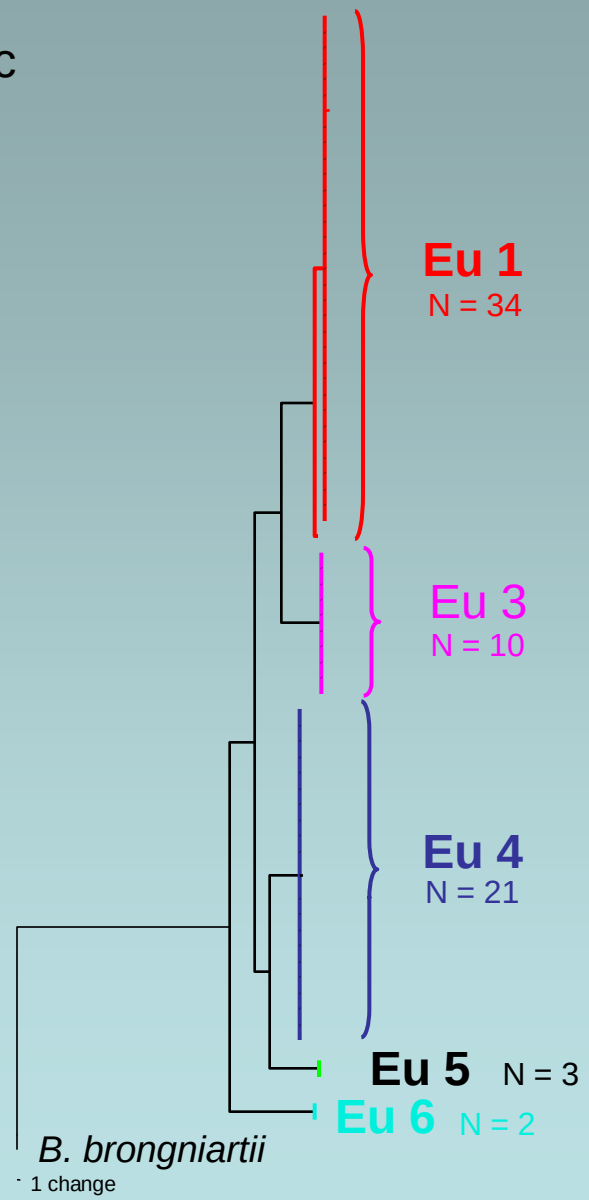
University of Copenhagen

Beauveria spp. at Bakkegaarden Site



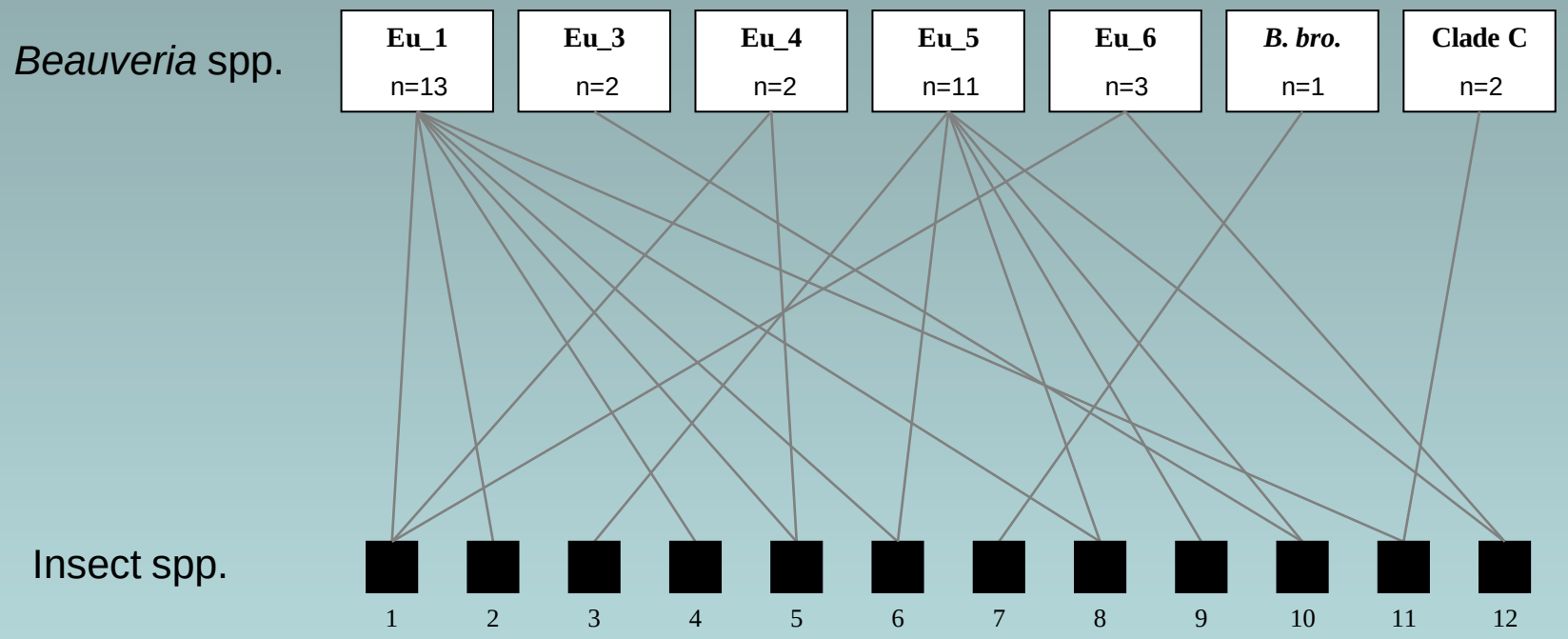
● = Reference Isolates

Environmental Distribution of *B. bassiana* Isolates



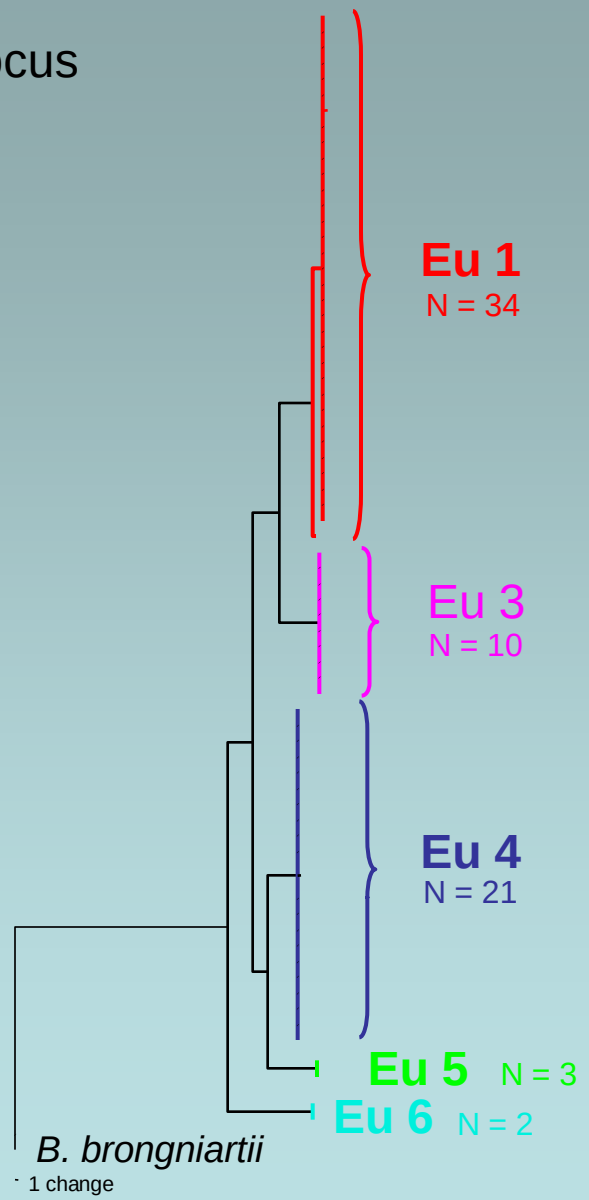
Insect	Soil/Bait	Leaf Surface
13	17/2 (Field / Hedgerow)	2
2	-/1	7
11	-/4	5
3	-	-
2	-	-

Beauveria infection web: hedgerow insects

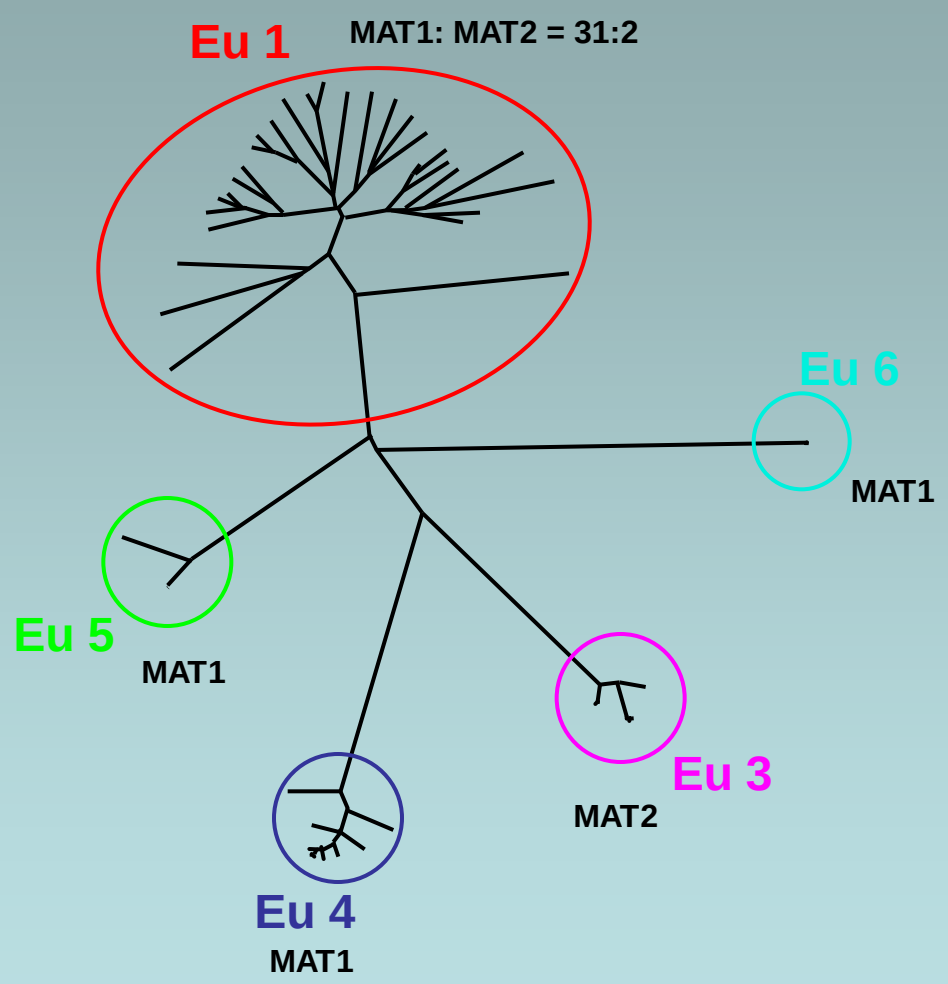


Gene Genealogy vs. Multilocus Microsatellite Genotyping

B locus



Microsatellites(n=18), Mating Type



Summary

***B. bassiana*:**

- includes numerous phylogenetic species
- most species have broad geographic distributions
- occur in multispecies communities, although 1 species usually dominates
- tillage affects community composition

Reproductive mode:

- despite outcrossing mating system, local population structure asexual
- extensive genotypic diversity among strains

Mycotrol:

- detected on 4 continents
- rare lineage, intraspecific recombination unlikely

Into the Future:

- Promote genome sequencing of agronomically important microbes

- Identification resources to enhance knowledge, communication, management

- Network to enhance biocontrol microbial ecology