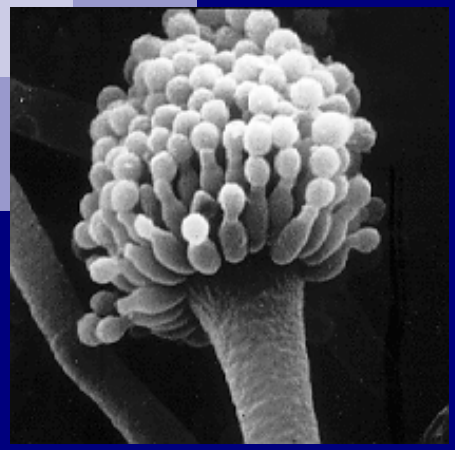
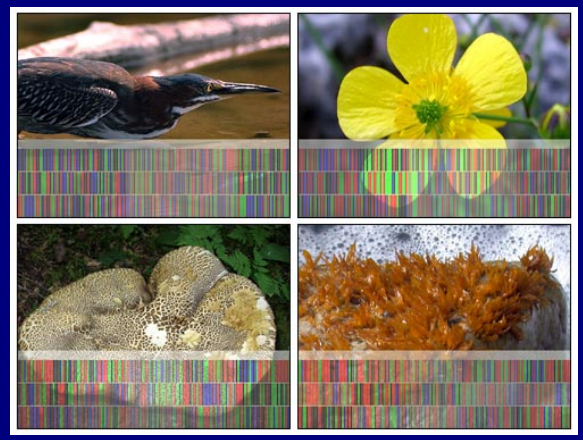
Aspergillus DNA barcoding – progress so far





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What is DNA barcoding?

- **"DNA barcoding** is a taxonomic method which uses a short genetic marker in an organism's (mitochondrial) DNA to quickly and easily identify it as belonging to a particular species"

Wikipedia

The Free Encyclopedia

Desirable attributes of a DNA barcode

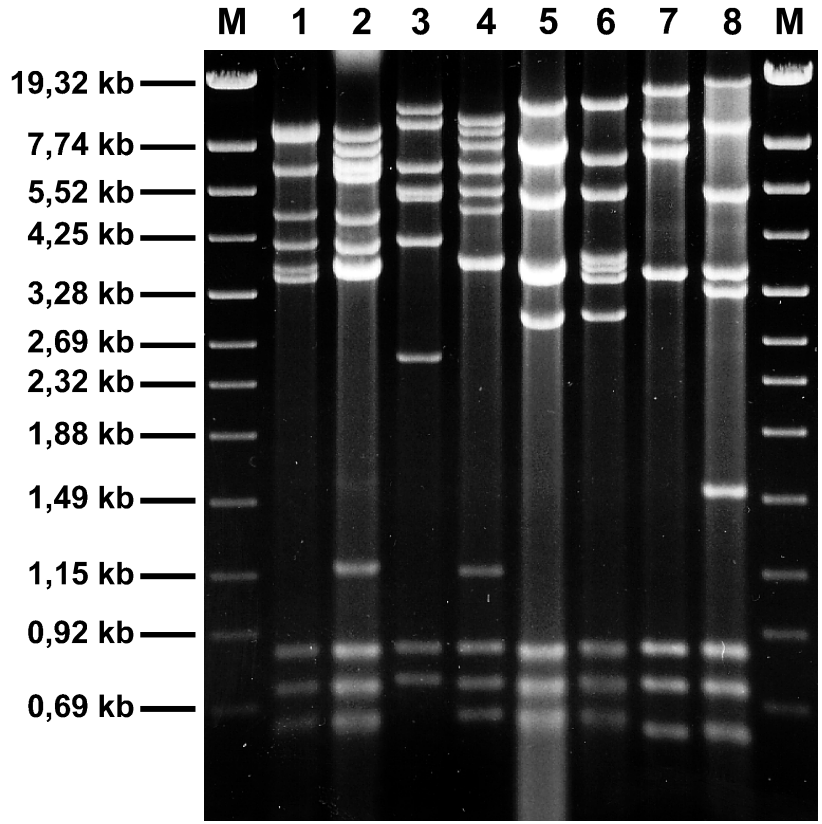
- Variable enough to allow species identification
- Very low levels of intraspecific variation
- Easily accessible (occurs universally, and can be amplified/sequenced by standardized primers from a wide set of organisms)
- Relatively short (≤5-600 bp), simple to sequence
- Easily alignable (can be overcome by using Composition Vector Tree analysis; Chu et al. 2006)
- Lack of recombination

Potential targets

- **Animals:** mitochondrial COI (*cox1*), D1D2 (?)(Sonnenberg et al. 2007)
  - Nematodes: LSU rRNA (De Ley et al. 2005)
- **Plants:**
  - *cox1* (red algae; Saunders 2005)
  - ITS + chloroplast genes (intergenic spacer, *rbcL*; Kress et al. 2005; Chase et al. 2005; Newmaster et al. 2006)
- **Protists:** ssu rRNA (Scicluna et al. 2006)
- **Fungi:** controversial
  - *Fusarium*: elongation factor 1α (TEF) (Geiser et al. 2004)
  - *Trichoderma*: ITS, TEF (Druzhinina et al. 2005)
  - Zygomycetes, dematiaceous fungi: ITS (Schwarz et al. 2006, Desmos-Ollivier et al. 2006, Pounder et al. 2007)
  - *Penicillium*: *cox1* (Seifert et al. 2007)
  - *Aspergillus*: *cox1*?

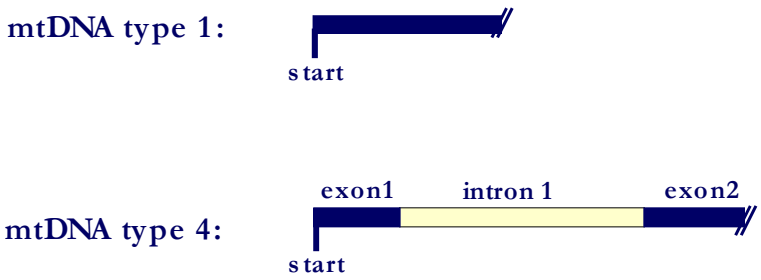
Problems with using *cox1* or other mtDNA genes as barcodes in Aspergilli 1.

- **Low intraspecific variation: not**
  - Black Aspergilli exhibit high levels of intraspecific variability not only in intron content, but also in exonic sequences (Hamari et al. 2003, Juhász et al. 2003, pers. comm.)

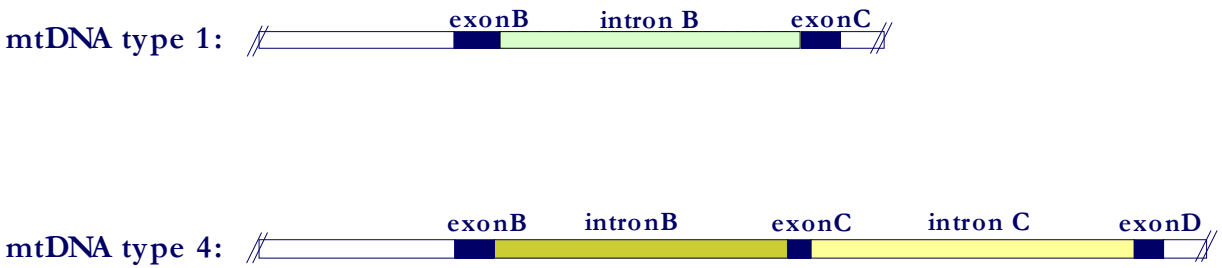


Altered intron content in *cob* and *cox*1 genes of *A. japonicus* mtDNA type 1 and 4

cob gene



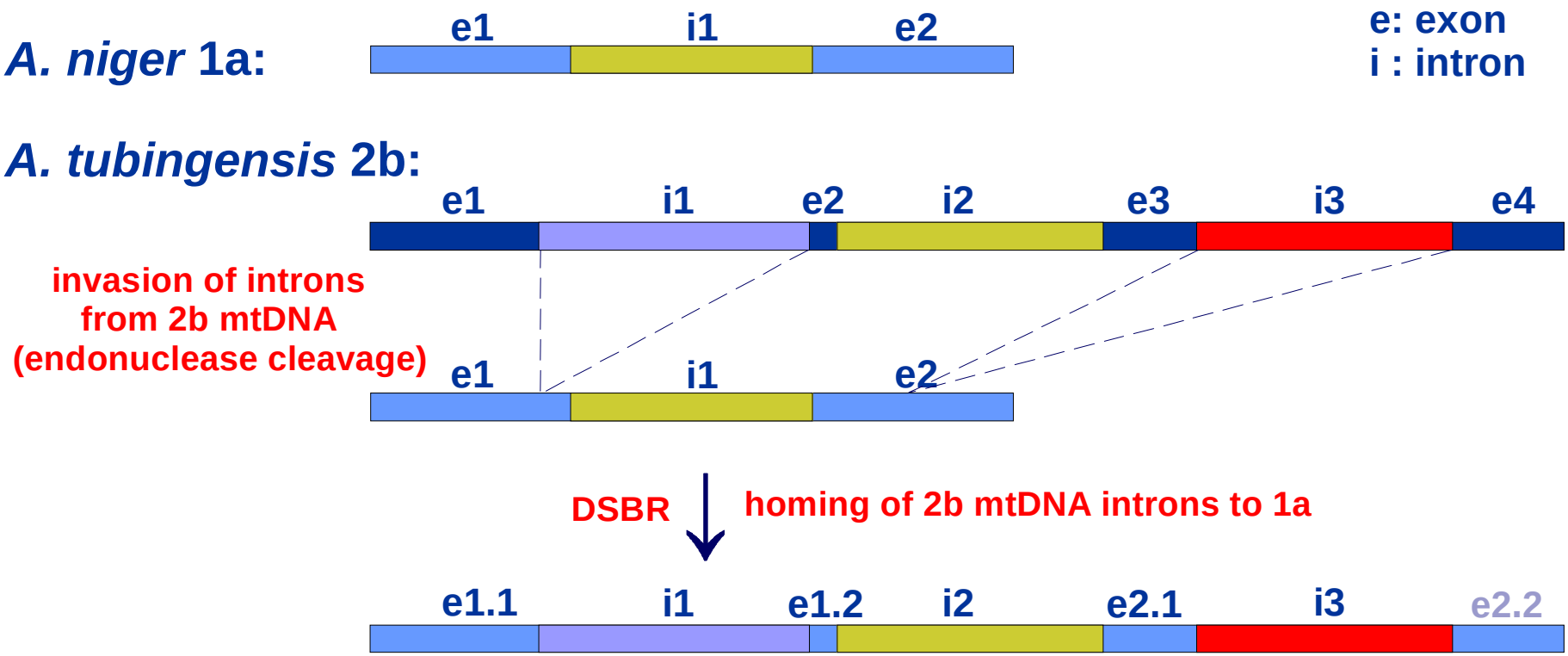
*cox*1 gene



Problems with using *cox1* or other mtDNA genes as barcodes in Aspergilli 2.

- **Lack of recombination: not**
  - Inter- and intraspecific recombination detected in several cases among *Aspergillus cox1* sequences even without selection pressure
    - Hamari et al. 2003 (*A. japonicus*)
    - Juhász et al. 2003 (*A. niger, A. tubingensis*)
    - Tóth et al. 1998 (*A. niger*)
    - Juhász Á. pers. comm. (*A. carbonarius*)
    - Varga & Croft 1995, etc. (*A. nidulans, A. quadrilineatus*)
  - Not only intron jumps, but exonic sequences also undergo changes

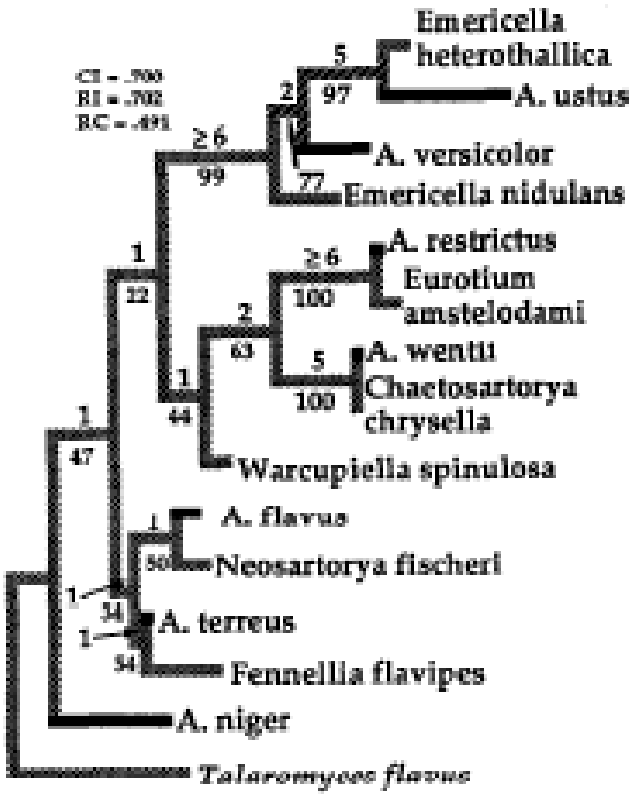
Intron movement in the *cox*1 gene after protoplast fusion of *A. niger* and *A. tubingensis*

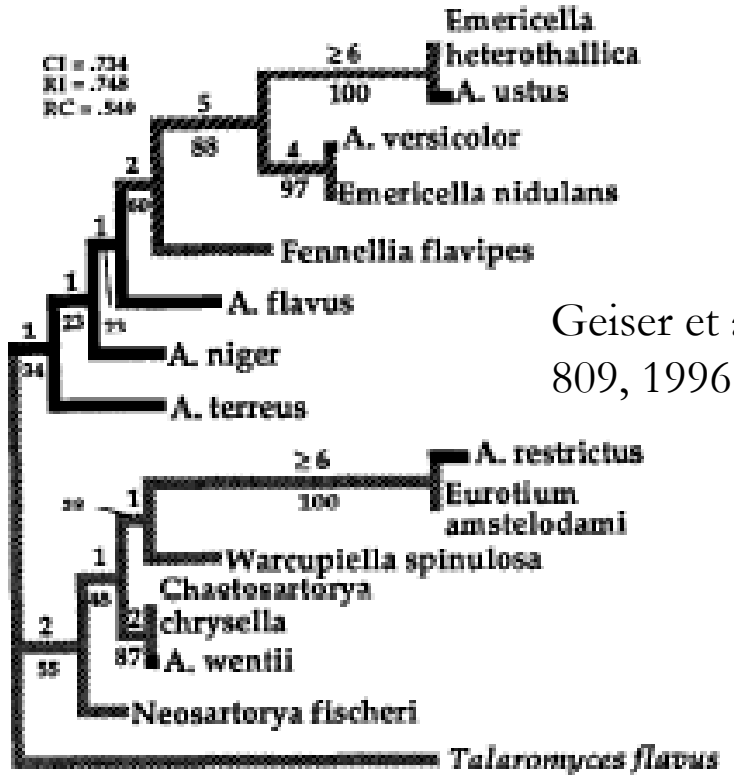


Problems with using *cox1* or other mtDNA genes as barcodes in Aspergilli 3.

- **Phylogenies based on nuclear and mitochondrial gene sequences are frequently incongruent**
  - Geiser et al. 1996: mt and nc rRNA genes
  - Wang et al. 2000: mt cytb genes

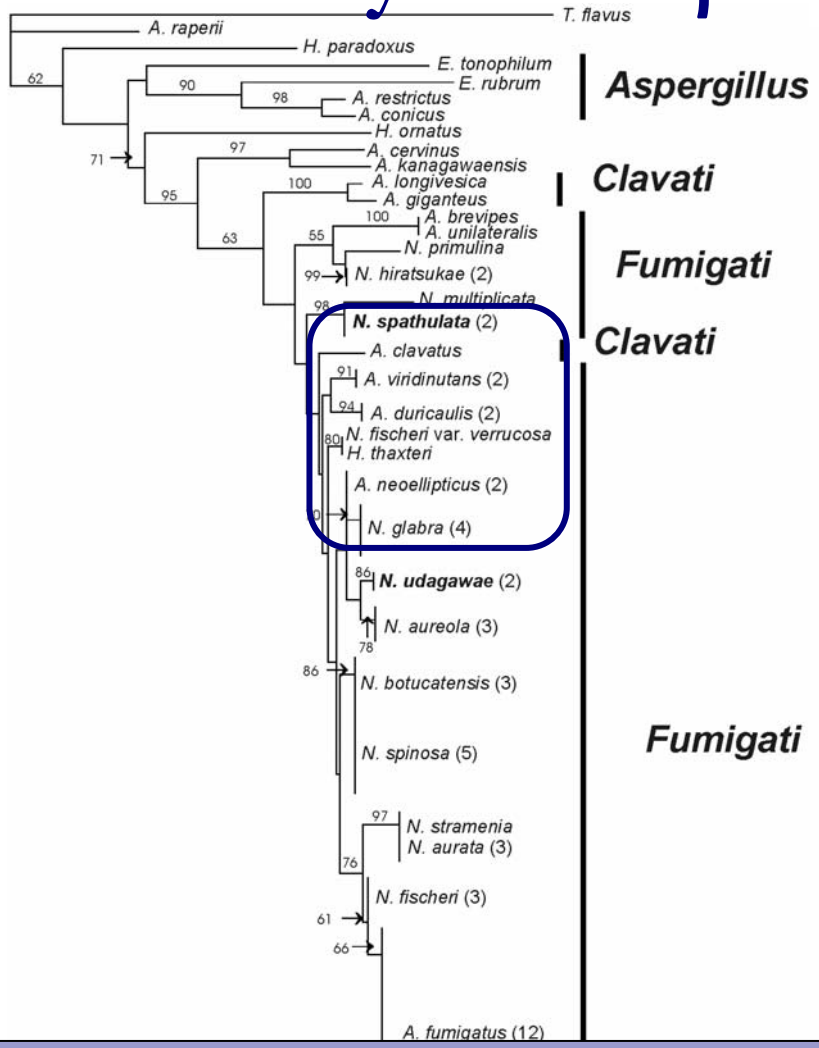
Trees based on mt and nc rRNA gene sequences

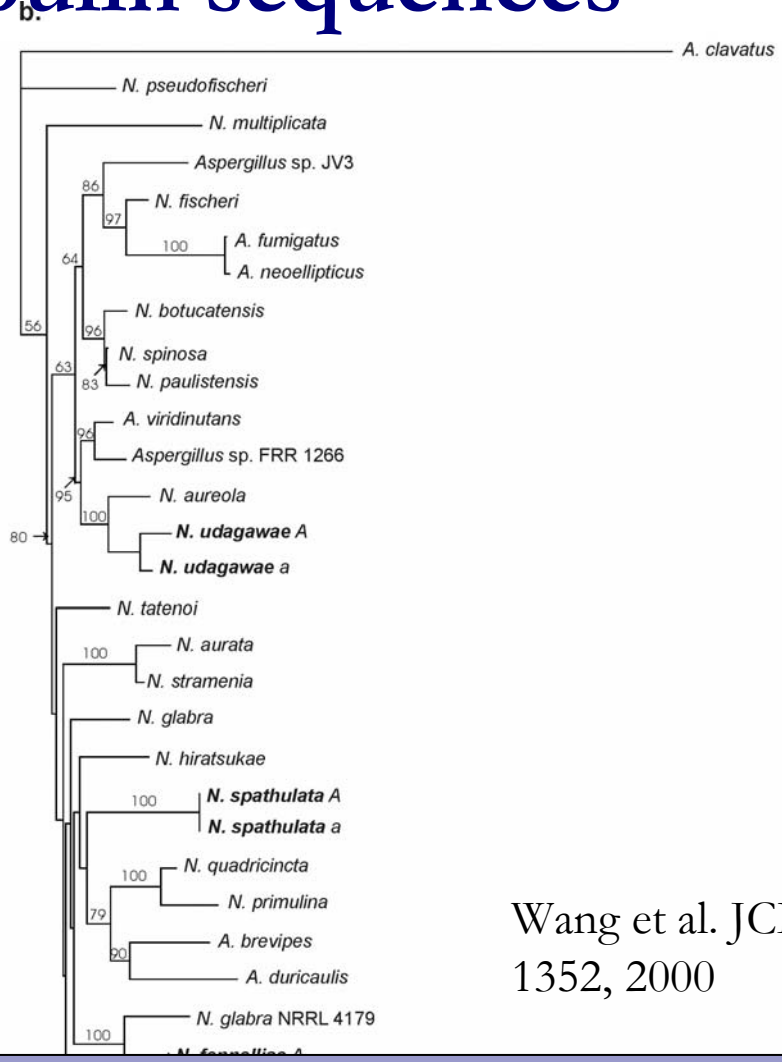




Geiser et al. MBE 13: 809, 1996

Trees of section *Fumigati* based on mt cytb and β-tubulin sequences





Wang et al. JCM 38: 1352, 2000

Problems with using *cox1* or other mtDNA genes as barcodes in Aspergilli 4.

- **Easily accessible: not**
  - *Cox1* sequences are available for 5 *Aspergillus* sp. (3 black Aspergilli, *A. nidulans* and *A. oryzae*)
  - Newly designed primers do not work for all Aspergilli (more to be designed and tested)
  - The *cox1* gene of several *Aspergillus* species carry numerous introns which could make further work tedious

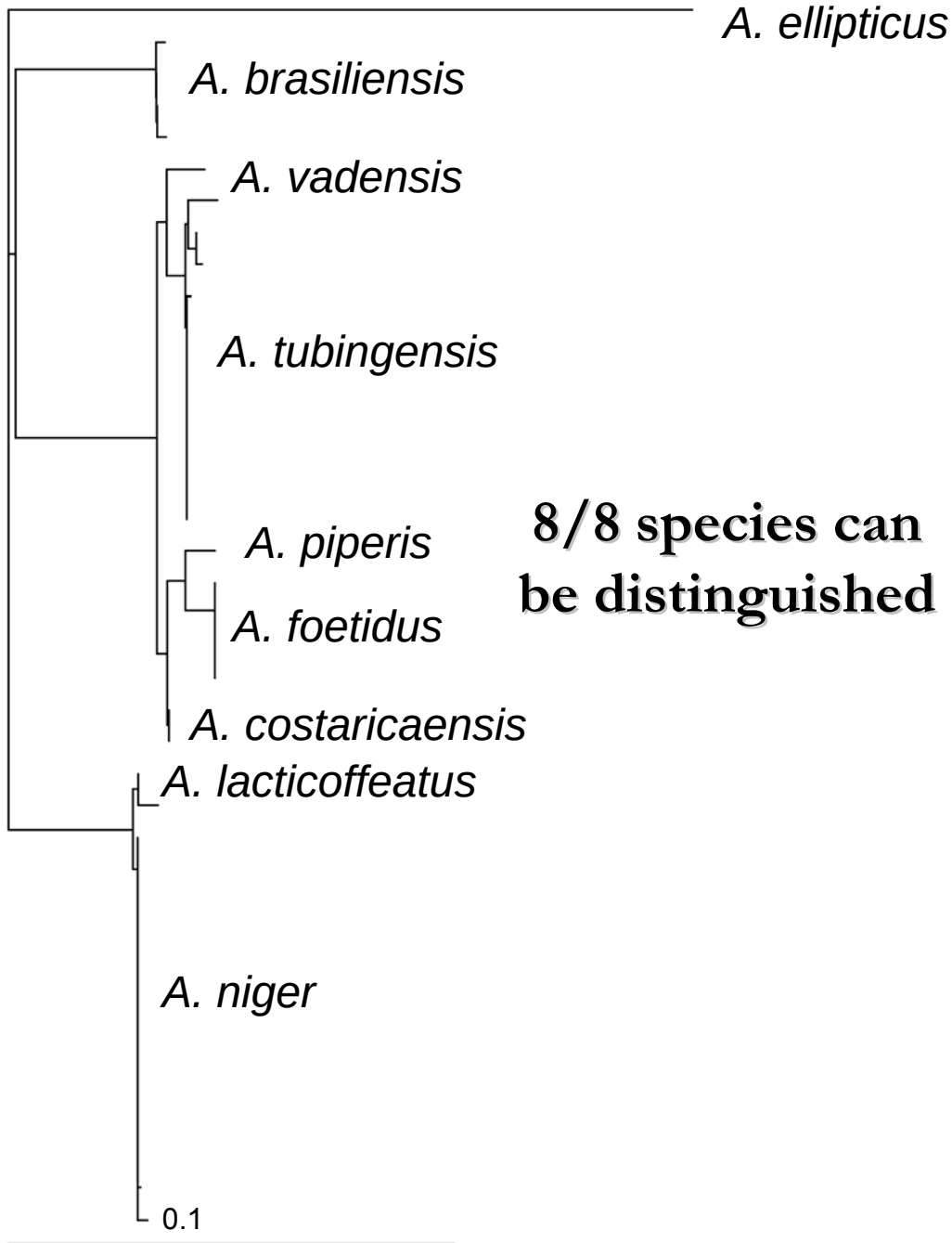
Problems with using *cox1* or other mtDNA genes as barcodes in Aspergilli 5.

- **Variable enough to allow species identification?**
  - Isolates of several *Penicillium* species cannot be differentiated using *cox1* sequences (Seifert et al. 2007)

Case study: the *Aspergillus niger* species complex (Al-Musallam, 1981)

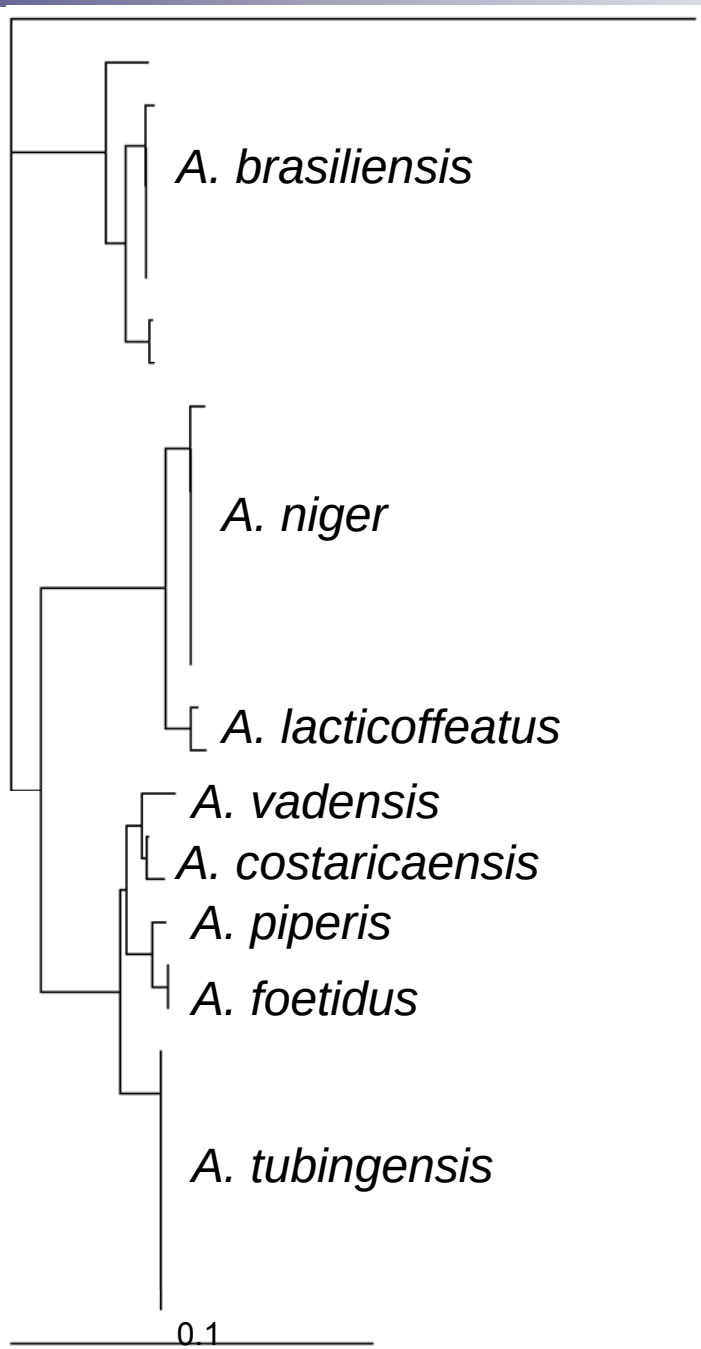
- Includes several species distinguishable by β-tubulin or calmodulin sequence data (most of them also by ITS):
  - *A. niger*
  - *A. tubingensis*
  - *A. foetidus*
  - *A. piperis*
  - *A. brasiliensis*
  - *A. vadensis*
  - *A. costaricensis*
  - *A. lacticoffeatus*

NJ tree based on β-tubulin sequences



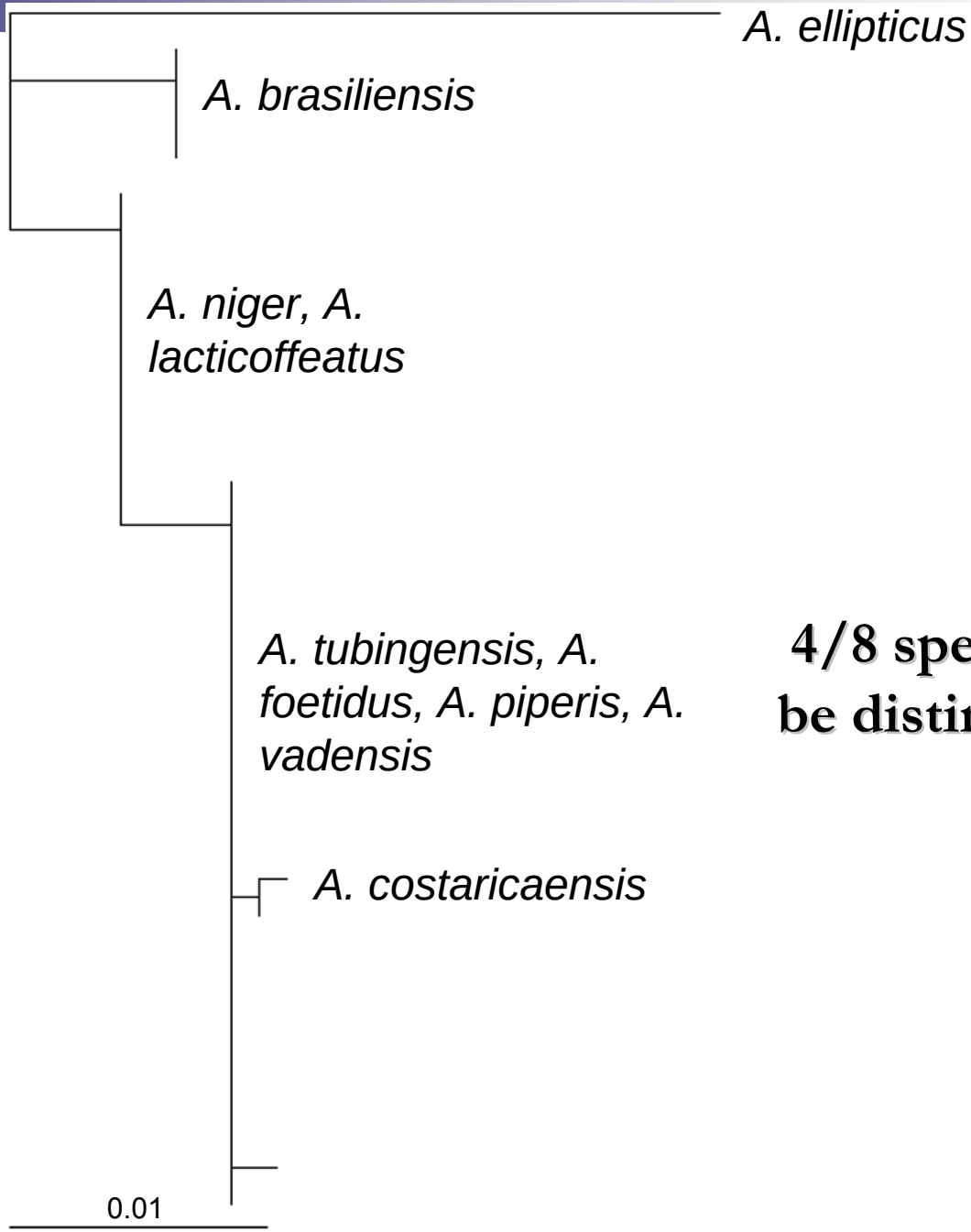
8/8 species can be distinguished

NJ tree based on calmodulin sequences



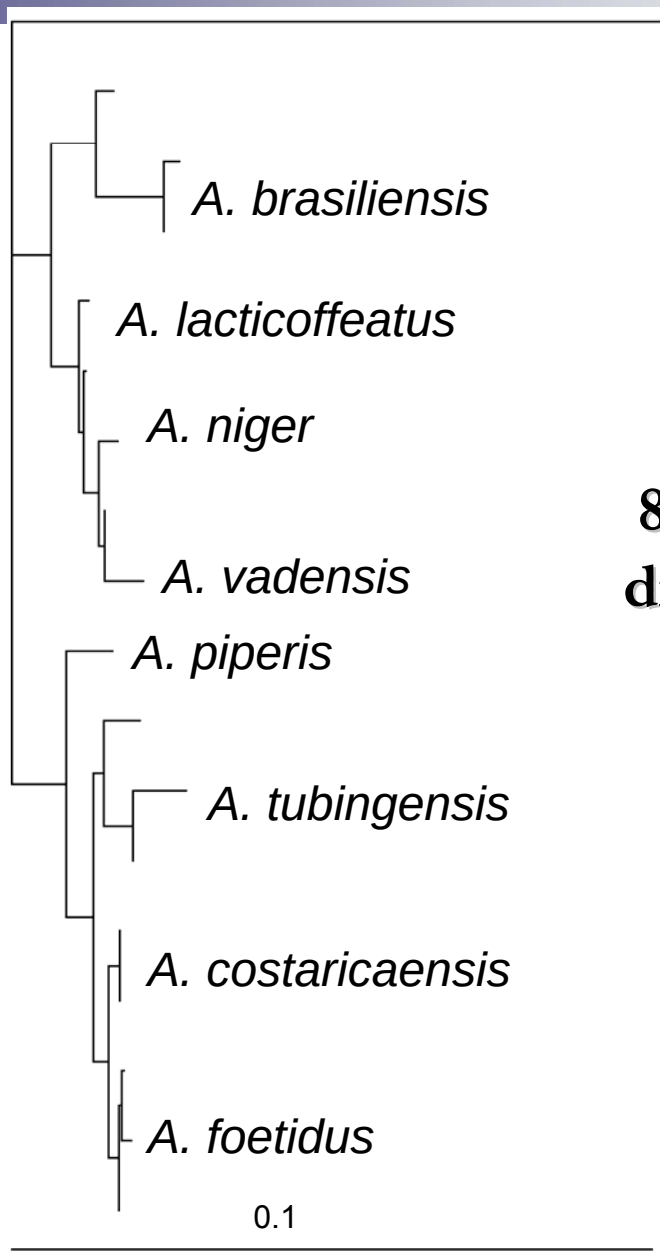
8/8 species can be distinguished

NJ tree based on ITS sequences



4/8 species can be distinguished

NJ tree based on IGS sequences

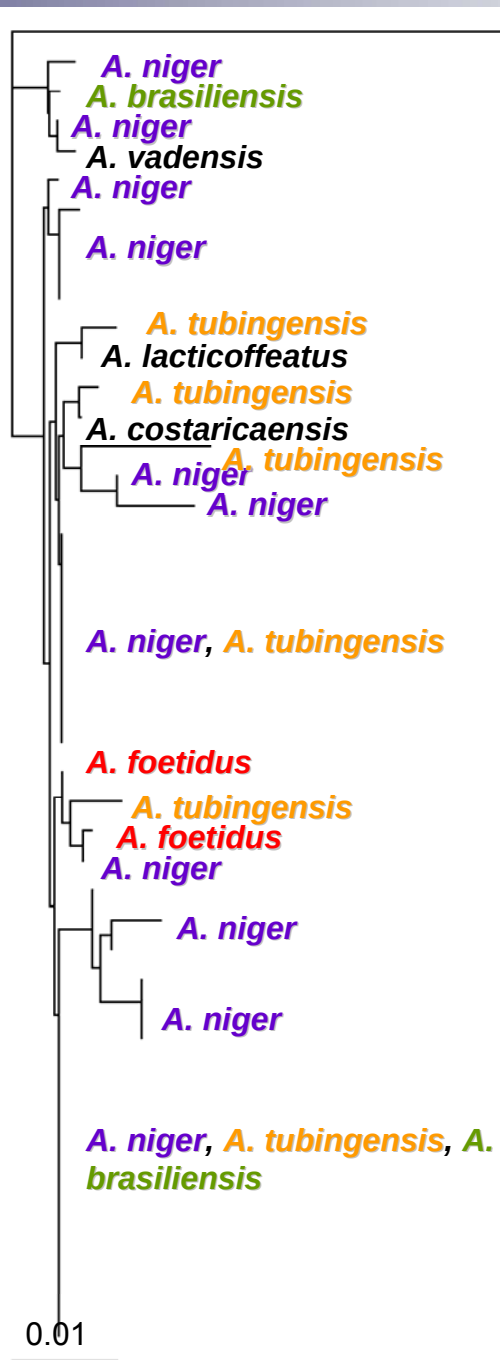


8/8 species can be distinguished (high intraspecific variability)

Other gene sequences able to distinguish between *A. niger* and *A. tubingensis*

- Pyruvate kinase, pectin lyase, polygalacturonase, arabinoxylan-arabinofuranohydrolase, etc. (J. Visser)
- Translation intitiation factor 2, pyruvate carboxylase, 70 kD heat shock protein, chaperonin complex component (TCP-1), ATPase (D. Geiser)
- Translation elongation factor 1-α, RNA polymerase, actin (S. Peterson)

NJ tree based on *cox1* sequences



2(?)/8 species can be distinguished; inter- and intraspecific variability overlaps

Problems with using *cox1* or other mitochondrial genes as barcodes in Aspergilli

- Variable enough to allow species identification: not
- Low intraspecific variation: not
- Lack of recombination: not
- Easily accessible: not
- Phylogenies based on nuclear and mitochondrial gene sequences are frequently incongruent

What to do?

- **Clarify if black Aspergilli represent an exception**
  - Gather *cox1* sequences for other Aspergilli (eg. for section *Flavi*)
- **Gather sequence data for several genes for a set of isolates (section *Nigri & Flavi*)**
  - *Cytb*, ITS, D1-D2, IGS, β-tubulin, actin, calmodulin, TEF, HSPs, etc.
- **Compare barcoding statistics for these genes**
- **Choose the appropriate target gene to be used as DNA barcode**

- “DNA barcoding, rather than being a 'master key' may be a 'master keyring', with different kingdoms of life requiring different keys”

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