

DNA barcoding mushroom species

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Outline

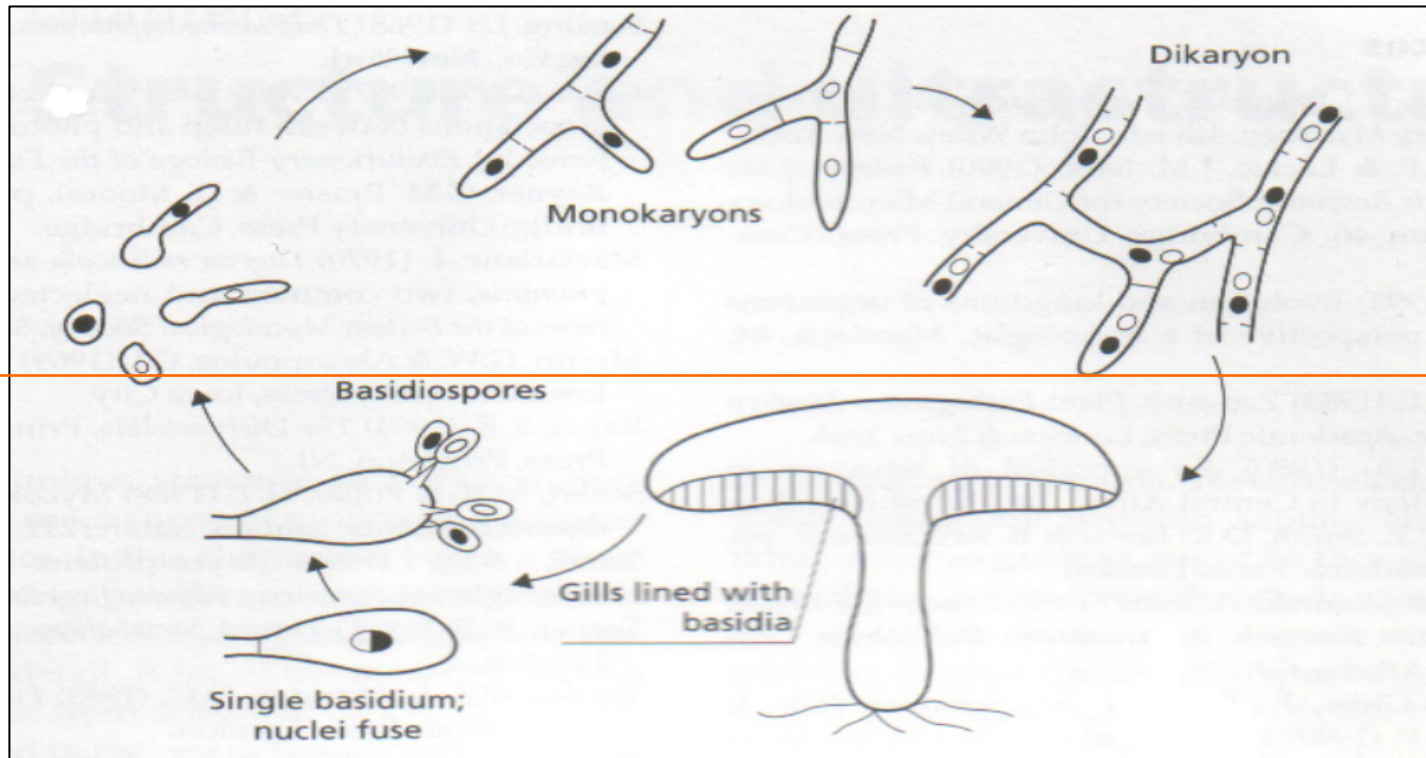
- Background
- Traditional molecular systematic markers
- COI
 - direct PCR amplification
 - RT-PCR

Mushroom diversity

Ca. 14,000 known species



Cryptic life cycle and sporadic fructification



below
ground

above
ground



Species concepts

Morphological

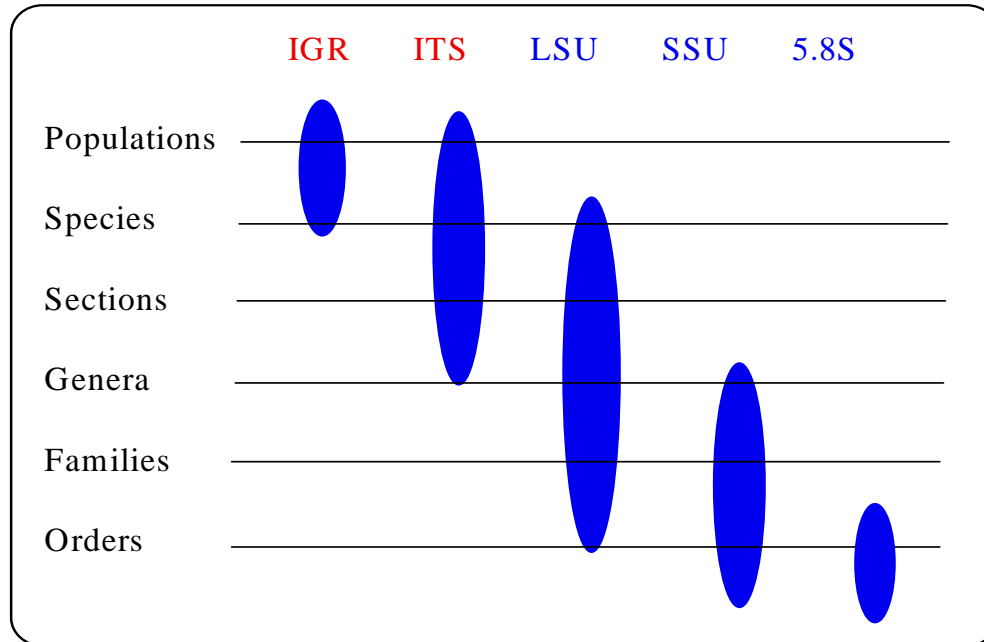
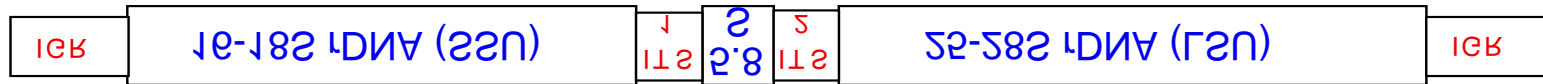
Ecological

Biological

Phylogenetic

Traditional molecular systematic markers

- nuclear ribosomal RNA genes





Traditional molecular systematic markers

- nuclear ribosomal RNA genes

- > 100,000 nuc rDNA fungal sequences currently in GenBank (Hibbett et al., Mycol. Res., in press)

- However.....

- their taxonomic identity is often questionable
 - many remain unclassified (e.g., data from environmental sampling)



Traditional molecular systematic markers

- other nuclear genes

RPB1 and 2

B-tub

EF1-a



Traditional molecular systematic markers

- other nuclear genes

RPB1 and 2

B-tub

EF1-a

- mitochondrial genes

mtSSU

mtLSU

ATP6

Traditional molecular systematic markers

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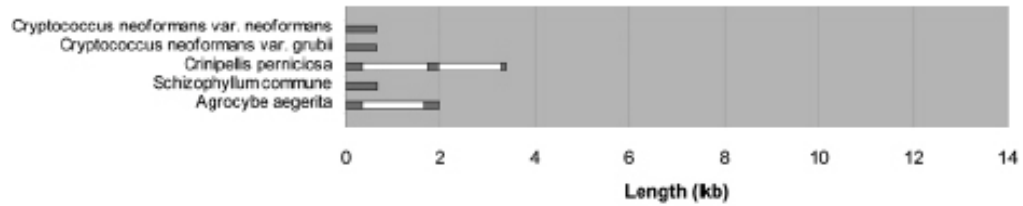
Molecular Ecology (1998) 7, 257–272

A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis

T. D. BRUNS,* T. M. SZARO,* M. GARDES,+ K. W. CULLINGS,‡ J. J. PAN,§ D. L. TAYLOR,**
T. R. HORTON,++ A. KRETZER,‡‡ M. GARBELOTTO,* and Y. LI§§

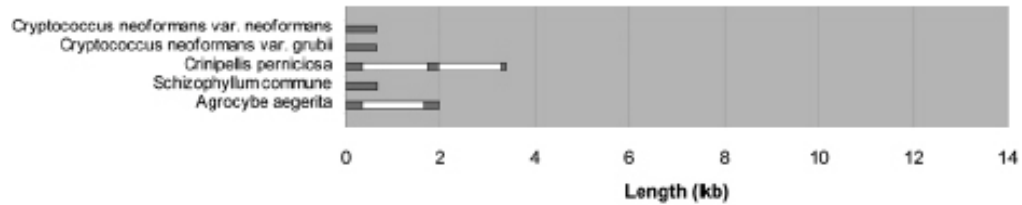
COI in mushrooms

- to begin with...



COI in mushrooms

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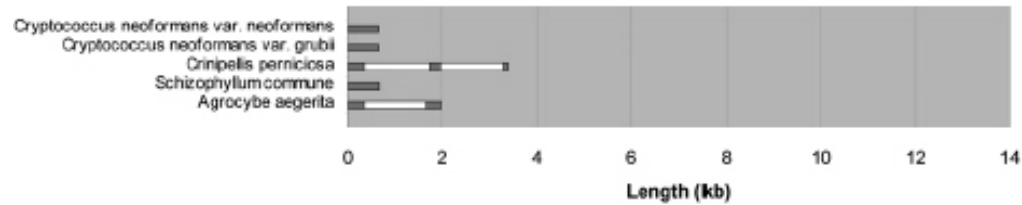


- direct PCR from genomic DNA

- dozen of primer tested
- over 80 species and multiple strains tested
- multiple PCR techniques
- cloning

COI in mushrooms

- to begin with...



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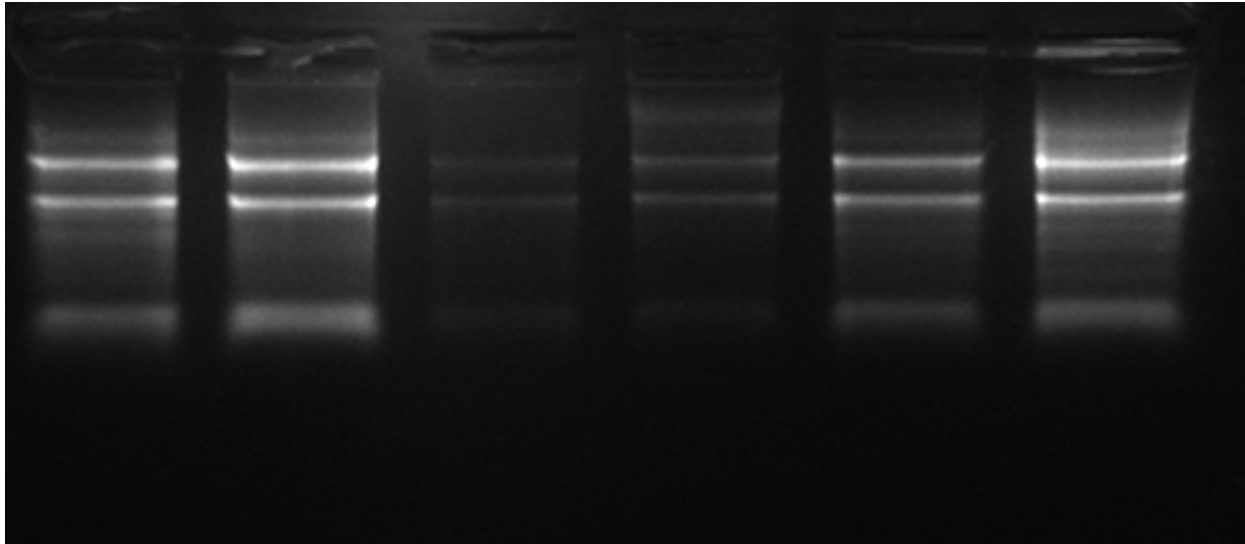
- results

- < 5% success
- two possible sites of insertion of app. 2000bp intron in the target region of CO1
- multiple copies per strain

Accessing COI sequence data

- RT-PCR

- RNA isolation



- create cDNAs using Reverse-Transcriptase
- PCR amplification using cDNAs as template

Accessing COI sequence data

- RT-PCR



Accessing COI sequence data

- RT-PCR



Images from www.freshmushrooms.us, soumon-mori.com, and bugs.bio.usyd.edu.au/Mycology/



Accessing COI sequence data

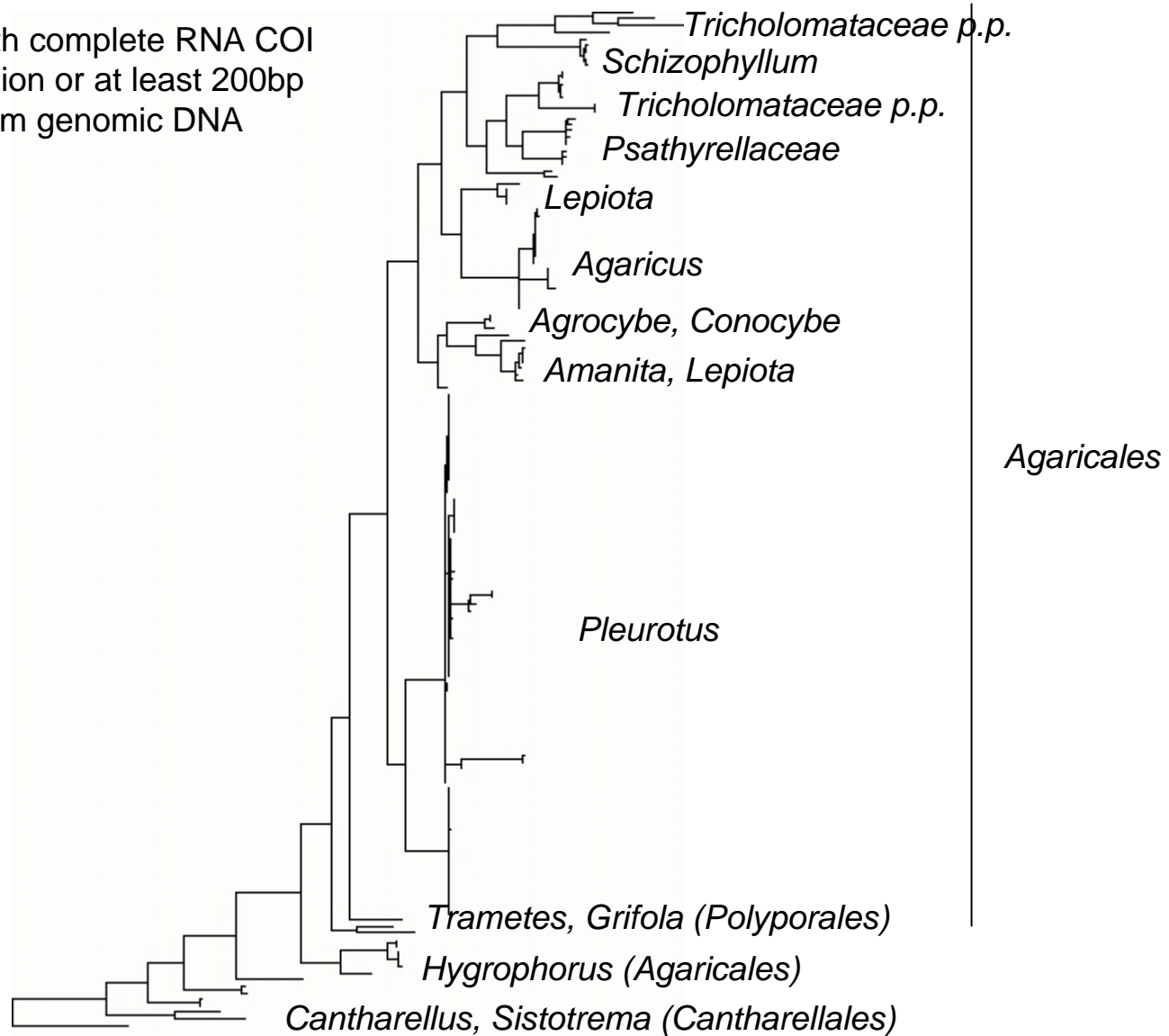
- RT-PCR

Results

- > 95% success
- primer pair developed for a broad taxonomic range

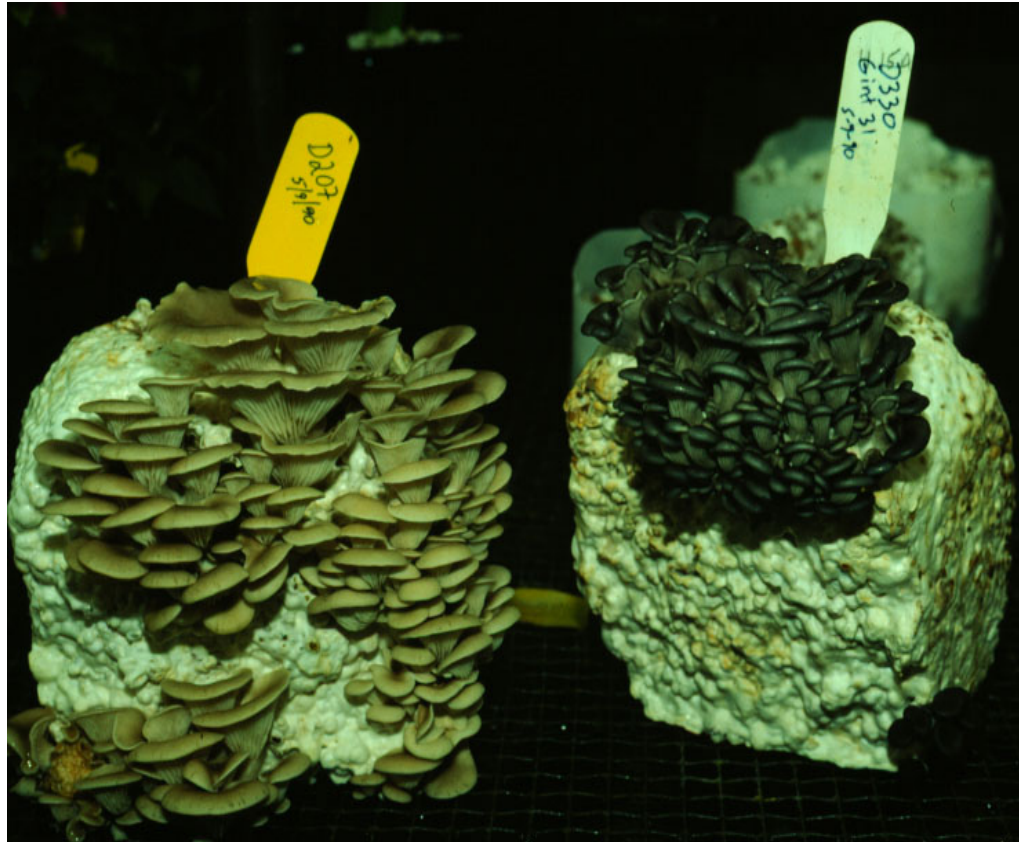
COI sequence groupings

155 taxa with complete RNA COI barcode region or at least 200bp obtained from genomic DNA



COI variation in *Pleurotus* (oyster mushrooms)

- RT-PCR



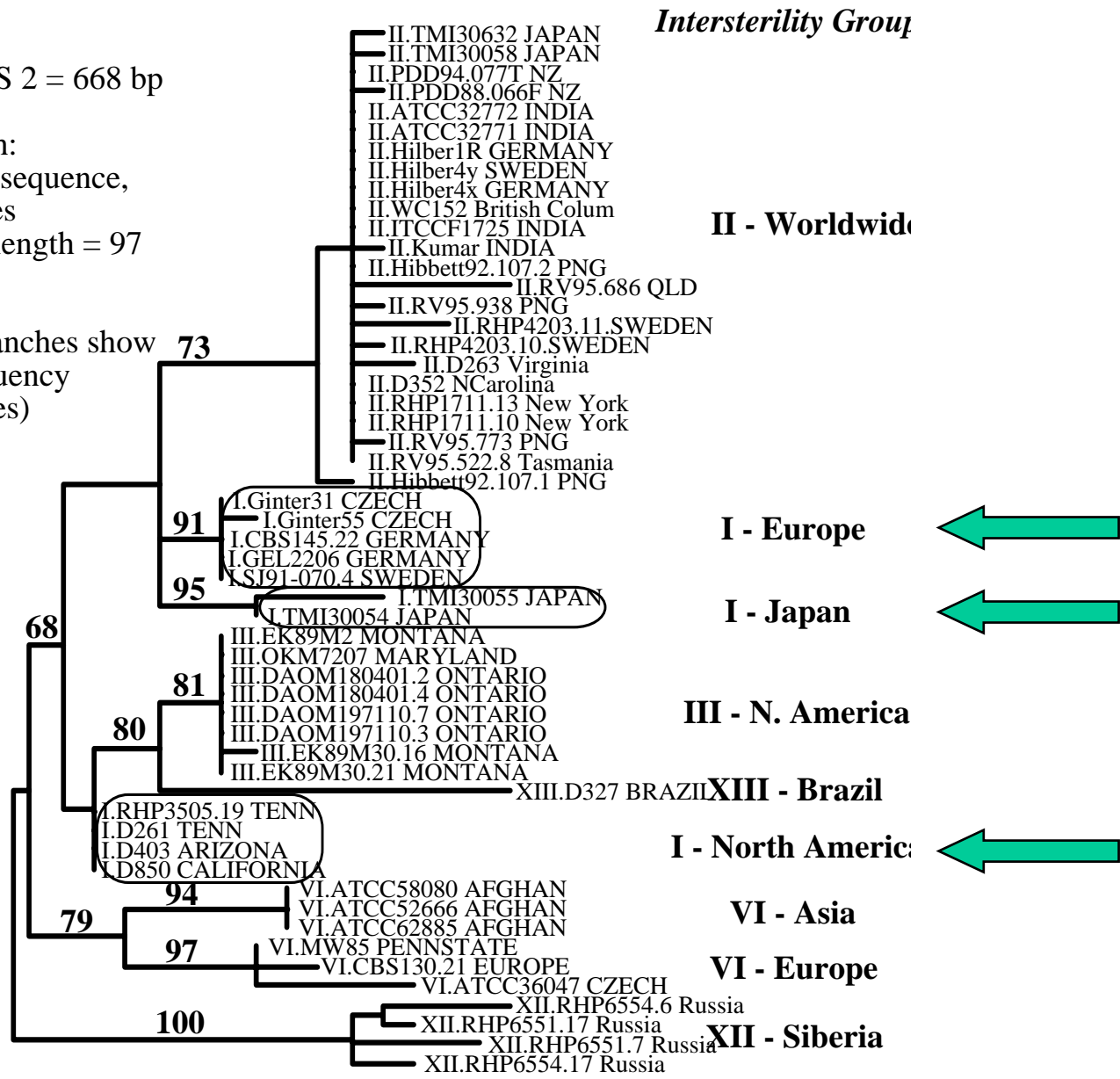
ITS phylogeography of the *Pleurotus ostreatus* group

Alignment for
ITS 1 - 5.8S - ITS 2 = 668 bp

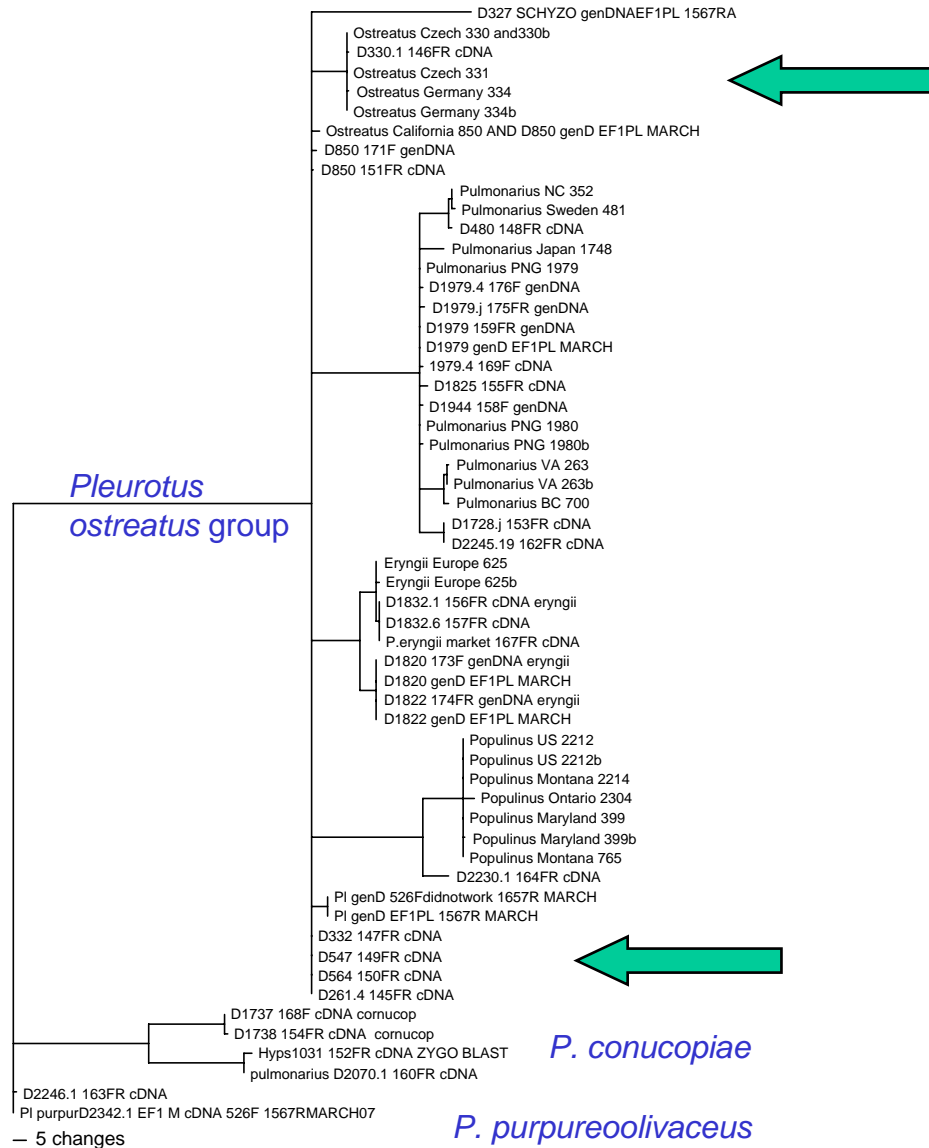
Parsimony search:
random addition sequence,
1000 replicates
>5000 mp tree, length = 97
CI = 0.8247

Values above branches show
bootstrap frequency
(1000 replicates)

Vilgalys and
Moncalvo, 2000

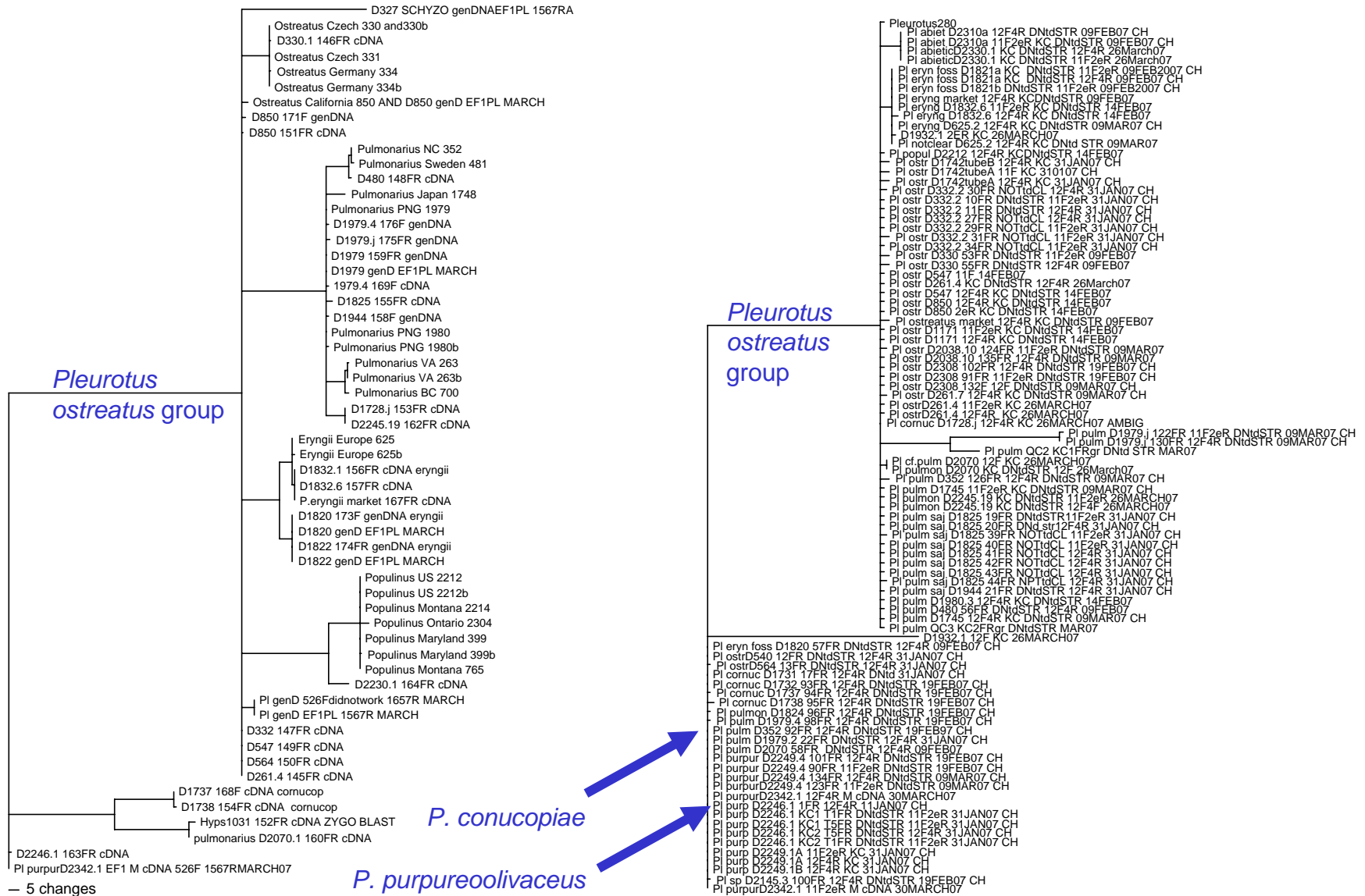


EF1-a (ca. 650 bp)



EF1-a (ca. 650 bp)

COI (ca. 550 bp)



COI variation in *Pleurotus* (oyster mushrooms)

P1 abiet D2310a 12F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 abiet D2310a 11F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 abieticD2330.1 F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 abieticD2330.1 F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryn foss D1820 TACAAGGAGATCATCAGTTATTTAAATGTAATTATATCAGCACACGCATTCATCATGATTTTCTTCATGGTTATGCCTGGATTAGTAGGA
P1 eryn foss D1821a TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryn foss D1821a TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryn foss D1821k TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryng market 12F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryng D1832.6 1J TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryng D1832.6 1Z TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 eryng D625.2 12F TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 popul D2212 12F4 TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D1742tubeB TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D1742tubeA TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D1742tubeA TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 30FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 10FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 11FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 27FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 29FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostr D332.2 31FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTTGGA
P1 ostr D332.2 34FF TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA
P1 ostrD540 12FR DM TACAAGGAGATCATCAGTTATTTAAATGTAATTATATCAGCACACGCATTCATCATGATTTTCTTCATGGTTATGCCTGGATTAGTAGGA
P1 ostrD564 13FR DM TACAAGGAGATCATCAGTTATTTAAATGTAATTATATCAGCACACGCATTCATCATGATTTTCTTCATGGTTATGCCTGGATTAGTAGGA
P1 ostr D330 53FR I TACAAGGAGATCATCAATTATTTAAATGTAATTATATCTGCTCACGCATTTATTTATGATTTTCTTTATGGTTATGCCTGGATTAGTAGGA

COI variation in *Hygrophorus*

(direct PCR)

574 positions

	34	33	70	71	72	35
H. picea 28ALG 34						
H. pudorinus 29ALG 33	67					
H. flavodiscus 49ALG 70	16	71				
H. fuliginus 52ALG 71	0	67	16			
H. flavodiscus 54ALG 72	16	71	0	16		
H. flavodiscus 54ALG 35	16	63	2	16	2	

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Barcoding mushrooms with COI

conclusions

- direct PCR: short regions only, although Long-PCR possible



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Barcoding mushrooms with COI

conclusions

- direct PCR: short regions only, although Long-PCR possible
- RT-PCR: feasible, including for sporocarps
- tissue storage in “RNALater” feasible
- multiple copies
- intra and interspecies variation: more investigations needed



Acknowledgements

- Project Leader: Paul Hebert
- CBOL fungal team: Donal Hickey, Concordia University, Montréal
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