

# **Evaluation of *CO1* as DNA barcode for Basidiomycota**

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# Is CO1 useful for barcoding fungi?

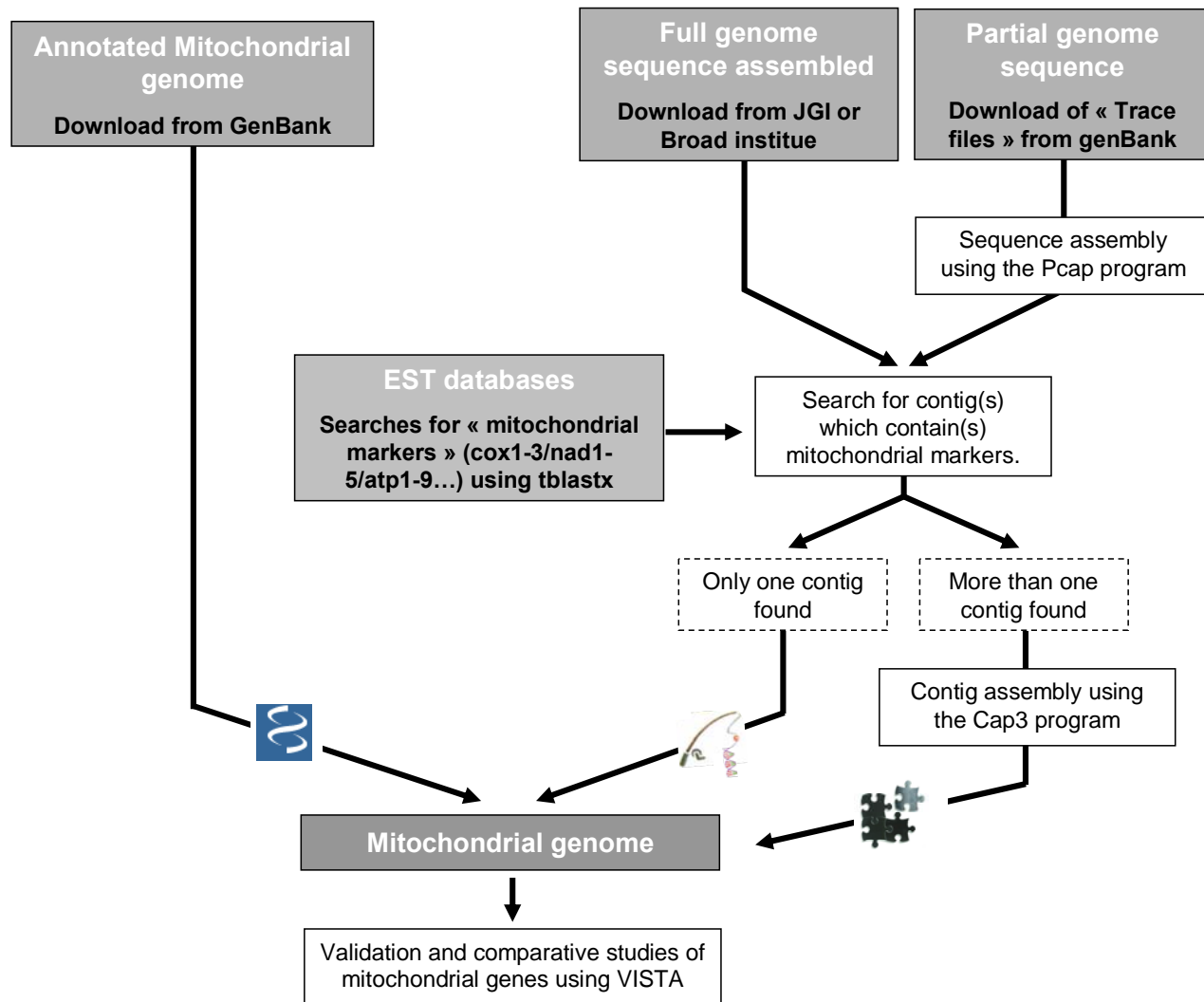
- Potential problems using CO1 in fungi
  - Introns
    - Group 1 introns in fungal mitochondria well documented
    - Presence not always predictable
    - Using « universal » CO1 primers, obtain fragments of different lengths, or no fragment
  - Copy numbers
    - Possibility of multiple copies, including nuclear copies of CO1
    - Yields ambiguous sequences
    - Have to clone and sort out copies
- Important to test whether or not CO1 holds some potential for DNA barcoding in fungi
  - Better characterization of CO1 and mitochondrial genomes
  - Compare with rDNA-ITS cluster, the work-horse in fungi

# Bioinformatics approach

Taxon	Description	Source
<i>Agrocybe aegerita</i>	cytochrome c oxidase subunit 1 (COI) gene	<a href="#">AF010257</a>
<i>Coprinus cinereus</i>	Full genome sequence	<a href="#">Broad institute</a>
<i>Cryptococcus neoformans</i>	Full genome sequence Annotated Mitochondrial genome	<a href="#">Broad Institute</a> <a href="#">NC_004336</a>
<i>Laccaria bicolor</i>	Full genome sequence (nuclear only) Trace files	<a href="#">Join Genome Institute (JGI)</a>
<i>Moniliophthora perniciosa</i>	Annotated mitochondrial genome	<a href="#">NC_005927</a>
<i>Phanerochaete chrysosporium</i>	Full genome sequence (nuclear only) <a href="#">Trace files</a>	<a href="#">JGI</a>
<i>Phakospora pachyrhizi</i>	<a href="#">Trace files</a>	
<i>Puccinia graminis</i>	Full genome sequence (nuclear + mitochondrion)	<a href="#">Broad institute</a>
<i>Schizophyllum commune</i>	Annotated mitochondrial genome	<a href="#">NC_003049</a>
<i>Ustilago maydis</i>	Full genome sequence Annotated mitochondrial genome	<a href="#">NC_008368</a>

- Several fungi with completely sequenced genomes
  - Some have genomes for multiple strains
- Can use this data to characterize CO1 in fungi
  - Map exons and introns
  - Determine copy number, locations
- Use the data to design primer pairs
  - Exclude regions with introns
  - Select against unwanted (nuclear) copies

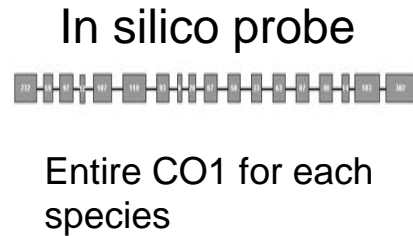
# Bioinformatics pipeline to retrieve mitochondrial genomes





# Copies of CO1 in the mitochondrial and nuclear genomes

## Targets



Mitochondrial genome (minus the copy used as a probe)

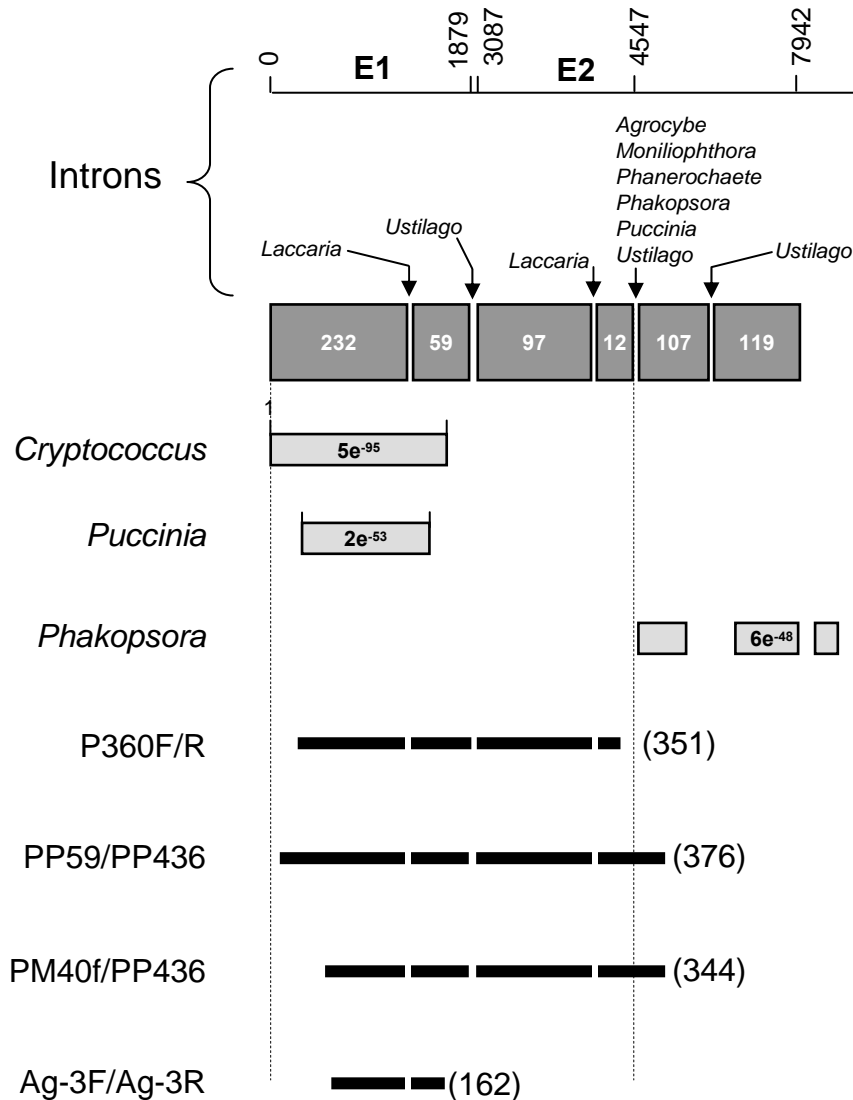
Nuclear genome

**Results  $\geq$  20 nt considered**

	Nuclear		Mitochondrial	
	Exons	Introns	Exons	Introns
<i>Coprinus</i>	1 (52)	-	-	-
<i>Cryptococcus</i>	5 (38-200)	-	1 (20)	-
<i>Laccaria</i>	-	1 (60)	-	-
<i>Moniliophthora</i>		ND	-	7 (21-30)
<i>Phakopsora</i>	1 (157)	1 (1065)	-	1 (24)
<i>Puccinia</i>	3 (87-121)	5 (108-728)	-	3 (24)
<i>Ustilago</i>	-	4 (37-56)	-	8 (20-132)



# DNA barcoding primers

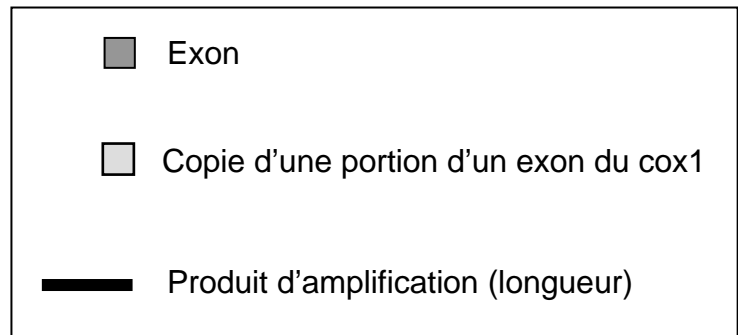


Primers Mathieu Allaire:

P360F 5'-GCTAAGGATATTGCCATTCTATAT-3'  
 P360R 5'-TCCATCCYGTTCCTGTCYCC-3'  
 PP59 5'-GATGAYTTTATAGYACBAAYGCTAAG-3'  
 PP436 5'-GACAGCWTATCACCTACHGTC-3'  
 PM40f 5'-TTGCMATTCTATATTTGTATTTGG-3'

Primers Mehrdad Hajibabaei:

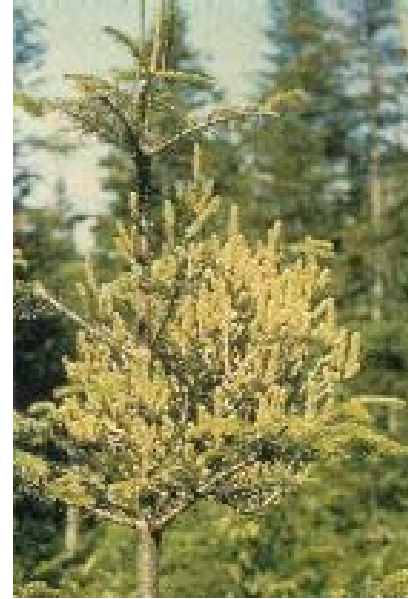
Ag-3F 5'-AGGTACCCTTTATTTAATTTTTGCTG-3'  
 Ag-3R 5'-GAAAATCATAATGAAGGCATGAGC3'



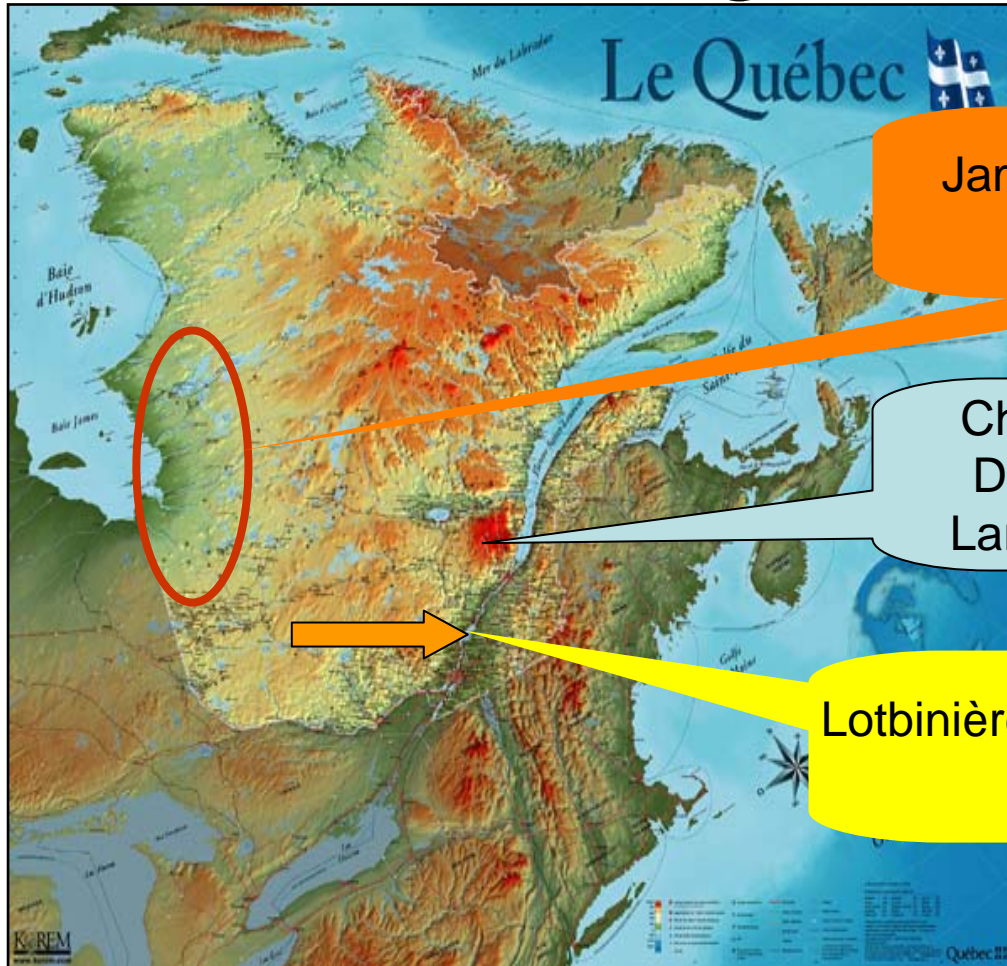


# Barcoding tree rusts

- Rusts cause some of the most severe plant disease epidemics
- Almost all trees are affected by a rust
- Rusts are heterotrophic parasites that steal resources from the host
- A variety of symptoms and host reactions on the different hosts
  - Witche's brooms
  - Cankers
  - Defoliation
  - Early needle sheds



# Rust sampling for barcoding

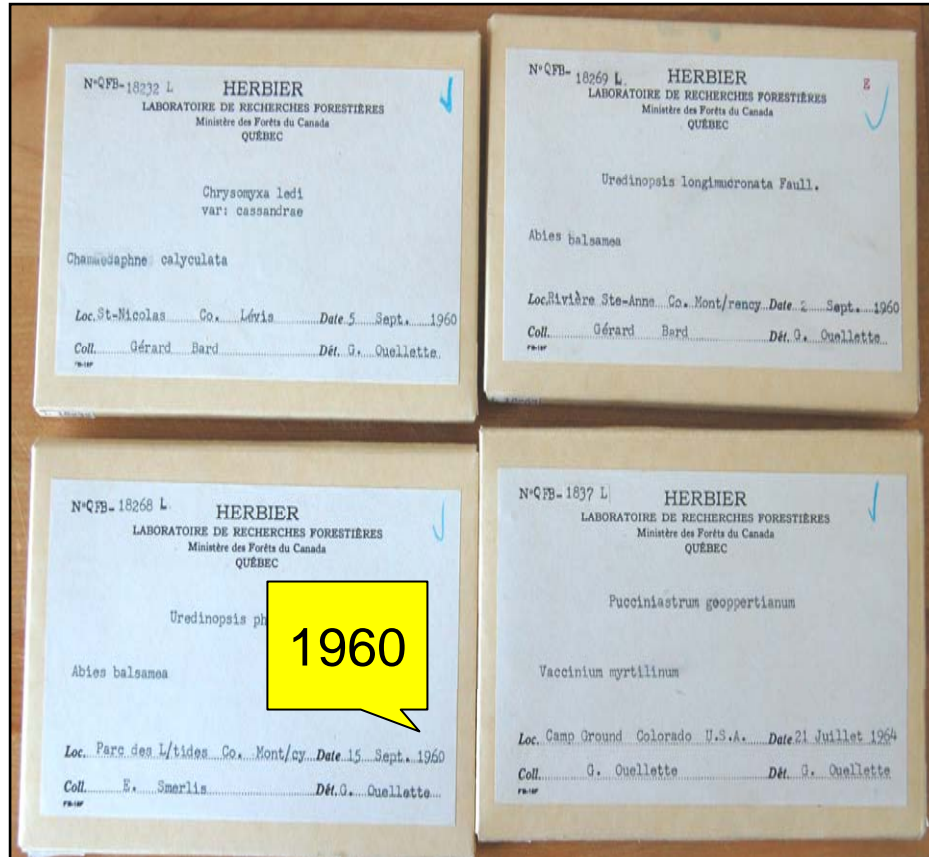


James Bay: boreal forest, bogs  
Jack pine+black spruce

Charlevoix: mountainous  
Diversity of ecosystems  
Larch, Pines, Spruce, etc.

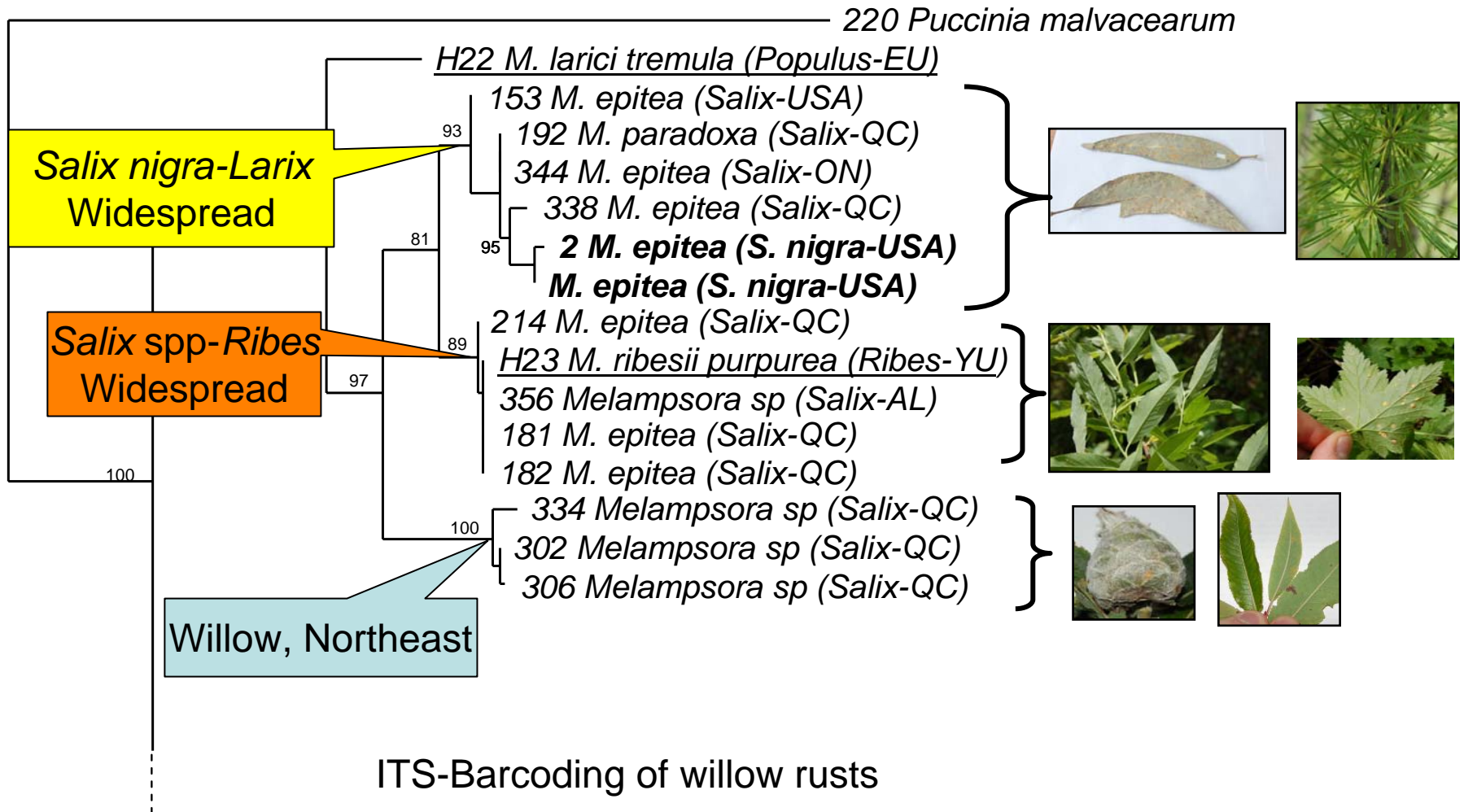
Lotbinière: St-Lawrence River Valley  
High diversity

# Herbarium samples



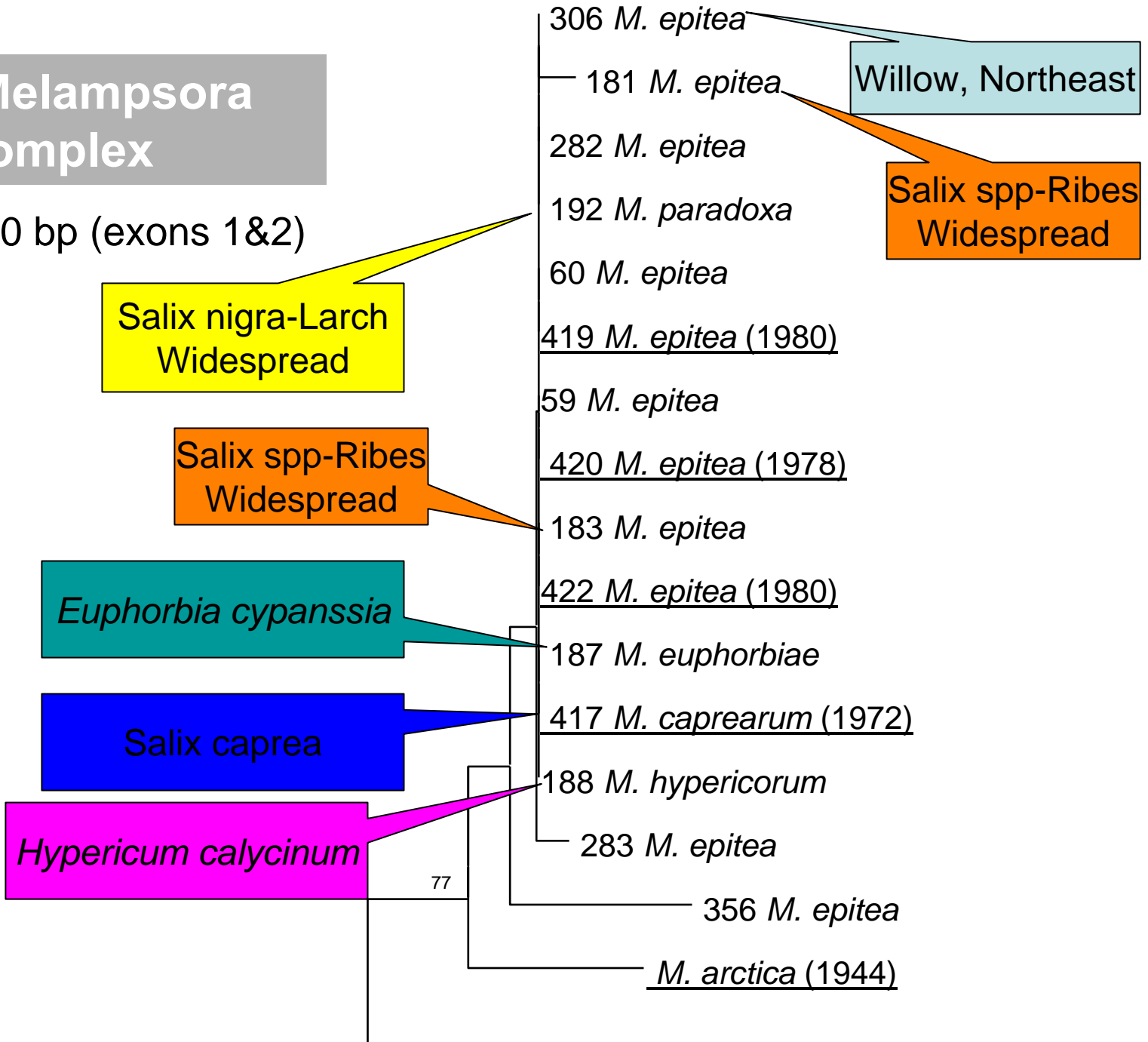
- Species from 10 different genera : *Cronartium*, *Pucciniastrum*, *Puccinia*, *Gymnosporangium*, *Melampsora*, *Melampsorella*, *Milesina*, *Hyalospora*, *Uredinopsis*, *Chrysomyxa*, *Kuehneola* and *Coleosporium*
- Collections from Northeastern North America, U.S., Europe
- Herbarium samples from Ottawa and Laurentian Forestry Center

# Willow rust: *Melampsora epitea*



# COI vs Melampsora epitea complex

COI with 380 bp (exons 1&2)



# BOL Rust Team

- Mathieu Allaire, research assistant
- Nicolas Feau, post-doctoral fellow
- Chantal Morin, post-doctoral fellow
- Agathe Vialle, student

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- Genome Canada
- Fungal BOL community

