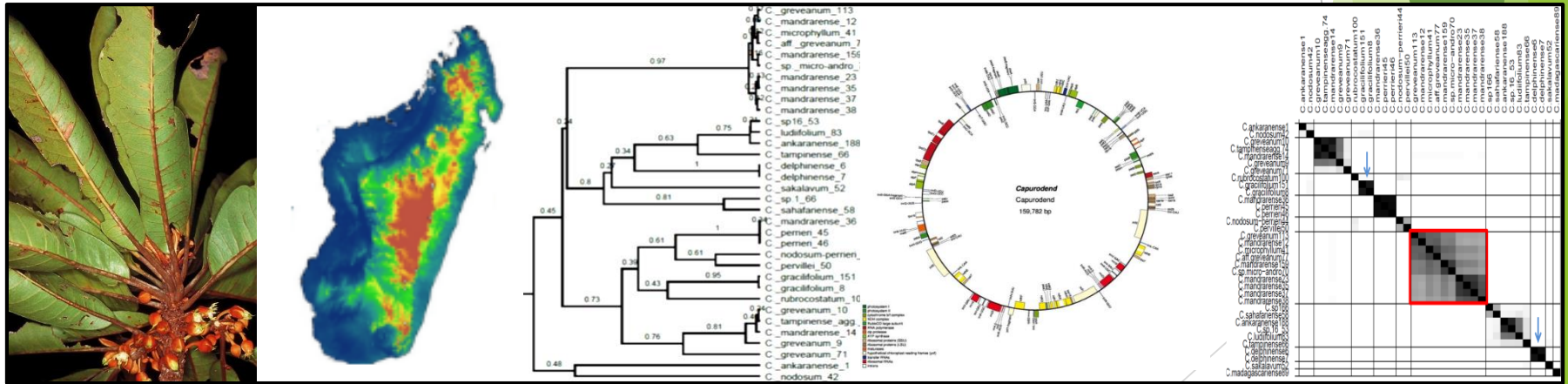


# A race against the extinction of *Capurodendron* trees in Madagascar: from phylogenomic diversity to conservation.

Carlos G. Boluda, Camille Christe, Laurent Gautier & Yamama Naciri  
Conservatoire et Jardin botaniques de la Ville de Genève



Biology19 meeting, University of Zurich-Irche,  
8-Feb-2019

# Introduction

## Genus *Capurodendron*:

- Sapotaceae family.
- Third-largest endemic genus in Madagascar (26 spp).
- Trees, rarely shrubs.
- From rainforests to arid lands.
- Critically endangered (deforestation + precious wood).
- Flowers homogeneous across species.
- Leaves, fruits and seeds characters variable.



*C. delphinense*



*C. greveanum*



*C. androyense*



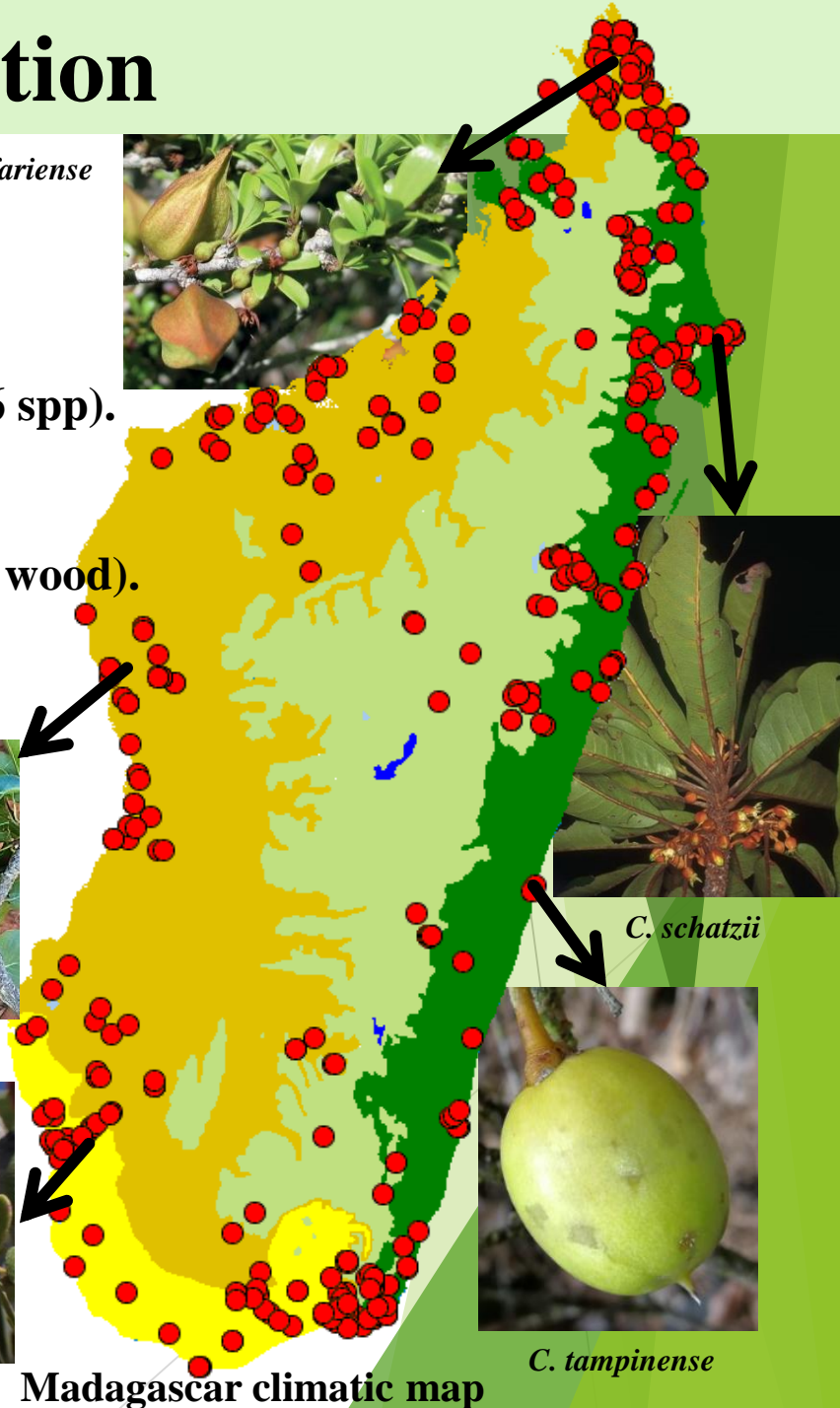
*C. sahafariense*



*C. schatzii*



*C. tampinense*



Madagascar climatic map



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Possibly extinct *Capurodendron* species

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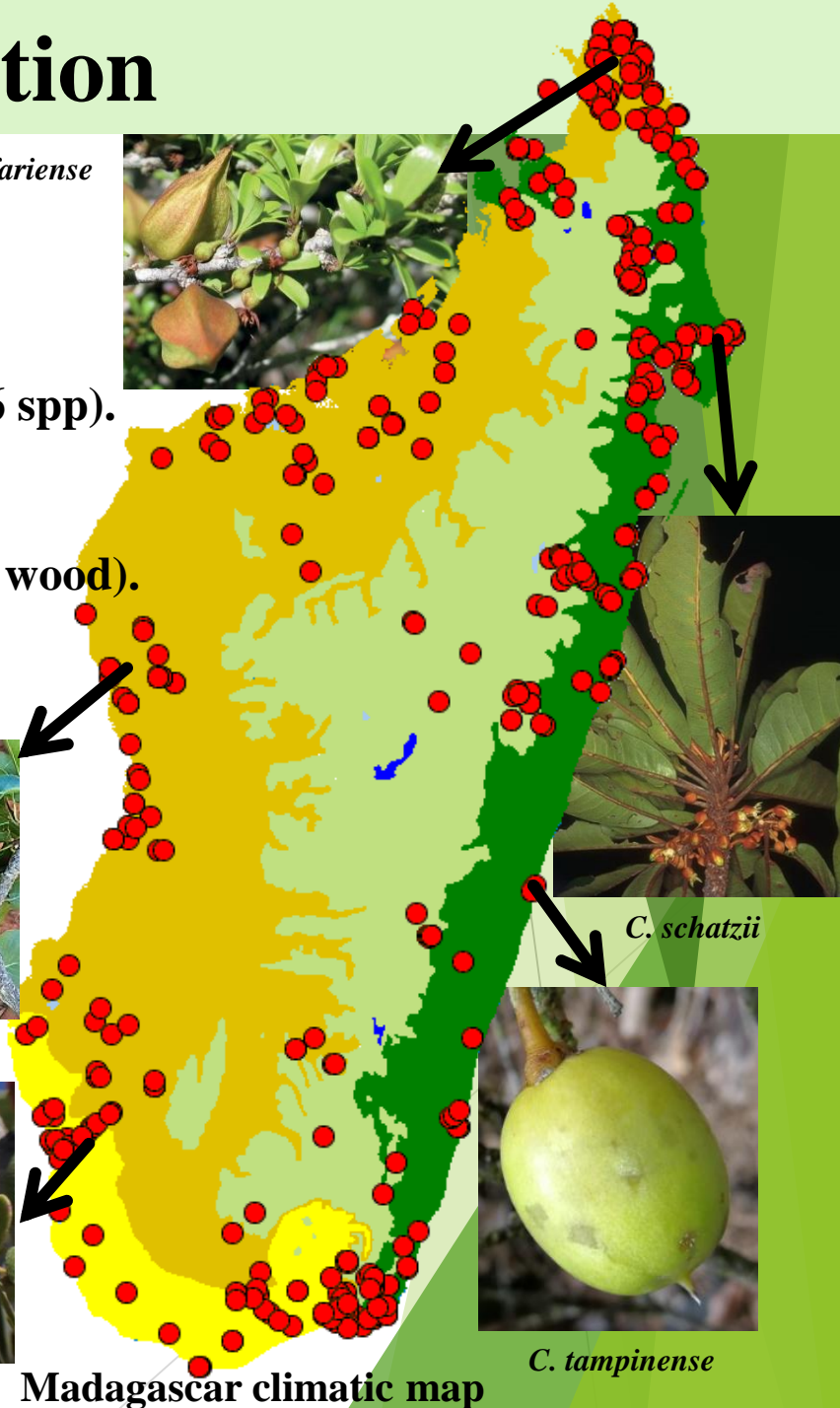
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Madagascar climatic map



# Introduction

## Genus *Capurodendron*:

Undescribed morphologies  
Intermediate morphologies  
Morphological species complexes



Undescribed species ?  
Hybridization  
Current speciation



*C. delphinense*



*C. greveanum*



*C. androyense*



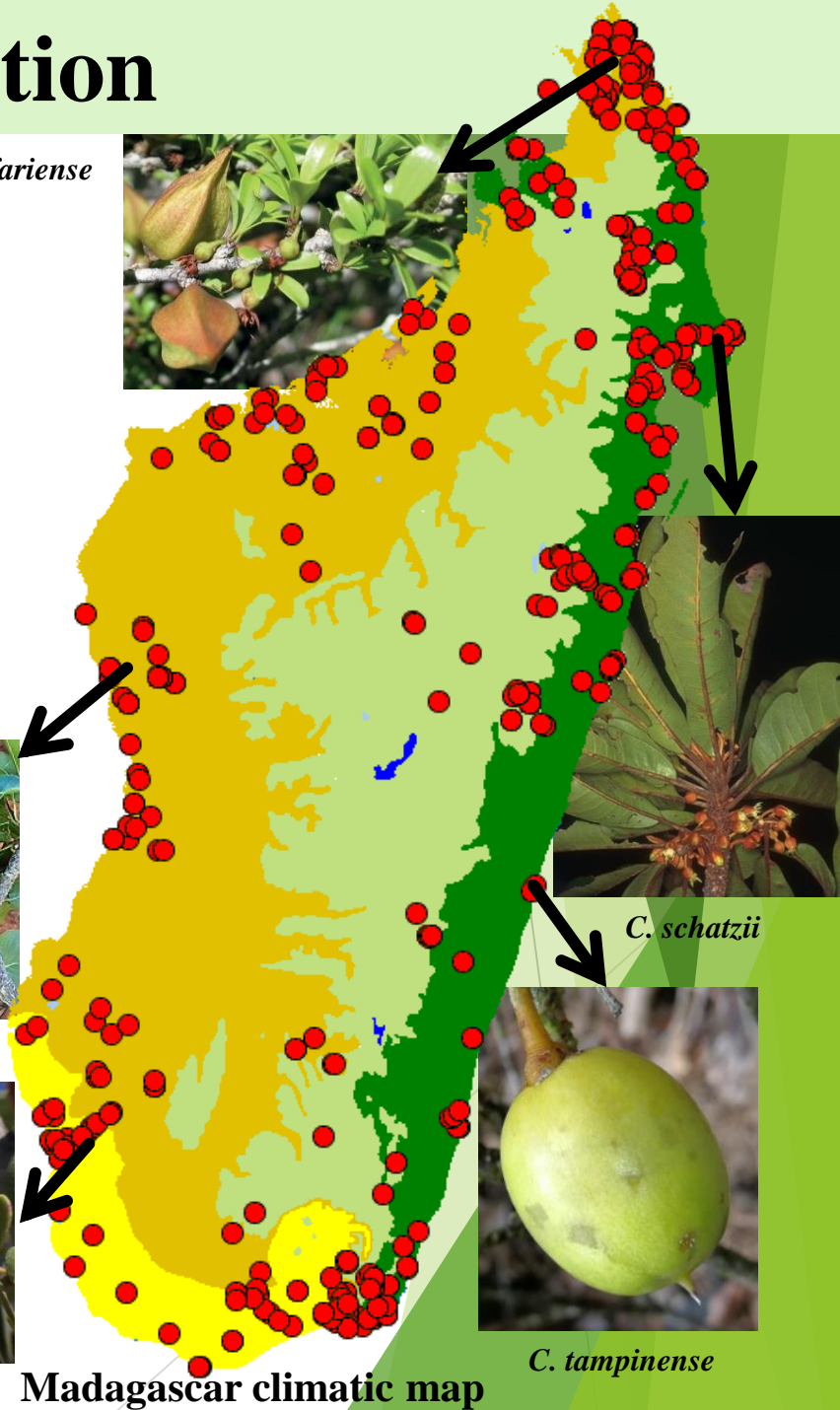
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Madagascar climatic map

# Introduction

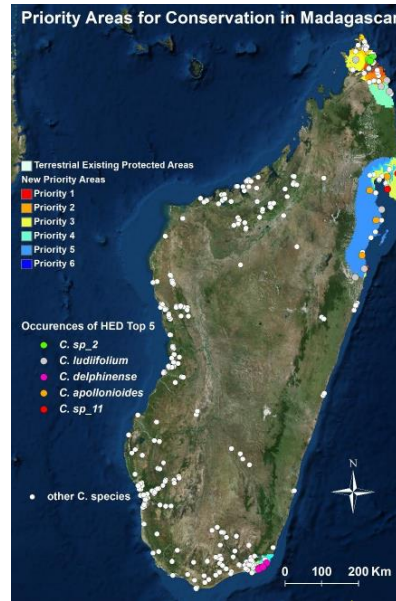
## Main objectives:

- 1° Delimit the *Capurodendron* species using phylogenomics.

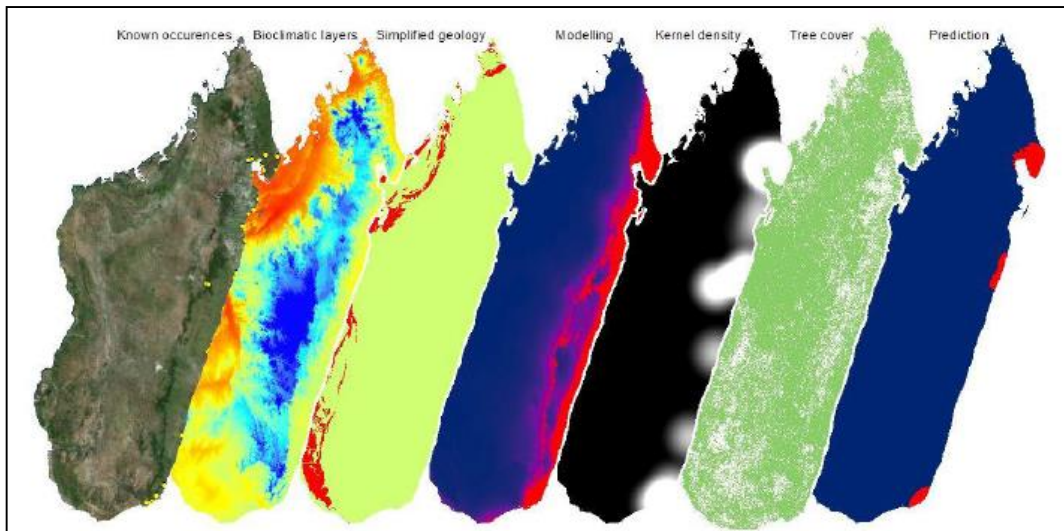
# Introduction

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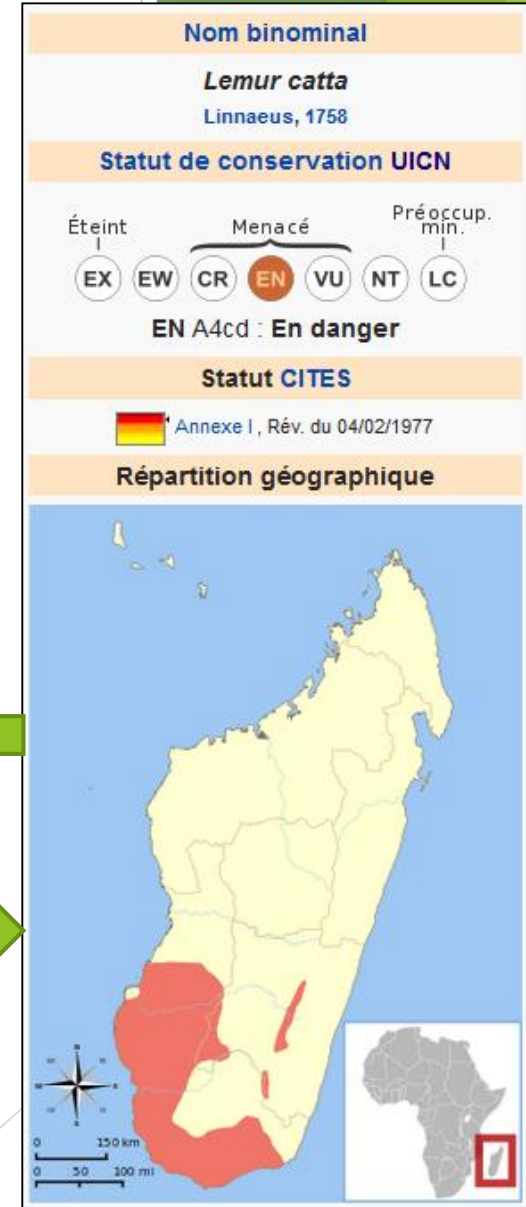
- 1° Delimit the *Capurodendron* species using phylogenomics.
- 2° Estimate the species potential distribution and establish IUCN protection categories.



Priority areas for conservation



Climatic, geographical, dispersal, ... map layers



Potential distribution, IUCN categories

# Materials and Methods

## Sequences obtention:

Fresh specimens      Up to 80 years old specimens



**281 specimens:**  
239 ingroup  
42 outgroup



**Library  
construction**



# Matterials and Methods

## Sequences obtention:

### GenBank Transcriptome



*Manilkara zapota*



### Newly sequenced incomplete genomes



*Bemangidia lowryi*

81 million reads

20x – 40x



*Capurodendron delphinense*

51 million reads

2x – 20x



### Baits design

#### Baits for 1020 loci

793 genes

227 microsatellites

Fresh specimens

Up to 80 years old specimens



281 specimens:

239 ingroup

42 outgroup



Library  
construction

**Baits:** small DNA sequences complementary to a target loci that allow us to capture these loci from a genomic DNA solution thorough an hybridization step.

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Baits design

**Baits for 1020 loci**

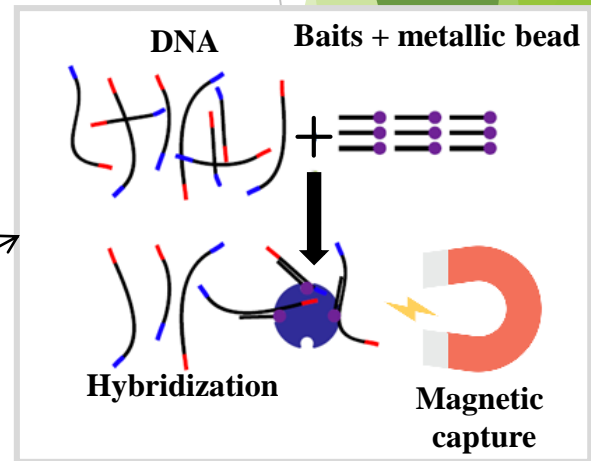
793 genes

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Library  
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Gene Capture

Captured  
loci



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### Baits design

#### Baits for 1020 loci

793 genes

227 microsatellites

### DNA Baits + metallic bead



### Sequences for phylogenomic analyses

Library construction

Gene Capture

Captured loci

Illumina sequencing

#### 281 specimens:

239 ingroup

42 outgroup

Fresh specimens

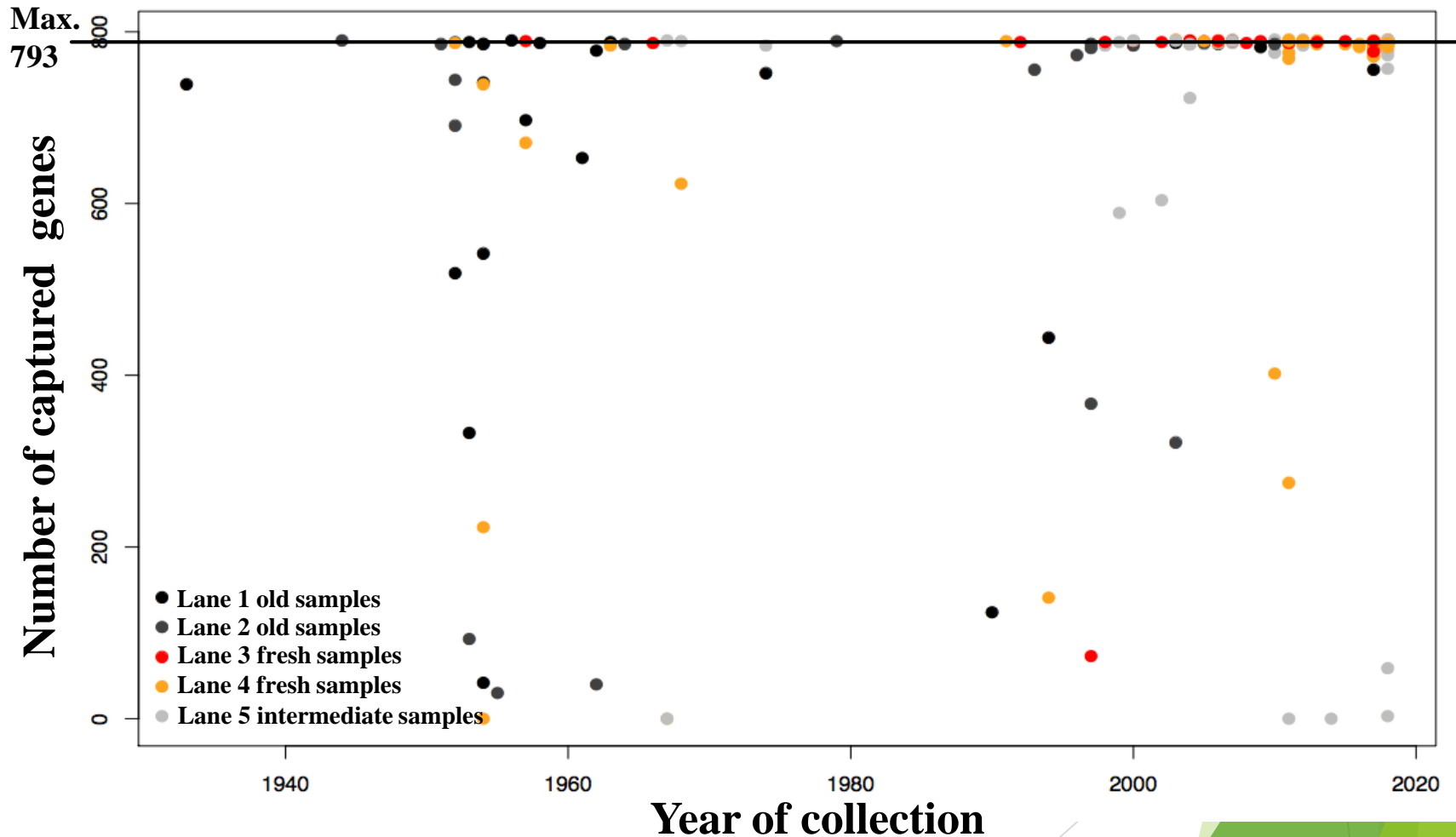
Up to 80 years old specimens





# Preliminary results

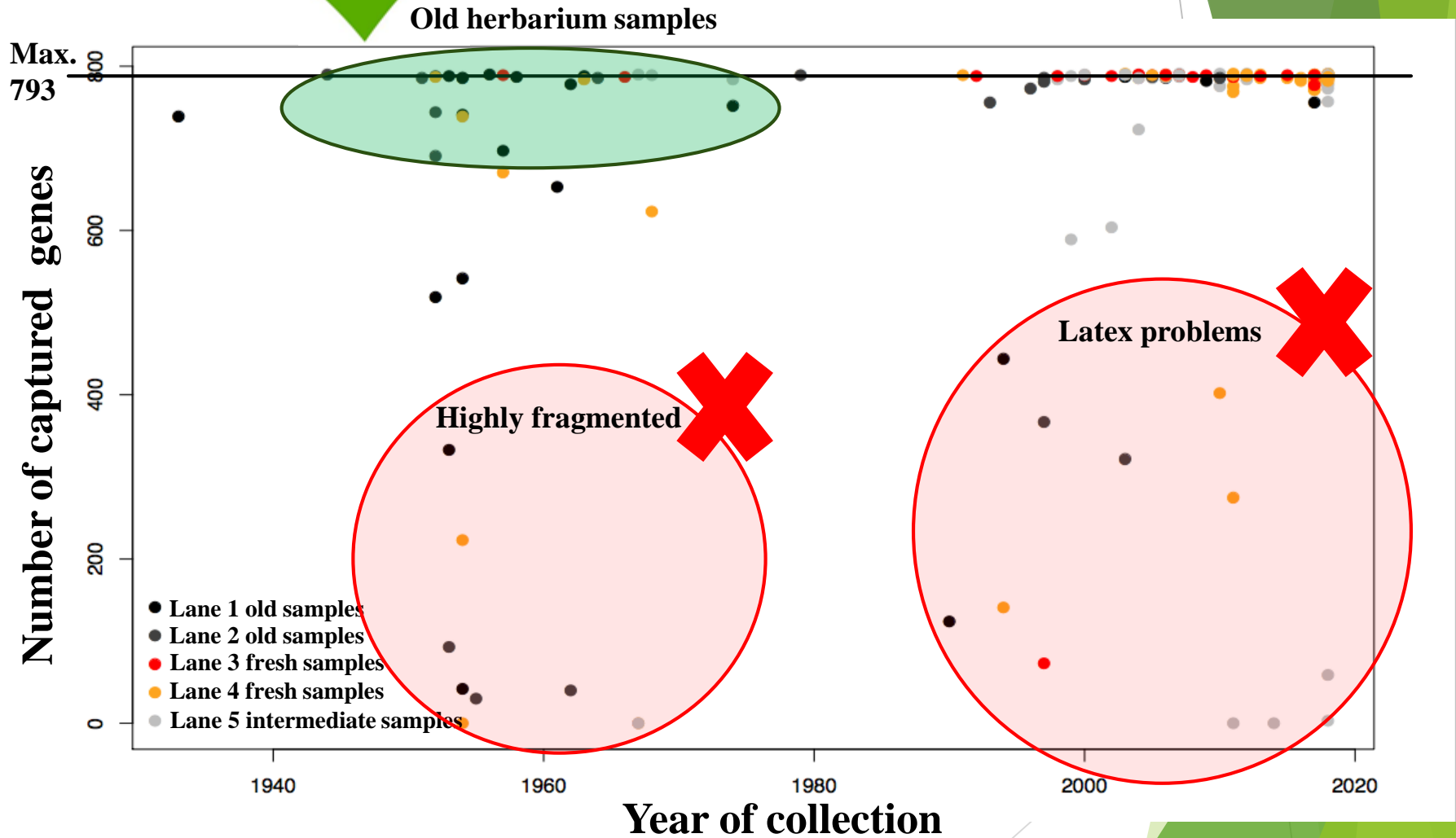
## Gene Capture efficiency



Gene Capture is efficient in fresh and old samples

# Preliminary results

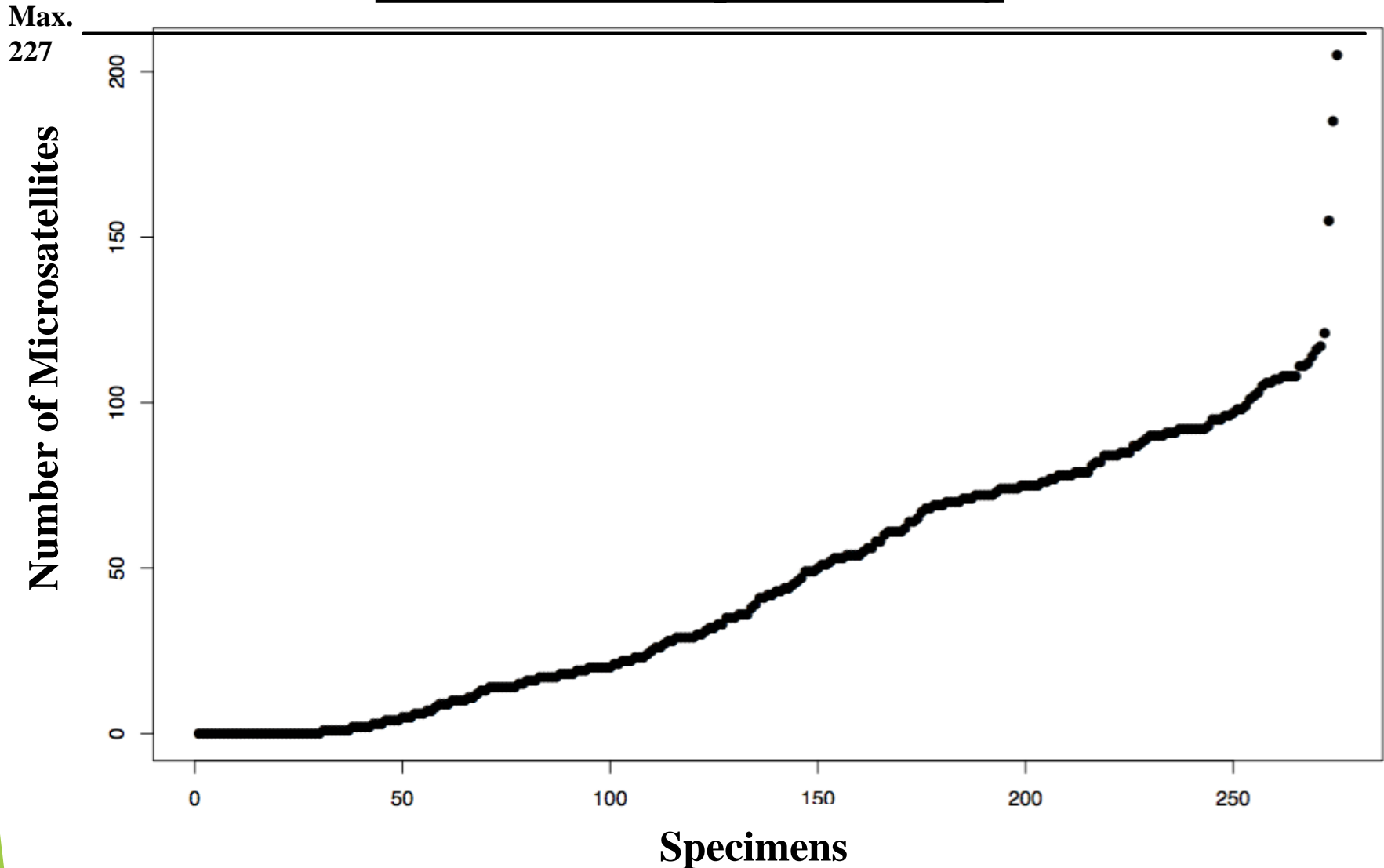
## Gene Capture efficiency



Gene Capture is efficient in fresh and old samples

# Preliminary results

## Microsatellite Capture efficiency

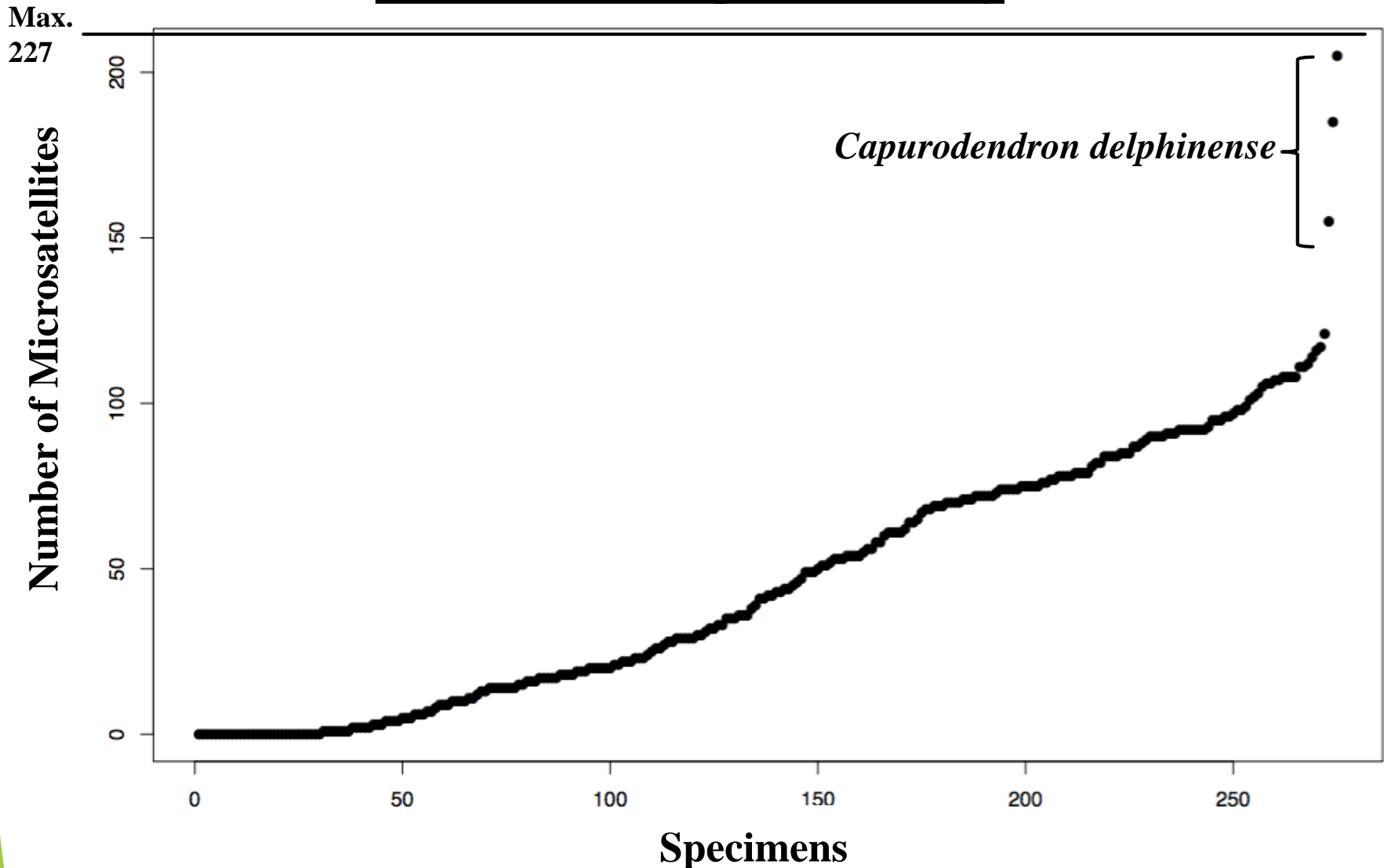


Less efficient than Gene Capture **➔** Highly variable sequences



# Preliminary results

## Microsatellite Capture efficiency



Less efficient than Gene Capture **➔** Highly variable sequences

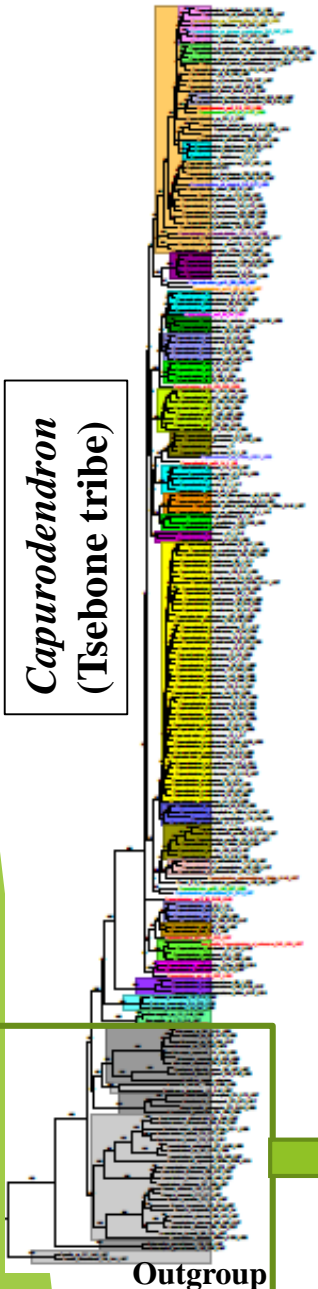
# Preliminary results

ML tree (3 genes, 12700 bp)

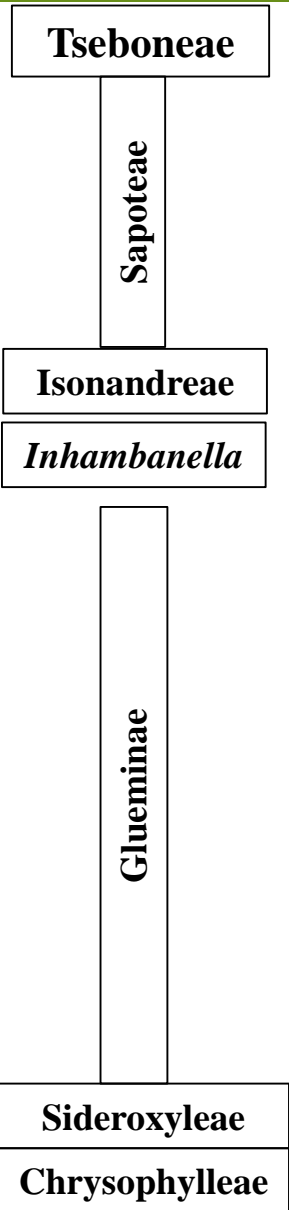
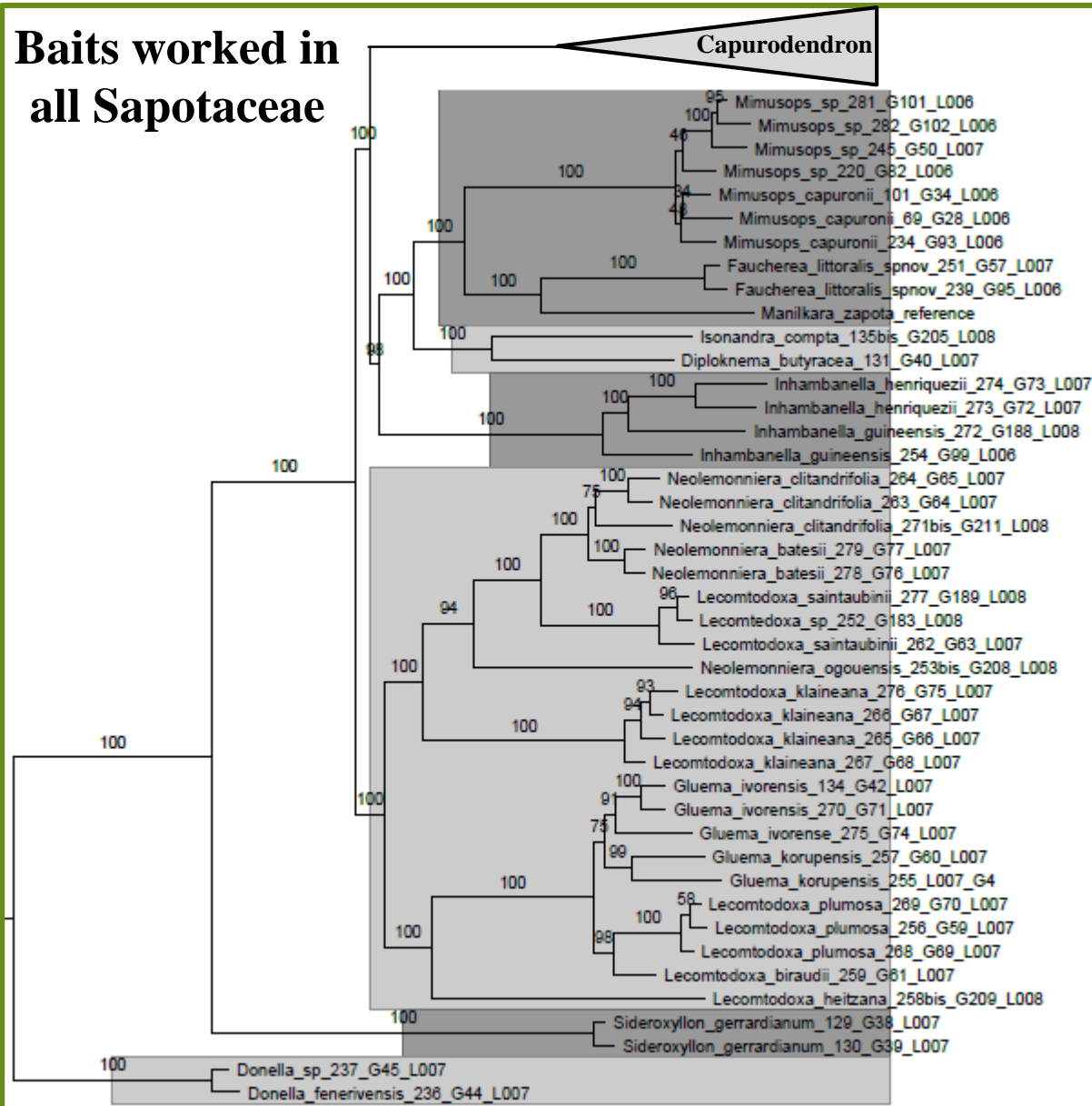
Tribes of Sapotaceae

Baits worked in all Sapotaceae

*Capurodendron*  
(Tsebhone tribe)



Outgroup



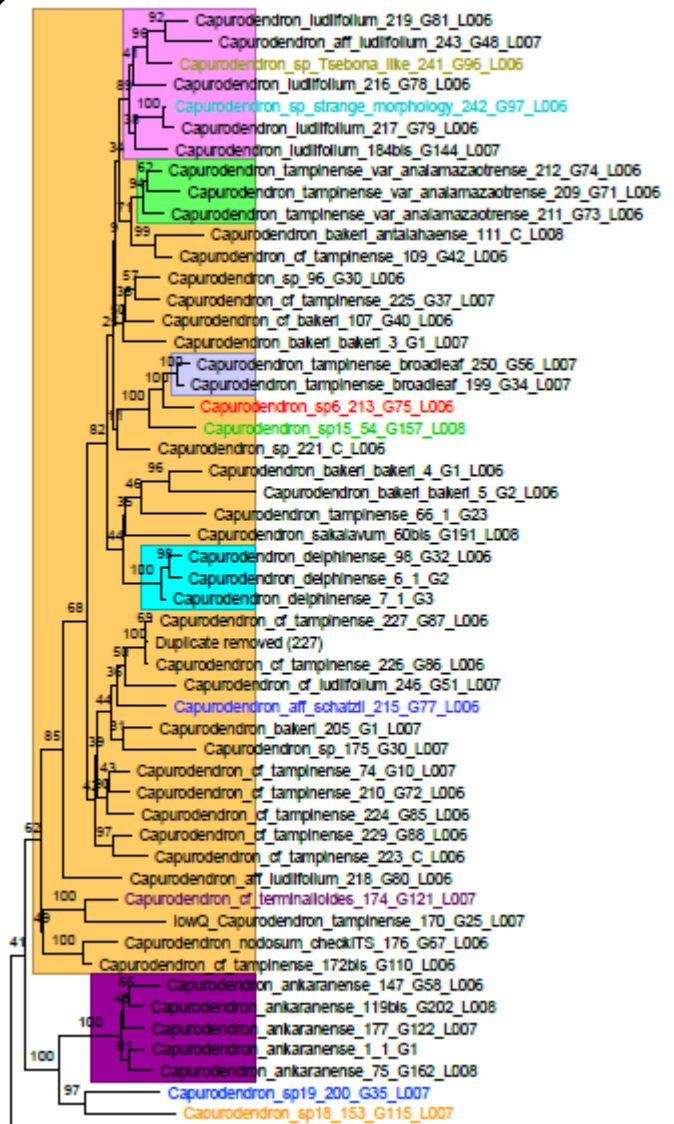
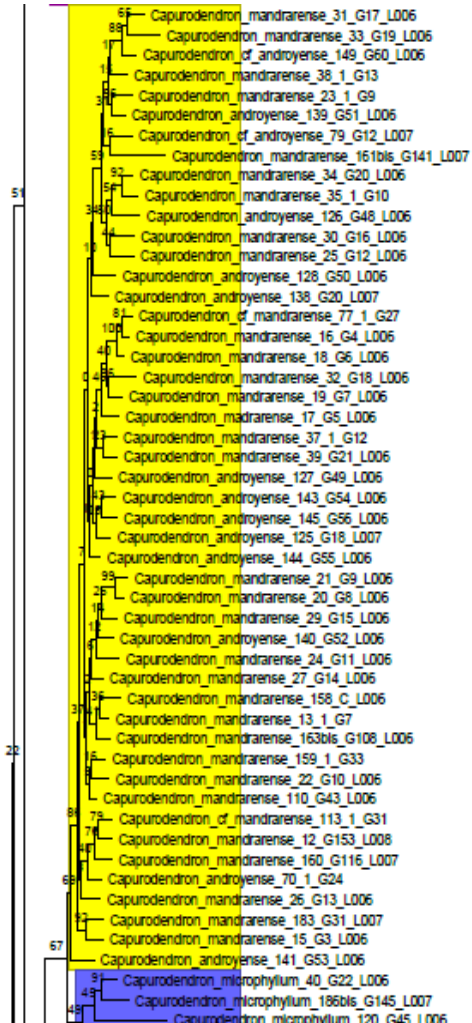
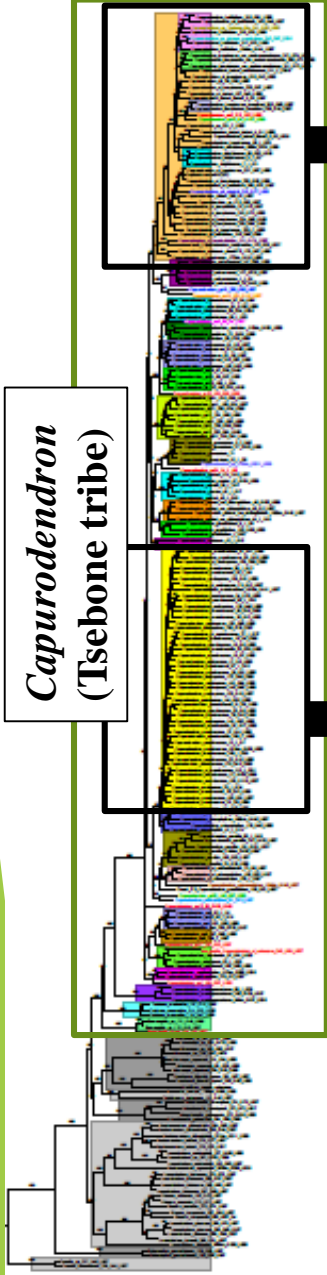
# Preliminary results

ML tree (3 genes, 12700 bp)

Eastern complex

Arid complex

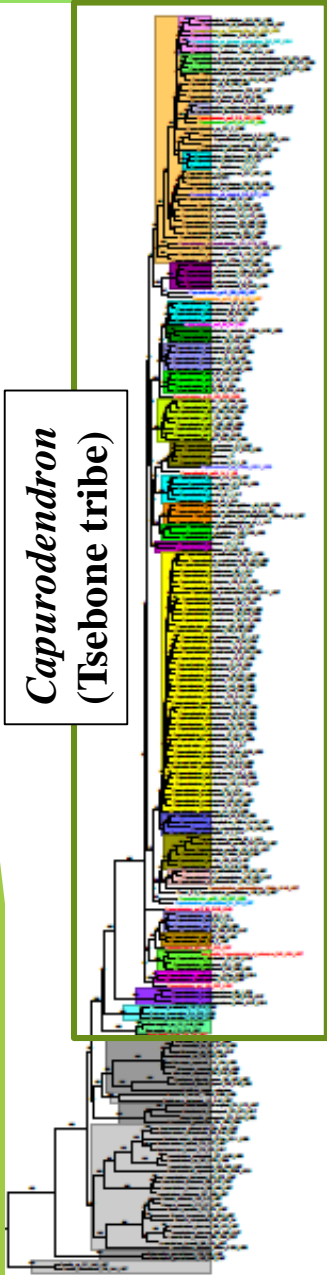
*Capurodendron*  
(Tsebone tribe)





# Preliminary results

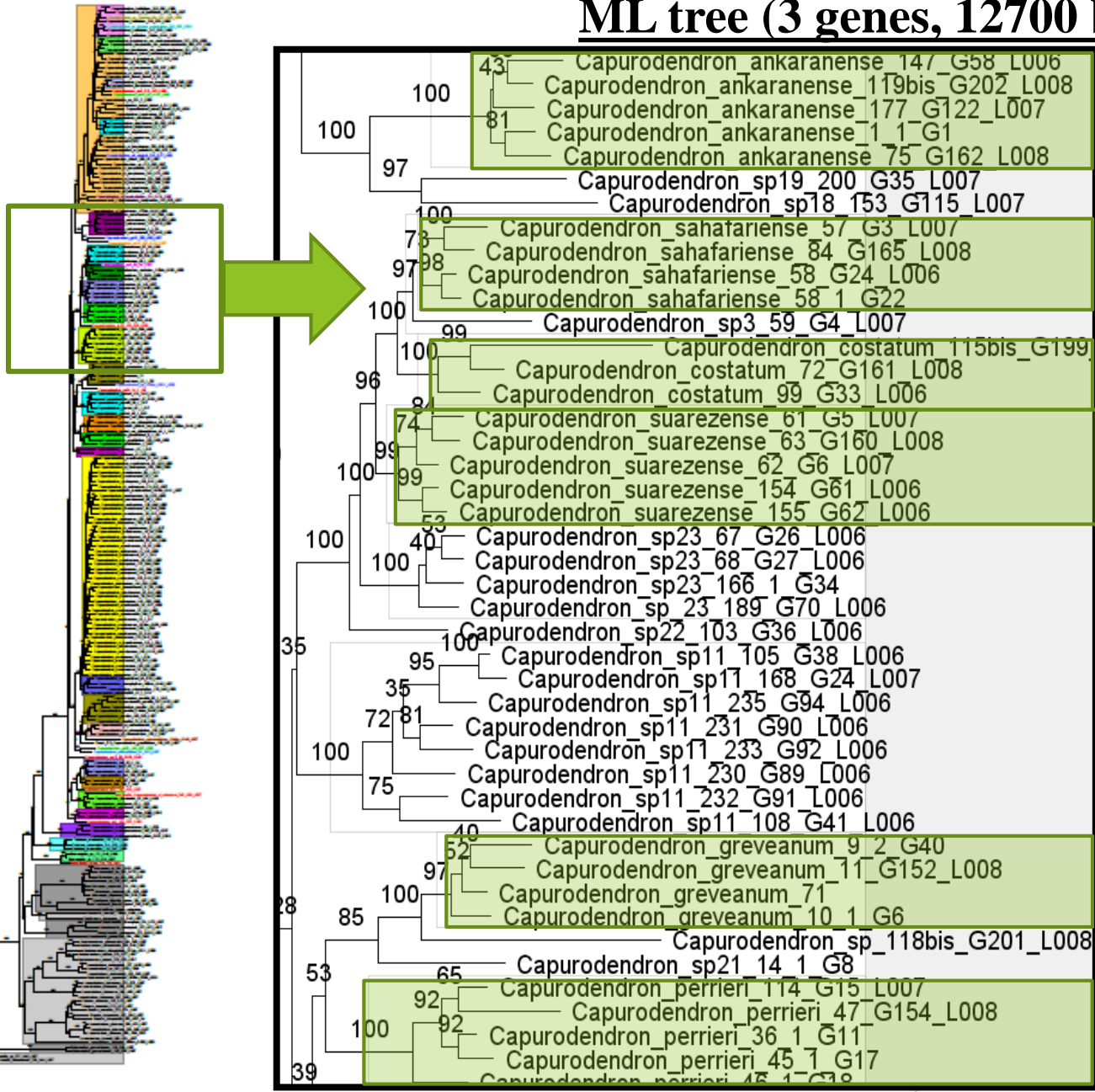
ML tree (3 genes, 12700 bp)



**~ 49 clades candidate to species**

# Preliminary results

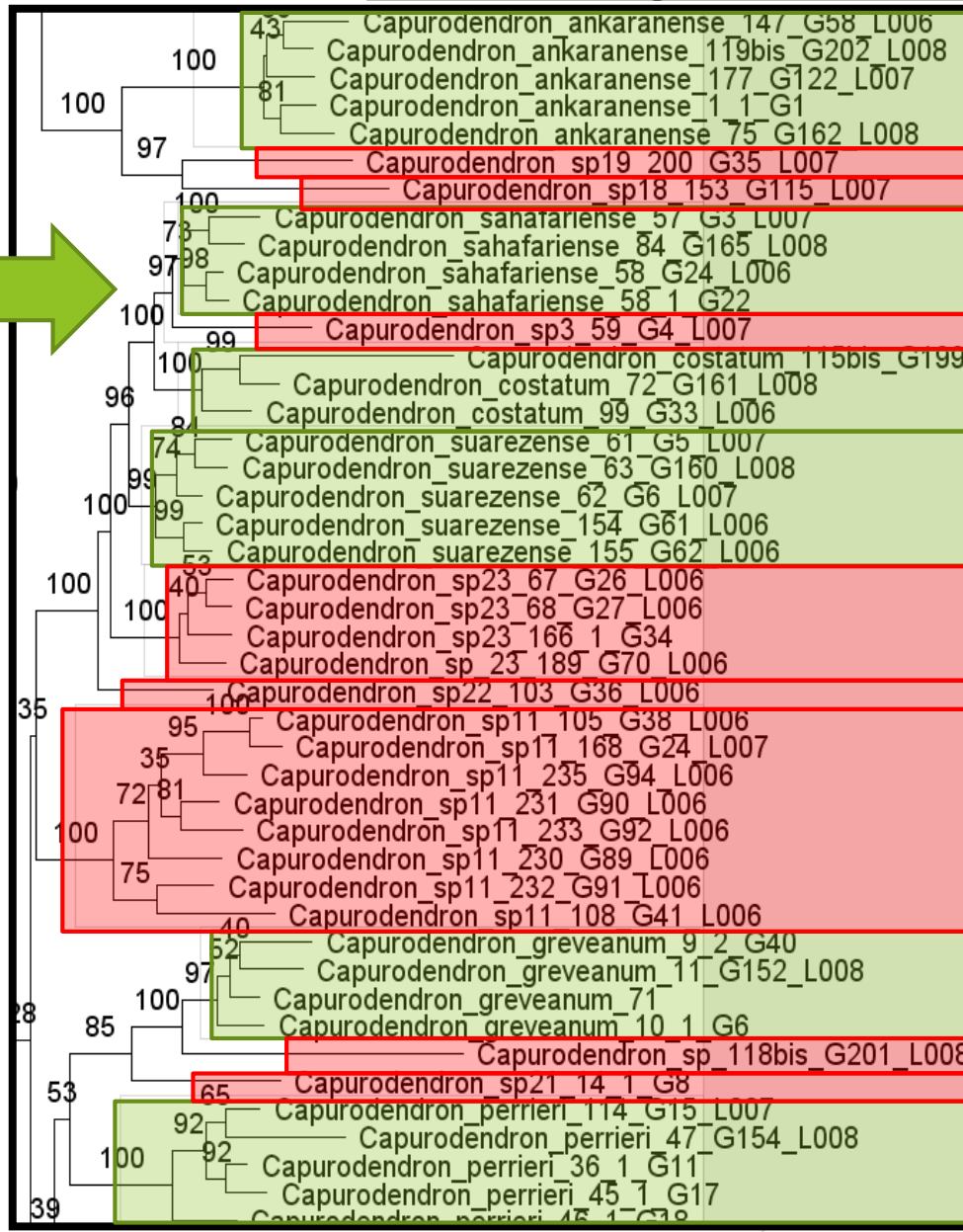
## ML tree (3 genes, 12700 bp)



# Preliminary results

**ML tree (3 genes, 12700 bp)**

**25 candidates  
to new species**



sp. nov. ?

sp. nov. ?

sp. nov. ?

sp. nov. ?

sp. nov. ?

sp. nov. ?

sp. nov. ?

sp. nov. ?



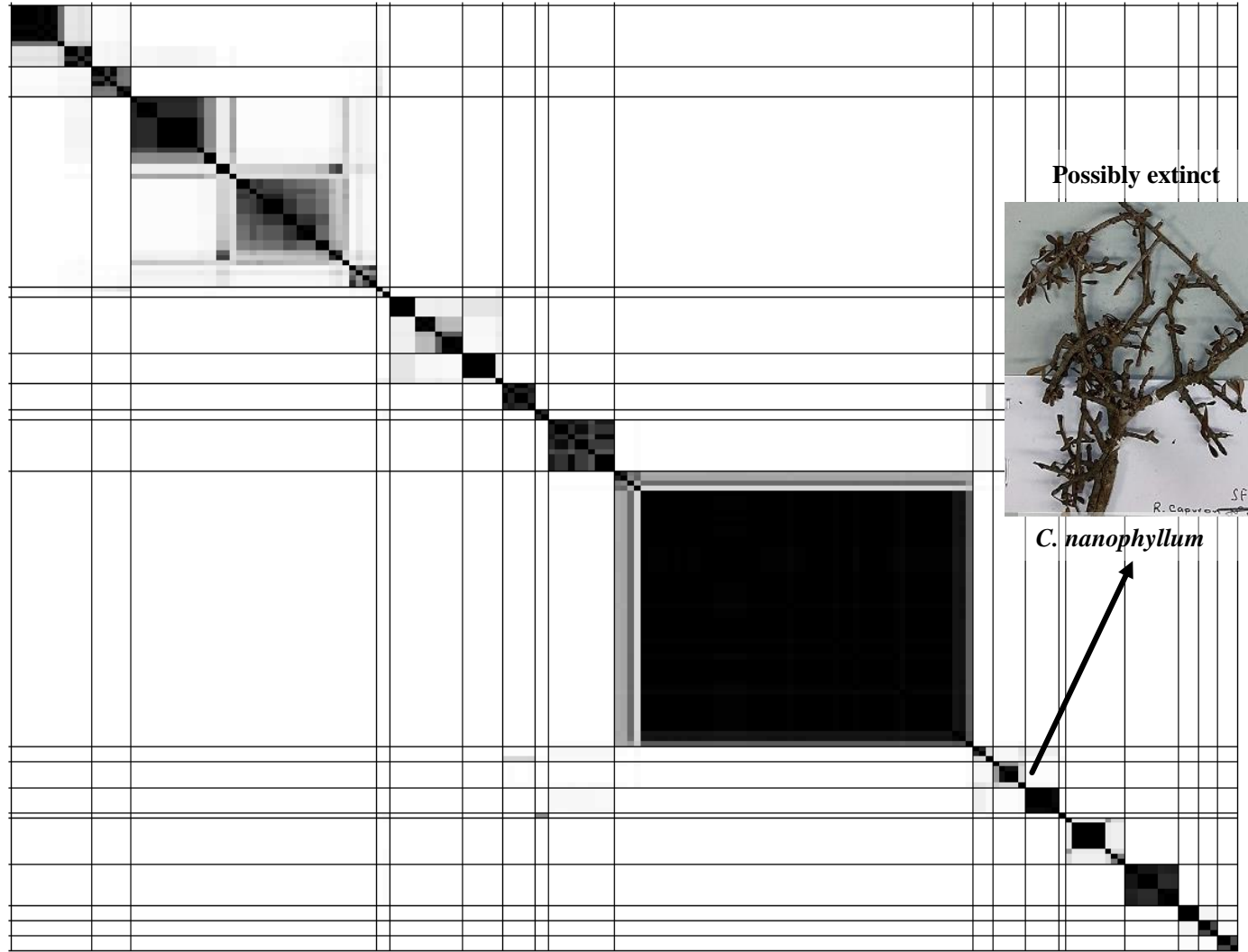
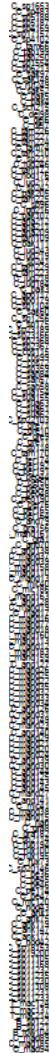
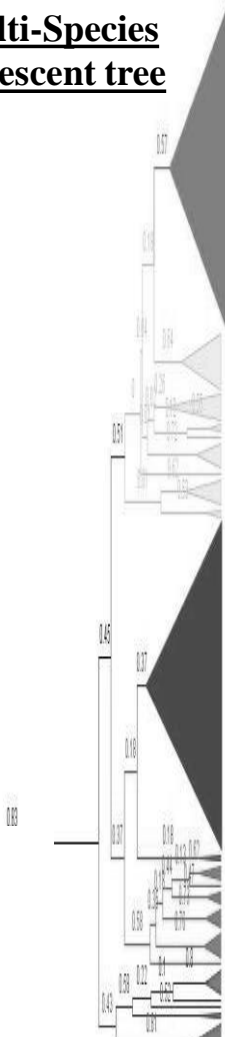




# Preliminary results

## STACEY species delimitation analysis

### Multi-Species coalescent tree



*C. nanophyllum*

Putative species are shown in black squares

Conspecificity: 0%  50%  100%

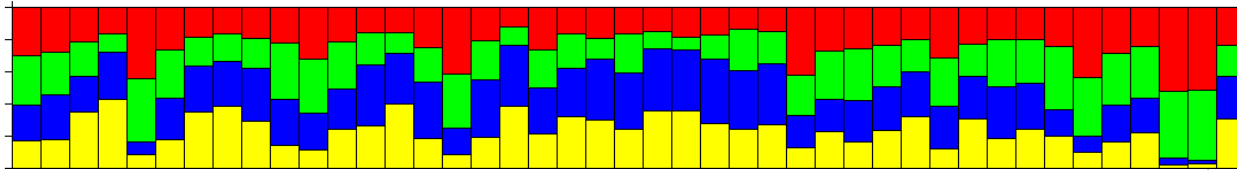


# Preliminary results

## Preliminary species delimitation using microsatellites

Using 30 microsatellites and  
Bayesian analyses:

- **Arid Complex: One “species”.**



STRUCTURE analysis at K4



*Capurodendron mandrarensis*

← **Conspecific???** →  
**Hybridization???**



*Capurodendron androyense*

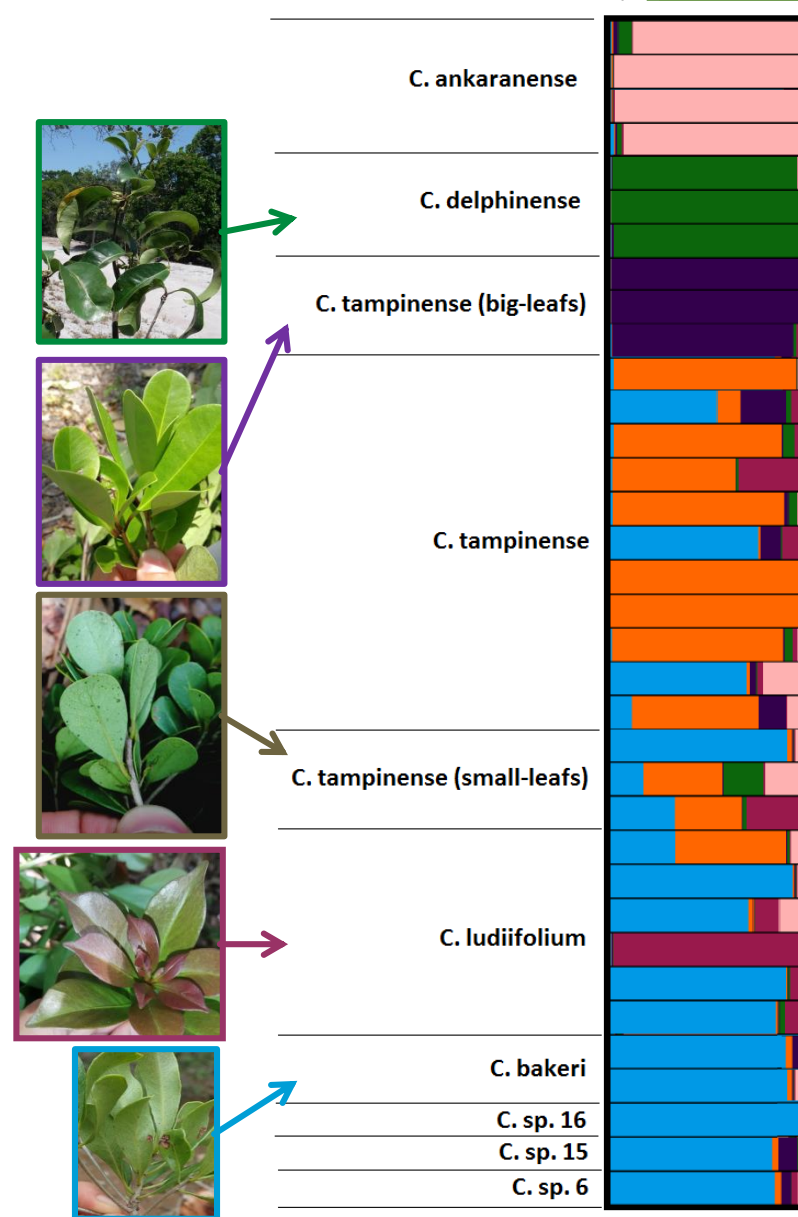
# Preliminary results

## Preliminary species delimitation using microsatellites

Using 30 microsatellites and Bayesian analyses:

- **Eastern complex: Species group with high levels of admixture**

Unbalanced sampling sizes  
Hybridization???



STRUCTURE results of part of the Eastern Complex



# Preliminar Conclusions

- ▶ **Gene capture works well in all Sapotaceae and even with old herbarium specimens**
- ▶ ***Capurodendron* contains much more species than initially thought**
- ▶ **Two species complexes with a mismatch between phenotypes and genotypes: Hybridization or incomplete lineage sorting?**
- ▶ **Microsatellites might not be as useful as expected**



# Acknowledgements



**Yamama Naciri**



**Richard Randrianaivo**

**Aina Randriarisoa**

**Carlos Galan Boluda**



**Camille Christe**



**Laurent Gautier**



**Thank you!**

