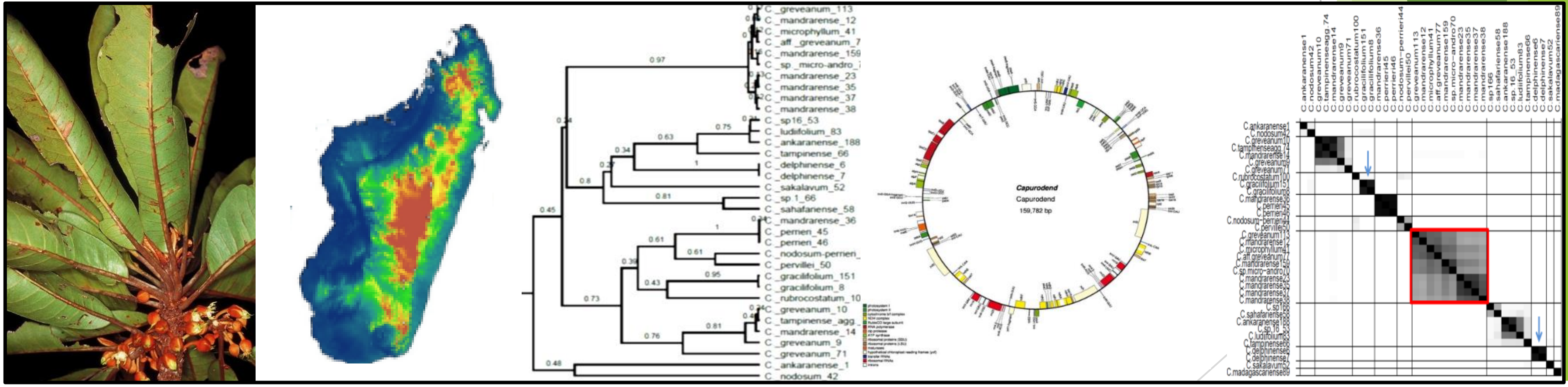
Establishing priority areas for conservation in Madagascar using phylogenomics and species potential distribution of Sapotaceae

Carlos G. Boluda

Conservatoire et Jardin Botaniques de la Ville de Genève



Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León, Linares (Nuevo León) Septiembre-2019

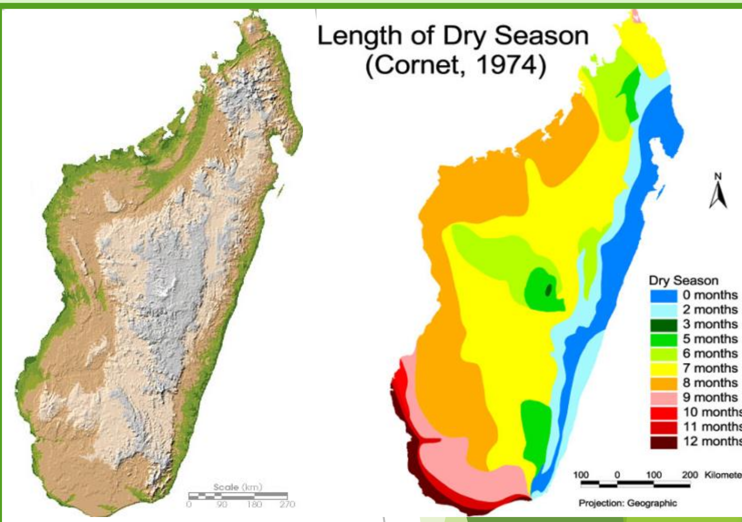
UNIVERSITÉ DE GENÈVE
Fondation Ernst et Lucie Schmidheiny
Fondation Franklinia
FNSNF Swiss National Science Foundation
Conservatoire et Jardin botaniques Genève

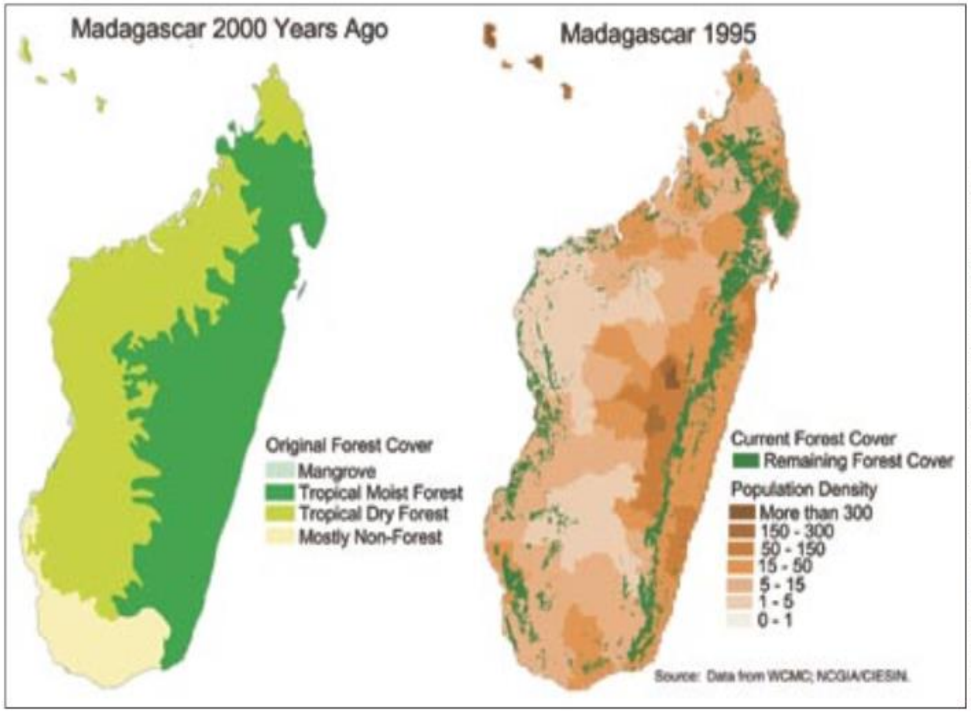
Introduction

Madagascar:

-Isolated from India ~ 88 mya. (Upper Cretacic).

- 82% of endemic vascular flora.





Source: Data from WCMC; NCGIA/CIESIN.

Cincotta, R.P. and R. Engelman. 2000. Nature's Place: Human Population and the Future of Biological Diversity. Washington, D.C: Population Action International.

Map of Madagascar's forest loss

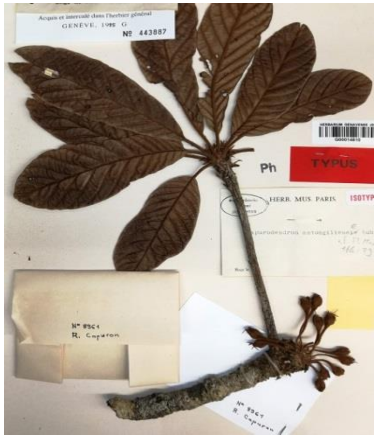
Introduction

Putative extinct species

Sartidia perrieri

Tinopsis tampoloensis

Capurodendron nanophyllum



Capurodendron antongiliense

Main causes of species extinction

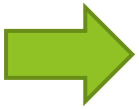
Deforestation

Wood felling

Introduction

How to decide which forest preserve?
How to know the quality of the forest?

Introduction

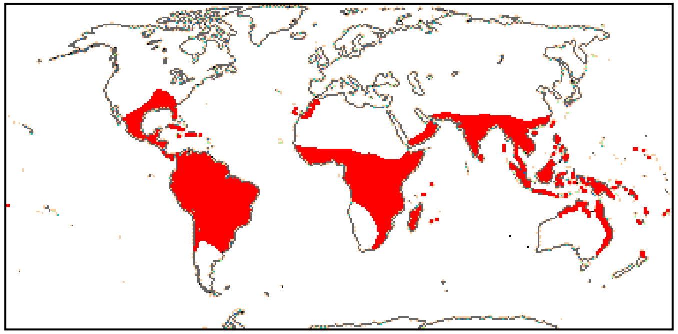


How to decide which forest preserve?
How to know the quality of the forest?

→ Sapotaceae as a model

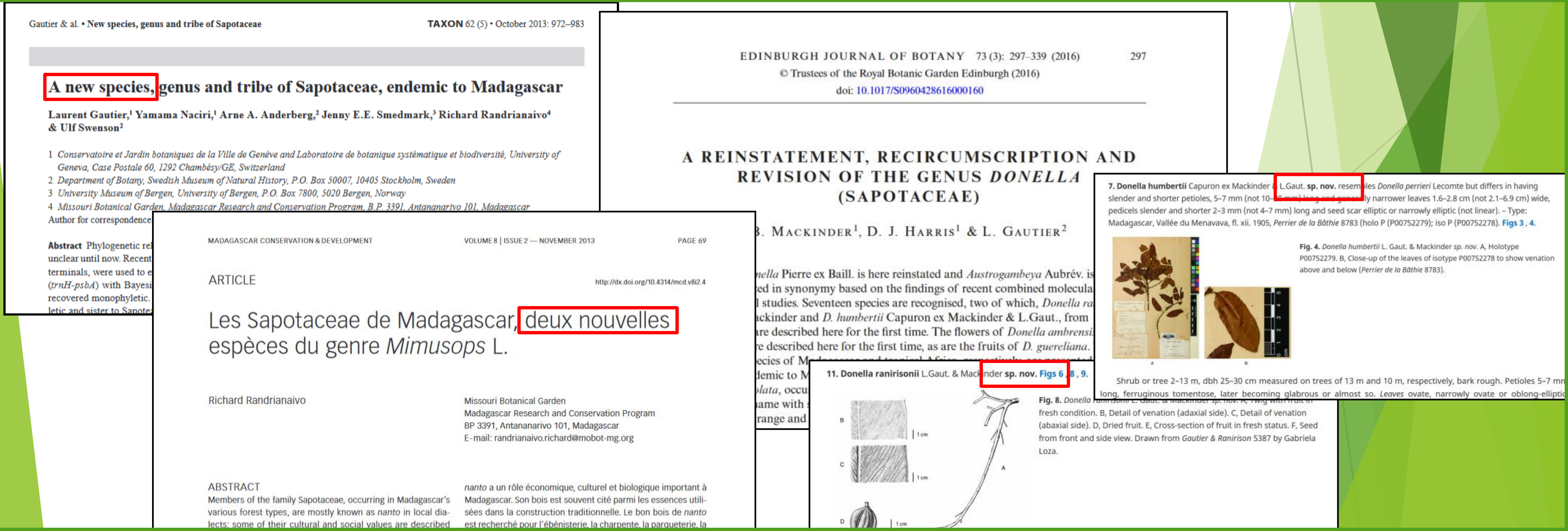
Introduction

The Sapotaceae family:



- Tropical trees.
- Appreciated wood.
- Slow growing trees from undisturbed areas
- Highly represented in Madagascar (10% of worldwide species).
- Understudied in Madagascar.

Type species of Sapotaceae:
Manilkara zapota



Gautier & al. • New species, genus and tribe of Sapotaceae TAXON 62 (5) • October 2013: 972–983

A new species, genus and tribe of Sapotaceae, endemic to Madagascar

Laurent Gautier,[1] Yamama Naciri,[1] Arne A. Anderberg,[2] Jenny E.E. Smedmark,[3] Richard Randrianaivo[4] & Ulf Swenson[2]

1 *Conservatoire et Jardin botaniques de la Ville de Genève and Laboratoire de botanique systématique et biodiversité, University of Geneva, Case Postale 60, 1292 Chambésy/GE, Switzerland*
2 *Department of Botany, Swedish Museum of Natural History, P.O. Box 50007, 10405 Stockholm, Sweden*
3 *University Museum of Bergen, University of Bergen, P.O. Box 7800, 5020 Bergen, Norway*
4 *Missouri Botanical Garden, Madagascar Research and Conservation Program, B.P. 3391, Antananarivo 101, Madagascar*
Author for correspondence

Abstract Phylogenetic rel
unclear until now. Recent
terminals, were used to e
(*trnH-psbA*) with Bayesi
recovered monophyletic.
letic and sister to Sapote

MADAGASCAR CONSERVATION & DEVELOPMENT VOLUME 8 | ISSUE 2 — NOVEMBER 2013 PAGE 69

ARTICLE http://dx.doi.org/10.4314/mcd.v8i2.4

Les Sapotaceae de Madagascar, deux nouvelles espèces du genre *Mimusops* L.

Richard Randrianaivo

Missouri Botanical Garden
Madagascar Research and Conservation Program
BP 3391, Antananarivo 101, Madagascar
E-mail: randrianaivo.richard@mobot-mg.org

ABSTRACT
Members of the family Sapotaceae, occurring in Madagascar's various forest types, are mostly known as *nanto* in local dialects; some of their cultural and social values are described

nanto a un rôle économique, culturel et biologique important à Madagascar. Son bois est souvent cité parmi les essences utilisées dans la construction traditionnelle. Le bon bois de *nanto* est recherché pour l'ébénisterie, la charpente, la parqueterie, la

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doi: 10.1017/S0960428616000160

A REINSTATEMENT, RECIRCUMSCRIPTION AND REVISION OF THE GENUS *DONELLA* (SAPOTACEAE)

B. MACKINDER[1], D. J. HARRIS[1] & L. GAUTIER[2]

nella Pierre ex Baill. is here reinstated and *Austrogambeya* Aubrév. is
ed in synonymy based on the findings of recent combined molecula
l studies. Seventeen species are recognised, two of which, *Donella ra*
ackinder and *D. humbertii* Capuron ex Mackinder & L.Gaut., from
are described here for the first time. The flowers of *Donella ambrensis*
re described here for the first time, as are the fruits of *D. guereliana*.
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demic to M
plata, occu
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7. Donella humbertii Capuron ex Mackinder & L.Gaut. **sp. nov.** resembles *Donella perrieri* Lecomte but differs in having slender and shorter petioles, 5–7 mm (not 10–… mm) long and generally narrower leaves 1.6–2.8 cm (not 2.1–6.9 cm) wide, pedicels slender and shorter 2–3 mm (not 4–7 mm) long and seed scar elliptic or narrowly elliptic (not linear). – Type: Madagascar, Vallée du Menavava, fl. xii. 1905, *Perrier de la Bâthie* 8783 (holo P (P00752279); iso P (P00752278). Figs 3 , 4.

Fig. 4. *Donella humbertii* L. Gaut. & Mackinder *sp. nov.* A, Holotype P00752279. B, Close-up of the leaves of isotype P00752278 to show venation above and below (*Perrier de la Bâthie* 8783).

Shrub or tree 2–13 m, dbh 25–30 cm measured on trees of 13 m and 10 m, respectively, bark rough. Petioles 5–7 mm long, ferruginous tomentose, later becoming glabrous or almost so. *Leaves* ovate, narrowly ovate or oblong-elliptic

---

11. Donella ranirisonii L.Gaut. & Mackinder **sp. nov.** Figs 6 , 8 , 9.

Fig. 8. *Donella* fresh condition. B, Detail of venation (adaxial side). C, Detail of venation (abaxial side). D, Dried fruit. E, Cross-section of fruit in fresh status. F, Seed from front and side view. Drawn from *Gautier & Ranirison* 5387 by Gabriela Loza.

Introduction

Some Sapotaceae species from Madagascar:

Faucherea littoralis sp. nov.

Labramia costata

Labramia costata

Mimusops coriacea

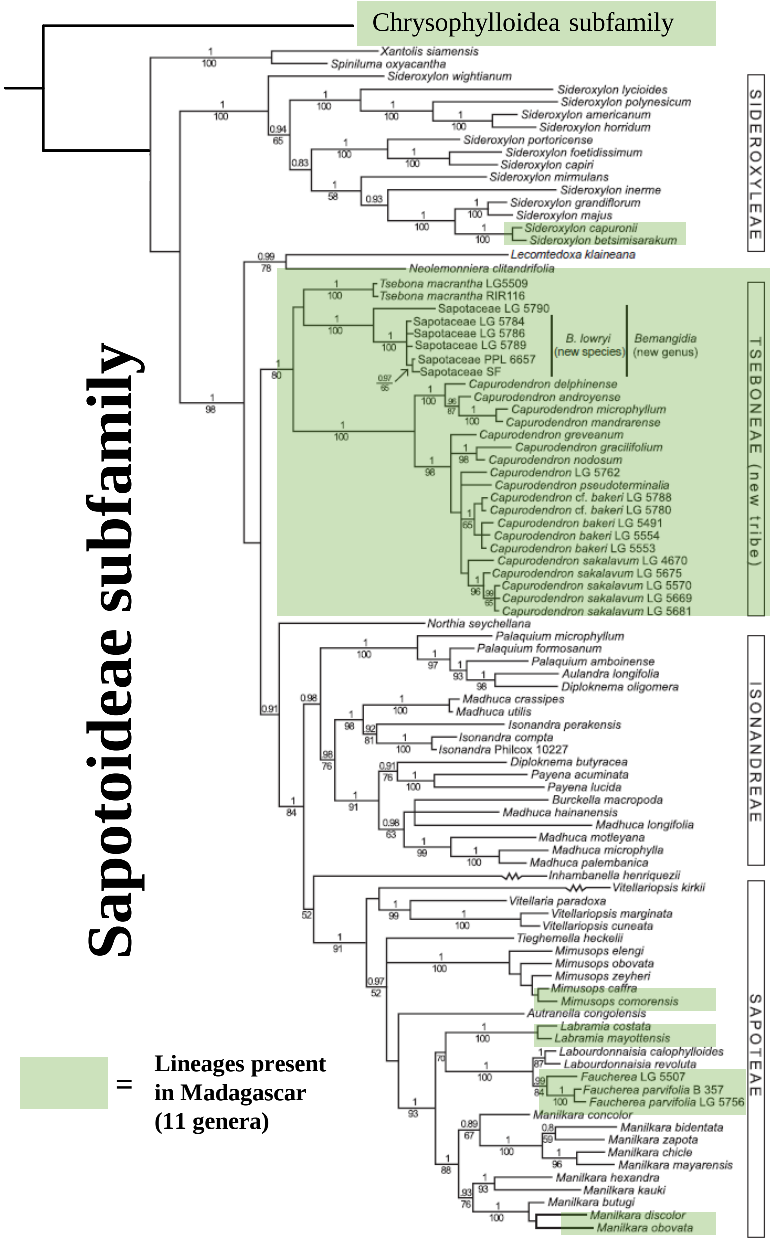
Labramia bojeri

Capurodendron androyense

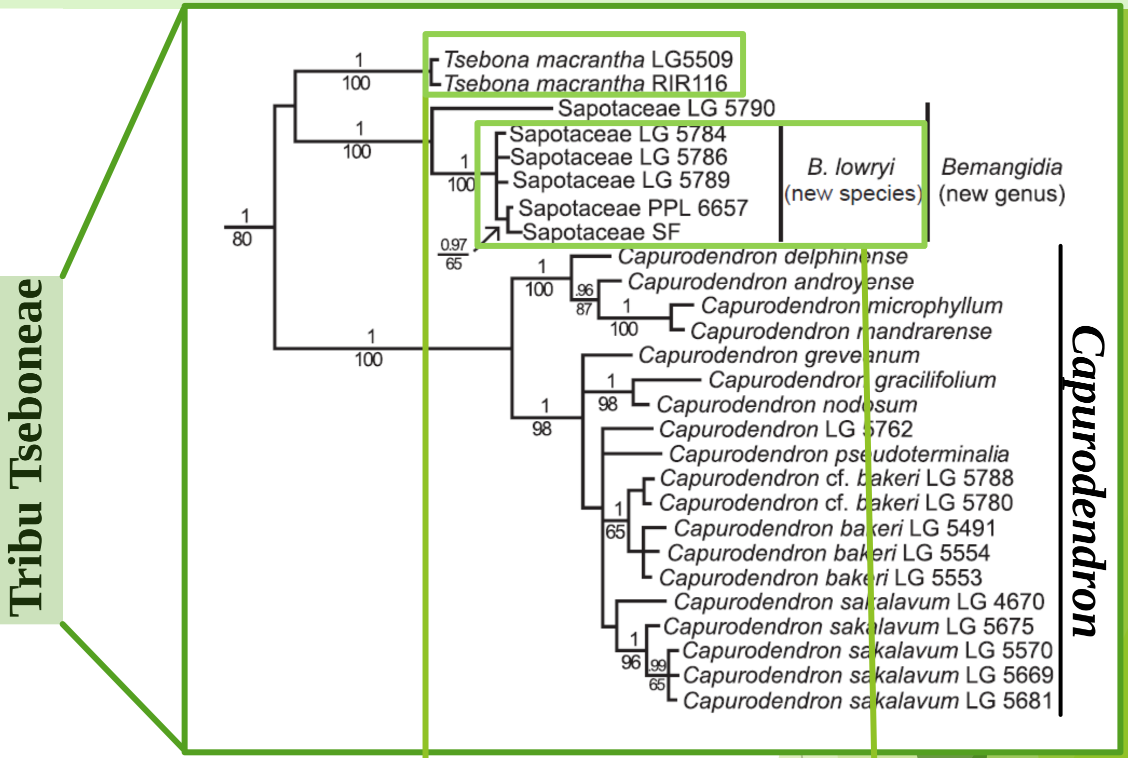
Mimusops coriacea

Sideroxylon betsimisarakum

Introduction



🟩 = **Lineages present in Madagascar (11 genera)**



Gautier *et al.* (2013) Taxon 62:972–983

Tsebona macrantha

Bemangidia lowryi

Introduction

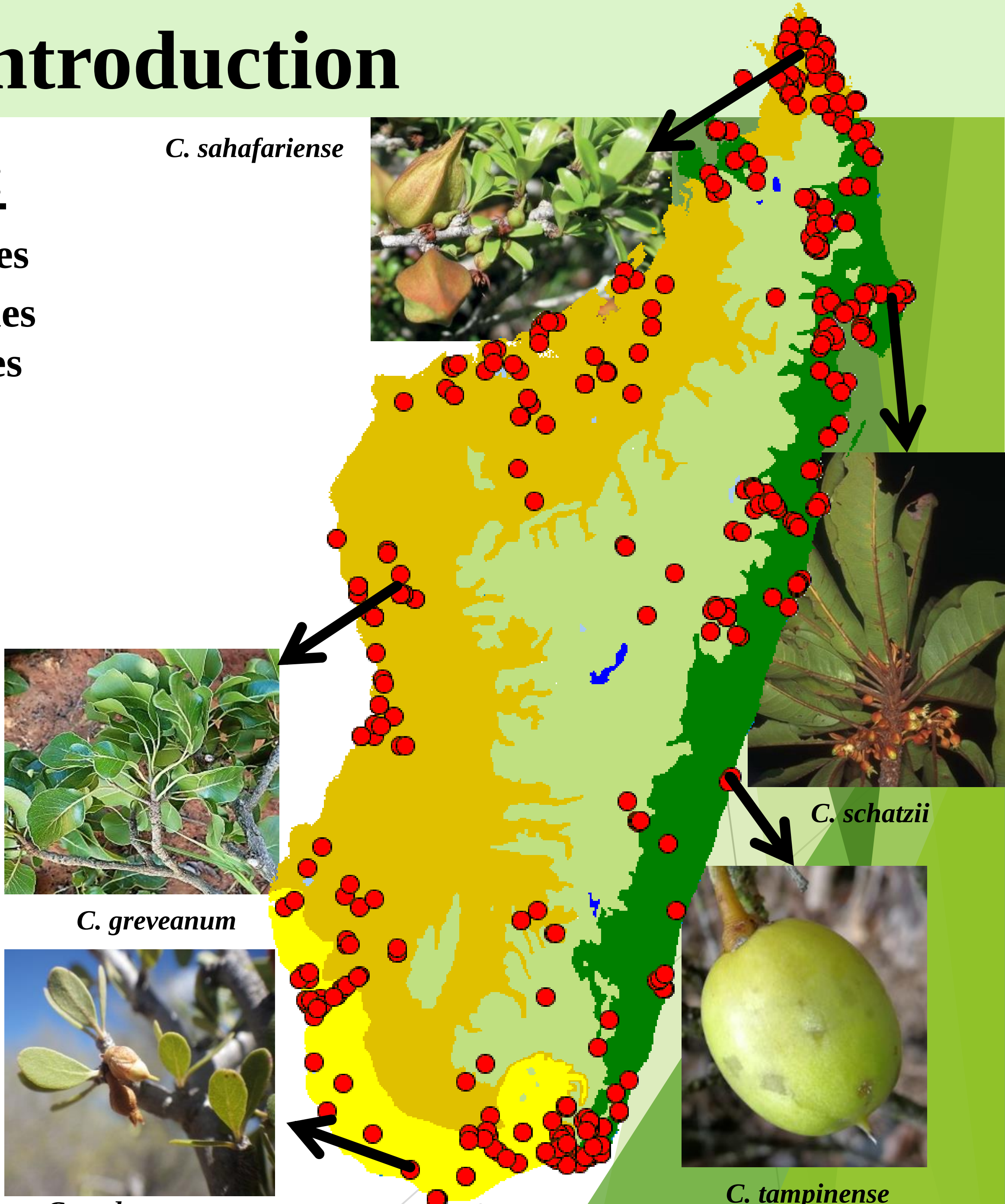
Genus *Capurodendron*:

Undescribed morphologies

Intermediate morphologies
morpho/species complexes

Undescribed species
Hybridization
Current speciation
?

C. delphinense



C. sahafariense

C. greveanum

C. androyense

C. schatzii

C. tampinense

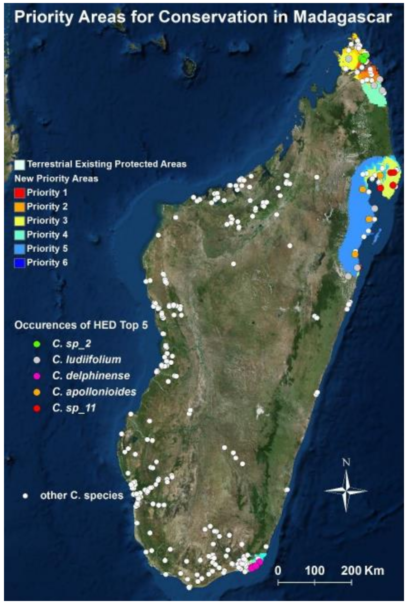
Climatic map of Madagascar

Introduction

Main objectifs:

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

- 2° Estimate the potential distribution of each species and establish the UICN protection categories.



Priority areas for conservation

Nom binominal

Lemur catta

Linnaeus, 1758

Statut de conservation UICN

Éteint — Menacé — Préoccup. min.

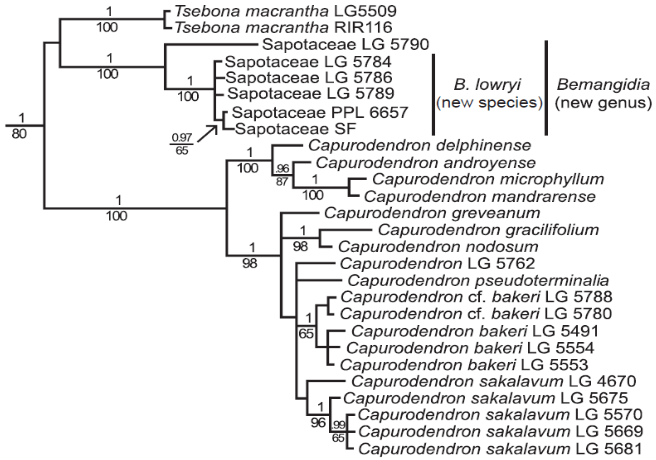
EX EW CR EN VU NT LC

EN A4cd : **En danger**

Statut CITES

🟥 Annexe I , Rév. du 04/02/1977

Répartition géographique

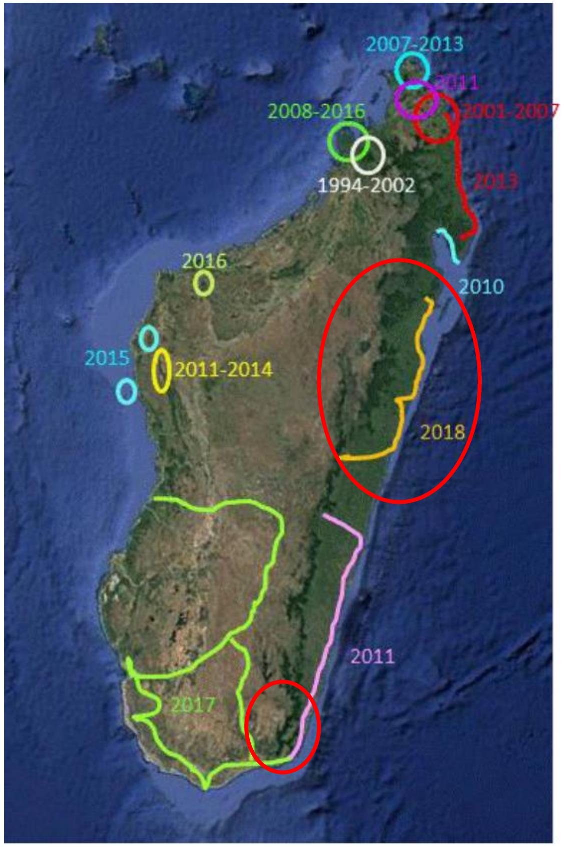


Species delimitation

Potential distribution, UICN category

Sampling

Madagascar 2018 :



Sampled areas

Sampling

Some field images:

Sampling

Some field images:

Sampling

Some field images:

Sampling

Recognize Sapotaceae in the field:

Labramia bojeri

Sampling

Recognize Sapotaceae in the field:

Labramia bojeri

Aubréville branching pattern

Faucherea sp.

Simple leaves, entire margin

Capurodendron sp. nov.

Leaves never opposite

Labramia bojeri

White latex always present

Faucherea sp. nov.

Sampling

Recognize Sapotaceae in the field:


Euphorbiaceae


Combretaceae


Sapotaceae


Sapotaceae

Sampling

Some field images:

Sampling

Some field images:

Sampling

Some field images:

Sampling

Some field images:

Sampling

Some field images:

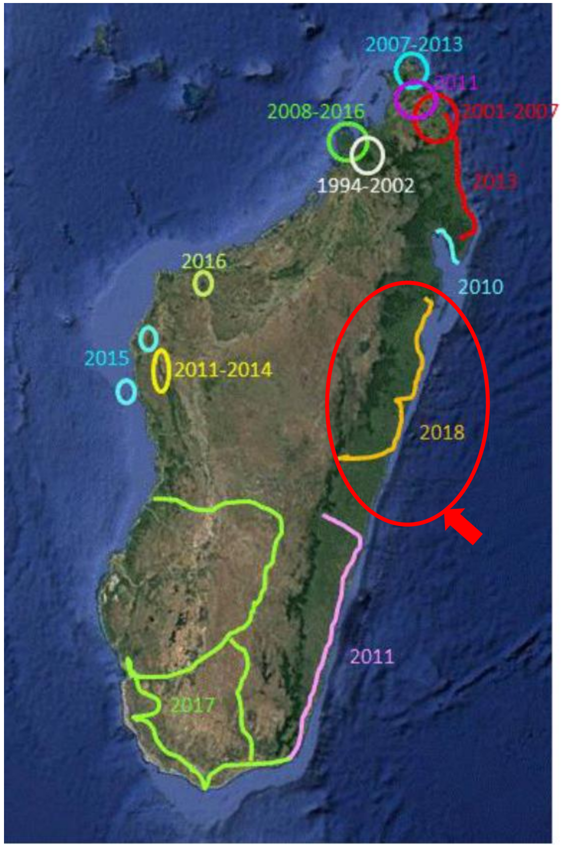
Sampling

Collecting:

Sampling

Some field images:

Sampling



**362 specimens collected,
~25 undescribed morphospecies
from a single trip**

Drying the samples

Matterials and Methods

Sequences obtention:

Hundreds of genes per specimen are wanted

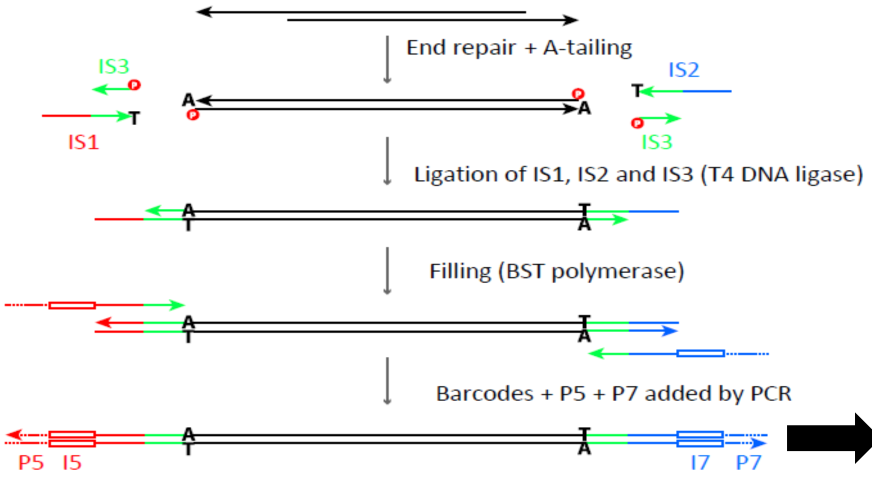
Silica gel samples

Herbarium samples up to 80 years old

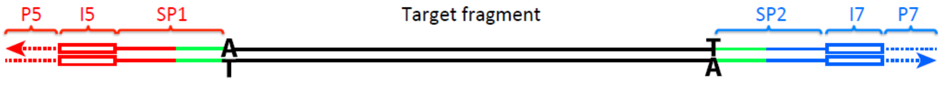
281 specimens:
239 ingroup
42 outgroup

Matterials and Methods

Library construction:



(Kircher 2011)



P5 = amplification primer
I5 = index, or barcode on P5 end
SP1 = First sequencing run primer

P7 = amplification primer
I7 = index, or barcode on P7 end
SP2 = second sequencing run primer

281 specimens:
239 ingroup
42 outgroup

281 Libraries

Matterials and Methods

Baits design:

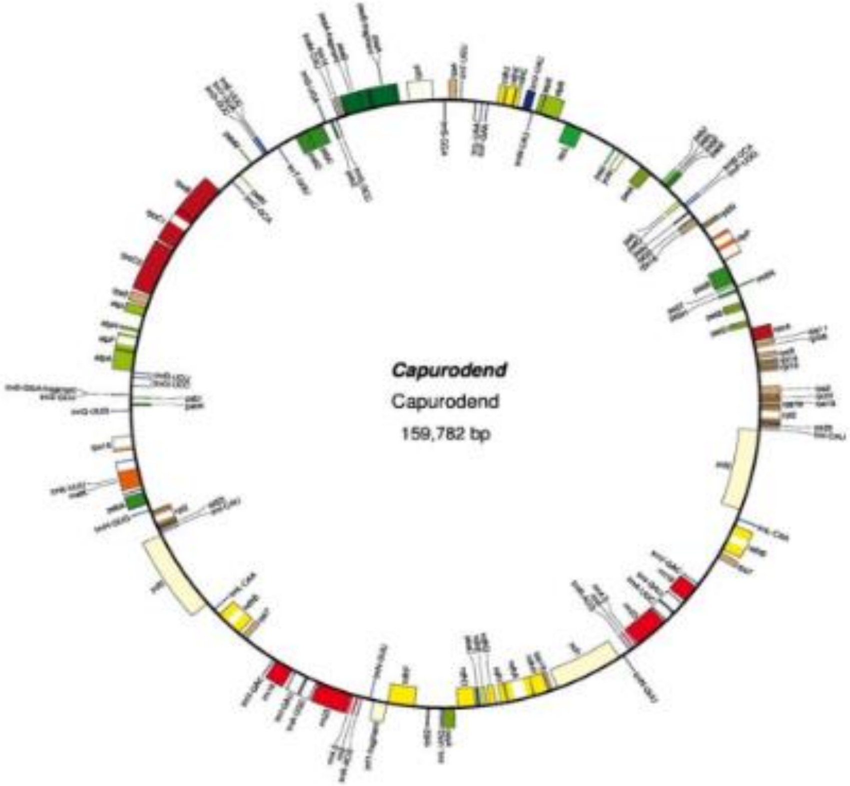
Newly sequenced genomes

Bemangidia lowryi
81 million reads
20x – 40x

C. delphinense
51 million reads
2x – 20x

281 specimens:
239 ingroup
42 outgroup

281 Libraries



Matterials and Methods

Baits design:

GenBank transcriptome

Newly sequenced genomes

Manilkara zapota

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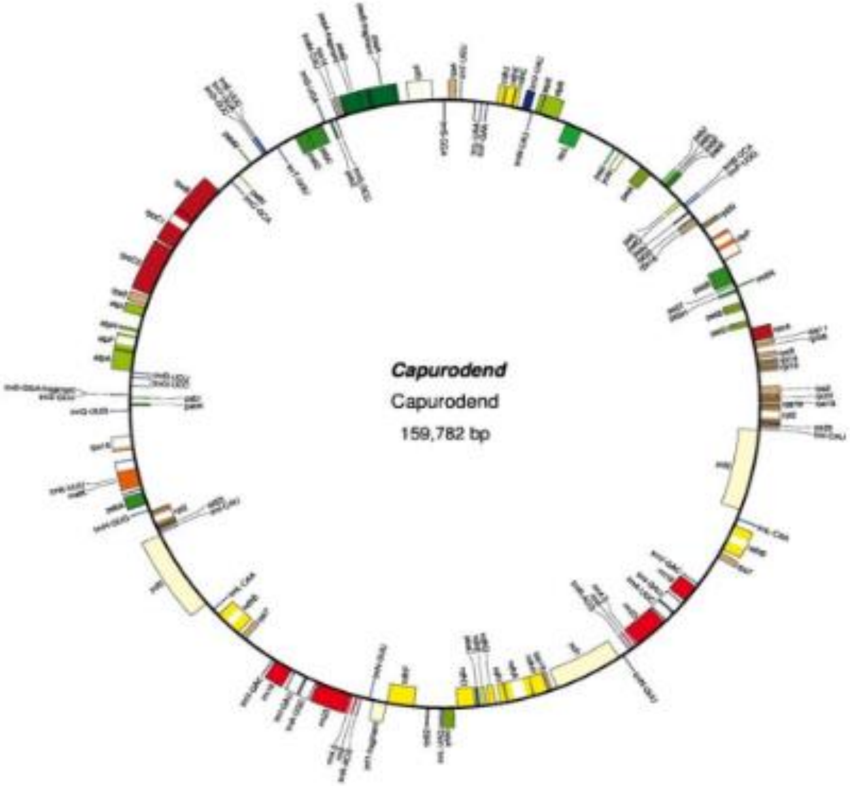

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Matterials and Methods

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GenBank transcriptome

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Baits design

Baits for 1020 loci:
793 genes
227 microsatellites

281 specimens:
239 ingroup
42 outgroup

281 Libraries

Baits: small DNA sequences complementary to a locus that allow us to capture these locus from a genomic DNA solution by hybridization

Baits designed for:

227 microsatellites → **Species complexes**

532 genes from *Tseboneae* → **Species level**

262 genes from Angiosperms → **Suprageneric level**
(Johnson *et al.* 2018)

Matterials and Methods

Gene Capture

GenBank transcriptome

Newly sequenced genomes

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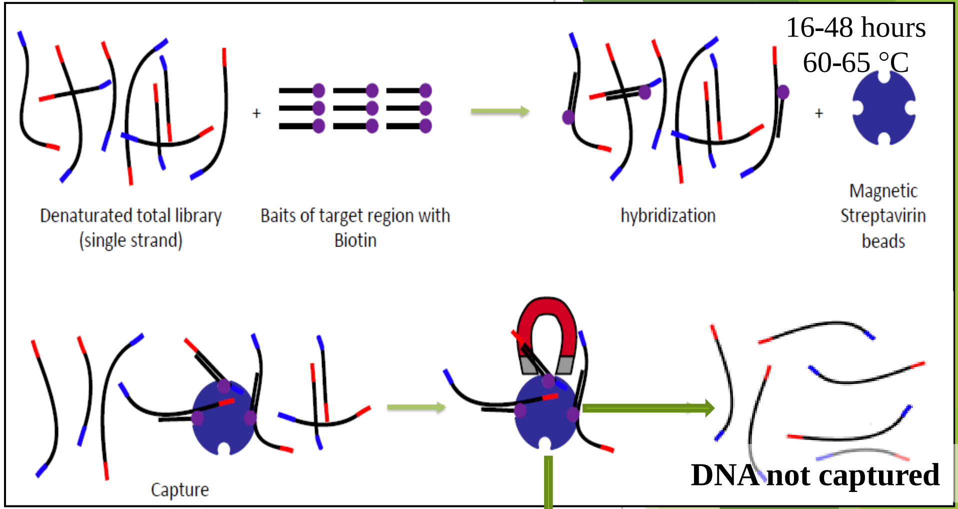
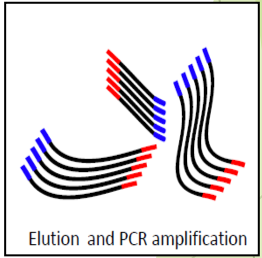
Gene Capture

Gene Capture





Captured genes

Matterials and Methods

Sequencing

GenBank transcriptome

Manilkara zapota

Newly sequenced genomes

Bemangidia lowryi
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20x – 40x

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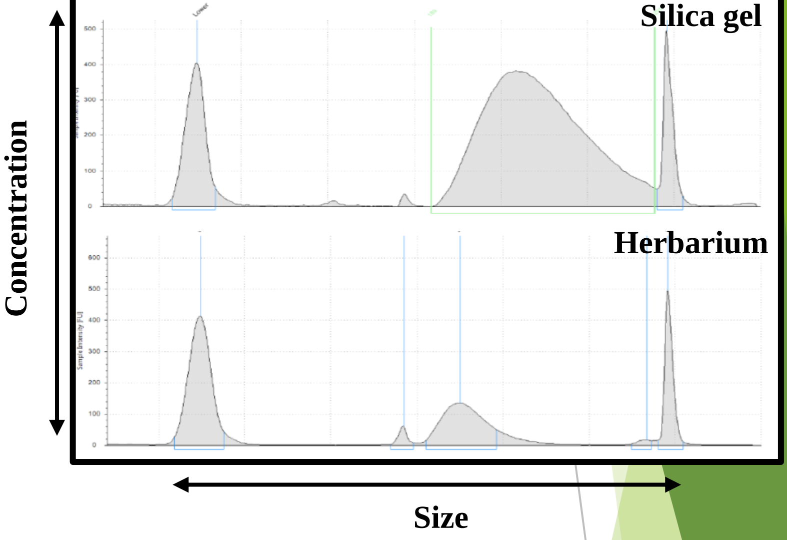
Example of tubes sent to sequencing



Baits design

Baits for 1020 loci:
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281 spécimens:
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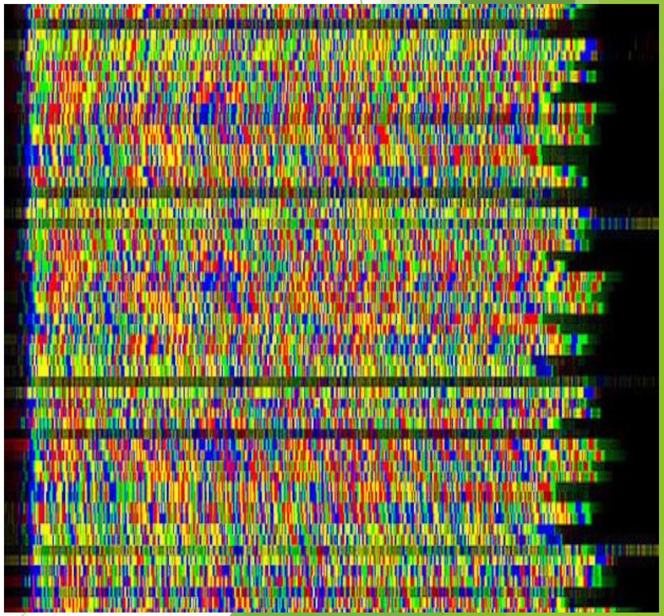
42 outgroup

281 Libraries

Gene Capture

Captured loci

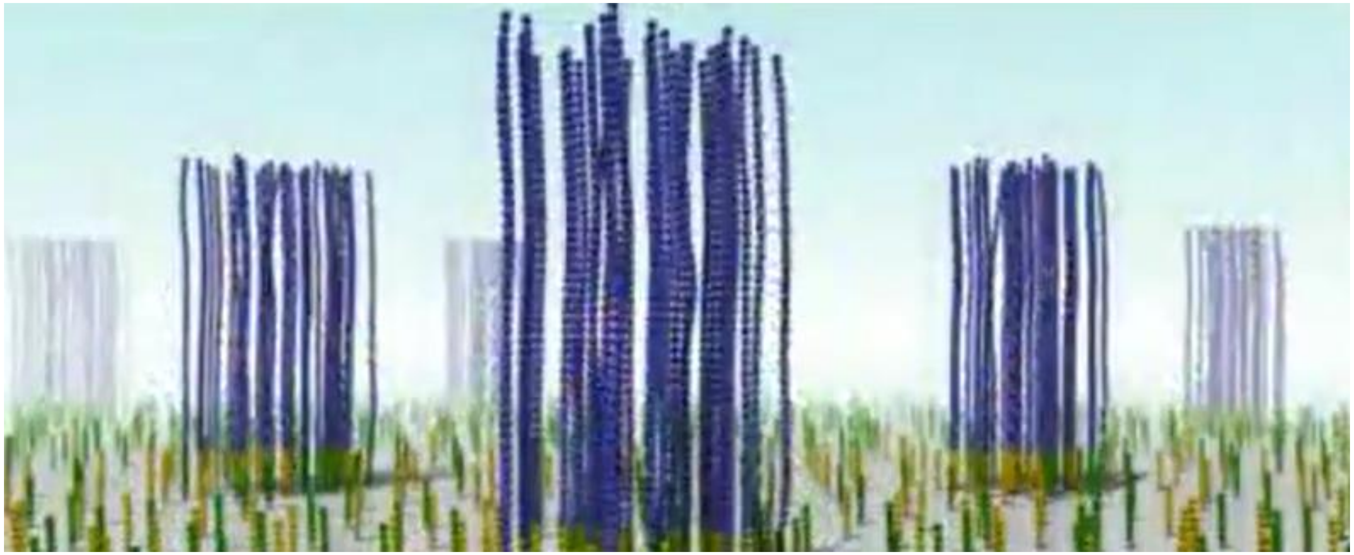
Illumina sequencing



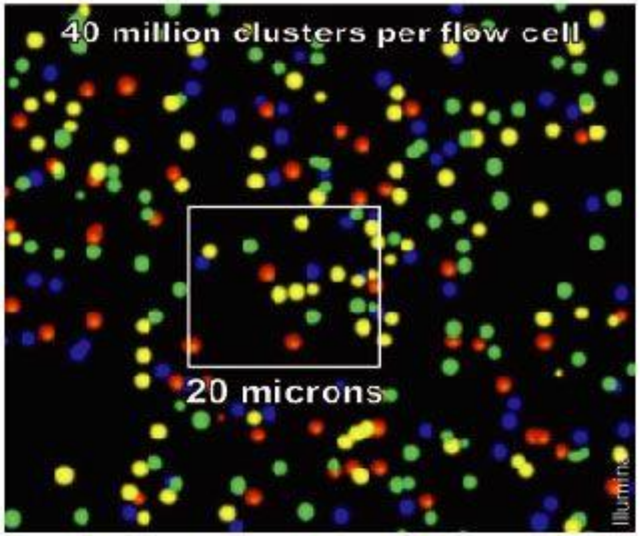
Matterials and Methods

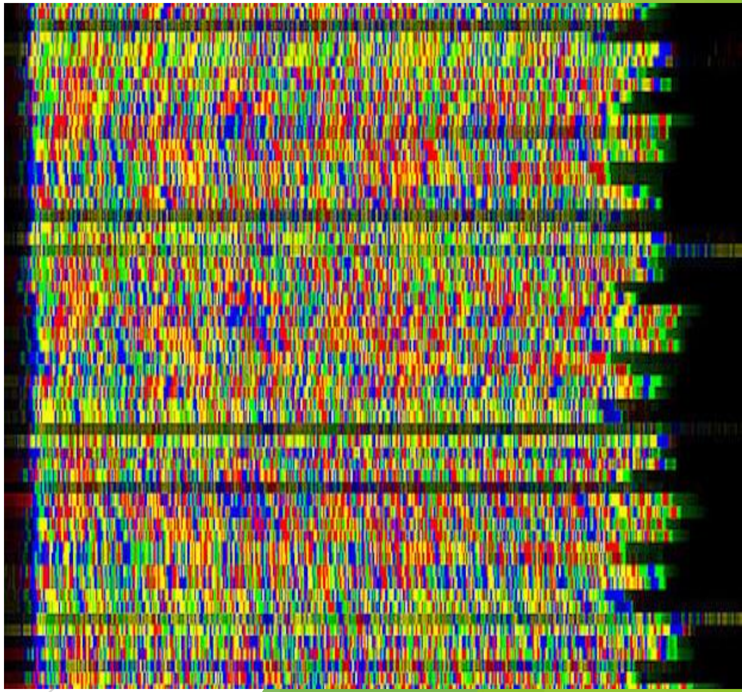
Sequencing (Illumina method)

Matterials and Methods



Sequencing





Matterials and Methods

Sequencing

GenBank transcriptome

Manilkara zapota

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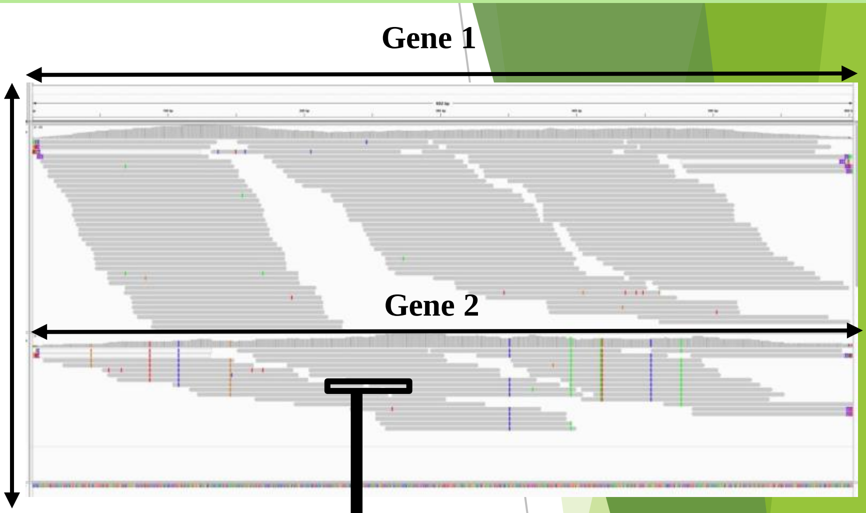
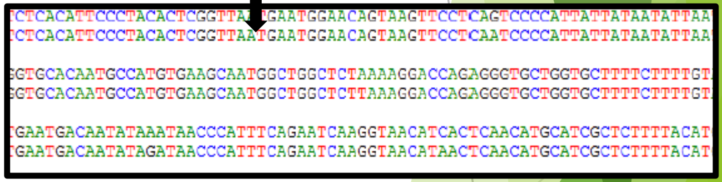




```
CTCACATTCCCTACACTCGGTTAATGAATGGAACAGTAAGTTCCTCAGTCCCCATTATTATAATATTAA
CTCACATTCCCTACACTCGGTTAATGAATGGAACAGTAAGTTCCTCAATCCCCATTATTATAATATTAA

GTGCACAATGCCATGTGAAGCAATGGCTGGCTCTAAAAGGACCAGAGGGTGCTGGTGCTTTTCTTTTGT
GTGCACAATGCCATGTGAAGCAATGGCTGGCTCTTAAAGGACCAGAGGGTGCTGGTGCTTTTCTTTTGT

GAATGACAATATAAATAACCCATTTCAGAATCAAGGTAACATCACTCAACATGCATCGCTCTTTTACAT
GAATGACAATATAGATAACCCATTTCAGAATCAAGGTAACATAACTCAACATGCATCGCTCTTTTACAT
```

Example of sequences

281 spécimens:
239 ingroup
42 outgroup

281 Libraries

Gene Capture

Captured loci

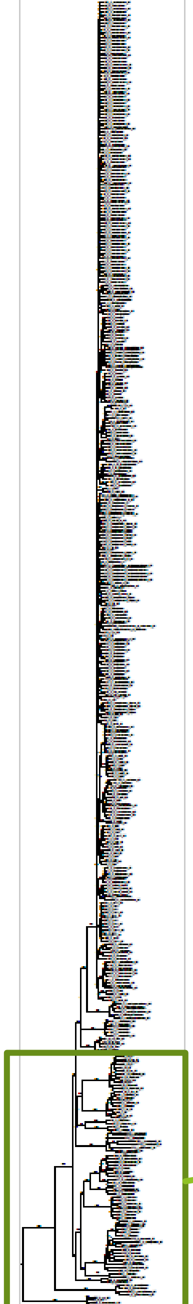
Illumina sequencing

Sequences for phylogenomic analyses

Results

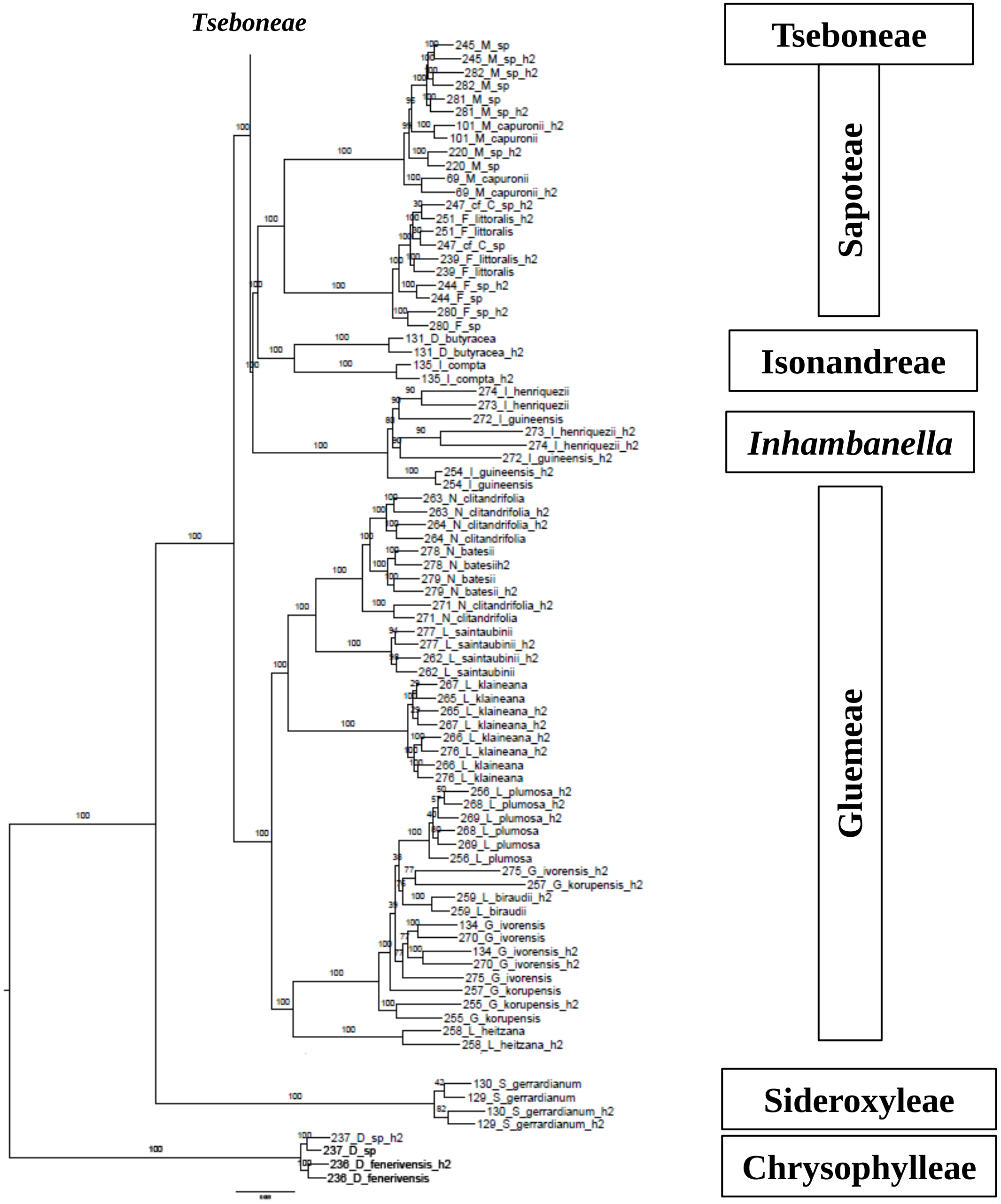
RaxML tree from SNPs

Results

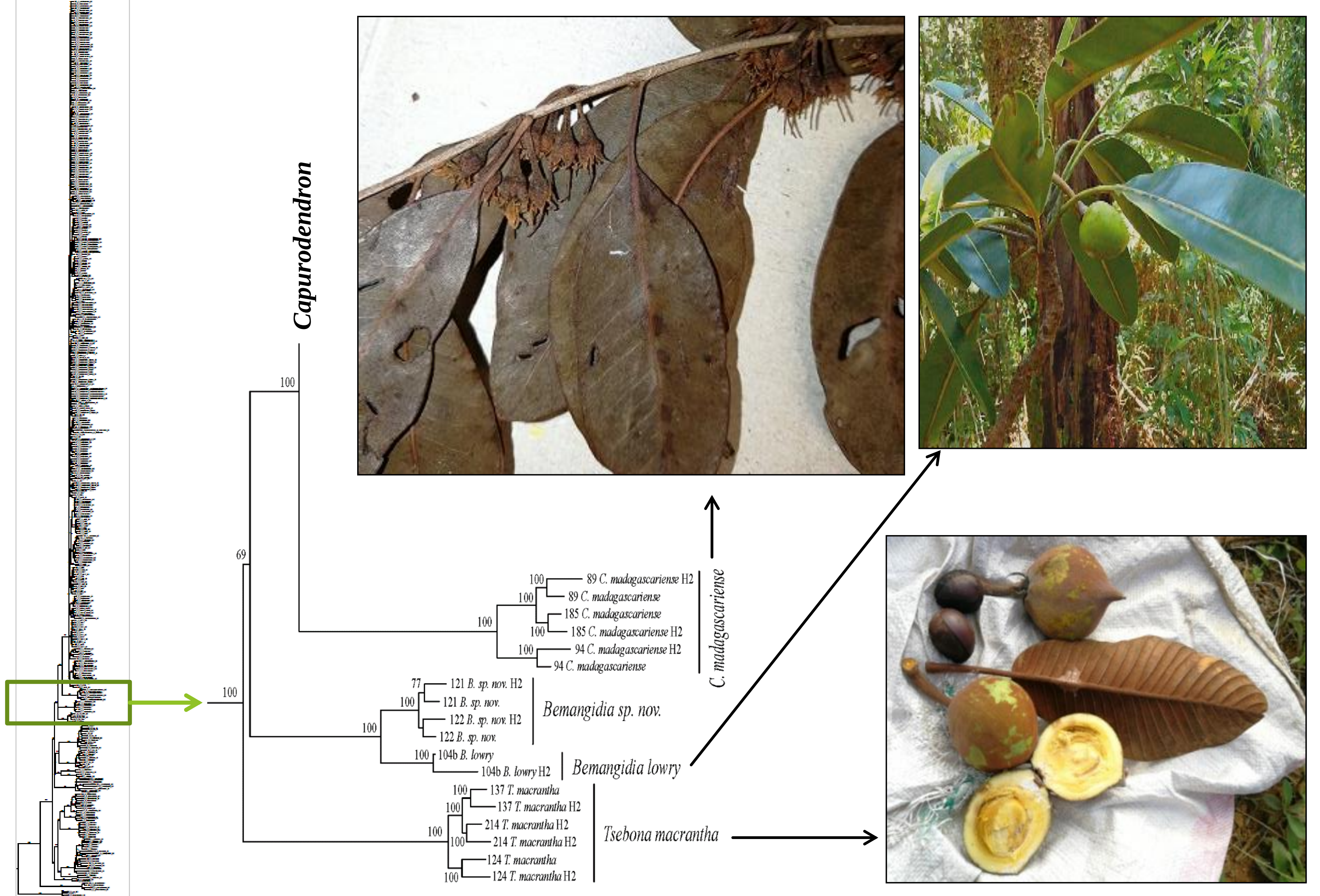


Baits worked well in all the tribes

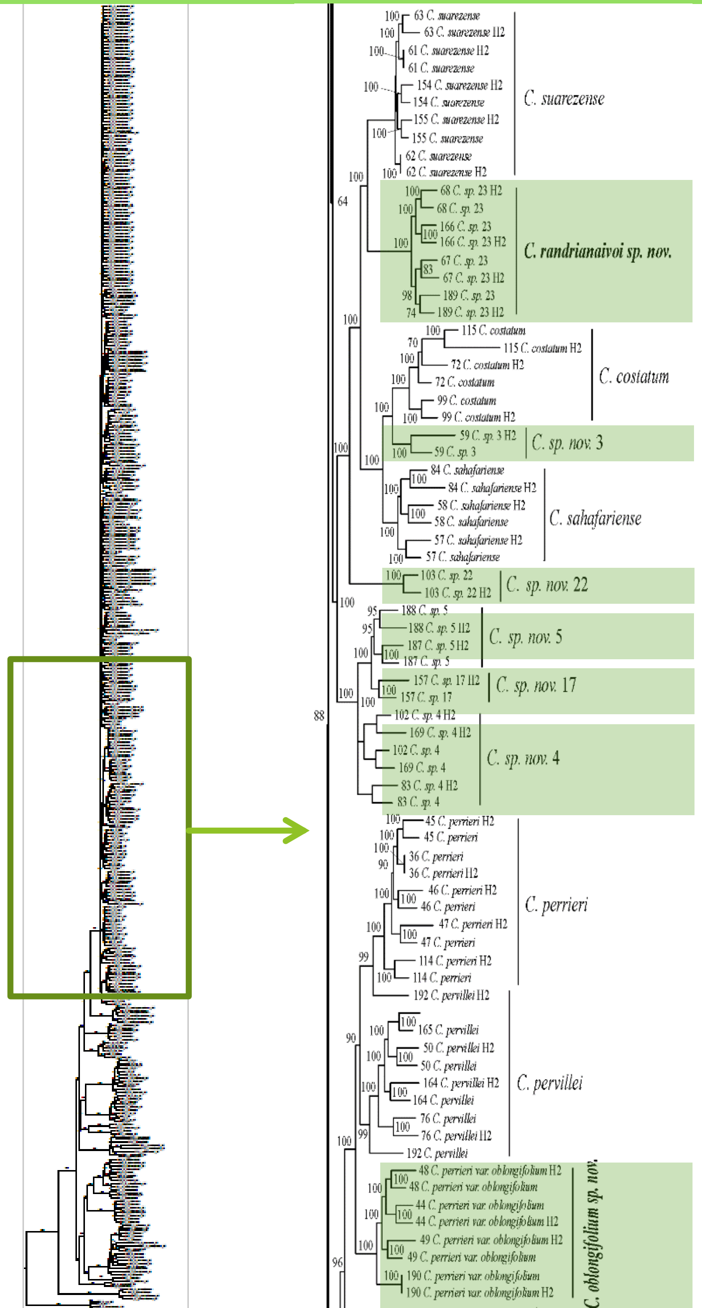
New projects has been started using the same baits



Results


Capurodendron
100
69
100
100
89 C. madagascariense H2
100
89 C. madagascariense
100
185 C. madagascariense
100
185 C. madagascariense H2
100
94 C. madagascariense H2
94 C. madagascariense
C. madagascariense
77
121 B. sp. nov. H2
100
121 B. sp. nov.
122 B. sp. nov. H2
100
122 B. sp. nov.
Bemangidia sp. nov.
100
104b B. lowry
104b B. lowry H2
Bemangidia lowry
100
137 T. macrantha
100
137 T. macrantha H2
214 T. macrantha H2
100
100
214 T. macrantha H2
124 T. macrantha
100
124 T. macrantha H2
Tsebona macrantha

Results



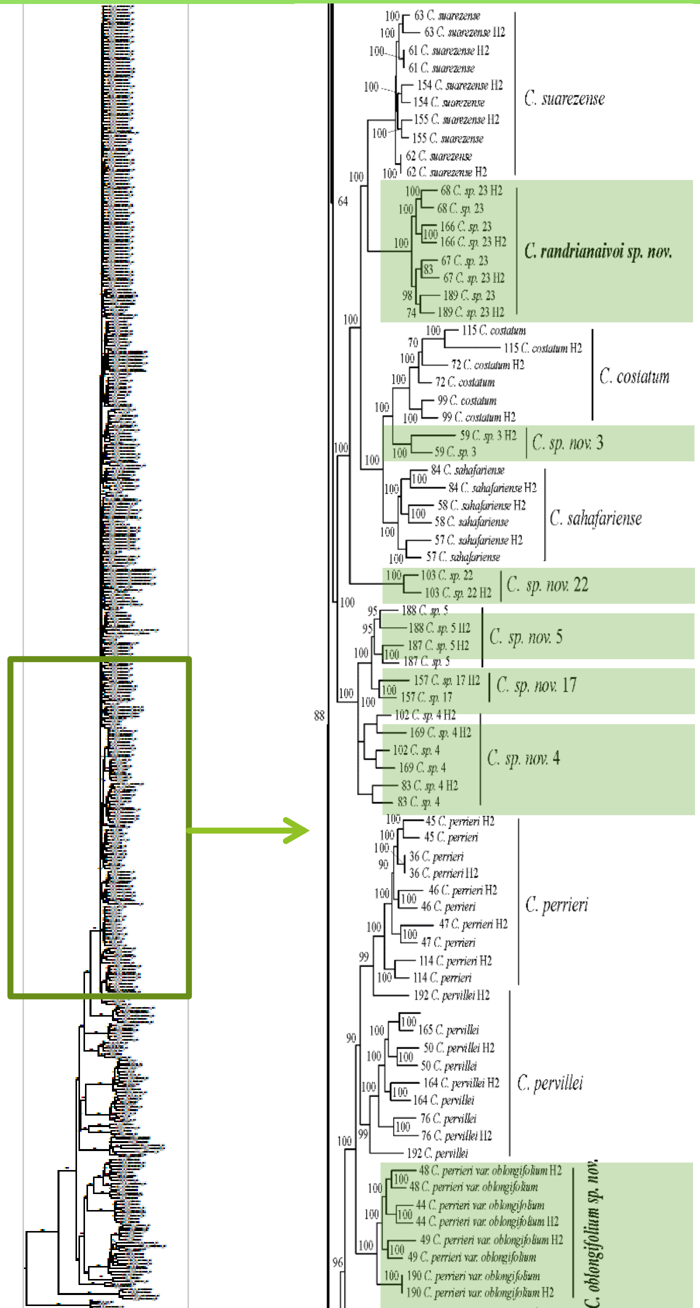
~ 49 clades/morphologies candidates for new species

Only 26 species described

23 new species?

🟩 **= new species**

Results



Described species

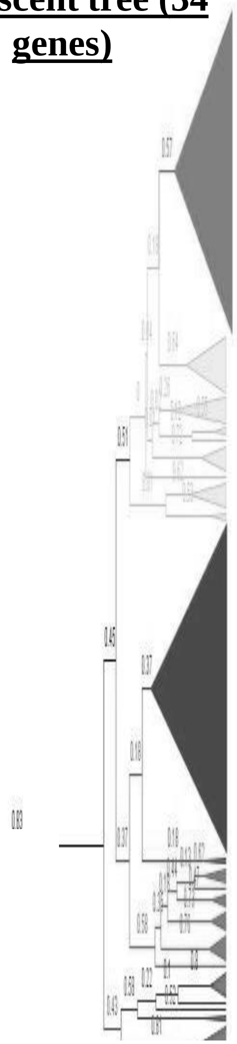
🟩 = new species

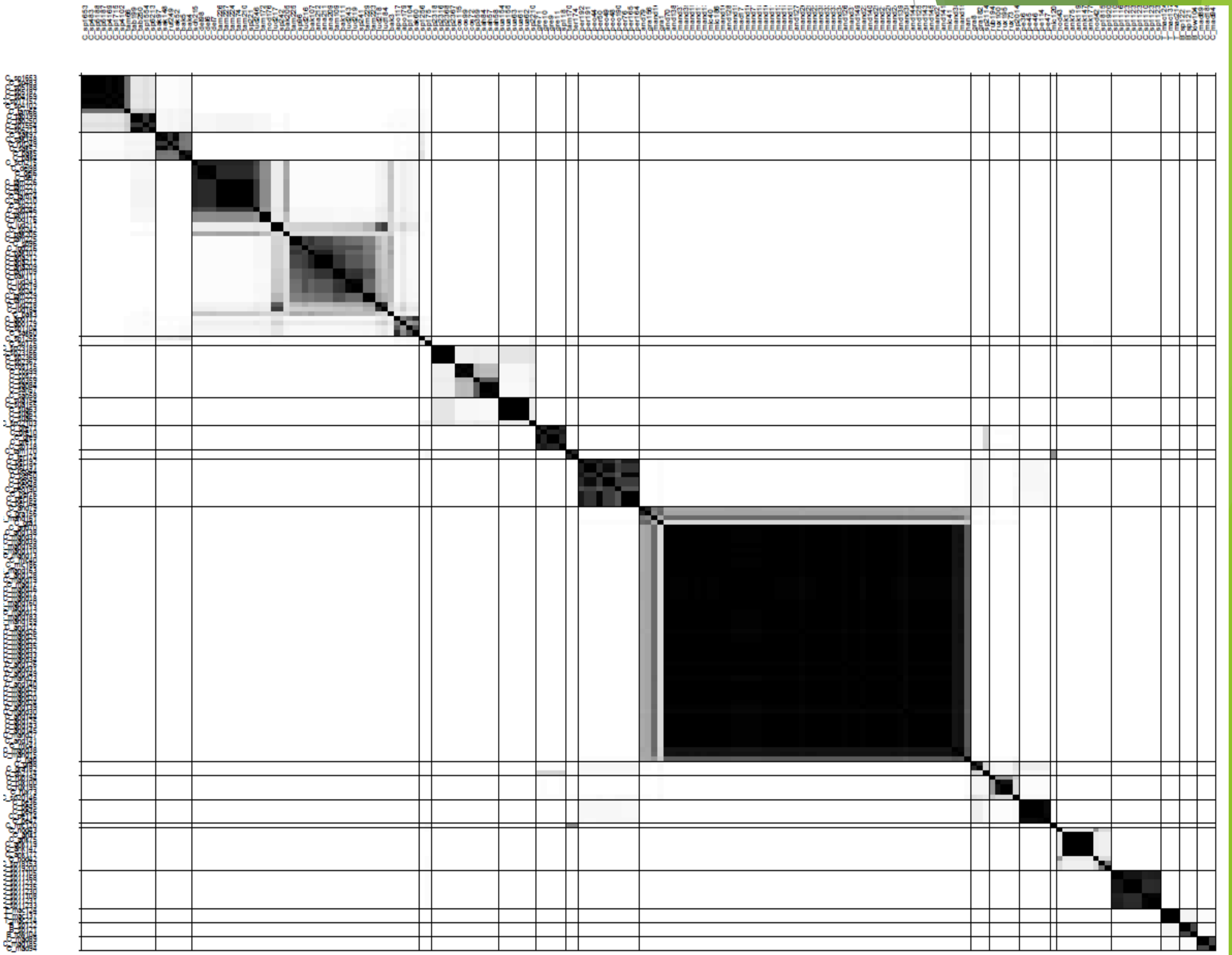
Undescribed species →

Results

STACEY analysis for species delimitation

Multi-species coalescent tree (34 genes)





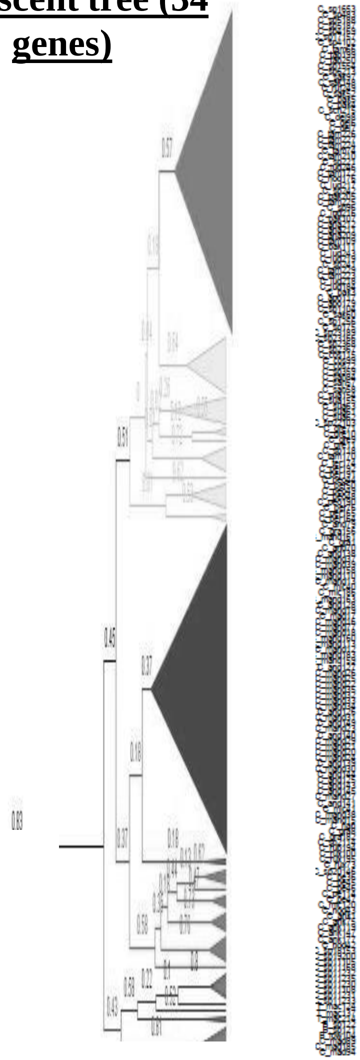
Putative species are indicated in black squares

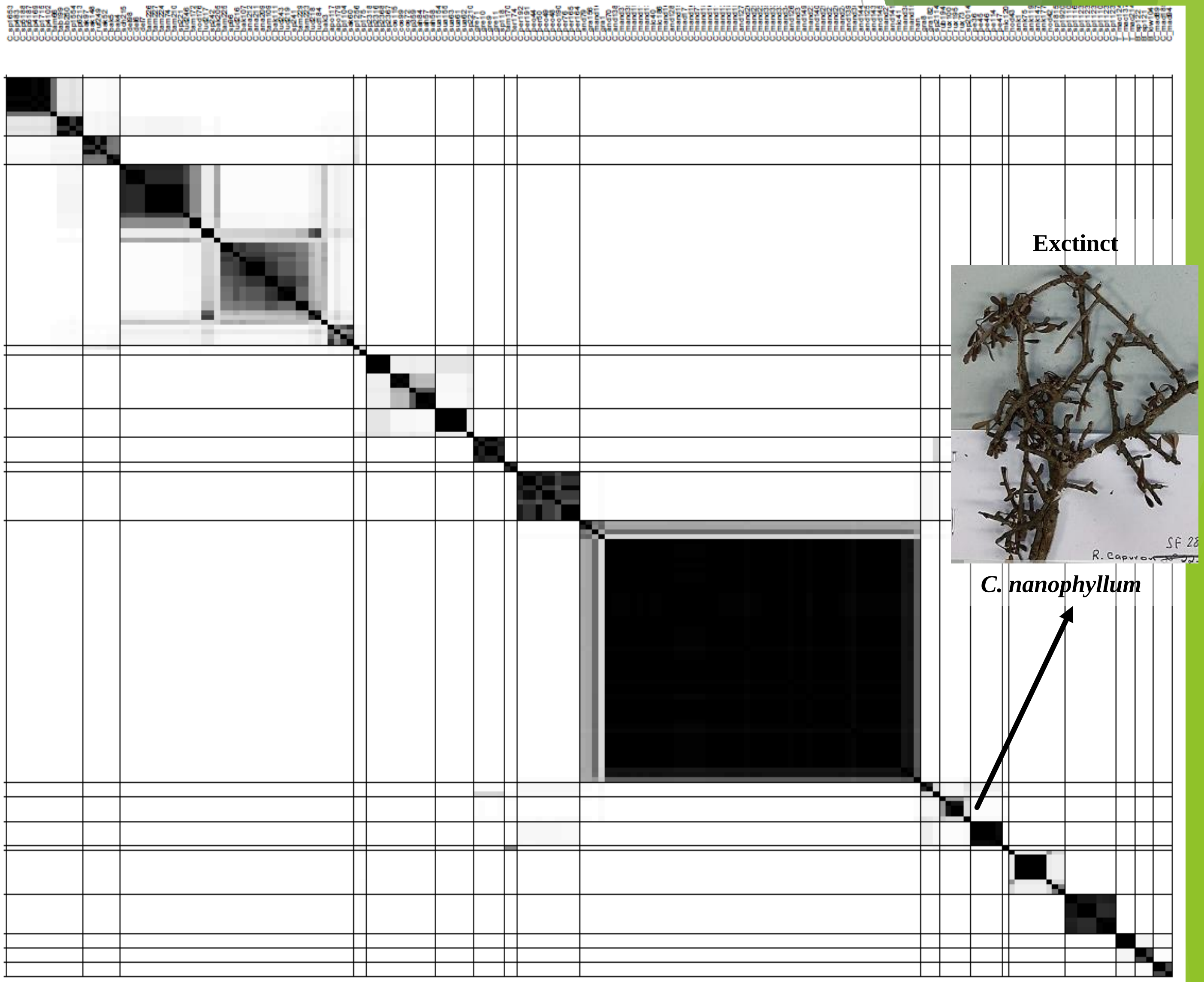
Conspecificity: 0% ☐ 50% ▨ 100% ■

Results

STACEY analysis for species delimitation

Multi-species coalescent tree (34 genes)





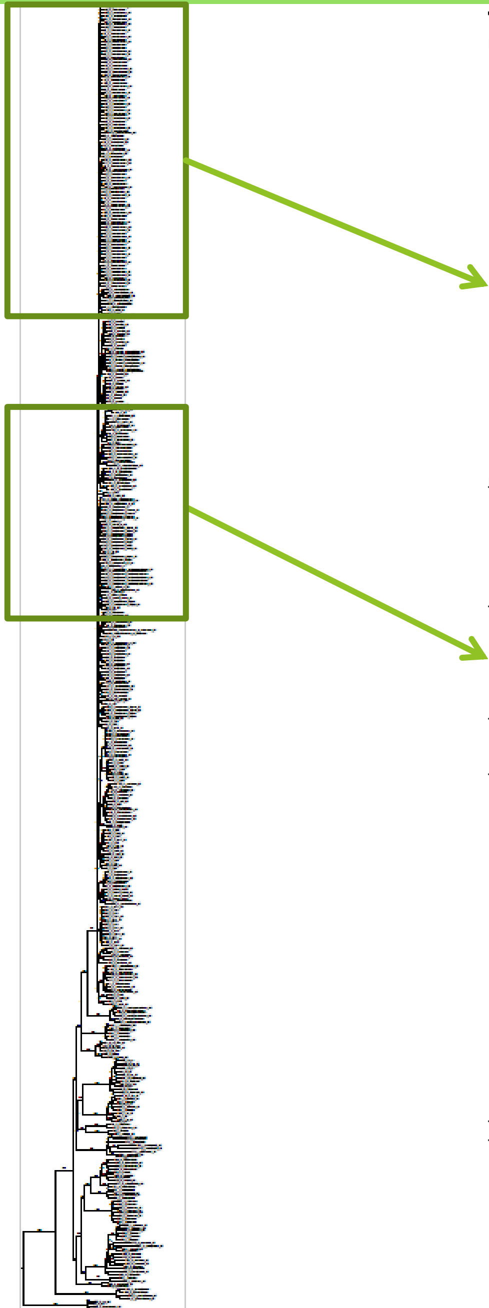
Exctinct

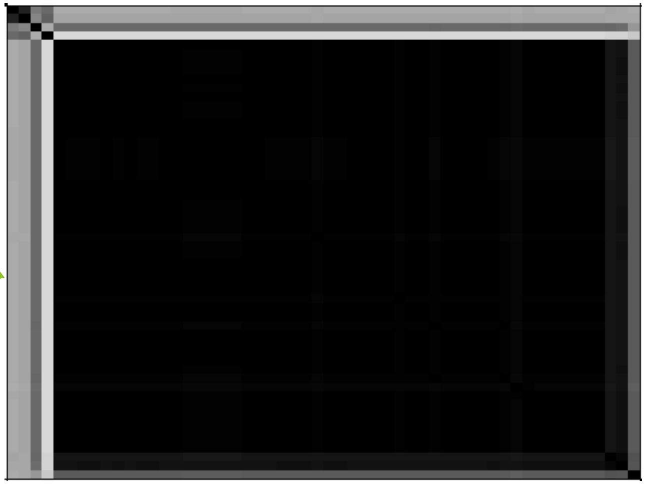
C. nanophyllum

Putative species are indicated in black squares

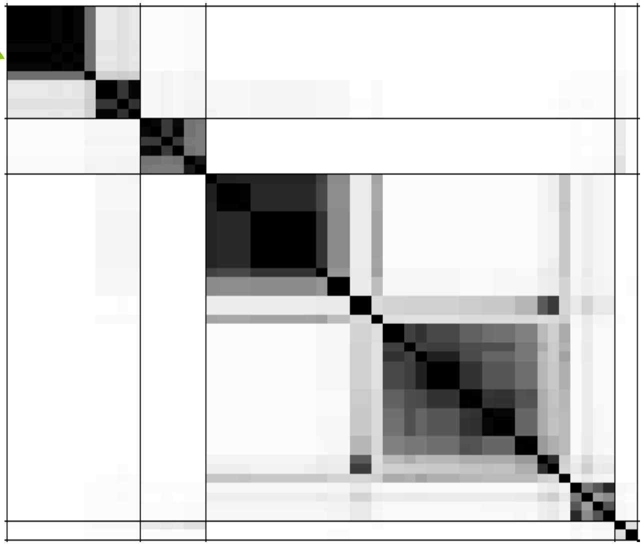
Conspecificity: 0% ☐ 50% ▨ 100% ■

Results



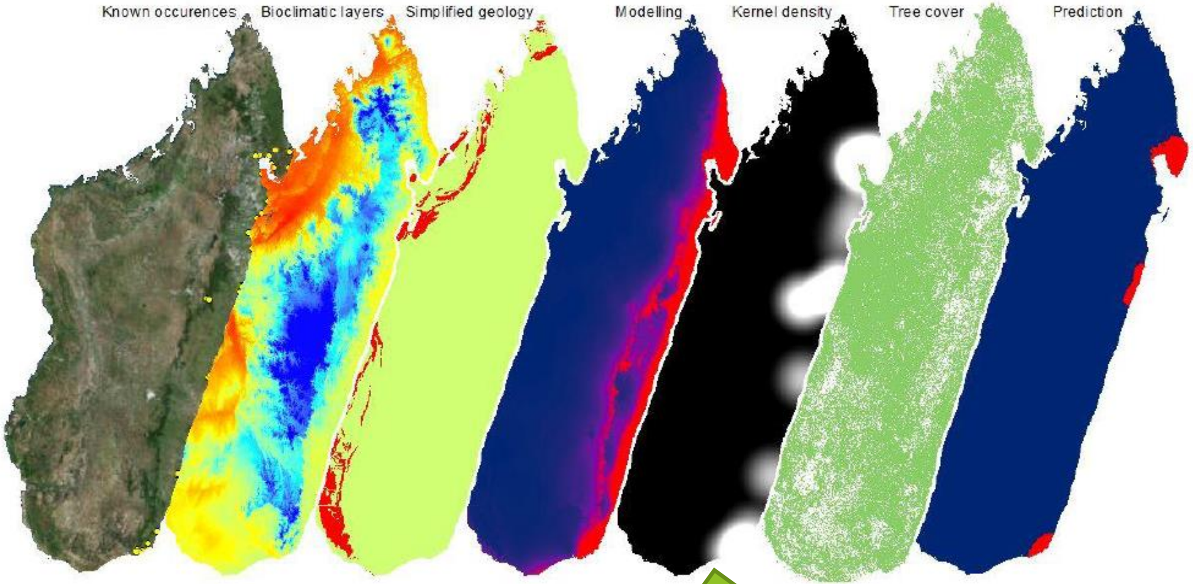


Arid species complex



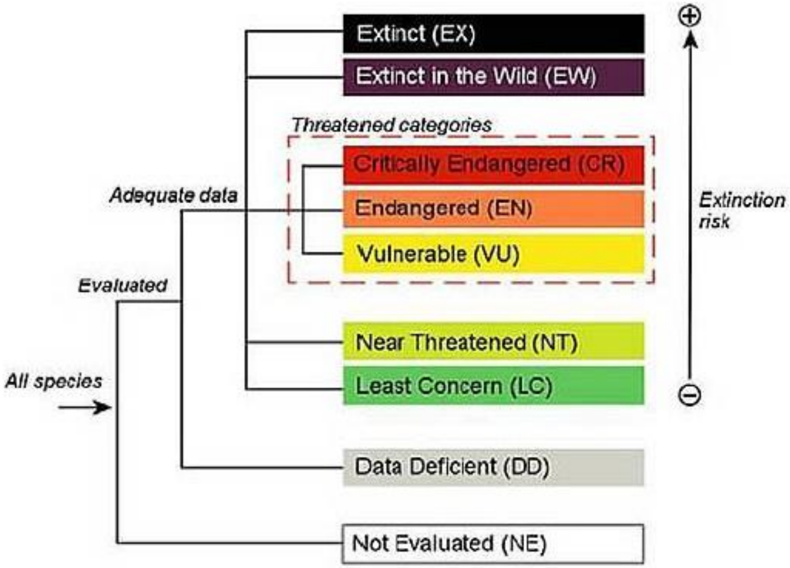
Eastern species complex

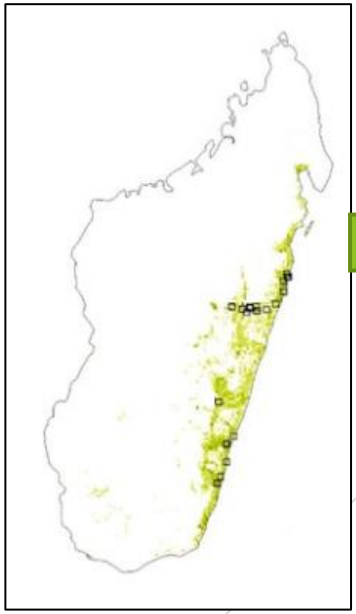
Selection of priority areas for conservation

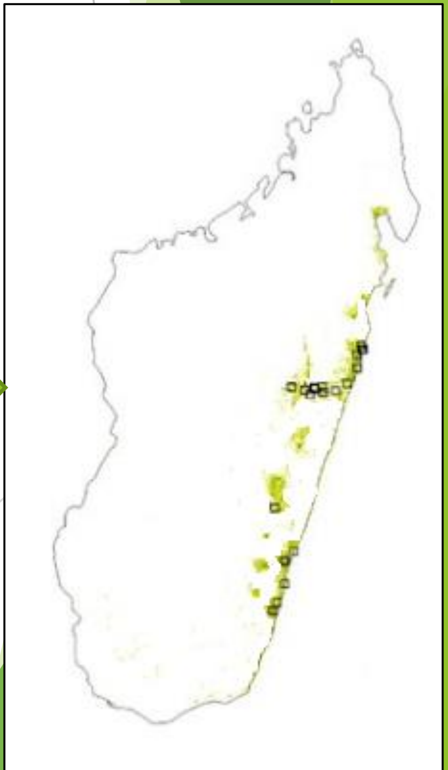


MaxEnt software

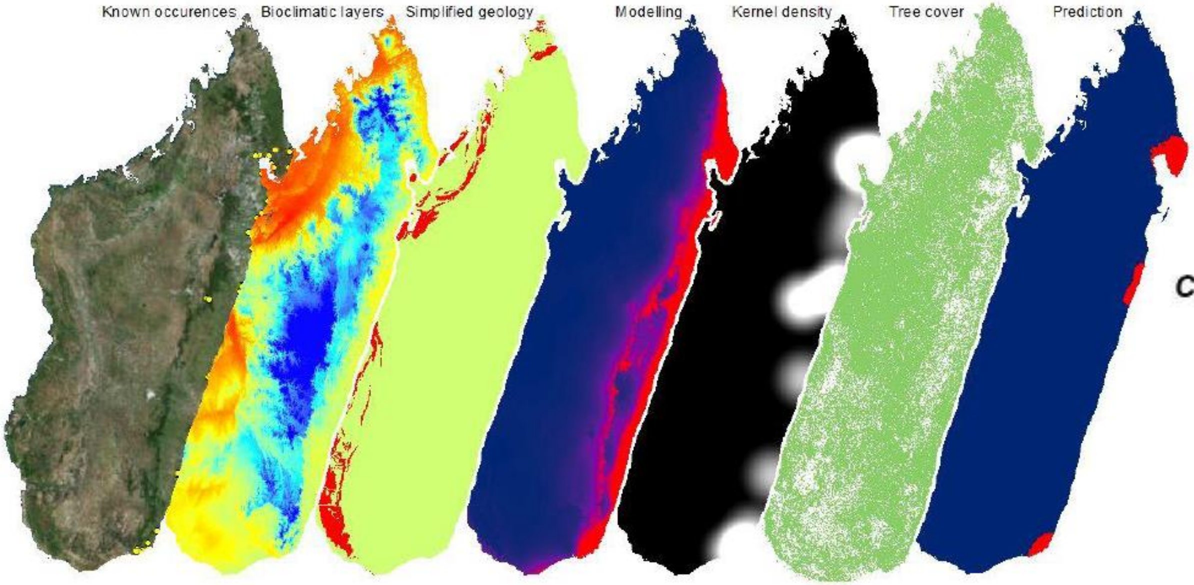
Predicted potential distribution

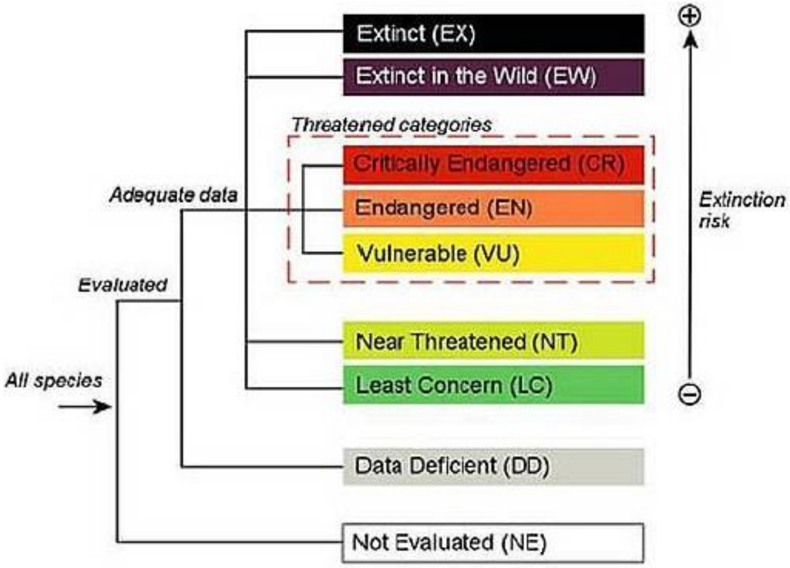


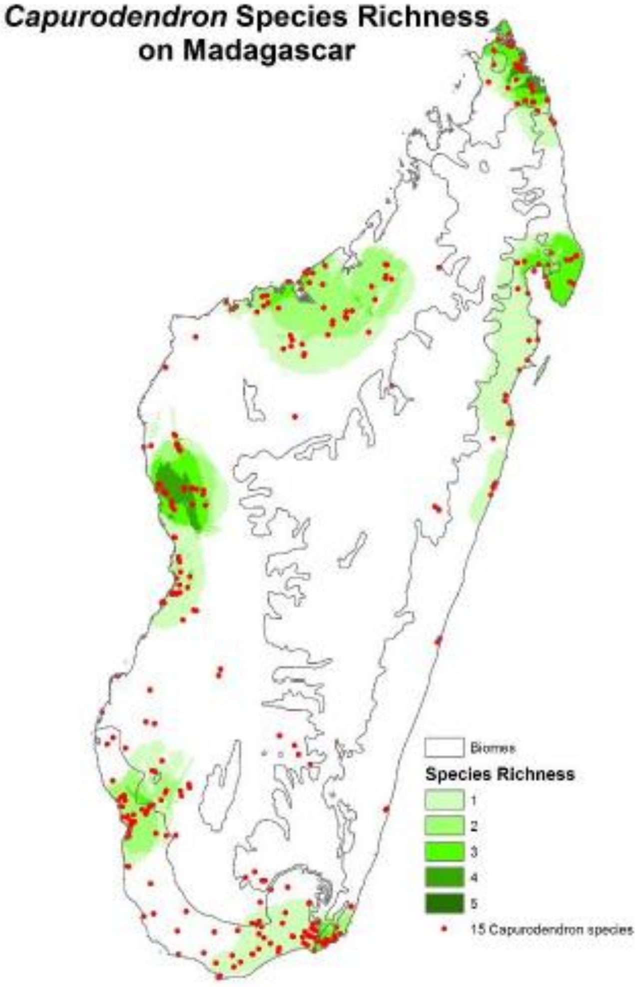




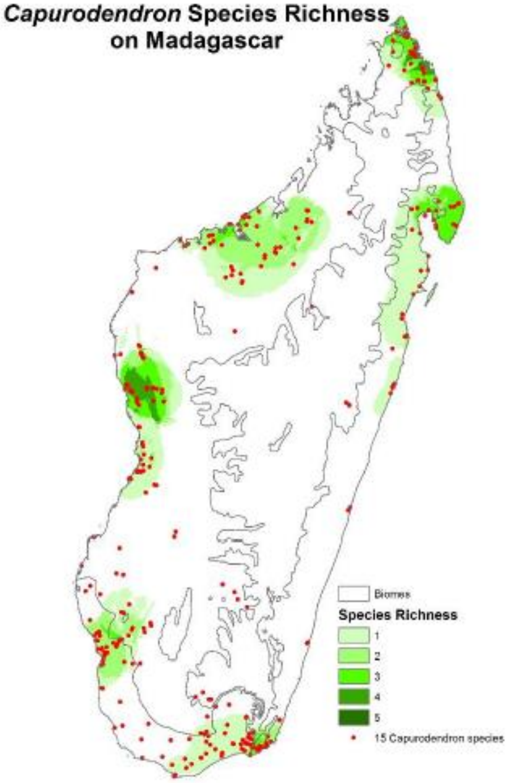
Selection of priority areas for conservation

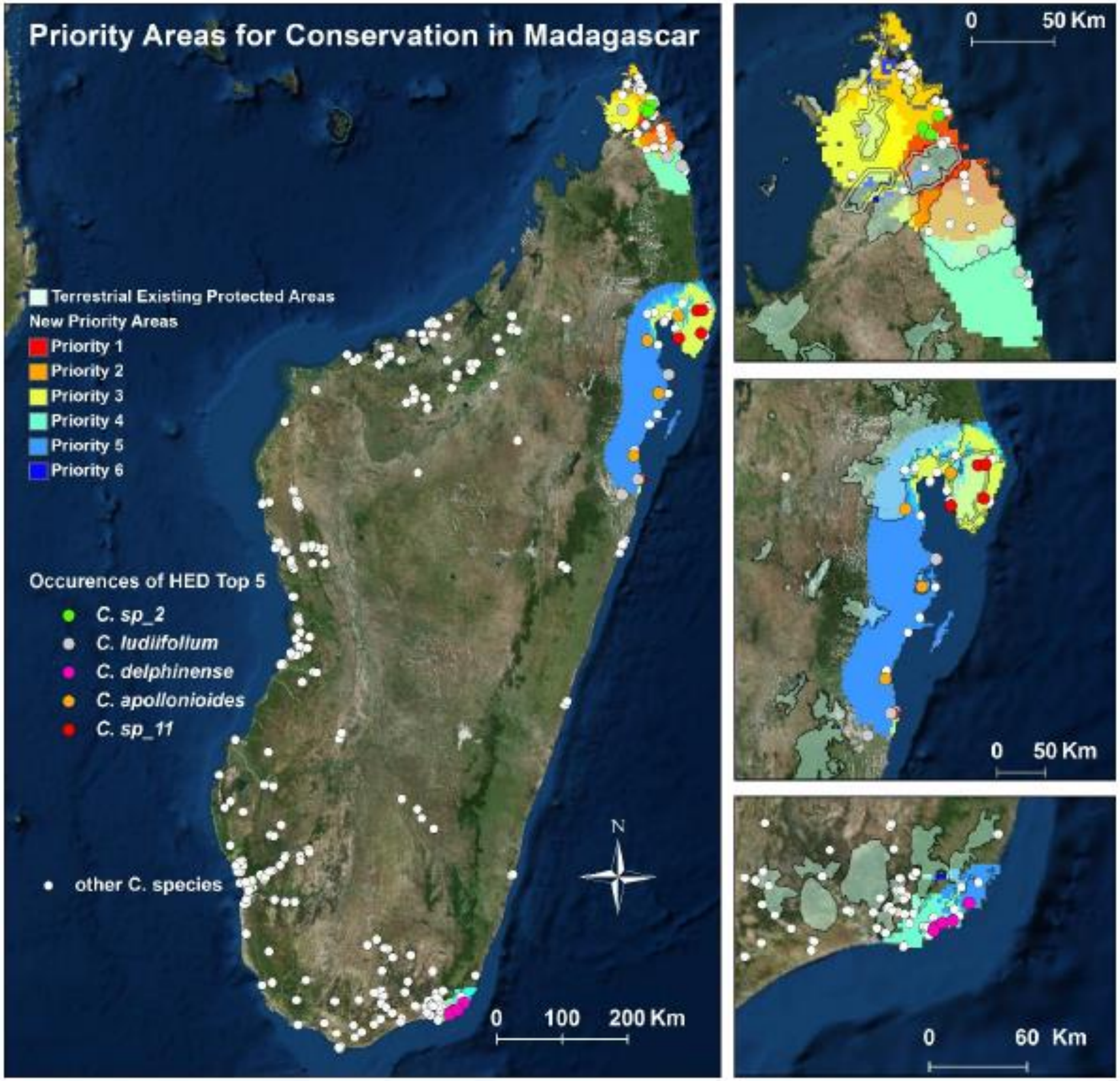





Capurodendron Species Richness on Madagascar

Selection of priority areas for conservation





Agradecimientos

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FNSNF Swiss National Science Foundation

Université de Genève

Fondation Ernst et Lucie Schmidheiny

Gracias!

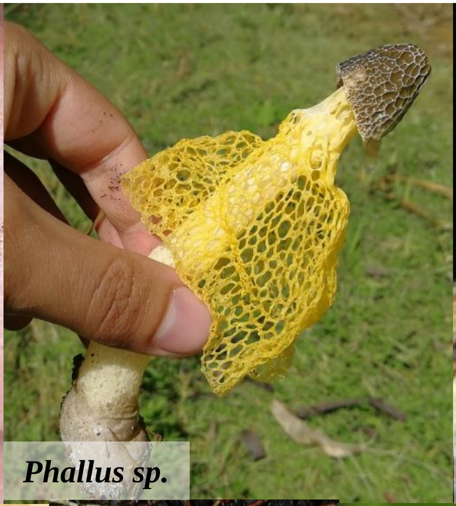
Contact: Carlos.g.boluda@gmail.com

Sampling

Some field images:


Clathrus sp.


Coenogonium sp.


Phallus sp.


Cookeina sp.


Sordariomycetes sp.


Astraeus aff. pteridis.


Mutinus aff. ravenelii.


Hypocreales sp.


Galiella sp.


Geastrum calceum


Dacrymyces sp. nov.

Sampling

Some field images:

Archispirostreptus sp.

Aphistogoniulus sp.

Zoosphaerium sp.

Zoosphaerium sp.

Zoosphaerium neptunus

Pelmatojulus sp.

Sampling

Some field images:

Caerostris sp. nov. 1

Caerostris sp. nov. 2

Gasterocantha sp.

Geoplanidae sp.

cf. Parectatosoma sp.

cf. Nepa sp.

Sampling

Some field images:

Sampling

Some field images:

Sampling

Some field images:

Tambourissa sp. (fruit)

Tambourissa sp. (fruit)

Tambourissa sp. (fleurs ♀)

Tambourissa sp. (flowers ♀)

Xylopia sp. (fruit)

Xylopia sp. (fruit)

Decarydendron sp. (fruit)

Tambourissa sp. (flowers ♂)

Sampling

Some field images:


Asteropeia sp.


Leptolaena sp.


Sarcolaena grandiflora


Rhopalocarpus sp.


Sarcolaena grandiflora

Sampling

Some field images:

Gymnosiphon sp.
(Burmanniaceae)

Sciaphila sp. (Triuridaceae)

Seychellaria sp.
(Triuridaceae)

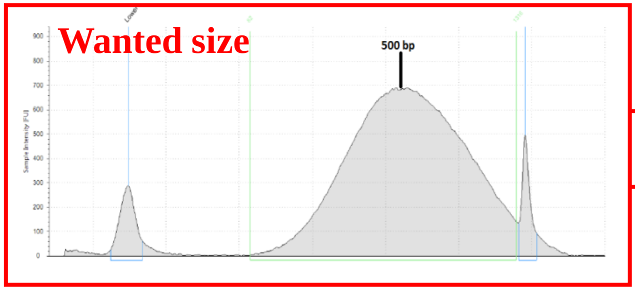
Plantes mycohétérotrophes

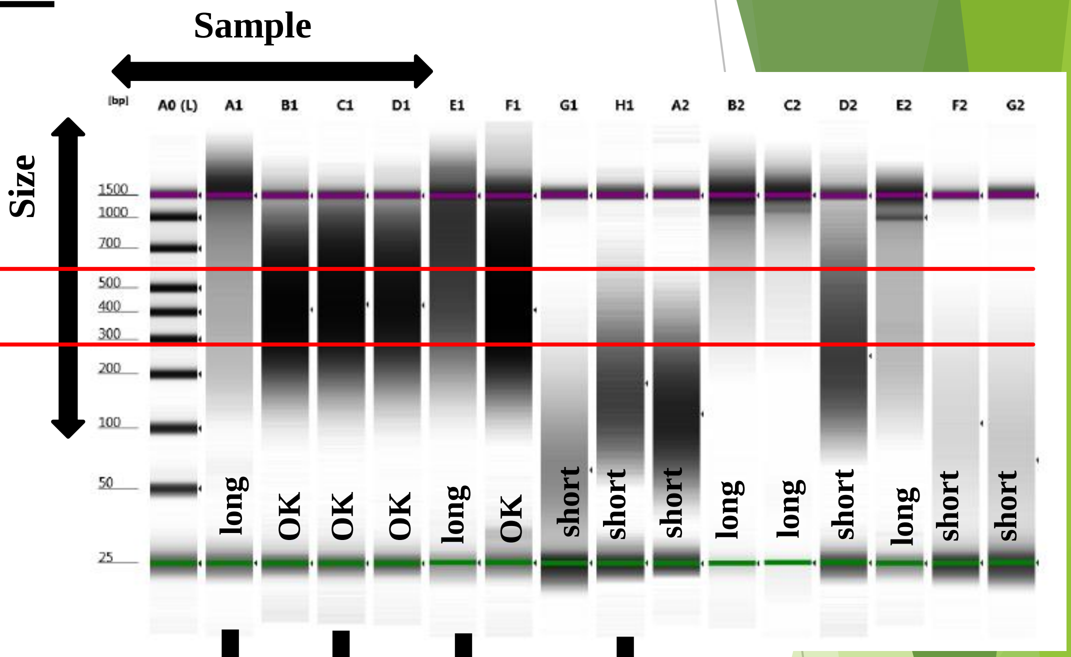
Sampling

Some field images:

Matterials and Methods

Library construction:





Year:	2018	2010	2010	1991
Sonication: cycles	8x		2x	

Silica gel samples

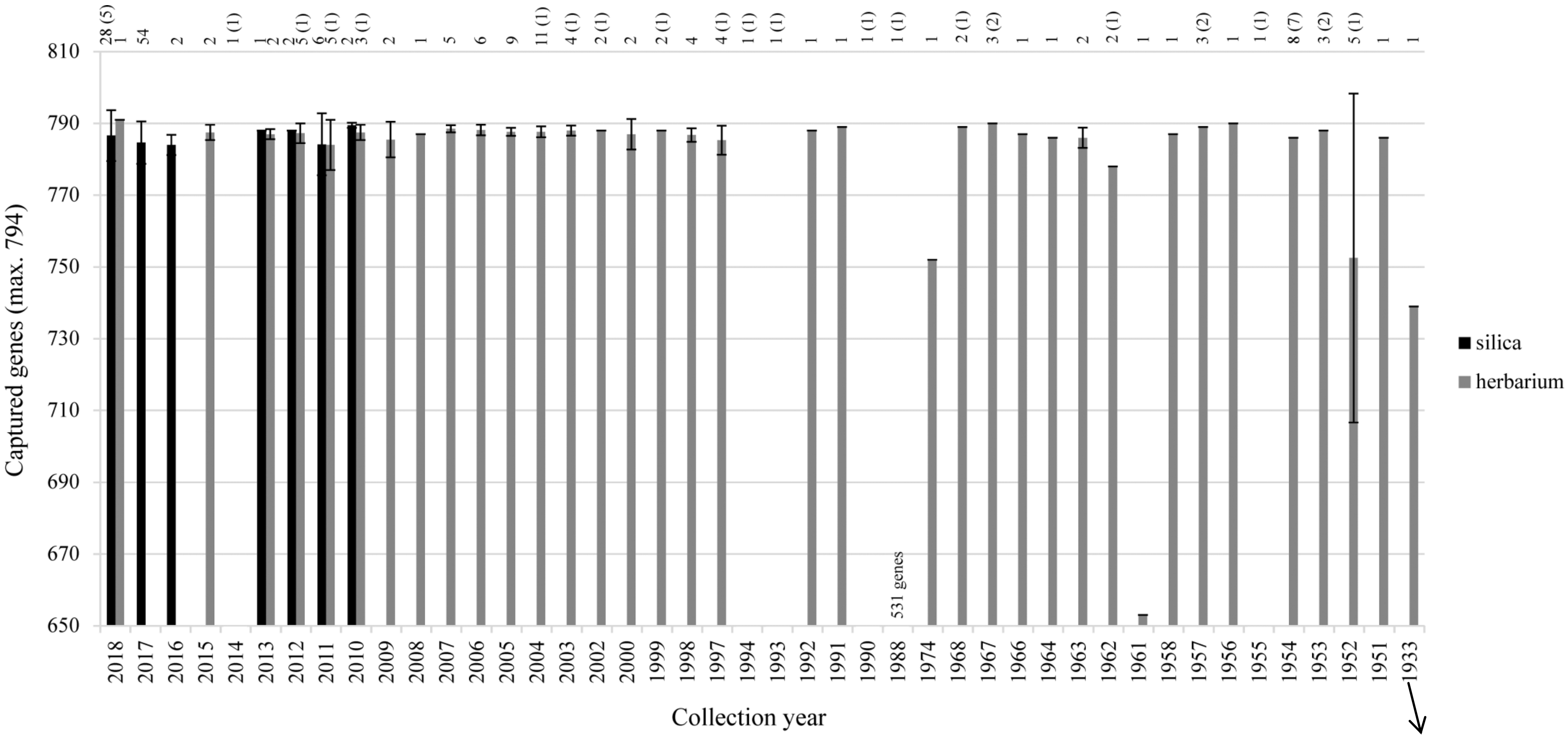
Herbarium samples up to 80 years old

281 specimens:
239 ingroup
42 outgroup

Bioruptor

Results

Gene Capture has been very efficient → Storing method and collection year had not a strong impact



86 years old

Fig. 2, Number of obtained genes according to the kind of sampling storage (silica-gel or herbarium) and collection year. Values in the upper part indicate the number of analysed samples per year, with the number of failed specimens between brackets.

Results

RAxML of SNP of 519 genes, 444 OTUs

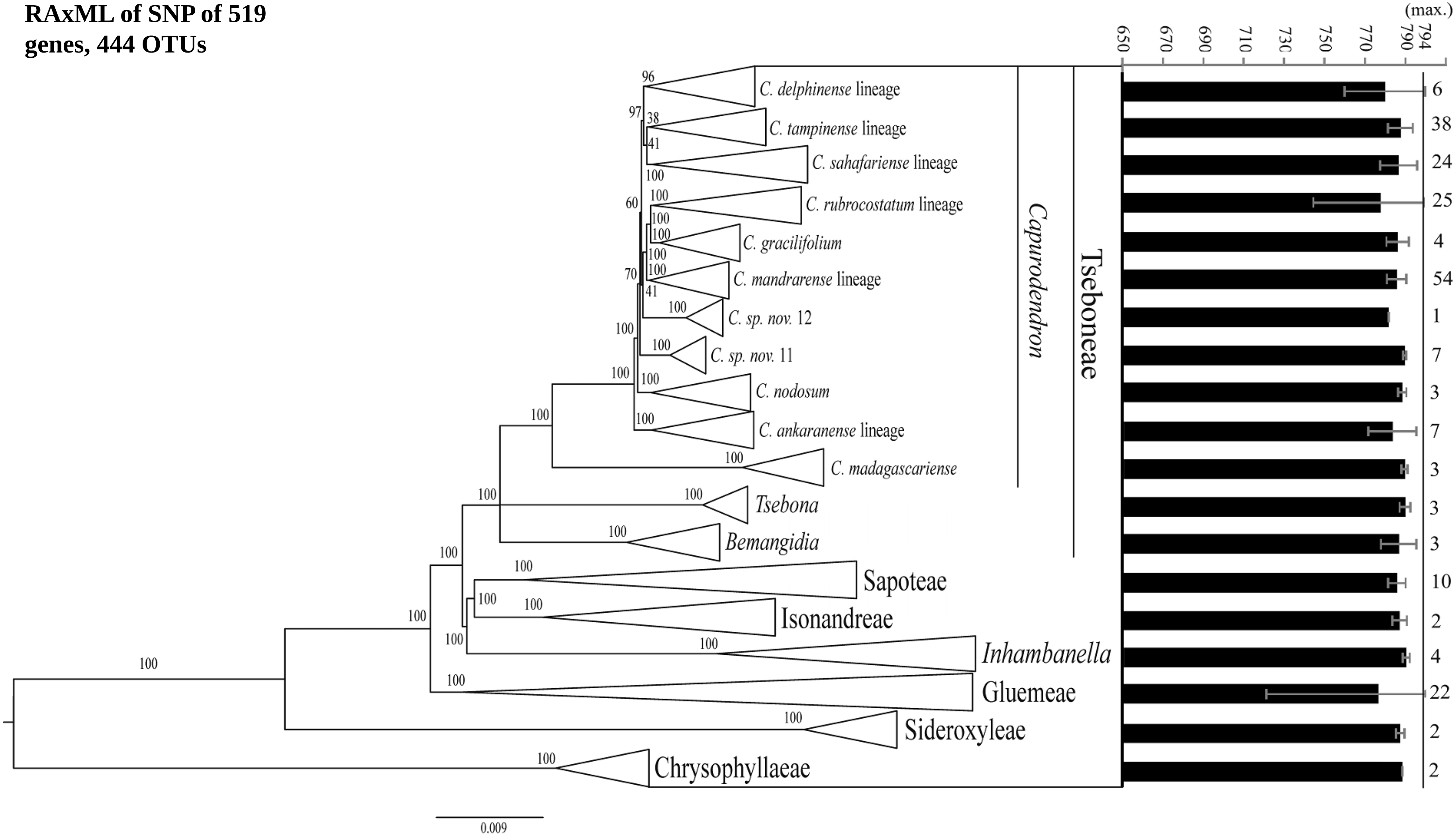


Fig.1 Maximum likelihood tree of 192192 SNPs from519 coding genes and 444 OTUs (222 specimens). The major clades have been collapsed at tribe, genus or infrageneric lineage levels. Bars in the right margin indicate the average number of captured genes per clade and their standard deviation. The number of specimens per clade is indicated at the right margin.

Results

Gene Capture has been very efficient in all the lineages

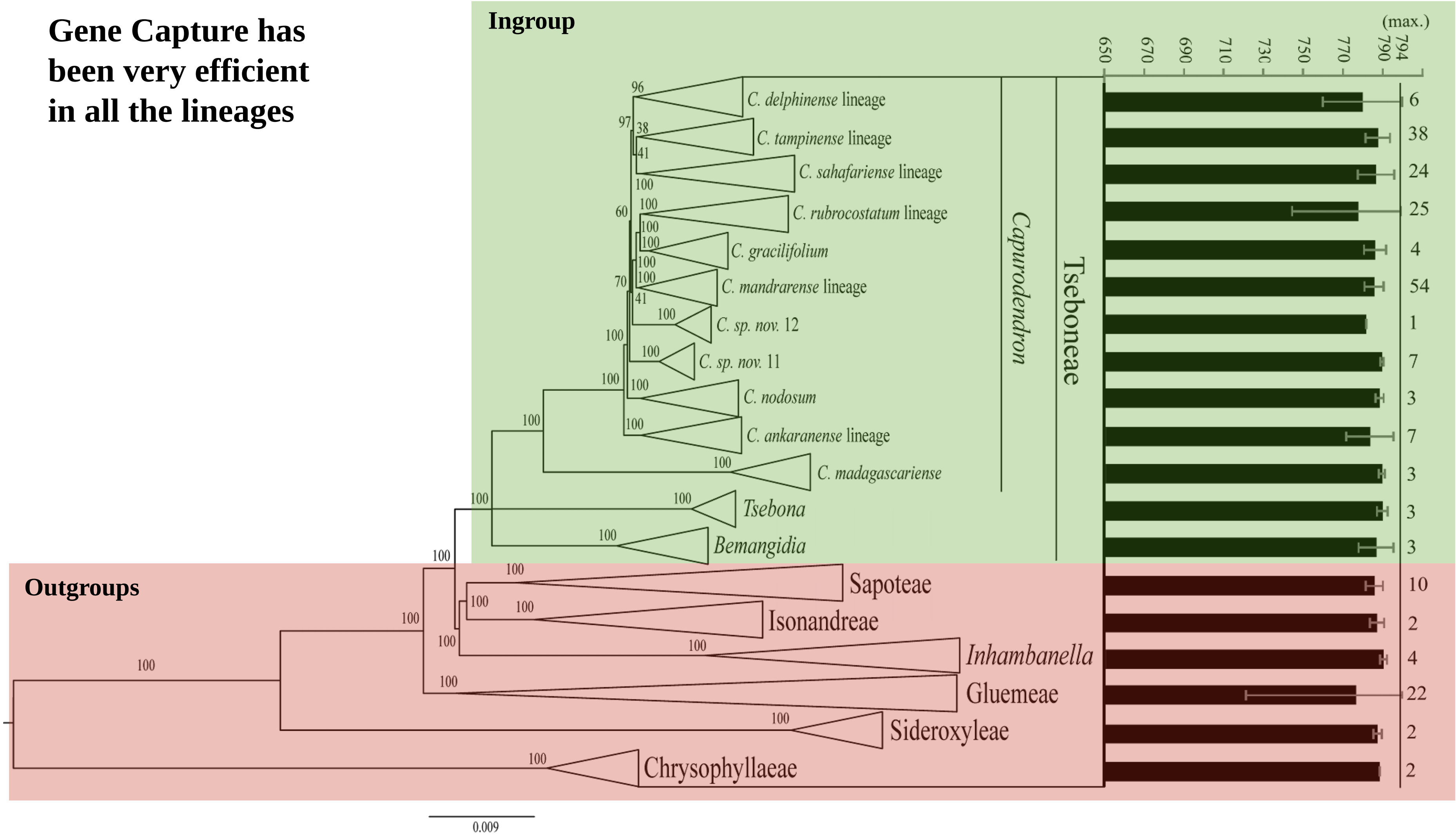


Fig.1 Maximum likelihood tree of 192192 SNPs from519 coding genes and 444 OTUs (222 specimens). The major clades have been collapsed at tribe, genus or infrageneric lineage levels. Bars in the right margin indicate the average number of captured genes per clade and their standard deviation. The number of specimens per clade is indicated at the right margin.

Results

Microsatellite Capture efficency is low → Independent of the number of reads

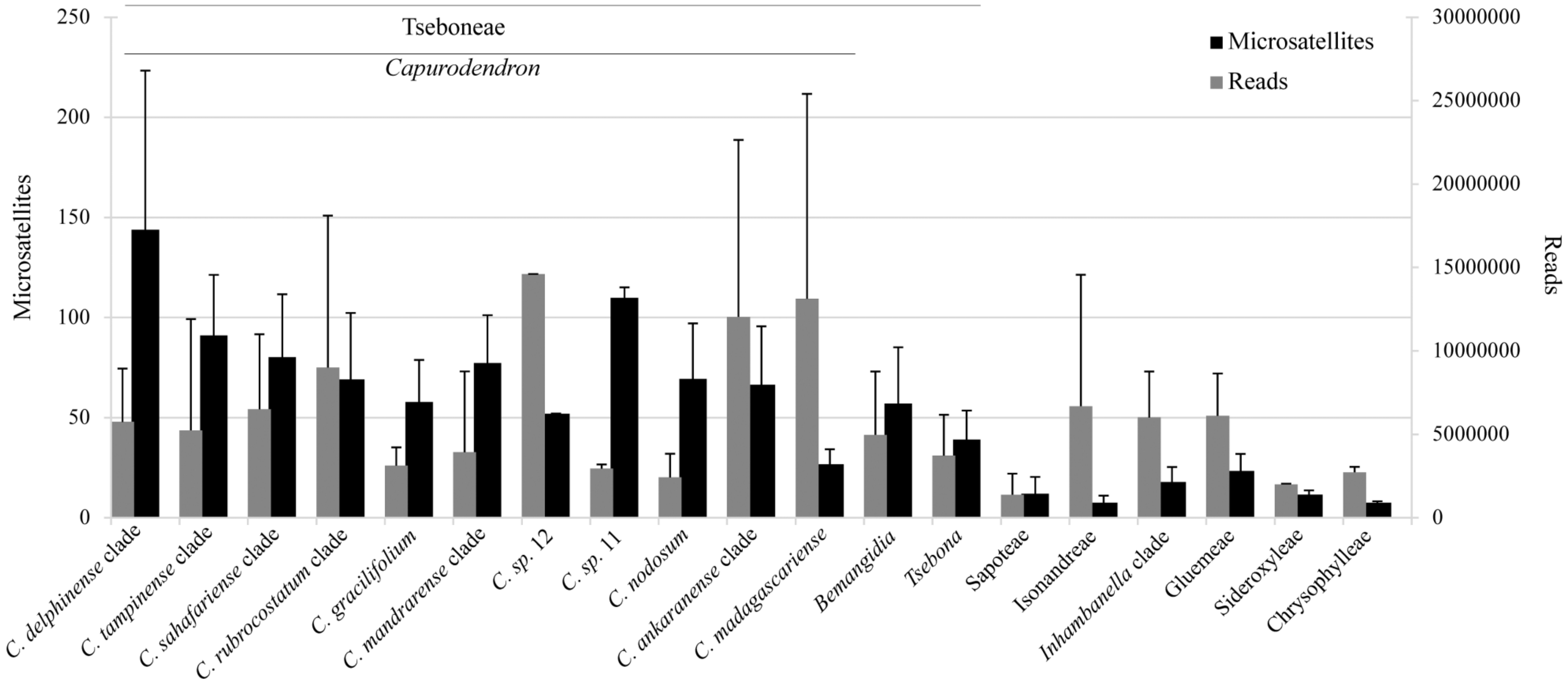


Fig. 3. Average number of captured microsatellites (black bars) and reads (gray bars) obtained for each clade. Vertical lines on bars indicate the standard deviation.

Results

Microsatellite Capture efficency is low → Strongly dependent of phylogenetic proximity

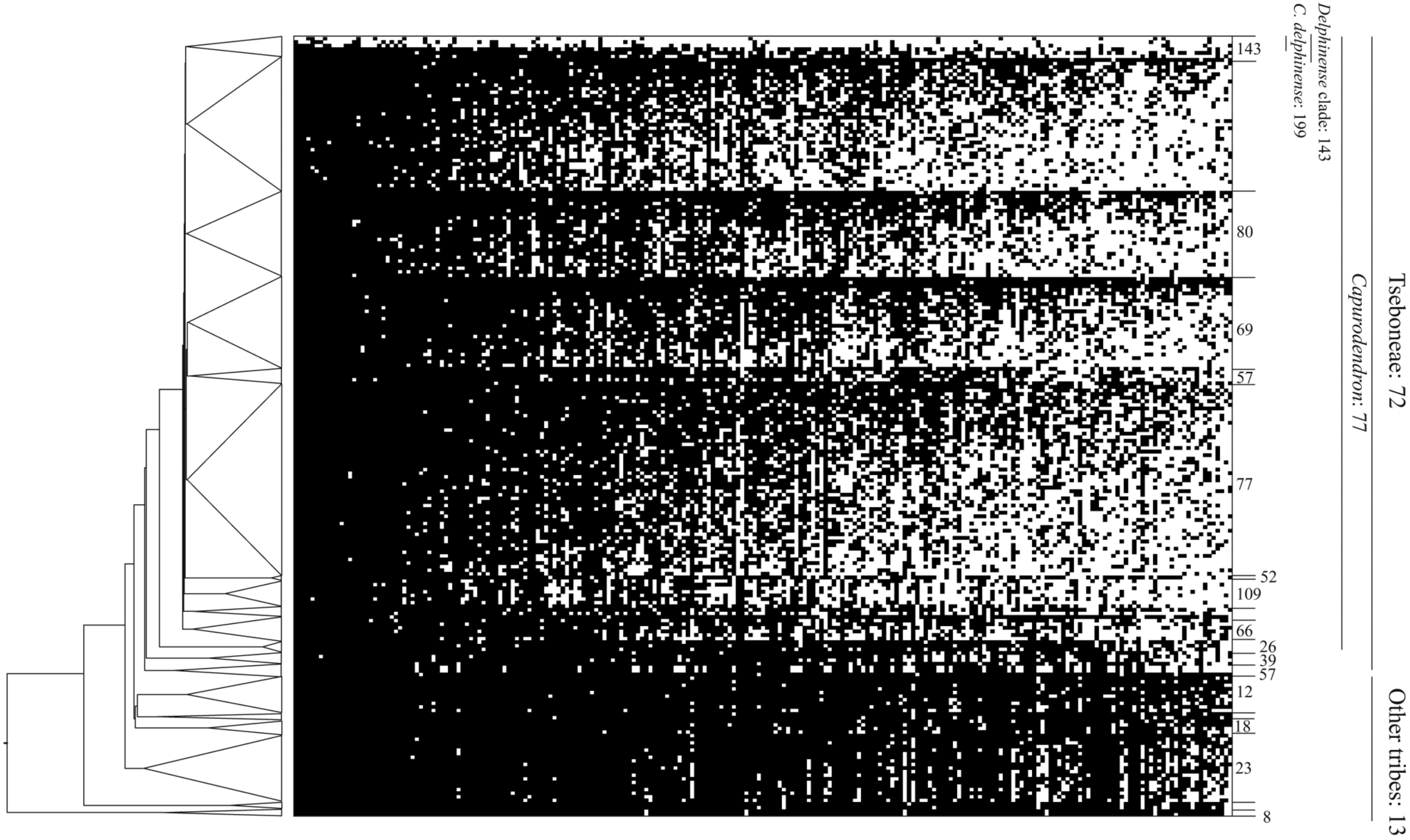


Fig. 4. Matrix showing falled microsatellites (black squares) and obtained (white squares) for each specimen and phylogenetic lineages. Numbers in the right margin indicate the average number of microsatellites succesfully obtained.

Matterials and Methods