Conservatoire et Jardin botaniques Genève

FNSNF Swiss National Science Foundation

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UNIVERSITÉ DE GENÈVE

Species delimitation in *Capurodendron* Aubrév. (Sapotaceae) using an integrative approach

Patterns of diversification in an endemic genus of Madagascar

Yamama Naciri, Carlos Galan Boluda, Camille Christe & Laurent Gautier

Context & Aims

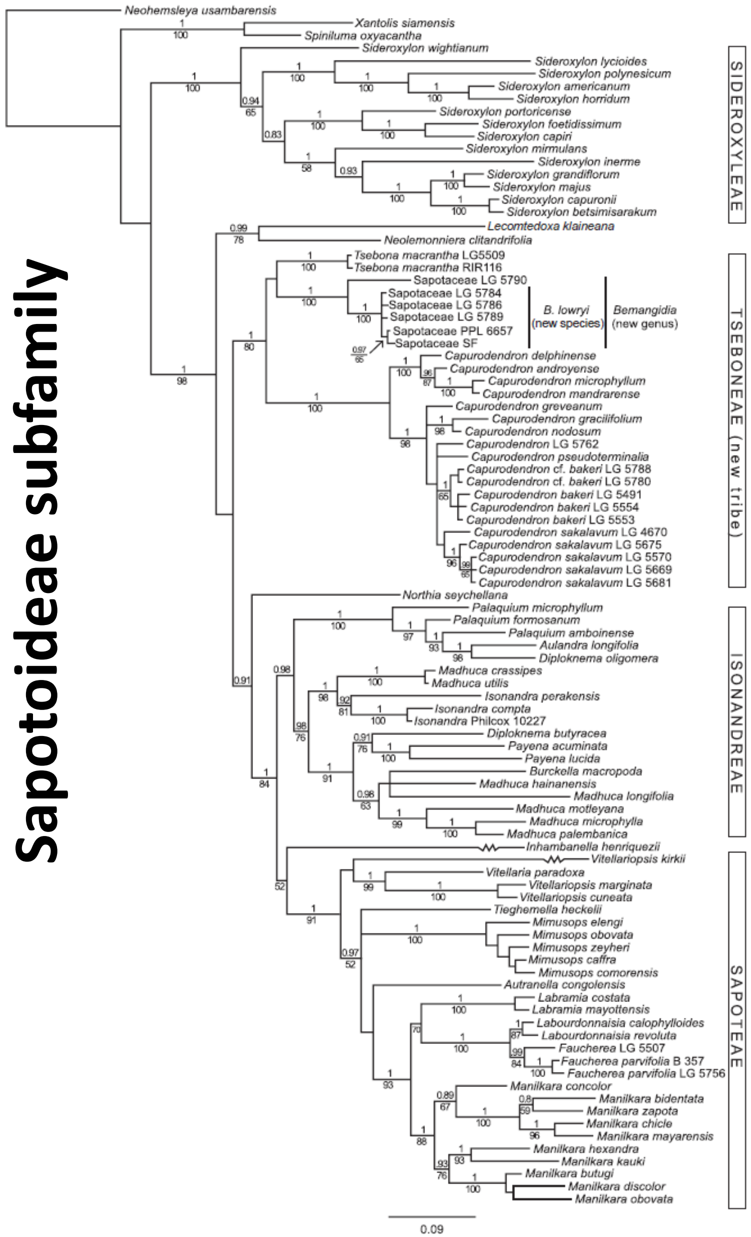
Tropical biodiversity is difficult to assess

- a difficult access to the field
- low number of individuals/species within a hectare
- scarcity of herbarium samples

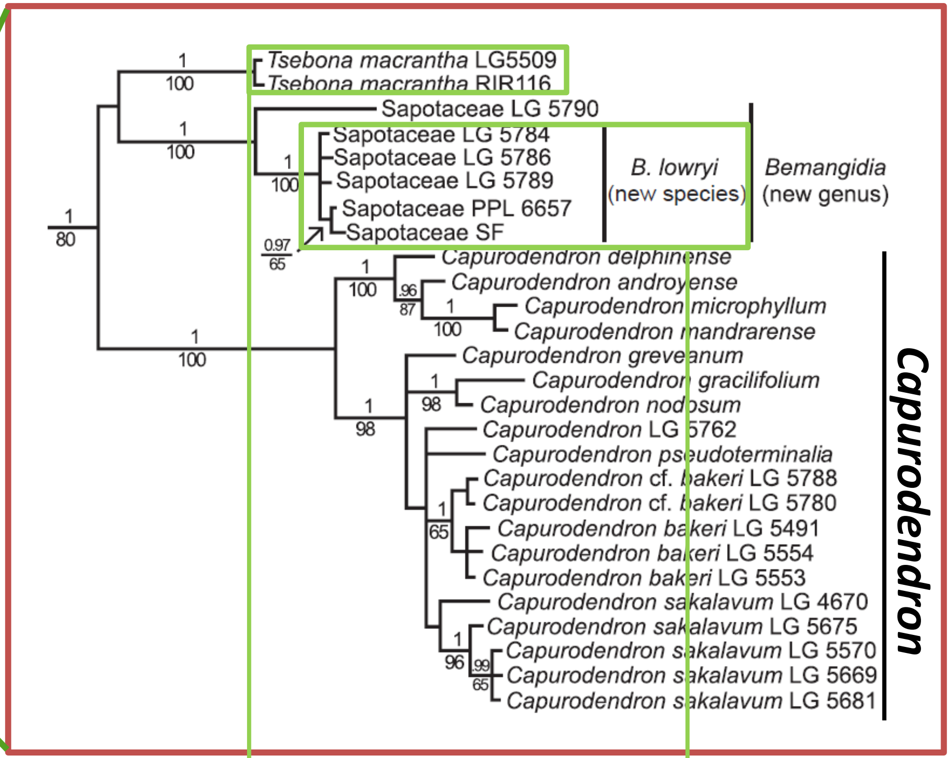
Difficult to delineate species within a particular genus

Especially the case with tree species because they are known to hybridize at higher rates than annual species

Sapotoideae subfamily



Tseboneae Tribe



Gautier, Naciri, Anderberg, Smedmark, Randrianaivo & Swenson (2013) *Taxon* 62:972–983

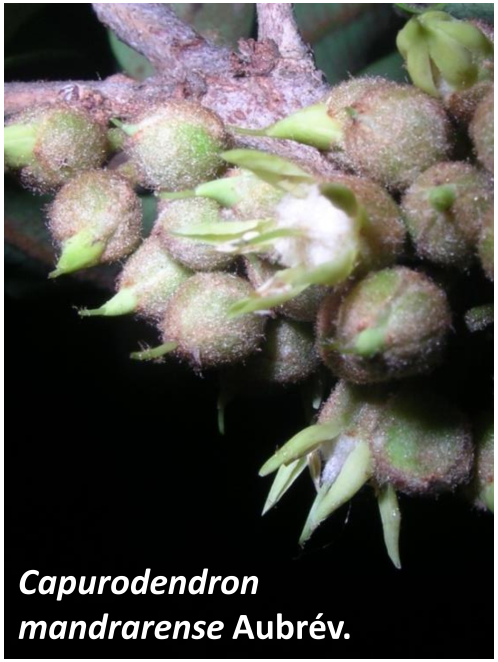
Capurodendron

5 sepals; gamopetal corolla 5-lobed, contorted; 5 stamens; 5 hairy connate staminodes closing a nectar chamber above the 5-celled ovary

Capurodendron delphinense Aubrév.

Capurodendron sakalavum Aubrév.

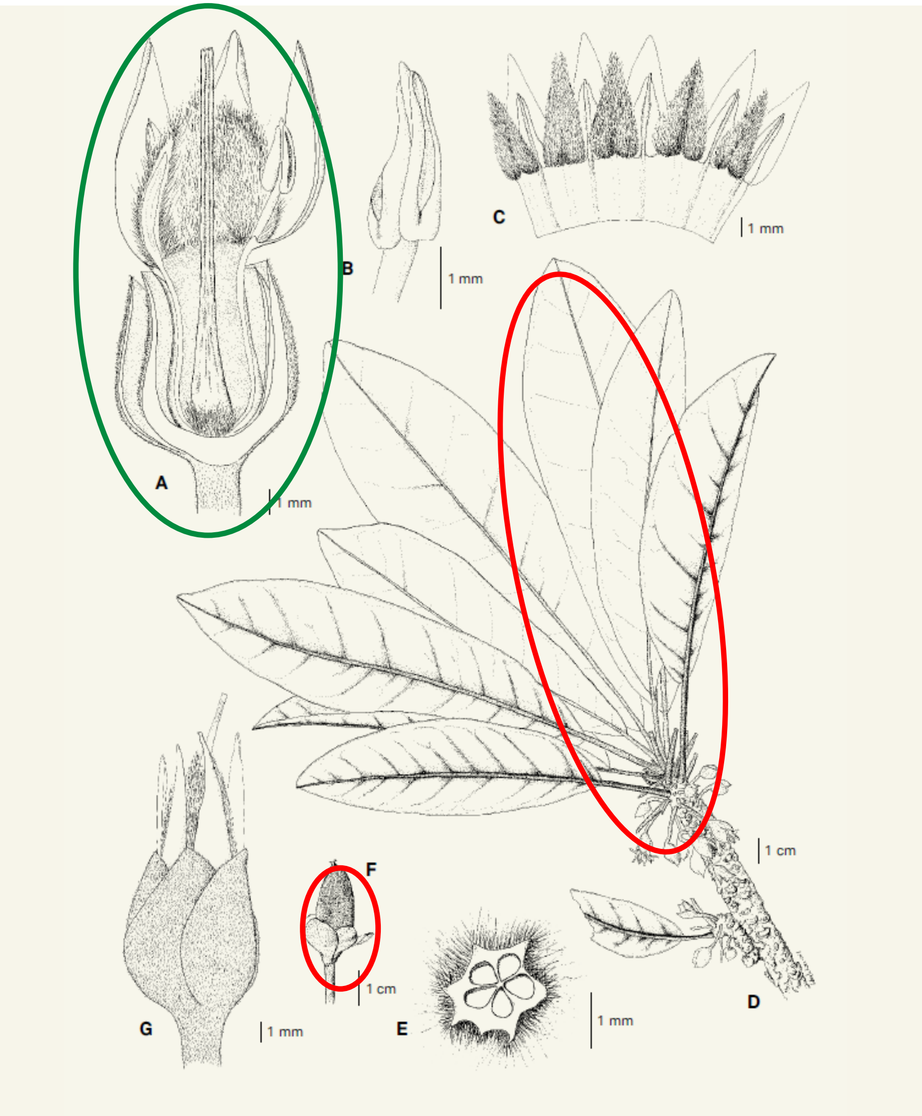
Capurodendronbakeri var. antalahense Aubrév.



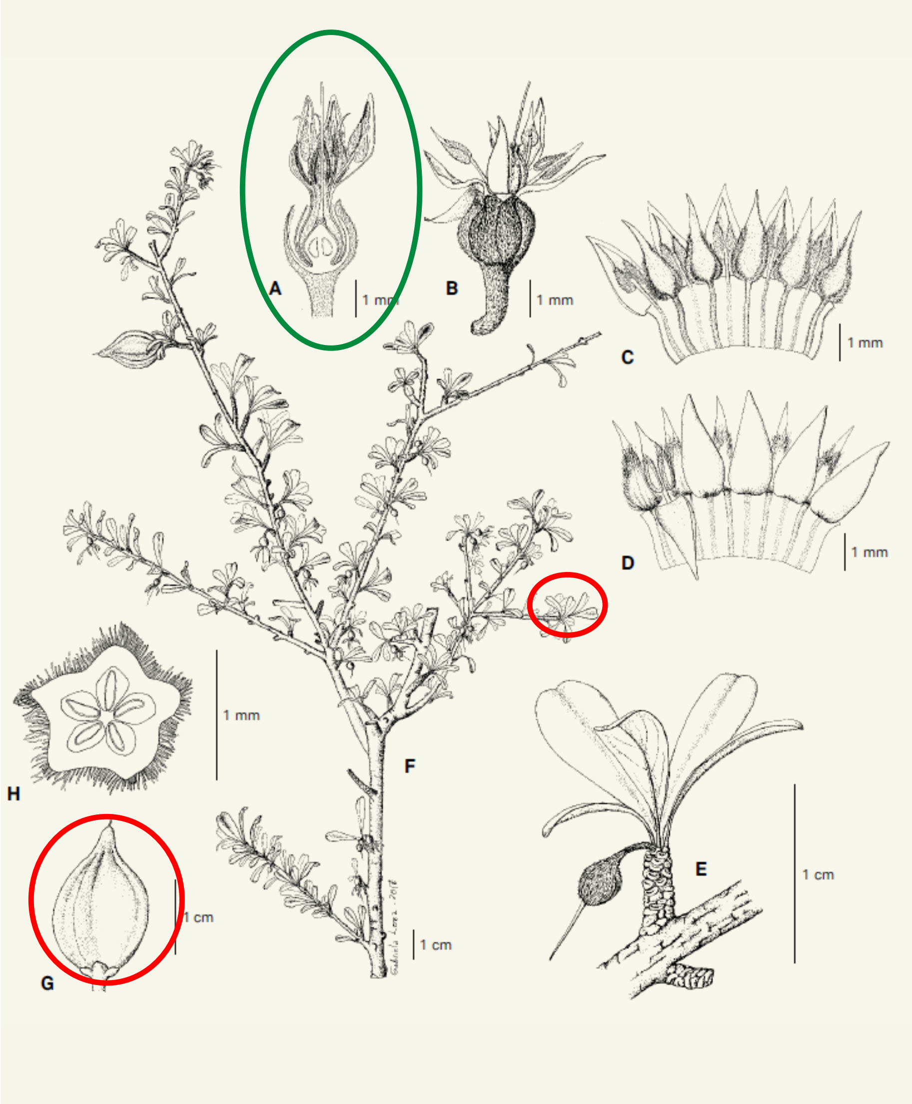
Capurodendron mandrarense Aubrév.

- Trees or shrubs
- 26 described species
- Very homogeneous flowers structure
- Differences in leaves, fruits and seeds morphology

Variability among *Capurodendron* species



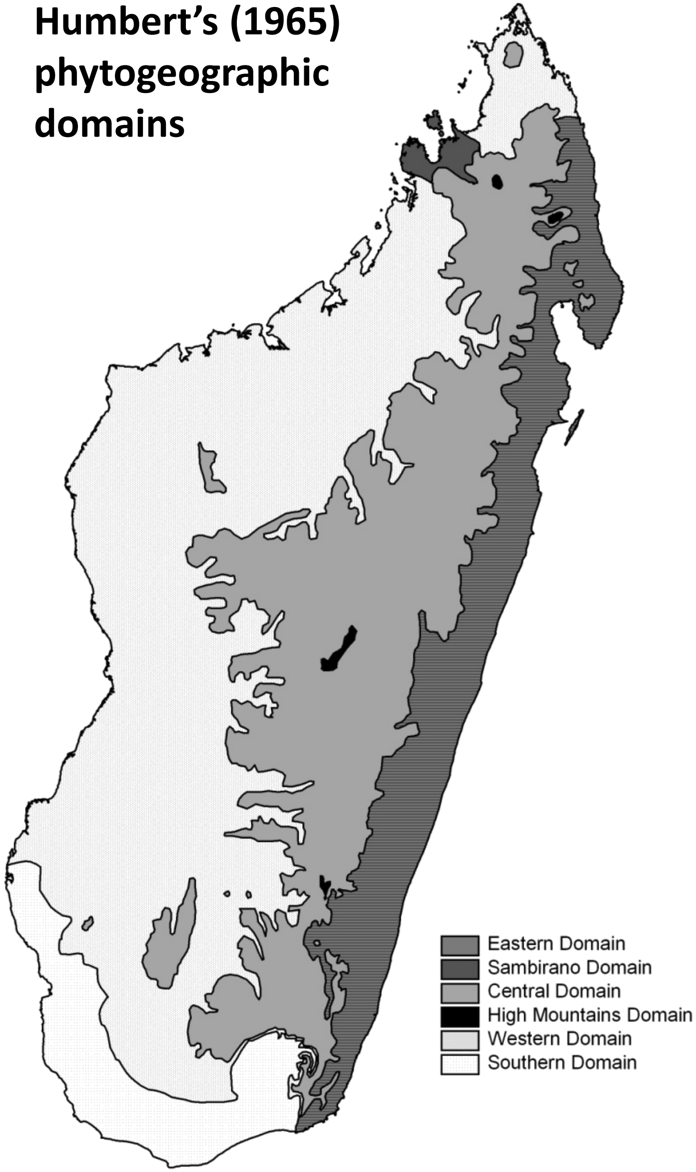
Capurodendron schatzii L. Gaut. & Naciri



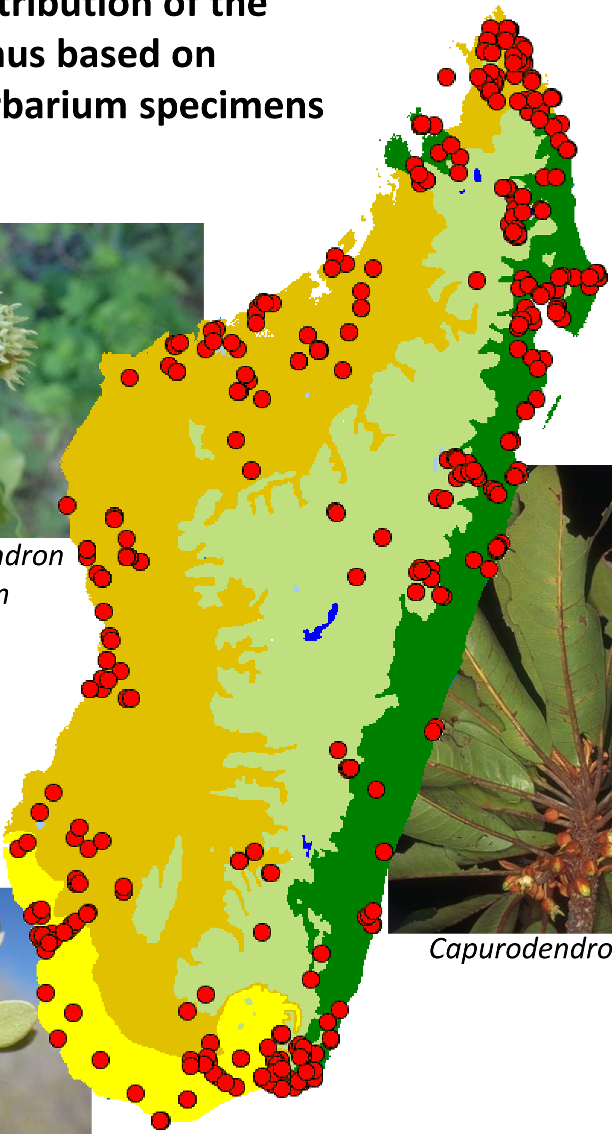
Capurodendron sahafariense L. Gaut. & Naciri

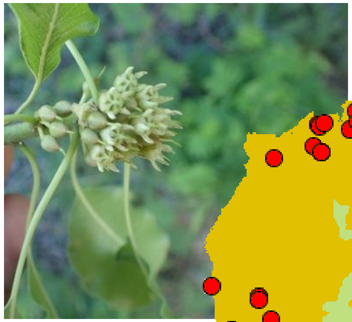
Capurodendron (Sapotaceae)

Humbert's (1965) phytogeographic domains



Distribution of the genus based on herbarium specimens





Capurodendron greveanum

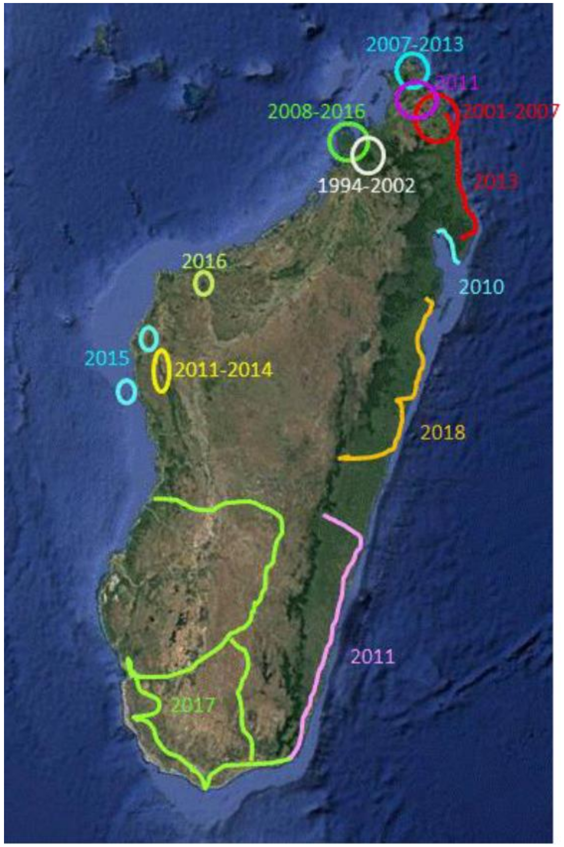
Capurodendron schatzii

Capurodendron androyense

Aims of the project

- Delimit species using morphology, anatomy and genetics
- Infer the genus history and assess its biogeography (dating)
- Infer the number of species and their known distribution using the samples at hand
- Model the species putative distributions using environmental factors (species modelling)
- Assess their threat categories according to IUCN criteria

Sampling (from the 1990ies)

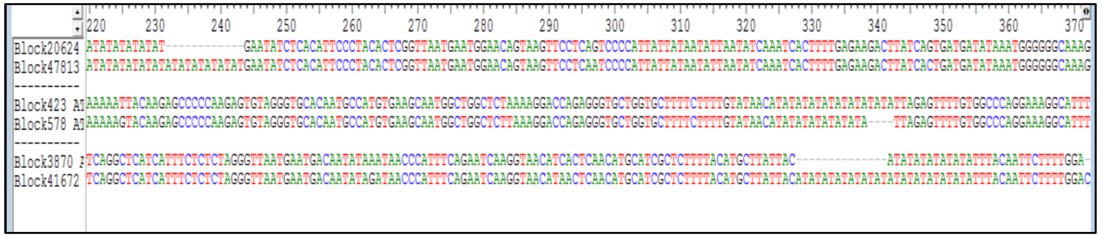


2017-2018: 178 new samples from South and East Madagascar

Targeting genes and microsatellites

We sequenced two genomes (*Capurodendron delphinense* and *Bemangidia lowryi*) and used them together with a published transcriptome (*Manilkara sapota*) to define baits targeting 1469 sequences totalling 1’034’731bp in order to fish:

- **227 microsatellite markers**, specific to *C. delphinense*



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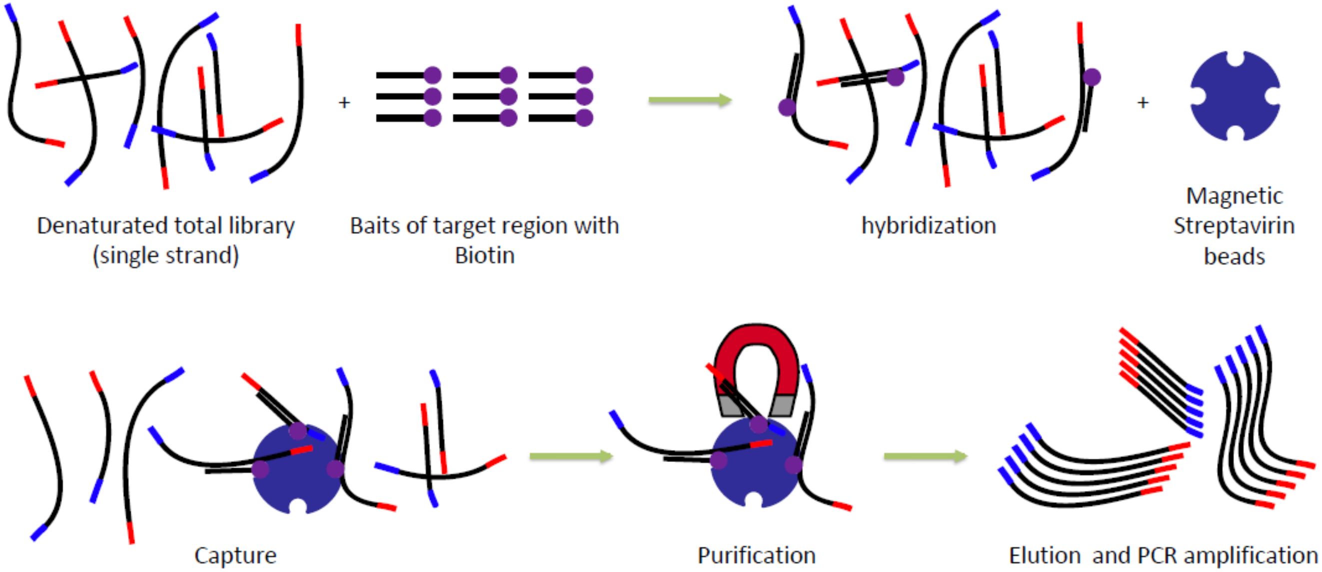
Block20624 ATATATATATAT------------GAATATCTCACATTCCCTACACTCGGTTAATGAATGGAACAGTAAGTTCCTCAGTCCCCATTATTATAATATTAATATCAAATCACTTTTGAGAAGACTTATCAGTGATGATATAAATGGGGGGCAAAG
Block47813 ATATATATATATATATATATATATGAATATCTCACATTCCCTACACTCGGTTAATGAATGGAACAGTAAGTTCCTCAATCCCCATTATTATAATATTAATATCAAATCACTTTTGAGAAGACTTATCACTGATGATATAAATGGGGGGCAAAG
----------
Block423 AT AAAAATTACAAGAGCCCCCAAGAGTGTAGGGTGCACAATGCCATGTGAAGCAATGGCTGGCTCTAAAAGGACCAGAGGGTGCTGGTGCTTTTCTTTTGTATAACATATATATATATATATATATTAGAGTTTTGTGGCCCAGGAAAGGCATTT
Block578 AT AAAAAGTACAAGAGCCCCCAAGAGTGTAGGGTGCACAATGCCATGTGAAGCAATGGCTGGCTCTTAAAGGACCAGAGGGTGCTGGTGCTTTTCTTTTGTATAACATATATATATATATA----TTAGAGTTTTGTGGCCCAGGAAAGGCATTT
----------
Block3870 A TCAGGCTCATCATTTCTCTCTAGGGTTAATGAATGACAATATAAATAACCCATTTCAGAATCAAGGTAACATCACTCAACATGCATCGCTCTTTTACATGCTTATTAC--------------ATATATATATATATTTACAATTCTTTTGGA-
Block41672 TCAGGCTCATCATTTCTCTCTAGGGTTAATGAATGACAATATAGATAACCCATTTCAGAATCAAGGTAACATAACTCAACATGCATCGCTCTTTTACATGCTTATTACATATATATATATATATATATATATATATATTTACAATTCTTTTGGAC
```

- **532 monocopy genes** (753 exons) specific to the Tseboneae tribe.
- **262 monocopy genes** (262 exons) from a pool suggested by Johnson & al. (2018) for the entire Angiosperm group.

Libraries and sequence capture

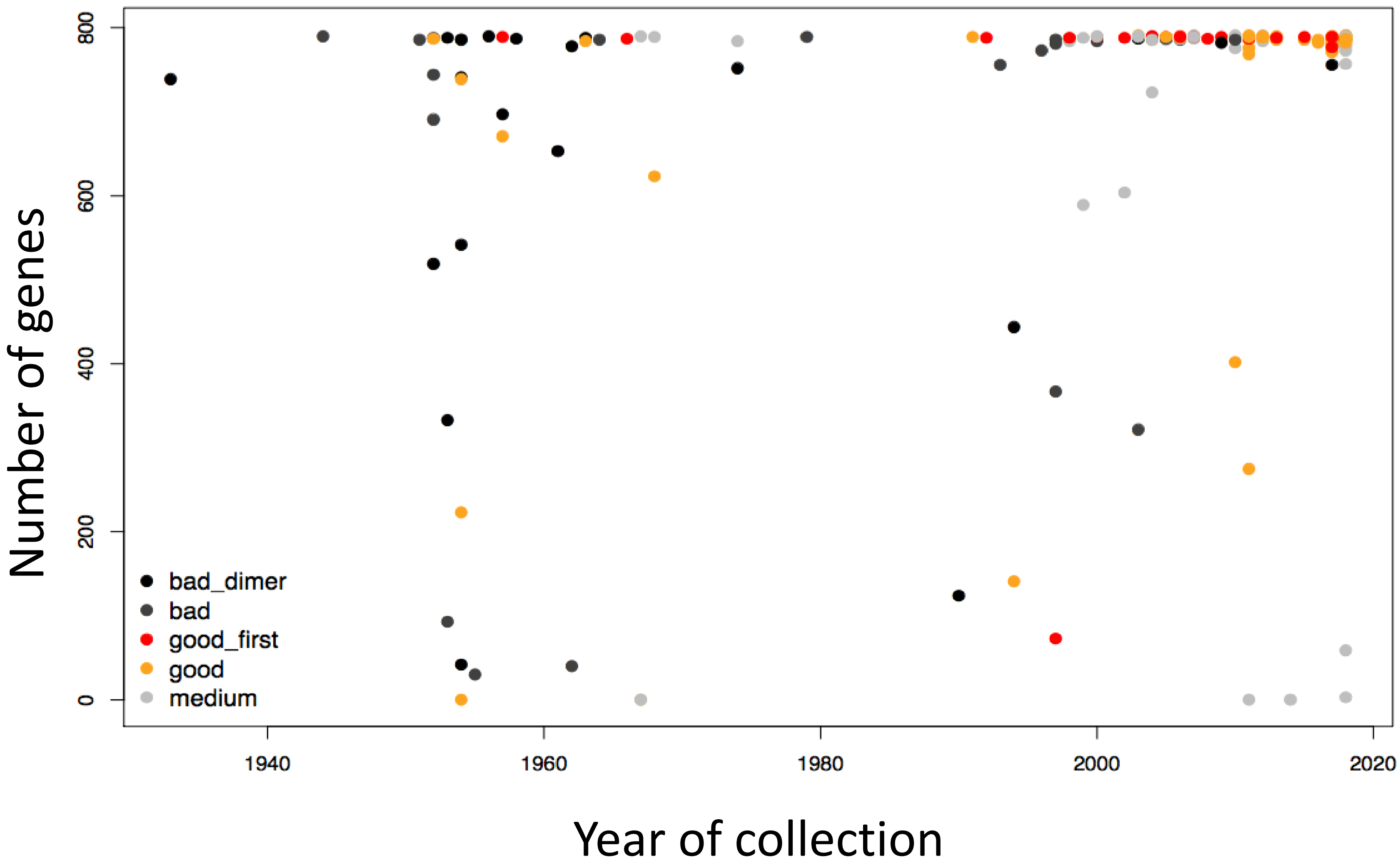
- 281 libraries for Illumina sequencing with fragments of 400bp on herbarium specimens and silagel dried leaves
- Gene capture using streptavidin beads



Picture by Romain Dewaele

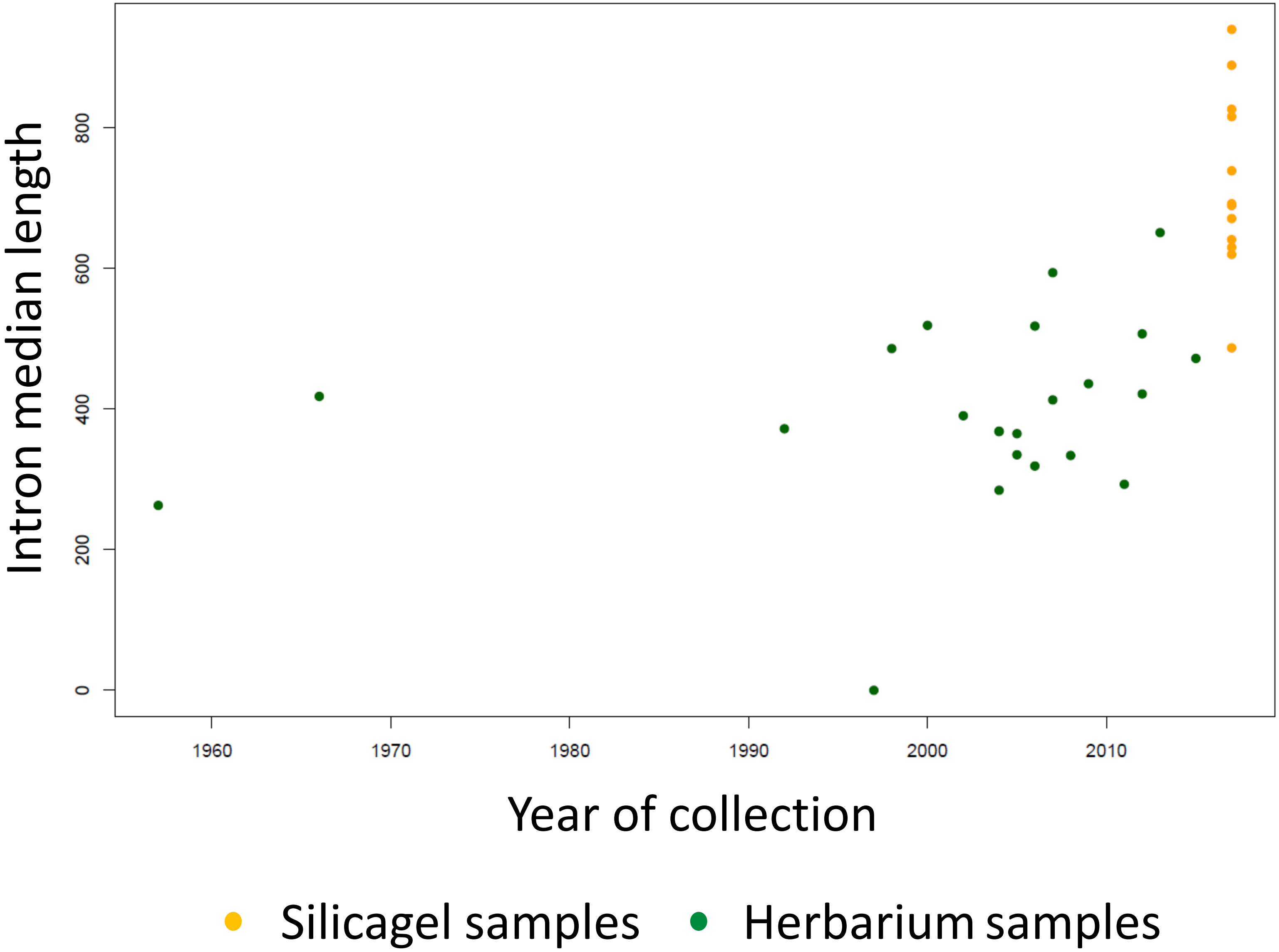
- Multiplexing in 5 different pools according to DNA quality and quantity

Gene capture efficiency: quality not age

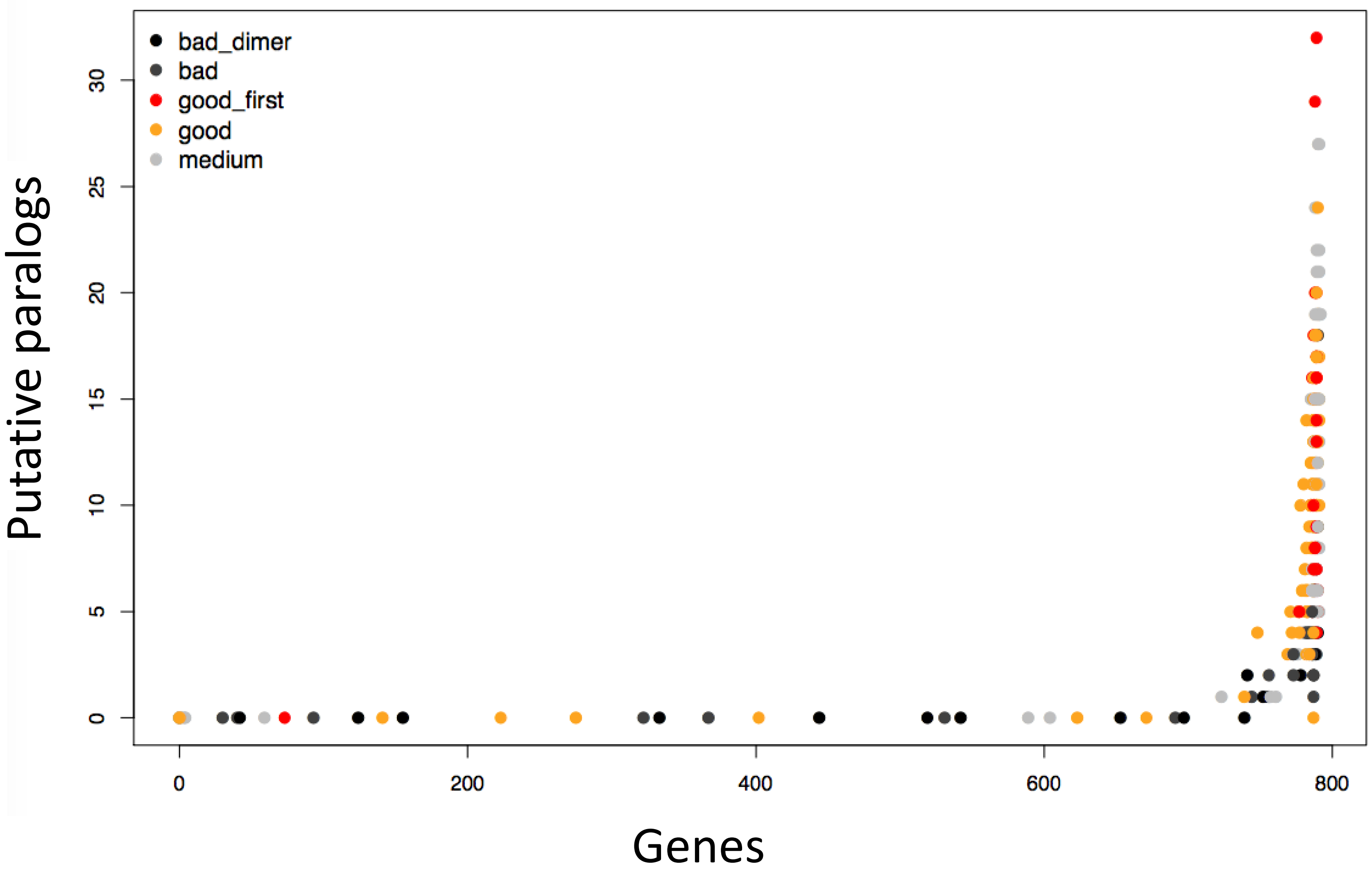


Problems: too fragmented DNA, latex-related products that bind with DNA in some samples

Gene capture efficiency: exons and introns



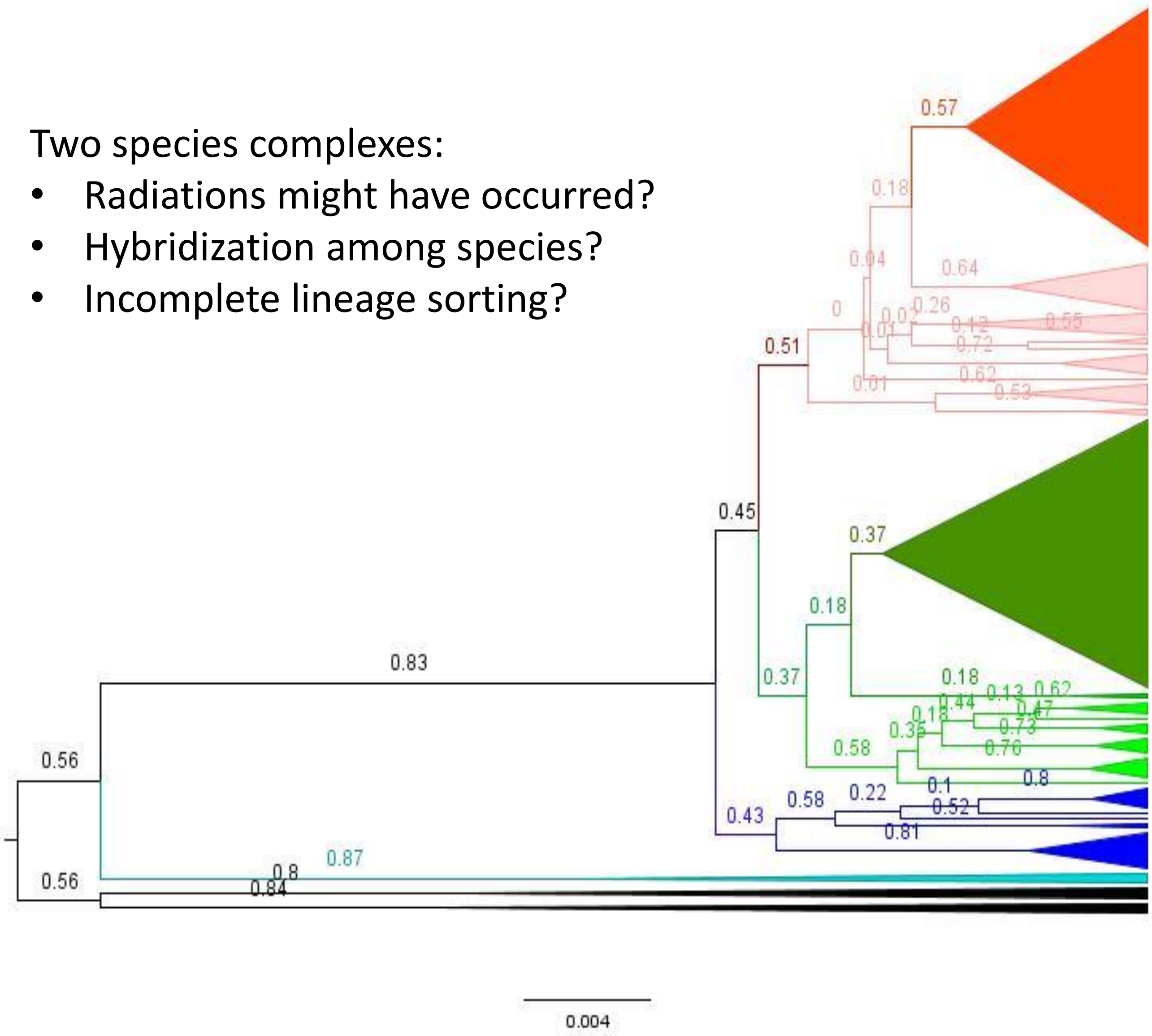
Gene capture efficiency: putative paralogs



Species delimitation using sequence data

Two species complexes:

- Radiations might have occurred?
- Hybridization among species?
- Incomplete lineage sorting?



Arid complex

C. mandrarense, C. androyense

C. microphyllum, C. nanophyllum

C. perrieri

C. rubrocostatum

C. pervillei

C. greveanum

Eastern complex

C. tampinense, C. bakeri, C. ludifollium,

C. delphinense, C. sakalavum

C. costatum

C. sahafariense

C. suarense

C. ankaranense

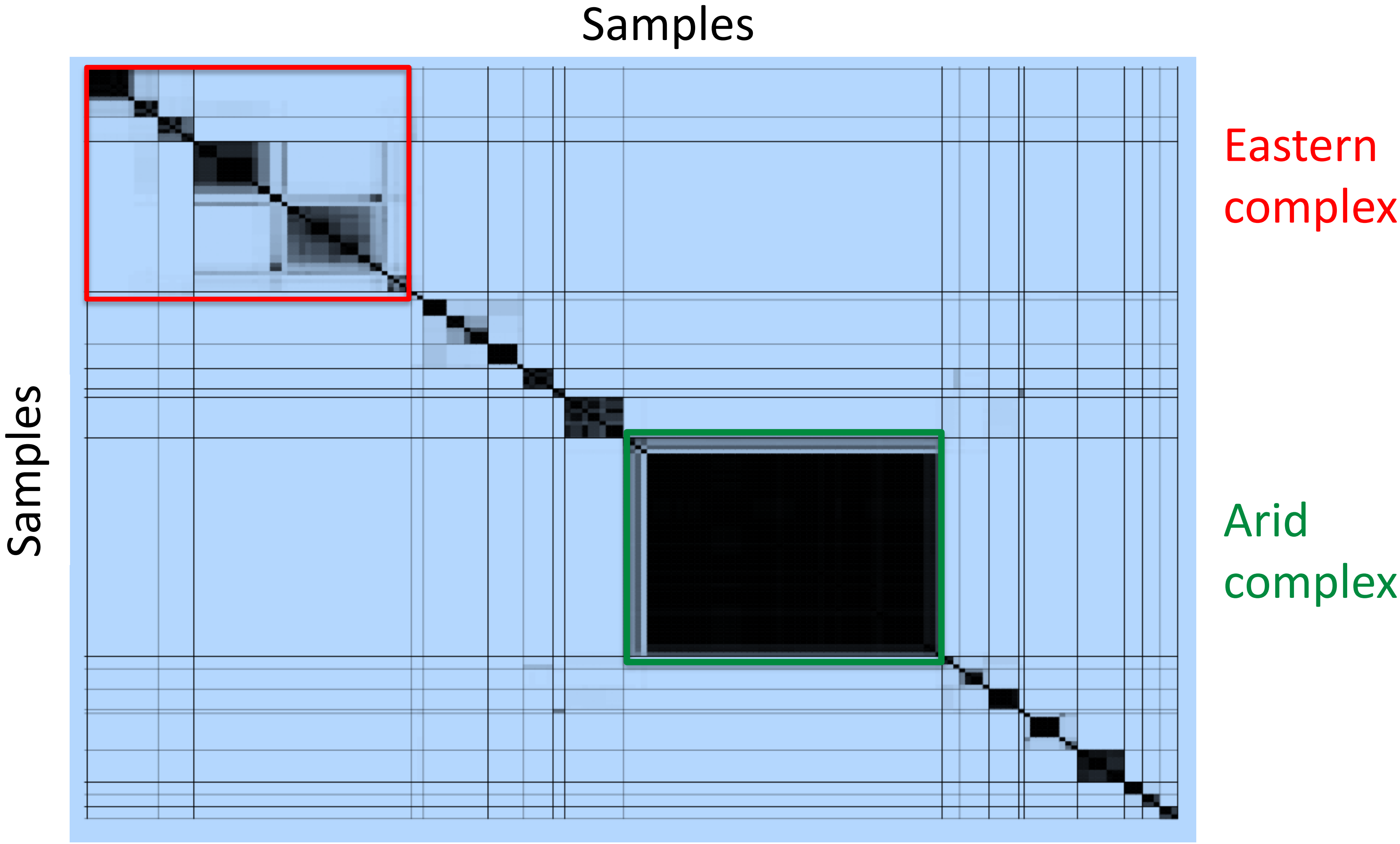
C. sp 11

C. madagascariense

Bemangidia & Tsebona

Multi-Species coalescent model, 2 curated genes, 8400bp

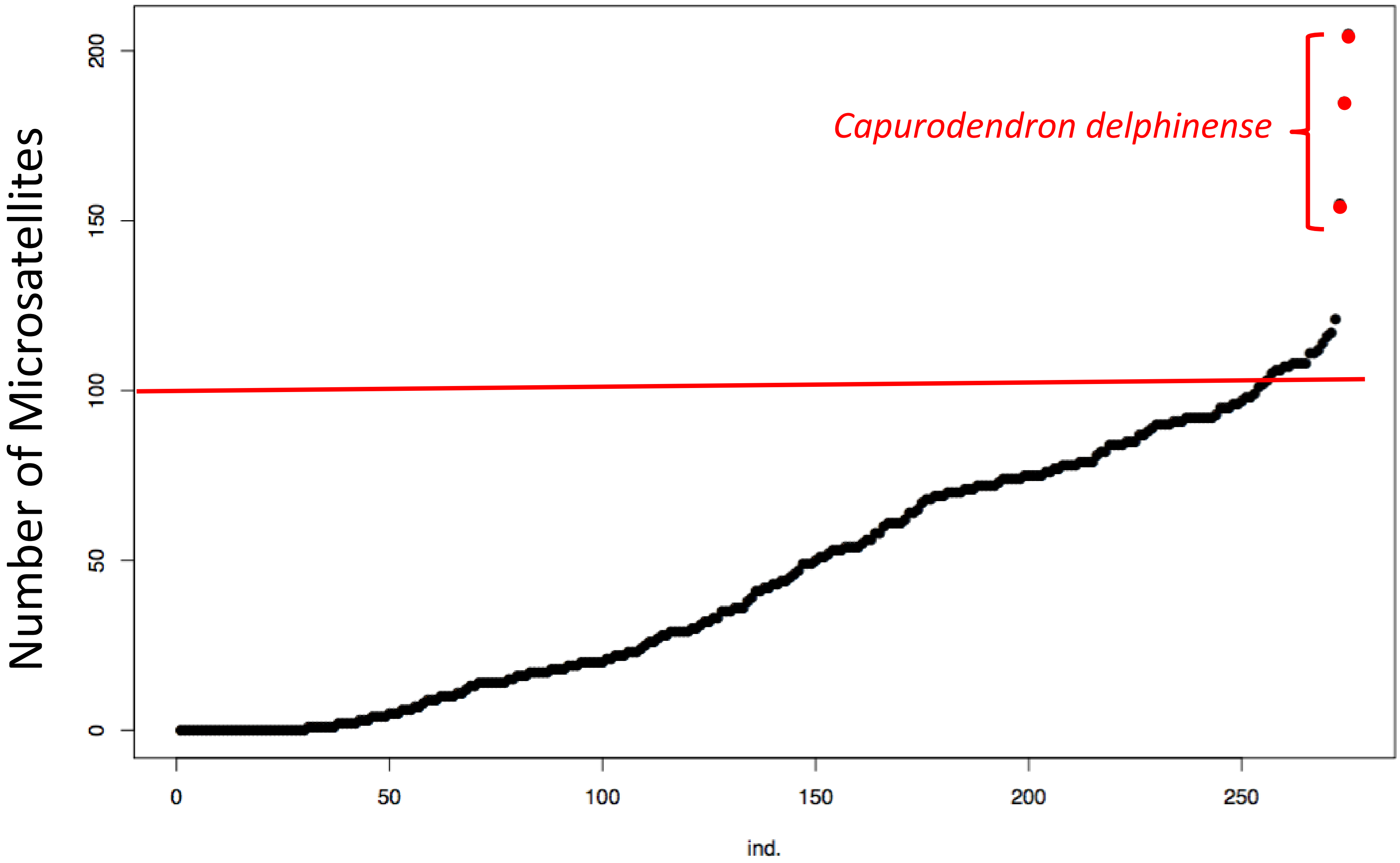
Species delimitation using sequence data



Similarity matrix

Black squares = very high probability of sample pairs to cluster together

Microsatellite capture efficiency

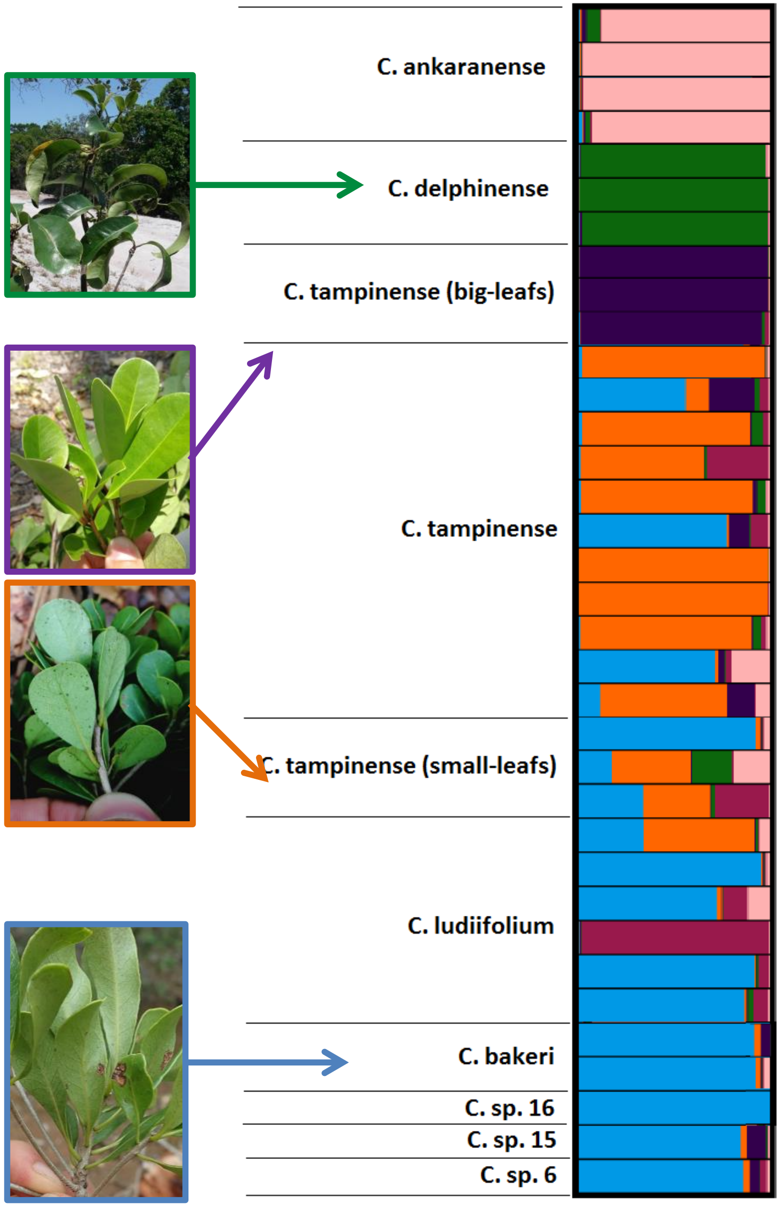


Species delimitation using microsatellite data

Using 30 microsatellites and Bayesian analyses:

- Only one group was found so far in the Arid complex despite contrasting morphologies

- Different putative species in the Eastern complex with high levels of admixture



Conclusions

- Gene capture works well with herbarium specimens
- Microsatellites might not be as useful as expected
- Some species are well delimited while others are not. Hybridization or incomplete lineage sorting seem to be pervasive
- At least two (recent?) radiations are suspected
- Much more species than initially thought
- Very preliminary results that need to be confirmed

Thank you for your attention


Carlos Boluda


Camille Christe


Laurent Gautier

Richard Randrianaivo

Aina Randriarisoa

Funding bodies

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New species described by scientists working in Switzerland

Capurodendron sahafariense L. Gaut. & Naciri

SWISS
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Species of the year 2019

Gautier & Naciri (2018) Three Critically Endangered new species of *Capurodendron* (Sapotaceae) from Madagascar. *Candollea* 73(1):121-129

Future directions

- Curate the genes (not all genes will be usable because of missing data, paralogous sequences, highly conserved sequences, etc…)
- Obtain a robust phylogeny and test biogeographic hypotheses
- Microsatellites might be usable, but in the Eastern complex only
- Model species distributions & assign conservation status