SNPs based timber tracking tools for African mahogany (*Khaya sp.*)

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OECD workshop "*Application of high throughput genotyping technologies for forest tree species identification and timber tracking*"

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African mahogany (*Khaya* sp.) species delimitation issues

- The online database CJBG
  - *K. grandifoliola, K. ivorensis, K. anthotheca*, *K. madagascariensis, K. nyasica*
  - *Khaya nyasica* & *K. anthotheca* as synonyms
- After Styles & White (1991)
  - *K. anthotheca* and *K. ivorensis* would be conspecific
  - *K. anthotheca* sensu lato might contain several species morphologically very similar

K. senegalensis *K. ivorensis* *K. anthotheca* or *K. nyasica* *K. grandifoliola*

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Overexploitation and illegal logging

"Threatened" or "Vulnerable"
(IUCN Redlist)

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Objectives

1. To develop a method for accurate species identification for African mahogany species
2. To generate genetic reference map to identify the country of origin
   - *Khaya senegalensis*
   - *Khaya grandifoliola*
   - *Khaya anthotheca*
   - *Khaya ivorensis*

NB: Only results for *K. senegalensis* & *K. grandifoliola* will be presented

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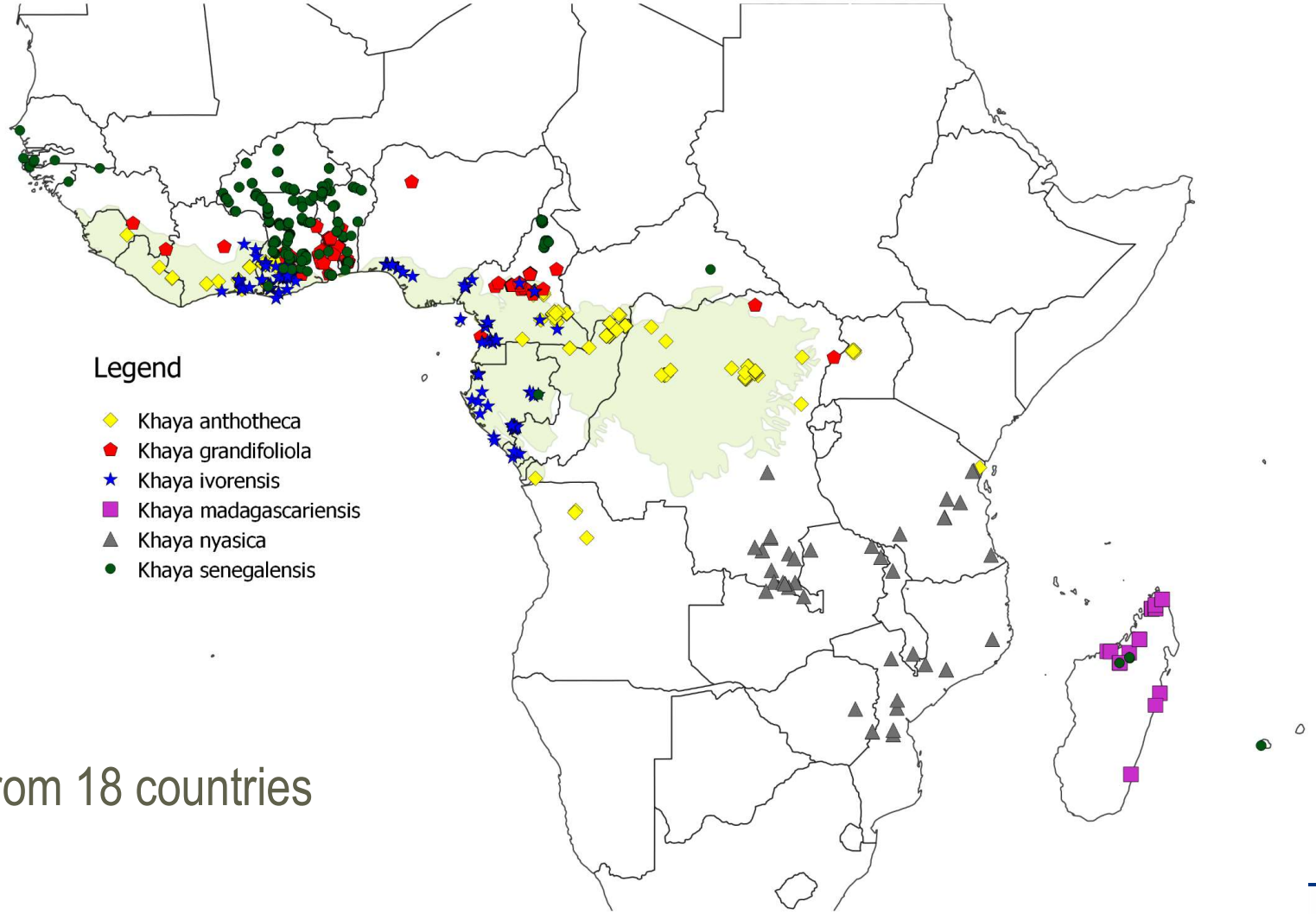
Species identification of African mahogany

Sampling



2,222 samples from 18 countries

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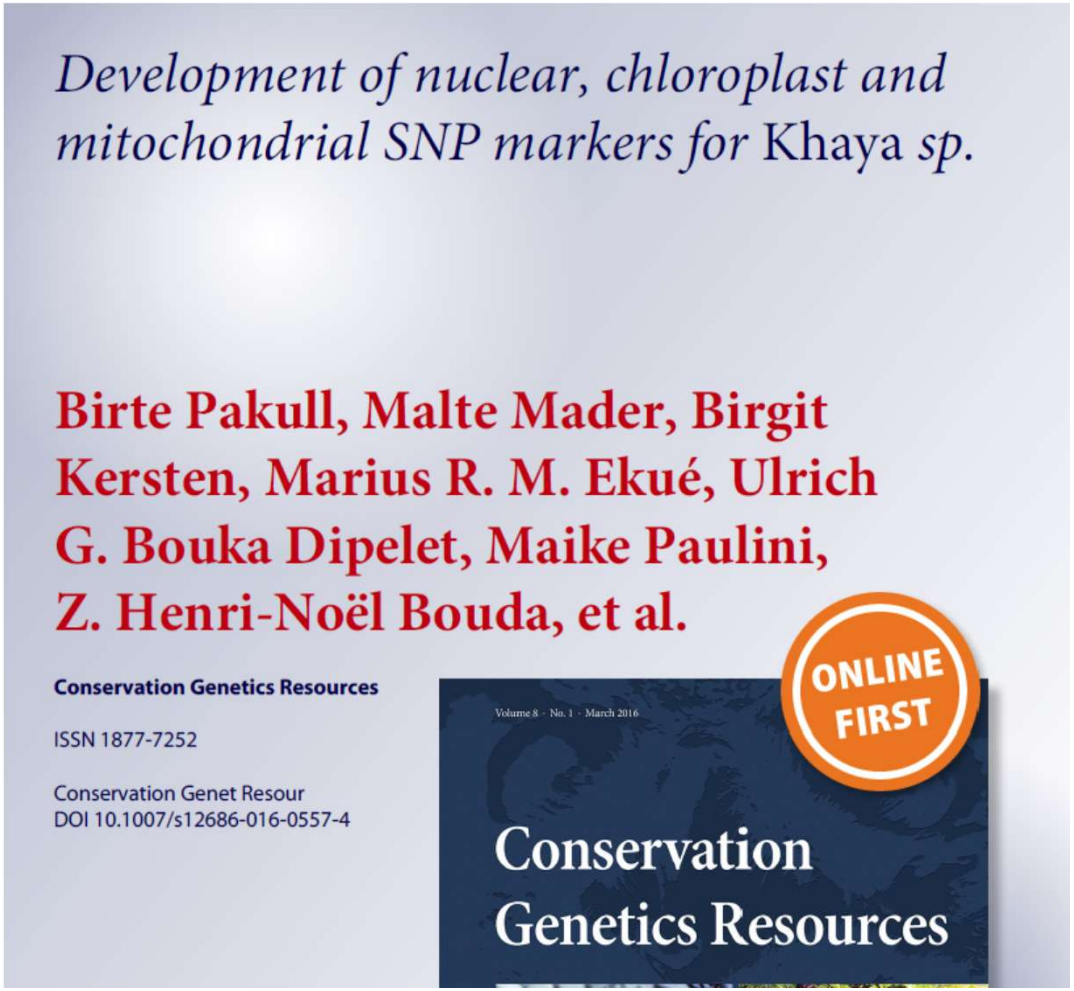
Gene marker development



- SNP identification was based on a combination of restriction associated DNA sequencing (RADseq) (Miller et al. 2007) for the identification of nuclear SNPs and low coverage MiSeq genome sequencing for the identification of chloroplast and mitochondrial SNPs (Straub et al. 2012).
- 101 SNP/Indel markers:
  - 67 nuclear SNPs
  - 11 chloroplast SNPs + 1 Indel
  - 22 mitochondrial SNPs

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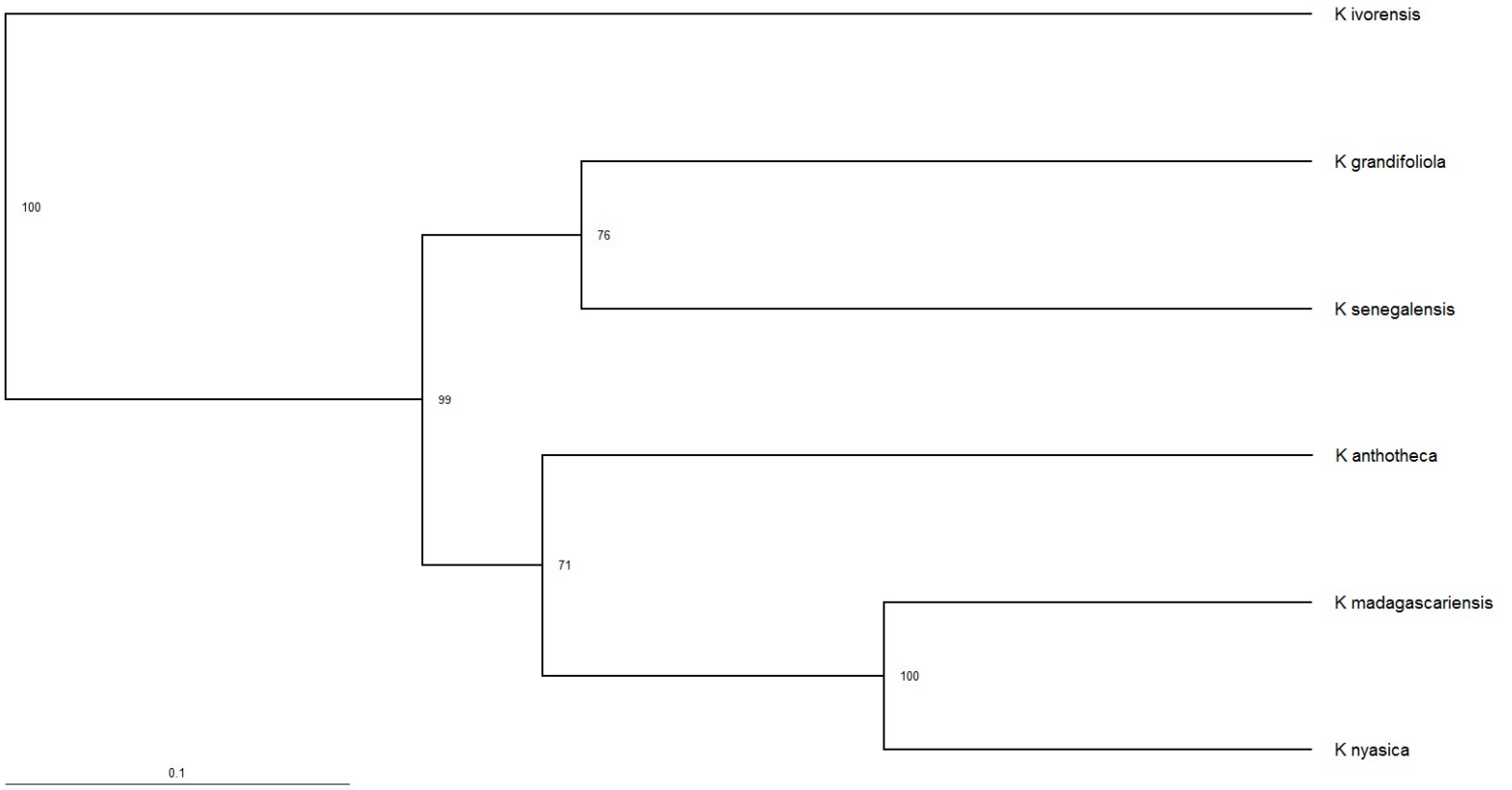
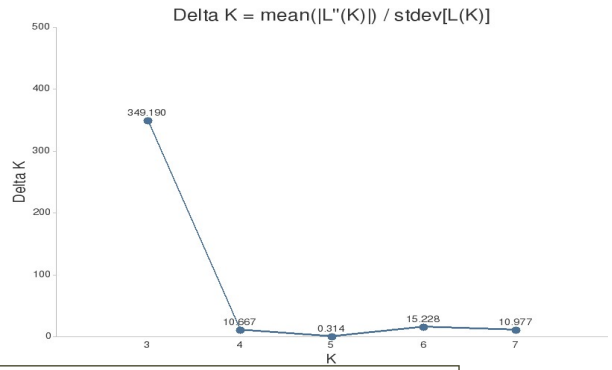
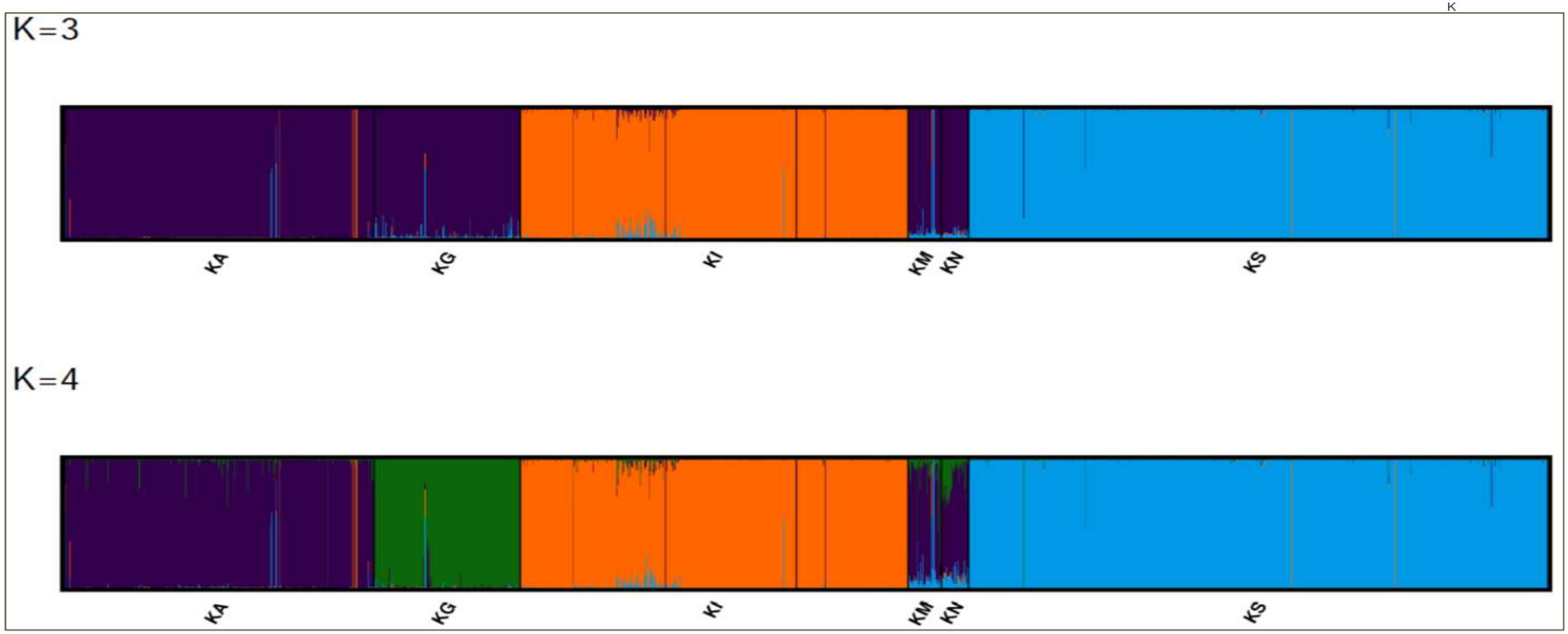
Genetic differences among *Khaya* species (all SNPs = 101; N = 2,222)



Delta = 0.26 / F_{ST} = 0.47

STRUCTURE (67 nSNPs, N= 2,222)





KA= *K. anthotheca*, KG= *K. grandifoliola*, KI= *K. ivorensis*, KM= *K. madagascariensis*, KN= *K. nyasica*, KS= *K. senegalensis*

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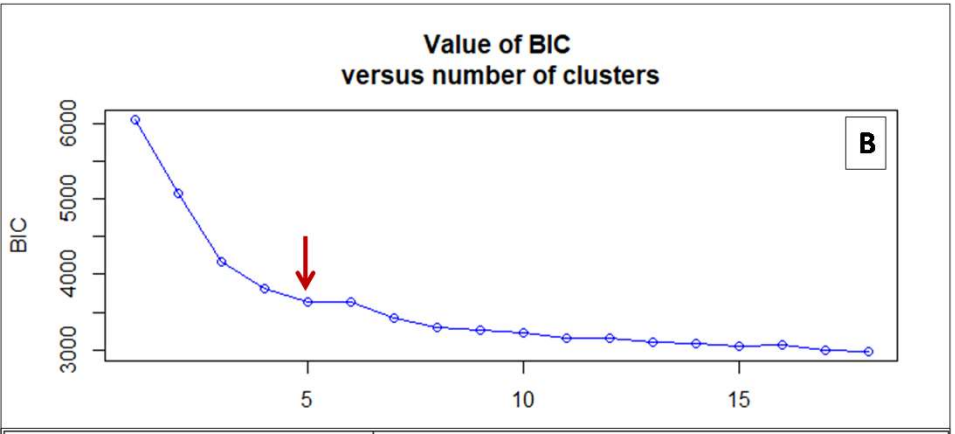
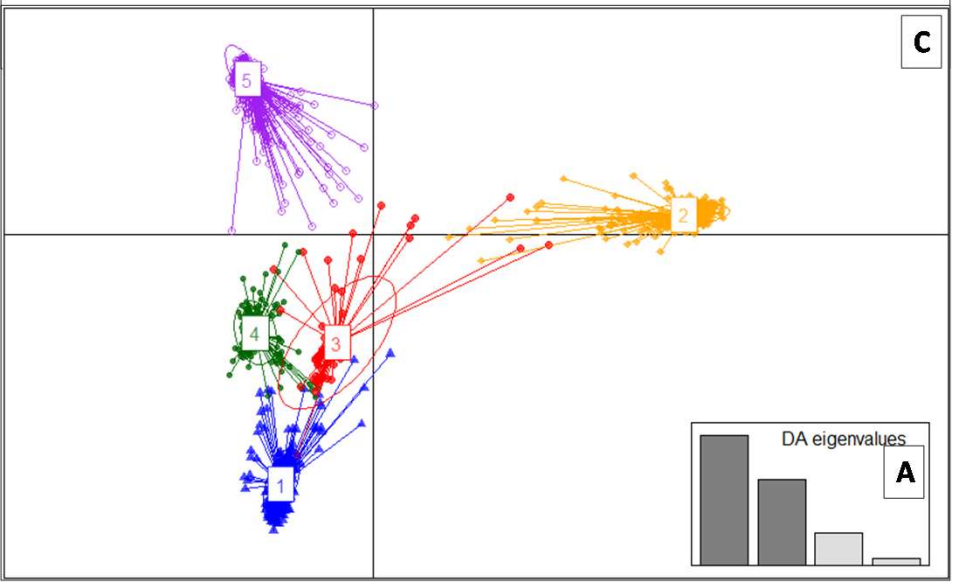
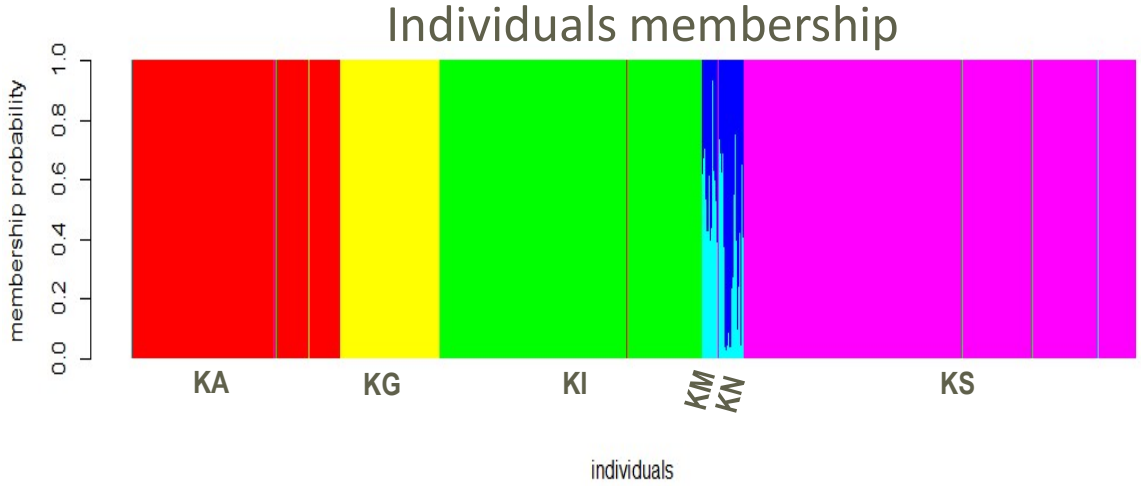
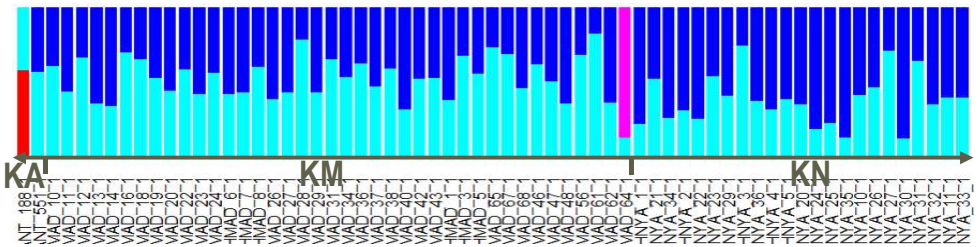
Discriminant Analysis of Principal Component (DAPC) (67 nSNPs, N=2,222)







65 admixed individuals mostly *K. madagascariensis* and *K. nyasica*



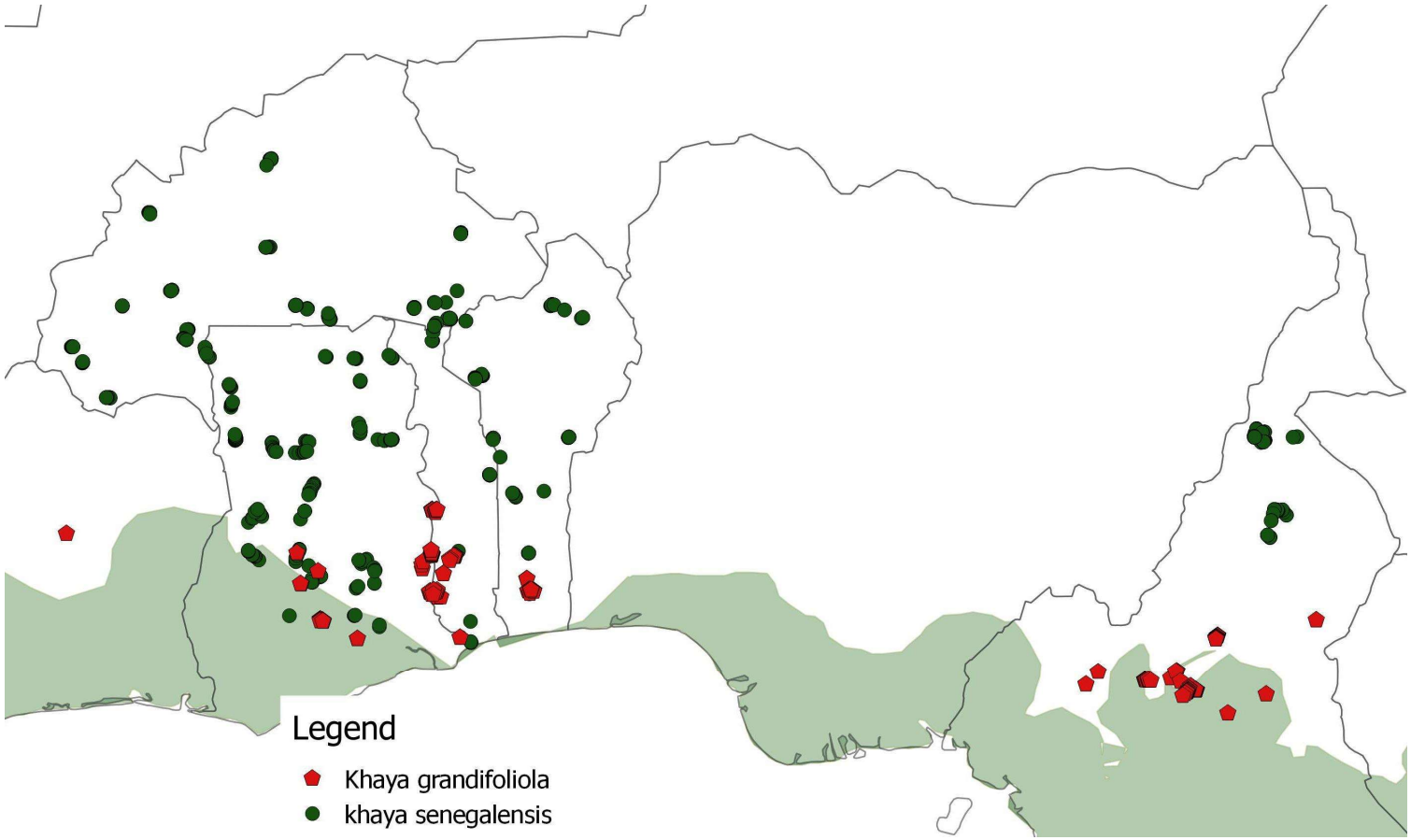
Self-assignment to species

Species	Percentage of correct self-assignment		
	Bayesian method		DAPC
	101 SNPs	15 golden SNPs	76 SNPs
K. anthotheca	97	97	97.8
K. grandifoliola	98	100	99.5
K. ivorensis	99	99	99.1
K. madagascariensis	92	12	89.1
K. nyasica	61	98	70.7
K. senegalensis	100	99	99.5

Genetic reference data to identify the country of origin

- Dry zone mahogany (*K. senegalensis*)
- Broad leaf mahogany (*K. grandifoliola*)

Samples used and countries



Ghana, Togo, Burkina Faso, Benin and Cameroon

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Khaya senegalensis

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Photo: P. Poilecot, CIRAD

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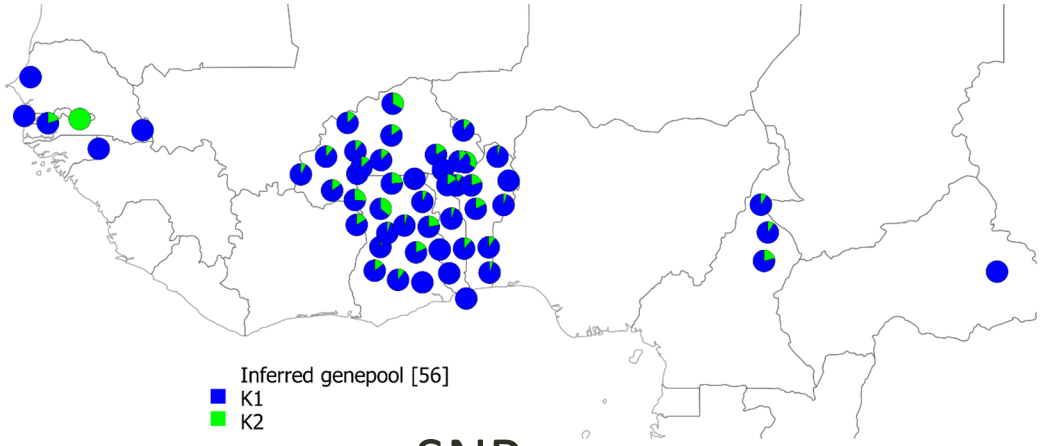
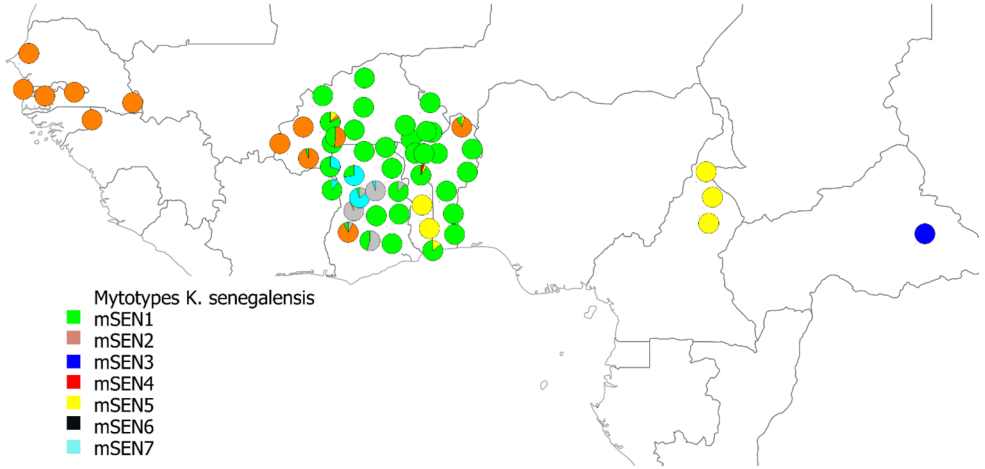
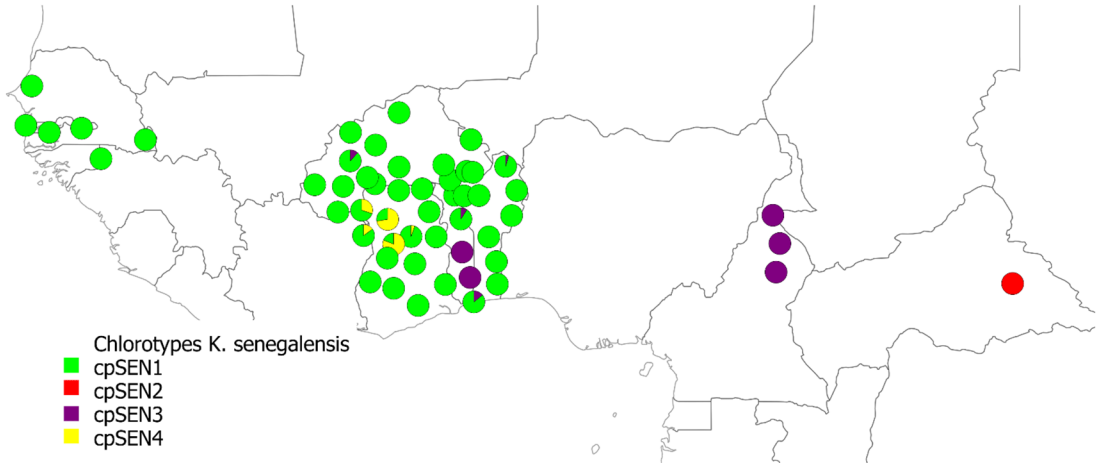


nSNPs



mSNPs



cpSNPs

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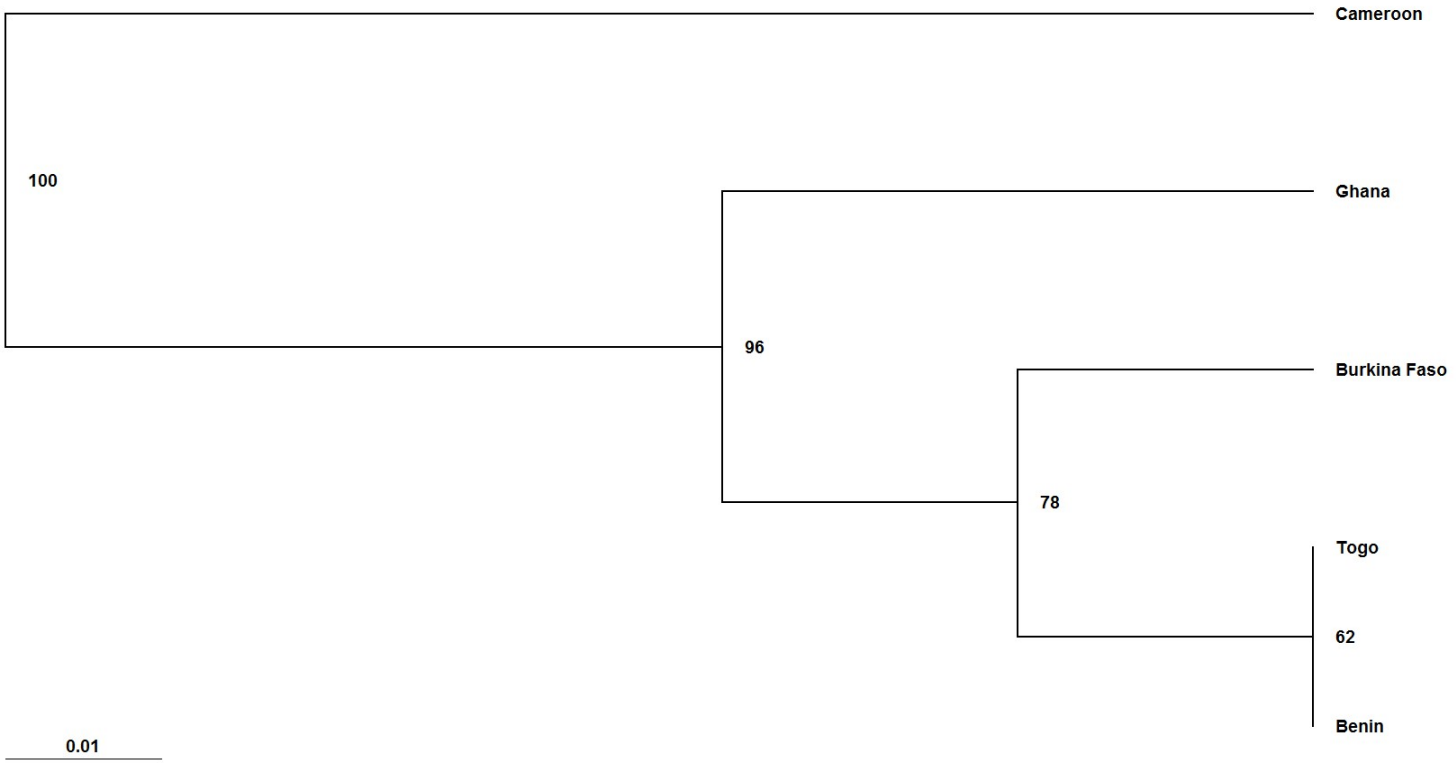
Genetic differentiation of *K. senegalensis*
(nSNPs = 101; N = 828)



Delta = 0.10 / F_{ST} = 0.18

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Self-assignment to species

Country	Sample size	% of correct assigned	
		Bayesian approach	Nearest neighbor approach*
Benin	154	6	52
Burkina Faso	243	70	53
Cameroon	126	79	100
Ghana	288	84	42
Togo	82	68	27
Mean	-	61	52

GeoAssign: Degen et al. (2017)

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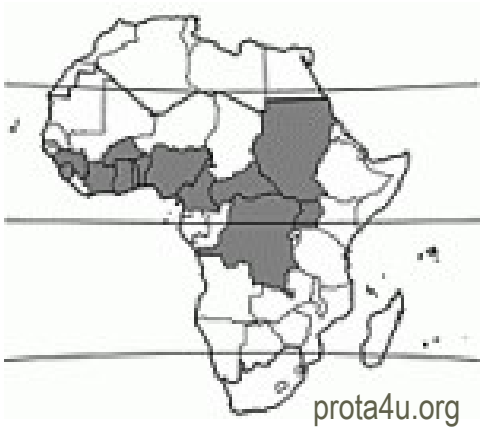
Khaya grandifoliola



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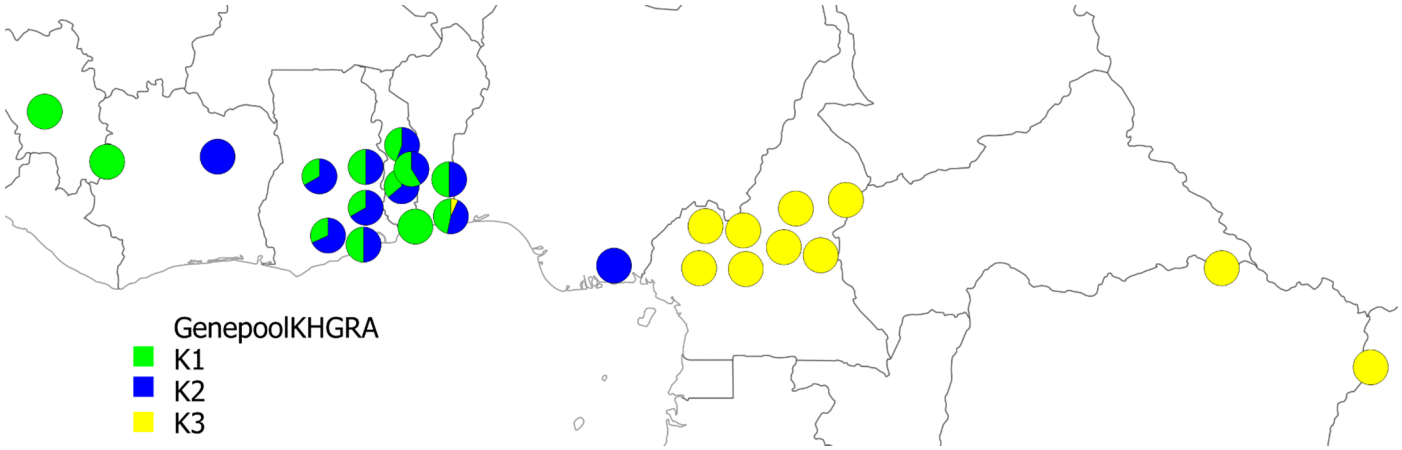
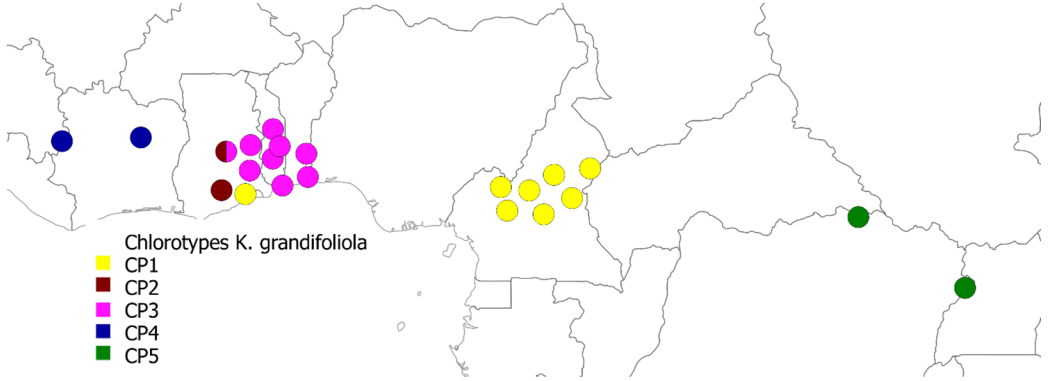
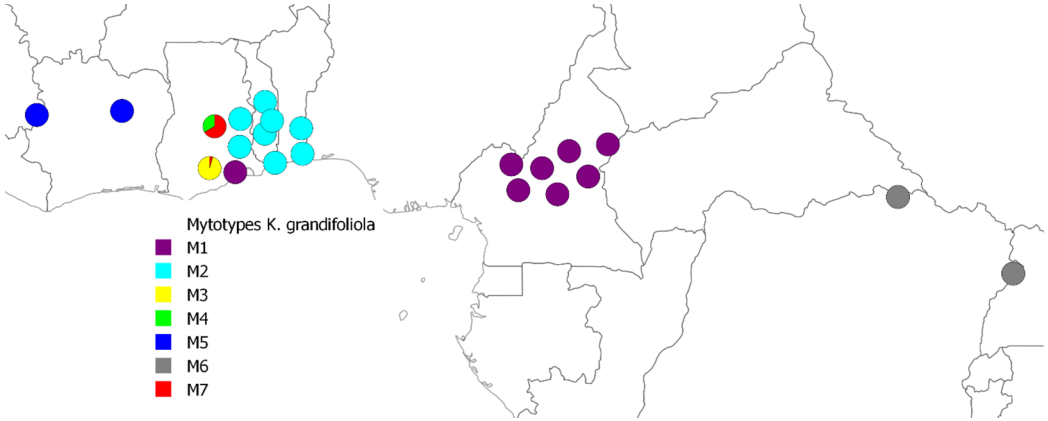


nSNPs



mSNPs



cpSNPs

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Self-assignment to species

Country	Sample size	% of correct assigned	
		Bayesian approach	Nearest neighbor approach*
Benin	20	71	25
Cameroon	65	100	100
Ghana	35	29	77
Togo	102	80	93
Mean	-	70	86

GeoAssign: Degen et al. (2017)

Blind tests

Species ID	Country of origin	
Khaya sp. (N = 15 samples)	*K. senegalensis* (N= 22 samples)	*K. grandifoliola* (N= 8 samples)
• **14 Correct (93.3%)** • 1 Wrong	• **14 Correct (63.6%)** • 6 Wrong • 2 Unsolved	• **7 Correct (87.5%)** • 1 Wrong

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Conclusions and outlook

- Species identification
  - Clear discrimination among African mahogany species
- Verification of the geographic origin
  - Very good differentiation between West and Central Africa region for *K. senegalensis* and *K. grandifoliola*
  - Verification of country of origin more pronounced for *K. grandifoliola* than *K. senegalensis*
  - Combination with results of stable isotopes fingerprinting will improve the precision
- Tools ready to be used for forensic timber tracking
- Genetic data generated are also useful to elaborate conservation strategies

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Financial supports

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Thank you

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