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Phylogenetic relationships in two African Cedreloideae tree genera (Meliaceae) reveal multiple rain/dry forest transitions

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Abstract

Resolving phylogenetic relationships allows the investigation of how species diversity has evolved in various ecosystems. The genera *Entandrophragma* and *Khaya* consist of tree species distributed in different African biomes (lowland rain forest, dry forest and savanna, montane forest), and are suitable to examine how (single or multiple events) and when the processes of diversification led to biome transitions. Based on the sequencing of plastome (pDNA: c. 160,000 bp), ribosomal DNA (rDNA: c. 5,300 bp), and habitat characteristic data for each species, we have: (i) reconstructed phylogenetic relationships between species and estimated the divergence period between the main lineages, and (ii) reconstructed ancestral states regarding biome preferences. The phylogenetic trees obtained with both markers support monophyly of the five sections of *Entandrophragma* previously defined based on fruit and floral characters. Nevertheless, the position of some species from the pDNA and rDNA tree topologies varied within sections. In *Khaya,* pDNA and rDNA show very divergent topologies, possibly due to a more recent diversification involving incomplete lineage sorting and/or recurrent hybridization events. *Khaya* diversified during the Pliocene and Pleistocene according to both markers; whereas, according to rDNA data, the *Entandrophragma* sections diverged during the early Miocene and species within sections diverged from the late Miocene to the Pleistocene. Divergence date estimates tended to be more recent using pDNA data. Biome transitions could not be reconstructed in *Khaya* as the species tree remains unresolved. Contrarily, three independent biome transitions were inferred in *Entandrophragma*. The first transition from rain to dry forests occurred during the Miocene and two other transitions were inferred during the Pleistocene, one from rain forest to dry forest and another from rain forest to mountain forest. Overall, we demonstrate that multiple biome transitions occurred, starting from a rain forest biome, possibly reflecting the global trend of aridification of Africa throughout the Cenozoic.

Keywords: Biome shift, Cedreloideae, Diversification, Mahogany, Molecular phylogeny, Plastome, Ribosome

1. Introduction

African tropical tree species occur in different biomes, but each species is often restricted to one biome characterized by particular climatic conditions, e.g. lowland rain forest (warm and humid), dry forest, woodland or savannas (warm and seasonally dry), or montane forest (cool and humid). The biogeographical history of these biomes and the origin of their flora is still discussed (e.g., Linder, 2014). To explain their species diversity, two main speciation models are generally invoked. Firstly, allopatric speciation is driven by geographical isolation of populations, causing a disruption of gene flow and genetic drift (Fjeldså and Lovett, 1997; Plana et al., 2004). Since the mid-Miocene, a global trend of aridification led to a reduction of African rain forests in favour of dry forests and savannas (Kissling et al., 2012), with more fluctuations between these biomes during the Plio-Pleistocene glacial / inter-glacial oscillations (Axelrod and Raven, 1978; Coetzee, 1993). African rain forests probably fragmented during more arid periods (Dupont, 2011), potentially contributing to allopatric speciation events. Several phylogeographical studies of African rain forest trees interpreted the occurrence of major genetic lineages within species as an imprint left by past population fragmentation induced by Pleistocene climate changes (e.g. Dainou et al., 2014, Duminil et al., 2015, Demenou et al., 2016). Secondly, ecological speciation is driven by natural selection across ecological gradients (Schneider et al. 1999; Smith et al., 2001), differentiating populations distributed in parapatry at local scale or at large scale. In this speciation model, new species emerge as a result of adaptations to new environmental conditions. However, during diversification, lineages are generally expected to retain their ecological niches (Wiens and Graham, 2005), so that plant species rarely cross biome boundaries, leading to a strong phylogenetic biome conservatism (Crisp et al., 2009). Even within the tropical rain forest biome, the climate adaptations of tropical tree lineages remain surprisingly correlated when measured on forests from opposite sides of the earth (Hardy et al., 2012). This leads to the prediction that lineages might rarely colonize new biomes (Wiens and Graham, 2005) and that species evolutionary success might have resulted not from an adaptation to new biomes but from an expansion of their biome as climate changed (Crisp et al., 2009). However, the evolutionary processes underlying such phylogenetic conservatism are still poorly understood, and the frequency of biome transitions needs to be better estimated at a continental level, where it seems to vary according to biome pairs (Crisp et al., 2009).

Despite an increasing number of molecular phylogenetic studies establishing diversification periods of African lineages (Couvreur et al., 2008; Davis et al., 2010), there are still few insights about biome transitions in tropical Africa. It remains indeed difficult to reconstruct well resolved species trees from molecular markers due to pitfalls such as: incongruence between gene trees from different molecular markers, complex species delimitation and incomplete lineage sorting (Duminil et al., 2012; Ikabanga et al., 2017). Nevertheless, recent studies have highlighted the role of biome shift in species

diversification processes. One transition from dry to rain forests was inferred during the diversification of the genus *Afzelia* Smith., during the Miocene (23 to 16 Ma; Donkpegan et al., 2017). Contrarily, one transition from rain to dry forests was inferred for the genus *Guibourtia* Benn. during the Pliocene (5.3 to 2.6 Ma; Tosso et al., 2018). Unexpectedly, six biome shifts among 27 semi-arid, woodland, and forest species of *Coccinia* (Cucurbitaceae) have been recorded since 6.9 Ma (Holstein and Renner, 2011). Similarly, 12 shifts from closed habitats (forest) to open habitats (grass and woodlands) were inferred for African Melastomateae during the Mid-Miocene and Pliocene (Veranso-Libalah et al., 2018). Conversely, Couvreur et al. (2011) did not detect substantial ecological shift between sister species of two Annonaceae genera.

Among Meliaceae, the subfamily Cedreloideae comprises 14 genera and 59 species (Koenen et al., 2015), widely distributed along a pantropical belt from the South-Central America to Sub-Saharan Africa, Madagascar, South-East Asia and Northern Australia. To understand phylogenetic relationships and target evolutionary trajectories of species between African biomes, we focus on two genera, *Entandrophragma* C.DC. and *Khaya* A.Juss., commonly known as African mahogany and exploited for their high timber quality and economic value (De Wasseige et al., 2013). The African *Khaya* and *Entandrophragma* species occur over the same ranges of forest types and biogeographical regions. *Entandrophragma* comprises 11 species (considering *E. congoense* as a distinct species from *E. angolense*, Monthe et al. 2018) growing in various tropical African biomes, in particular rain forest, dry forest (in a broad sense, including wooded savannas and dry woodlands), montane forests and swamp forests. They occur in the Sudano-Guinean, Guineo-Congolian and Zambezian biogeographical regions (Fig. 1) (Linder et al., 2012; White, 1983). This genus has been subdivided into five main sections (*Neoentandrophragma* Harms; *Pseudoentandrophragma* Harms; *Choriandra* Harms; *Euentandrophragma* Harms; *Wulfhorstia* (C.DC.) Sprague) based on fruit and floral traits (Harms, 1940; Louis and Fouarge, 1947) but the monophyly of these sections has never been tested. The genus *Khaya* comprises nine species distributed in Africa and Madagascar (Fig. 1). Despite some overlap in their distribution ranges, each *Khaya* species displays restricted ecological requirements along an ecological gradient from moist evergreen forest to semi-deciduous forest and savanna. Species delimitation within *Khaya* has been contentious and we here rely on the latest revisions (Bouka, 2017; Bouka et al., 2017).

In this study, we use both plastid DNA (whole plastome, pDNA) and ribosomal DNA (rDNA) sequences after high throughput sequencing to infer the infrageneric and interspecific phylogenetic relationships of *Entandrophragma* and *Khaya* and deduce the role of biome transitions in the diversification of these genera. We address the following specific questions: (i) What are the phylogenetic relationships within each genus, and are morphologically-based sections of *Entandrophragma* supported

by molecular phylogenetic evidence? (ii) How many biome transitions can be detected? (iii) What is the tempo and mode of diversification for these genera, in relation with past climate changes?

2. Materials and Methods

2.1. Taxa sampling, DNA extraction and genomic libraries sequencing

We used nine samples of *Khaya* representing eight of the nine species recognized by Bouka (2017; *K. nyasica* Stapf ex Baker was missing): *K. agboensis* A. Chev., *K. anthotheca* (Welw.) C. DC., *K. euryphylla* Harms., *K. grandifoliola* C. DC., *K. ivorensis* A.Chev., *K. madagascariensis* Jum. & H. Perrier, *K. senegalensis* (Desr.) A. Juss., *K. sp. nov.*; and 28 samples of *Entandrophragma* representing all 11 species currently recognized (Table 1): *E. angolense* (Welw.) C.DC., *E. bussei* Harms ex Engl., *E. candollei* Harms, *E. caudatum* (Sprague) Sprague, *E. congoense* (De Wild.) A.Chev., *E. cylindricum* (Sprague) Sprague, *E. delevoyi* De Wild., *E. utile* (Dawe & Sprague) Sprague, *E. excelsum* (Dawe & Sprague) Sprague, *E. palustre* Staner, *E.spicatum* (C.DC.) Sprague. DNA was extracted from dry leaves collected in the field or from herbarium specimens (National Herbarium of the Netherlands Wageningen, WAG; African Botanical Library of Université Libre de Bruxelles, BRLU; Botanic Garden Meise, BR, and the Missouri Botanical Garden, MBG).

For each sample, total genomic DNA was extracted using a CTAB protocol (Doyle and Doyle, 1987), before using the QIAquick purification method (Qiagen, Venlo, Netherlands). We then quantified and controlled the quality of DNA using a Qubit® 2.0 Fluorometer (Life Technologies, Invitrogen, Foster City, USA) and a QIAxcel (Qiagen). For genomic library construction, we first sheared 5-10 µg of DNA per sonication using a Bioruptor® Pico (Diagenode SA., Liège, Belgium) to target a mean DNA fragment size of c. 400 bp per sample, except for herbarium samples. We then proceeded by sizing to remove larger (> 600 bp) and shorter (< 200 bp) DNA fragments using the AMPure XP protocol (Agencourt). Then, using NEB enzymes (New England Biolabs, Beverly, USA), we proceeded to the end-repair, adenylation, and ligation steps. The tagging of each sample was done using 6-bp indexed adapters, following the protocol of Rohland and Reich (2012). After checking for DNA quality, the genomic libraries were finally amplified on a StepOnePlus (Applied Biosystems, Foster City, USA) real-time PCR thermocycler, using Kapa kit (Kapa Biosystems, Boston, USA), to generate the final genomic libraries. At the end, we pooled libraries according to their concentration for a final quantitative PCR. The paired-end sequencing (2 × 150 bp) was performed on an Illumina NextSeq instrument at the GIGA platform (Liège, Belgium).

2.2. Plastome and ribosomal DNA reconstruction, mapping, and SNP calling

Paired-end reads were checked for quality control using FastQC 0.11.5 software (Andrews, 2010). The demultiplexing step was based on the 6-bp barcodes ligated with adapters using the sabre script (https://github.com/najoshi/sabre). Reads with low quality (Q ≤ 20) were trimmed with Trimmomatic

(Bolger et al., 2014). We reconstructed the reference plastomes for each genus using Mitobim 1.8 software (Hahn et al., 2013) that integrates the program MIRA 3.4.1.1. We used the trimmed reads from one sample of *Entandrophragma caudatum* (Sprague) Sprague (2,264,944 reads) and of *Khaya madagascariensis* Jum. & H. Perrier (2,027,826 reads; Table 1) in combination with one of seven closely-related and published plastomes (*Azadirachta indica* A.Juss. , *Boswellia sacra* Flueck., *Sapindus mukorossi* Gaertn., *Leitneria floridana* Chapm*., Citrus platymamma* hort. ex Tanaka, *Acer davidii* Franch. and *Spondias tuberosa* Arruda) used as initial references to run Mitobim. We then kept the reconstructed plastomes showing the lowest number of missing data: the ones initiated from the plastome of *Citrus platymamma* (*Rutaceae*; GenBank accession NC030194.1) in the case of *Entandrophragma,* and of *Sapindus mukorossi* (*Sapindaceae*; KM454982.1) in the case of *Khaya.* Reads of all the samples were then mapped on these reconstructed reference plastomes, using the Burrows-Wheeler Aligner BWA mem 0.7.5a-r405 (Li et al., 2009) with -M and -B 4 options. We followed the recommendations of Scarcelli et al. (2016) to call the variants using Samtools 1.1 (Li et al., 2009) to generate a mpileup file for each genus separately, and Varscan 2.3.9 (Koboldt et al., 2012) with the option --min-var-freq set to 50 for calling Single Nucleotide Polymorphism (SNP). The VCF file finally obtained was filtered to remove the sites with less than 80 % of bases available per sample and a fasta file was produced.

We also reconstructed ribosomal DNA sequences from the *E. caudatum* and *K. madagascariensis* samples using the assembler NOVOplasty (Dierckxsens et al., 2016). As this assembler requires a seed DNA sequence, we used as seeds the 5.8S ribosomal gene and internal transcribed spacer 2 from *E. utile* (Dawe & Sprague) Sprague (GenBank accession FJ518896.1) and *K. grandifoliola* C. DC. (KF840425.1, including the 28S ribosomal gene), respectively. We used the same approach as described above for the mapping, SNP calling, and VCF filtering, relying on a reconstructed consensus reference rDNA of each genus.

2.3. Phylogenetic analyses and estimation of divergence times

All the sequences were aligned using MAFFT 7 (Katoh and Standley, 2013). We considered two separate datasets: the first containing all samples (including multiple samples per species, mostly in the case of *Entandrophragma*); and the second considering only one sample per species. For the first dataset we applied Maximum Likelihood (ML) and Bayesian Inference (BI) methods considering independently whole plastome and rDNA data to perform phylogenetic analyses. Maximum Likelihood analyses were conducted with default parameters and 100 bootstrap replicates using RAxML 7.2.6 (Stamatakis, 2014) through the CIPRES Portal 2.1 (*www.phylo.org;* Miller, 2009). BI analyses were conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001), after using jModeltest 2.1.10 (Darriba et al., 2012) to select the best nucleotide substitution model: TVM+G for plastomes and TrN+I+G for ribosome data. The Metropolis Coupled Markov Chains (MCMC) with Monte Carlo simulations were run for 1,000,000 generations, with

a tree sampled every 1,000 generations. The different phylogenetic trees were visualised in FigTree 1.4.3 (Rambaut, 2007).

For estimating divergence times, we relied on the second dataset to avoid overestimating diversification periods due to unequal sample sizes between species and genera. We estimated the divergence times using the Bayesian MCMC analysis implemented in BEAST 1.7.4 (Drummond et al., 2012). We calibrated both phylogenies by setting the divergence date between *Entandrophragma* and *Khaya* as a normal distributed random variable (mean = 36.9 Ma, StDev = 4.0), following a recent dated Meliaceae phylogeny which took into account three different fossil calibrations in the Cedreloideae subfamily (Koenen et al., 2015). Furthermore, Bayesian analyses were done separately on plastome and ribosomal data using these priors: an uncorrelated lognormal relaxed clock model which allows an independent optimization of mutation rates for each branch of the tree, a Yule process of speciation which is adequate to analyse data at the interspecific level (Heled and Drummond, 2015; Yule, 1925), and the selected nucleotide substitution model. The MCMC analyses were run for 100,000,000 generations, sampling trees every 10,000 generations. BEAST output files were examined through Tracer 1.6 (Rambaut and Drummond, 2016) to evaluate convergence, and ensure sufficient effective sample sizes (ESS values) for all parameters. TreeAnnotator 1.8 was then used to produce a Maximum Clade Credibility tree (Rambaut and Drummond, 2007).

2.4. Ancestral states reconstruction

To infer ancestral states of biome preference, we first characterized the climate niche of each species to determine species groups sharing similar niches. We extracted (i) occurrences data for each species from the RAINBIO database (Dauby et al., 2016), keeping only one individual per 10x10 km^2 grid cell for species represented by at least 30 occurrences, and (ii) bioclimatic variables corresponding to each occurrence from the WORLDCLIM database using the R package 'raster' (Hijmans et al., 2016). We then applied a principal component analysis (PCA) on the table of bioclimatic variables, keeping at most 30 random samples per species, and reported the centroid of each species. From this PCA, we could assign each species to one of three climatic niches corresponding to the following biomes: (i) lowland rain forest (warm and humid), (ii) dry forest, woodland and savannah (warm and seasonally dry), (iii) montane forest (cool and humid; Linder et al., 2005). We then mapped the assigned biome on the phylogenetic tree obtained with ribosomal data, keeping only one individual per species. Assuming that each ancestral species was adapted to a single biome, we applied a Maximum Likelihood approach implemented in the R package 'phytools' (Revell, 2012) to estimate for each ancestral lineage the probability that it was adapted to a particular biome.

3. Results

3.1. Reference genome construction, mapping and SNP calling

The reference plastomes for each genus were reconstructed after 12 to 13 iterations of Mitobim, resulting in plastomes with a length of 163,180 bp for *Entandrophragma* and 163,739 bp for *Khaya* (Genbank accessions MK058683 and MK058684). On average, across libraries, 146,218 ± 92,930 SD and 83,171 ± 54,507 SD reads were correctly mapped on the reference plastomes for *Entandrophragma* and *Khaya* samples*,* respectively, making on average 7 % of the total reads. The mapping and SNP calling resulted in a mean genome coverage of 118x and the identification of 1,480 SNPs and 380 indels for *Entandrophragma*, and 70x, 1,068 SNPs and 177 indels for *Khaya*.

For ribosomal DNA (rDNA), we obtained a contig of 5,389 bp for *Entandrophragma* and 4,084 bp for *Khaya.* They were annotated by blasting on GenBank database, recovering 28S, 26S, 18S and 5.8S rRNA genes, ITS1 and ITS2 (Genbank accessions MK058685 and MK058686). The mapping of genomic libraries on these ribosome contigs reached on average (± SD) 13,564 ± 9,212 reads for *Entandrophragma* and 4,750 ± 3,345 reads for *Khaya*, resulting in mean genome coverages of 249x and 143x*,* respectively. This represented around 0.5 % of the total reads sequenced. Finally, we detected 891 SNPs plus 70 indels for *Entandrophragma*, and 116 SNPs plus 6 indels for *Khaya.*

3.2. Phylogenetic relationships and haplotypes distribution

The MAFFT alignment combining sequences from *Entandrophragma* and *Khaya* provided finally 169,732 bp of pDNA and 7,515 bp of rDNA. For species represented by multiple samples (essentially in *Entandrophragma*), all of them appeared monophyletic in the rDNA tree (Fig. 2) and all except one in the pDNA tree (Fig. S1). The *Entandrophragma* pDNA and rDNA phylogenetic trees congruently supported the monophyly of the five sections, but they show differences in their topologies (Fig. 2 and S1). According to pDNA data, sections *Wulfhorstia* (*E. spicatum, E. bussei* . and *E. caudatum*) and *Neoentandrophragma* (*E. utile* are sister, as well as sections *Euentandrophragma* (*E. delevoyi ., E. congoense , E. angolense and E. excelsum* and *Pseudoentandrophragma* (*E. cylindricum*), while section *Choriandra* (*E. candollei* and *E. palustre*) would be the first branching lineage of *Entandrophragma* (Fig. S1 and 3a). There is by contrast much less support in the rDNA data to group sections so that all of them would appear in a polytomy if we considered a minimum bootstrap support above 70% (Fig. 2). The topologies of the pDNA and rDNA trees differ within some sections. In the *Euentandrophragma* section, according to pDNA *E. delevoyi* is sister to *E. angolense* and *E. excelsum* is sister to all other species (Fig. S1 and 3a) while, according to rDNA *E. delevoyi* is sister to *E. congoense* and *E. excelsum* is sister to *E. angolense* (Fig. 2). Similarly, within section *Wulfhorstia*, *E. bussei* is sister to *E. caudatum* according to pDNA but sister to *E. spicatum* according to rDNA.

The pDNA and rDNA-based phylogenies of the genus *Khaya* were not congruent. For instance, *K. grandifoliola* and *K. senegalensis* from Guinean and Sudanian domains appeared as sister species in the pDNA tree, as was the case for *K. agboensis* and *K. euryphylla*. From the rDNA tree, *K. grandifoliola*, *K. agboensis* and *K. euryphylla* are closely related and well-supported in the same clade. However, for both markers, *K. anthotheca* and *K. sp. nov.* are considered as sister species (Fig. 2 and S1).

3.3. Timing of diversification and habitat characterisation

The posterior mean rate of evolution calculated by BEAST reached 1.97×10^{-4} substitutions per site per Ma for the plastomes and 1.41×10^{-3} substitutions per site per Ma for rDNA sequences, values that appear within the range of documented evolution rates in tree species for these genomes (e.g., Palmer, 1991, Savard et al., 1993, Smith and Donoghue, 2008).

Entandrophragma started to diversify during the early or mid-Miocene (between 10 and 20 Ma according to pDNA and rDNA phylogenies, respectively; Fig. 3) while *Khaya* diversified in the Plio-Pleistocene, a period where diversification within three *Entandrophragma* sections also occurred according to pDNA (Fig. 3a). However, the rDNA phylogeny indicates that diversification within *Entandrophragma* sections would have occurred earlier (late Miocene to Pleistocene; Fig. 3b).

Niche characterisation and ancestral state reconstruction were done only for *Entandrophragma* species whose plastome and ribosome phylogenies were globally congruent, contrary to the case of *Khaya* species. The principal component analysis (PCA) on bioclimatic variables for occurrences data revealed three main groups of *Entandrophragma* species (see Fig. S2) corresponding to the following biomes: (i) lowland rain forest (six species: *E. angolense*, *E. candollei*, *E. congoense*, *E. cylindricum*, *E. palustre*, and *E. utile*), (ii) dry forest, woodland and savanna (four species: *E. bussei*, *E. caudatum*, *E. delevoyi*, and *E. spicatum*) and (iii) montane forest (*E. excelsum*). Using these three states to reconstruct ancestral characters on the ribosomal phylogeny, lowland rain forest appeared as the most likely ancestral biome of *Entandrophragma* (probability of 0.80 for rain forest, 0.12 for dry forest, 0.07 for montane forest). During the diversification of the genus, at least three biome transitions occurred, with two transitions from rain forest to dry forest, and one transition from lowland rain forest to montane forest (see arrows in Fig. 4).

4. Discussion

4.1. Phylogenetic relationships within Entandrophragma *and* Khaya *genera*

By sequencing non-enriched genomic libraries, we detected high levels of polymorphism with 1,480 and 891 SNPs in *Entandrophragma* and 1,068 and 116 SNPs in *Khaya,* for pDNA and rDNA regions, respectively. We can thus expect reliable phylogenetic reconstructions within these two Cedreloideae-Meliaceae genera, as already highlighted from plastome captures (Li et al., 2017; Migliore et al., 2018;

Tosso et al., 2017; Yang et al., 2013). Moreover, combining rDNA and pDNA allows assessing marker incongruences and discussing how to interpret gene trees versus species trees (Eidesen et al., 2015; Donkpegan et al., 2017). Our phylogenies support a clear species delineation within the genus *Entandrophragma*, where each species represented by multiple samples appeared monophyletic, except for the closely related *E. candollei* and *E. palustre* using the pDNA data (Fig. 2 & S1). By contrast, within the genus *Khaya* the rDNA phylogeny is not well resolved, and the tree topologies are not congruent between plastid and ribosomal markers (Fig. 2 & S1). This result is consistent with an ongoing morphological and genetic delineation of *Khaya* species showing that plastid haplotypes can be shared between species, indicating that pDNA is not reliable to identify *Khaya* species (Bouka, 2017). In this context, more individuals per species and additional nuclear markers are needed to establish the phylogenetic relationships among *Khaya* species, and thus test if shared plastome markers result from plastome captures and/or incomplete lineage sorting (Bouka, 2017; Dainou et al., 2014). Nevertheless, our dated phylogenies allowed assessing the period of diversification of *Khaya*. The more recent diversification of *Khaya* compared to *Entandrophragma* might explain why its species are less straightforward to separate.

Regarding *Entandrophragma* genus, five clades identified in both pDNA and rDNA phylogenies correspond to the sections previously described according to fruits (apical or basal dehiscence; Harms, 1940) and flowers characters (presence or absence of a more or less partitioned disc at the base of the ovary; Louis and Fouarge, 1947). The latter morphological feature is exclusive to the genus *Entandrophragma* within the Meliaceae family, yet it is absent from the section *Wulfhorstia* (*E. bussei*, *E. caudatum*, and *E. spicatum*) growing in Sudano-Zambesian dryland areas. However, species were classified based on this flower characteristic from the least to the most partitioned ovaries in the four other sections: *Pseudoentandrophragma* (*E. cylindricum)*, *Choriandra* (*E. candollei*, *E. palustre*), *Neoentandrophragma* (*E. utile*), and *Euentandrophragma* (*E. angolense*, *E. congoense*, *E. delevoyi*, and *E. excelsum*). This congruence between morphological characteristics and molecular phylogeny emphasizes the reliability of reproductive features in species delimitation of *Entandrophragma*. Therefore, these reproductive traits could explain the position of *E. delevoyi*, a mid-altitude dry forest species, as part of a clade including three lowland rain forest species (*E. angolense, E. congoense*, and *E. excelsum*) in the section *Euentandrophragma.* This also suggests that these traits in *Entandrophragma* sections could pre-date the expansion of taxa to very contrasted habitats.

Furthermore, as the genus *Entandrophragma* has undergone many taxonomic studies resulting in a high number of synonyms (44 synonyms for 11 species), mainly for forest species, this phylogenetic study provides new insights in species delineation. *Entandrophragma congoense* and *E. angolense* were sometimes put in synonymy while they appear reciprocally monophyletic, and their differentiation was also confirmed when combining morphological and nuclear microsatellite markers (Monthe et al., 2018).

It is important to note that *E. candollei* and *E. palustre* are sister species in both pDNA and rDNA trees (Fig. 2); however, their divergence time estimates are quite different, 0.46 Ma from pDNA and 6.21 Ma from rDNA data. This main incongruence could be explained by chloroplast capture, especially since both species co-occur in Congolese forests, which highlights the importance to develop multi-markers phylogenies for reconstructing species history and not only markers history (Duminil et al., 2013).

4.2. Tempo of speciation and lineage sorting

Two main diversification periods have been detected within Cedreloideae. First, the diversification leading to the five sections of *Entandrophragma* occurred during the Oligocene and/or Miocene (between 7.47 and 13.15 Ma according to 95% HPD intervals on pDNA and between 12.2 and 28.9 Ma according to rDNA). This is concomitant to a period of global cooling that led to the drying of the climate in equatorial Africa and fragmentation of tropical rain forests (Coetzee, 1993). No diversification event was detected during this timeframe for *Khaya*. Secondly, another diversification period during the late Miocene to Pleistocene characterized the divergence of species within the *Entandrophragma* sections and in parallel the divergence of extant *Khaya* species. These pre-Pleistocene diversifications of *Entandrophragma* and *Khaya* are similar to what was obtained in the whole Meliaceae phylogeny (Koenen et al., 2015) and congruent with numerous phylogenetic studies performed in Ancistrophyllinae (Arecaceae, Faye et al., 2016), Annonaceae (*Isolona* and *Monodora*, Couvreur et al., 2011; *Greenwayodendron,* Migliore et al., 2018), Detarioideae (*Erythrophleum*, Duminil et al., 2013; *Afzelia*, Donkpegan et al., 2017; *Guibourtia*, Tosso et al., 2018; Detarioideae, de la Estrella et al., 2017), Melastomateae (Melastomataceae, Veranso-Libalah et al., 2018), Moraceae (*Milicia*, Dainou et al., 2014), Sapotaceae (*Manilkara*, Armstrong et al., 2014), Zingiberaceae (*Afromomum*, Auvray et al., 2010). Thus, during the Miocene, an increasing number of phylogenetic studies tend to confirm that major environmental changes could have promoted speciation in many plant and animal genera in tropical Africa.

The incongruent phylogenies between markers for *Khaya* suggest that gene trees and species trees are not congruent, possibly due to incomplete lineage sorting. A similar pattern was reported among African rain forest trees of the genus *Afzelia* that also started diversifying at the end of the Miocene (Donkpegan et al., 2017). This may be due to the time required to reach reciprocal monophyly (c. 10**Ne* generations if sister species maintain effective population sizes equal to *Ne*; Hudson and Coyne, 2002) which can potentially reach millions of years in long living organisms such as trees (e.g., if the generation time is 100 years and *Ne* > 1000). This pattern of incomplete lineage sorting seems therefore common in the recent diversification of several African rain forest species (Dainou et al., 2014; Donkpegan et al., 2017; Duminil et al., 2013).

4.3. *Multiple transitions across African biomes*

The phylogenetic discrepancy between pDNA and rDNA markers in *Khaya* does not allow us to investigate patterns of biome shifts in this genus. However, as both pDNA and rDNA phylogenies indicate that the diversification of *Khaya* started about 4.1 Ma ago, biome transitions in *Khaya* should have occurred in the late Pliocene and/or Pleistocene. By reconstructing ancestral biomes in *Entandrophragma*, we recorded three biome shifts. The first transition from rain to dry forests led to the diversification of the section *Wulfhorstia* (*E. bussei*, *E. caudatum*, *E. spicatum*). This biome shift occurred between 4.7 and 15.7 Ma according to rDNA (Fig. 3b) and between 0.6 and 6.7 Ma according to pDNA (Fig. 3a), so that the overlap of the two ranges points toward the end Miocene and early Pliocene, an interval including aridification episodes (Partridge, 1993). After this biome shift, the diversification of the *Wulfhorstia* section was probably driven by allopatric speciation as its three species are allopatrically distributed (Fig. 1). The two other biome transitions occurred in the *Euentandrophragma* section, leading to *E. delevoyi* (rain to dry forest shift) and to *E. excelsum* (lowland rain to montane forest shift). These transitions occurred during the last 5 to 6 Ma according to rDNA, and during the last 3.4 Ma for *E. excelsum* and the last 0.8 Ma for *E. delevoyi* according to pDNA (Fig. 3). Hence, these biome transitions might have been driven by general cooling and drying during the Pliocene, and climatic oscillations at the beginning of the Pleistocene (Morley, 2011).

Such multiple biome transitions have been poorly documented in African rain forests, while they might be common in African taxa, as several genera are characterized by wide distribution ranges and their species occur in different biomes (Couvreur et al., 2011, 2008). As for ancestral African Melastomateae (Veranso-Libalah et al., 2018) and *Guibourtia* species (Tosso et al., 2017), *Entandrophragma* taxa were adapted to closed rainforest habitats with shifts to open habitats during the Miocene or Pliocene. Multiple transitions between rain and dry biomes have also been suggested in the South American cerrados to explain the development of an endemic woody flora to these fire-prone habitats (Simon and Pennington, 2012). We can thus follow Veranso-Libalah et al. (2018) suggesting that adaptation to open habitats during the Neogene is an important driver of African plant diversity. As discussed by Holstein and Renner (2011), steeper ecological gradients in East Africa and South Africa appear to have resulted in allopatric speciation.

Inferring the relative role of allopatric and ecological (parapatric) speciation is always difficult and often speculative because current species distributions do not necessarily reflect distributions during speciation processes, and the discrepancy is expected to increase with time. In the case of *Entandrophragma* it is worth noting that rain forest species are essentially distributed in sympatry whereas dry and montane forest species are distributed in parapatry (Fig. 1). Three speciation events were correlated to biome shifts from rain forest to dry or montane forest (Fig. 4), so that ecological speciation

in parapatry (or allopatry) was likely involved, although we cannot exclude that allopatric speciation occurred first and was later followed by biome shift. Three dry forest species of section Wulforsia (*E. caudatum, E. spicatum, E. bussei*) diverged relatively recently (Plio-Pleistocene according to rDNA, Pleistocene according to pDNA) and are found in allopatry, so that an allopatric mode of speciation seems here likely. Six rain forest species are largely sympatric, but while five of them occur in *terra firme* forest, *E. palustre* a swamp forest species is closely related to *E. candollei*. Hence, an ecological speciation in local parapatry could be at the origin of the divergence between the latter species. The five *terra firme* rain forest species share very similar ecological niches but belong to different sections, except *E. angolense* and *E. congoense*, and have diverged early from each other. It is thus plausible that these species have diverged following an allopatric mode of speciation and had ample time to subsequently expand throughout the Central and West African rainforests (except *E. congoense* absent from West Africa).

5. Conclusion

Our study highlights the important role of environmental changes in species diversification processes within two genera of the Cedreloideae subfamily. Phylogenetic analyses with both plastome and ribosomal DNA markers support current species delimitation within the genus *Entandrophragma*. Our analyses also confirm monophyly of the current morphological infrageneric classification of *Entandrophragma* based on fruit and floral characteristics. Despite similar species richness and environmental diversity characterizing both *Entandrophragma* and *Khaya* genera, it appears that phylogenetic relationships among *Khaya* are not resolved, due to inconsistencies between pDNA and rDNA markers. These results reinforce the need to use several nuclear molecular markers to reconstruct reliably the phylogenetic relationships and evolutionary history between species. In addition, our study shows that *Entandrophragma* had two periods of diversification, one concomitant with the diversification of *Khaya*, associated with at least three biome shifts. Hence, our results highlight the capacity of tree species to adapt to new biomes. Nevertheless, most speciation events detected in *Entandrophragma* were not directly associated with a biome transition, indicating that species diversification is probably only marginally driven by environmental gradients.

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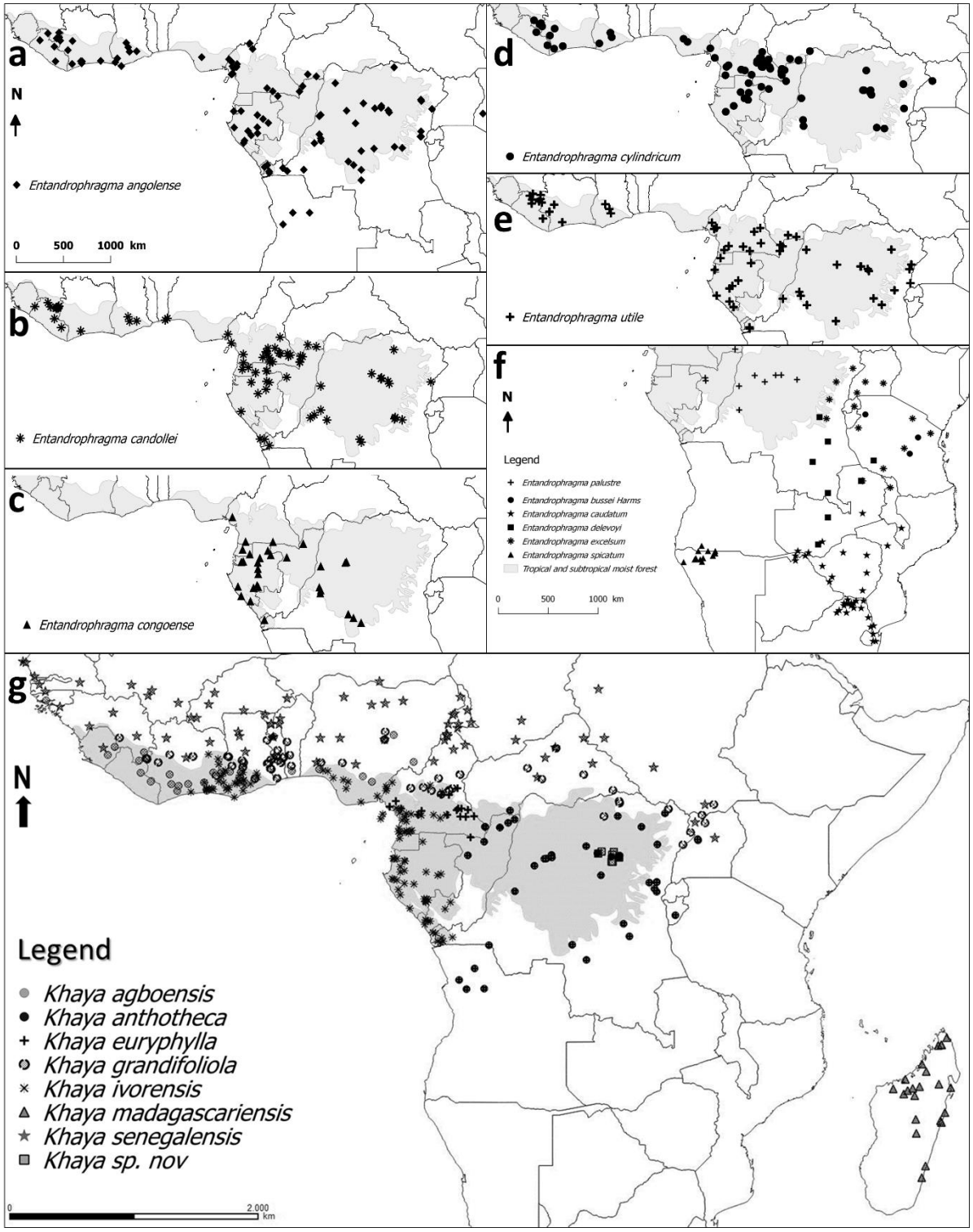

Fig. 1. Geographical distribution of: (a-e) five sympatric rain forest *Entandrophragma* species, (f) six allopatric or parapatric *Entandrophragma* species from dry, montane or swamp forests and (g) eight of the nine *Khaya* species recognized by Bouka (2017).

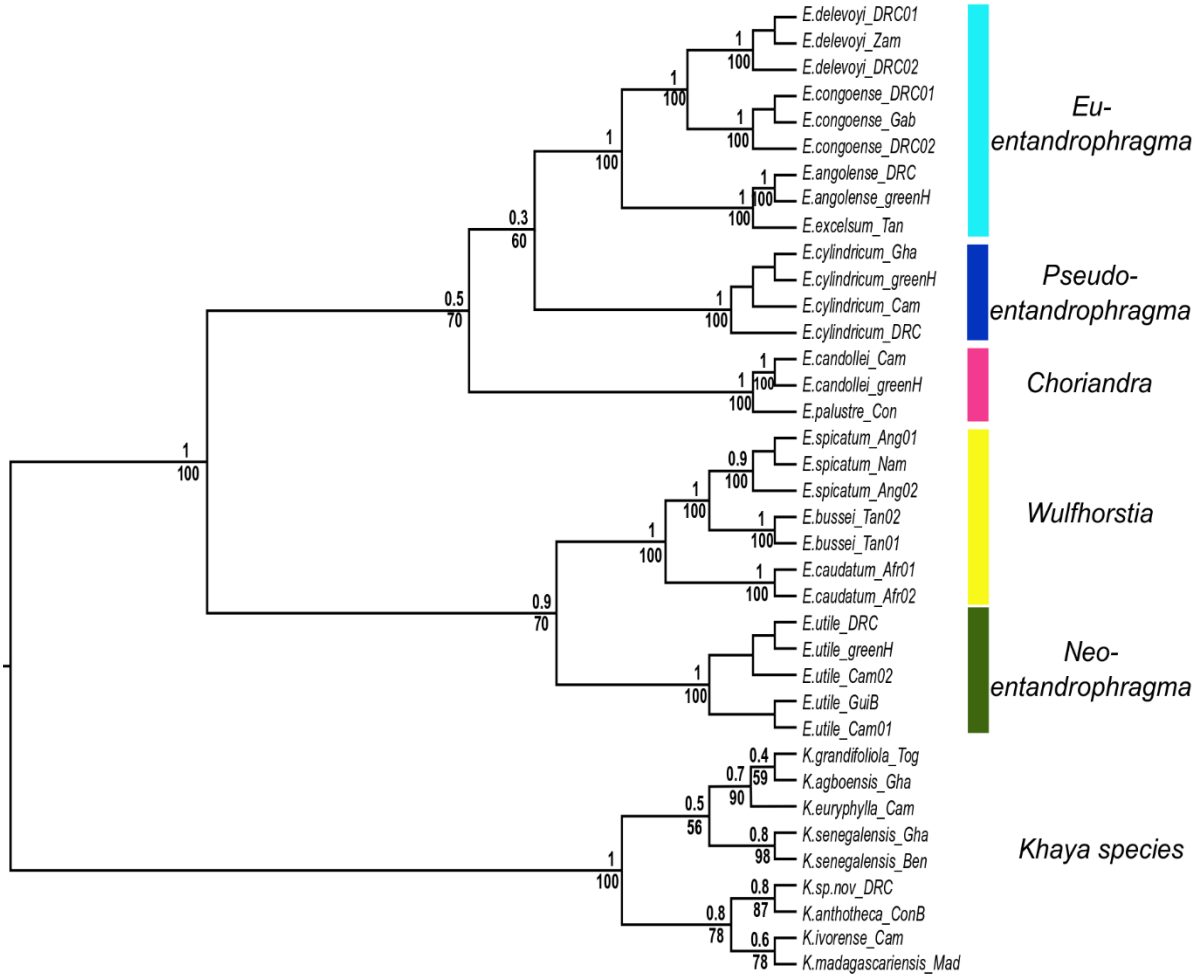

Fig. 2. Maximum clade credibility tree based on the rDNA data set for *Entandrophragma* (*n* = 28) and *Khaya* (*n* = 9) samples highlighting the relationship between sections (*Wulfhorstia*, *Pseudoentandrophragma*, *Choriandra*, *Neoentandrophragma* and *Euentandrophragma*) as defined by Louis and Fouarge (1947). Numbers above branches are Bayesian posterior probabilities, and numbers below branches are bootstrap percentages from the Maximum Likelihood analysis.

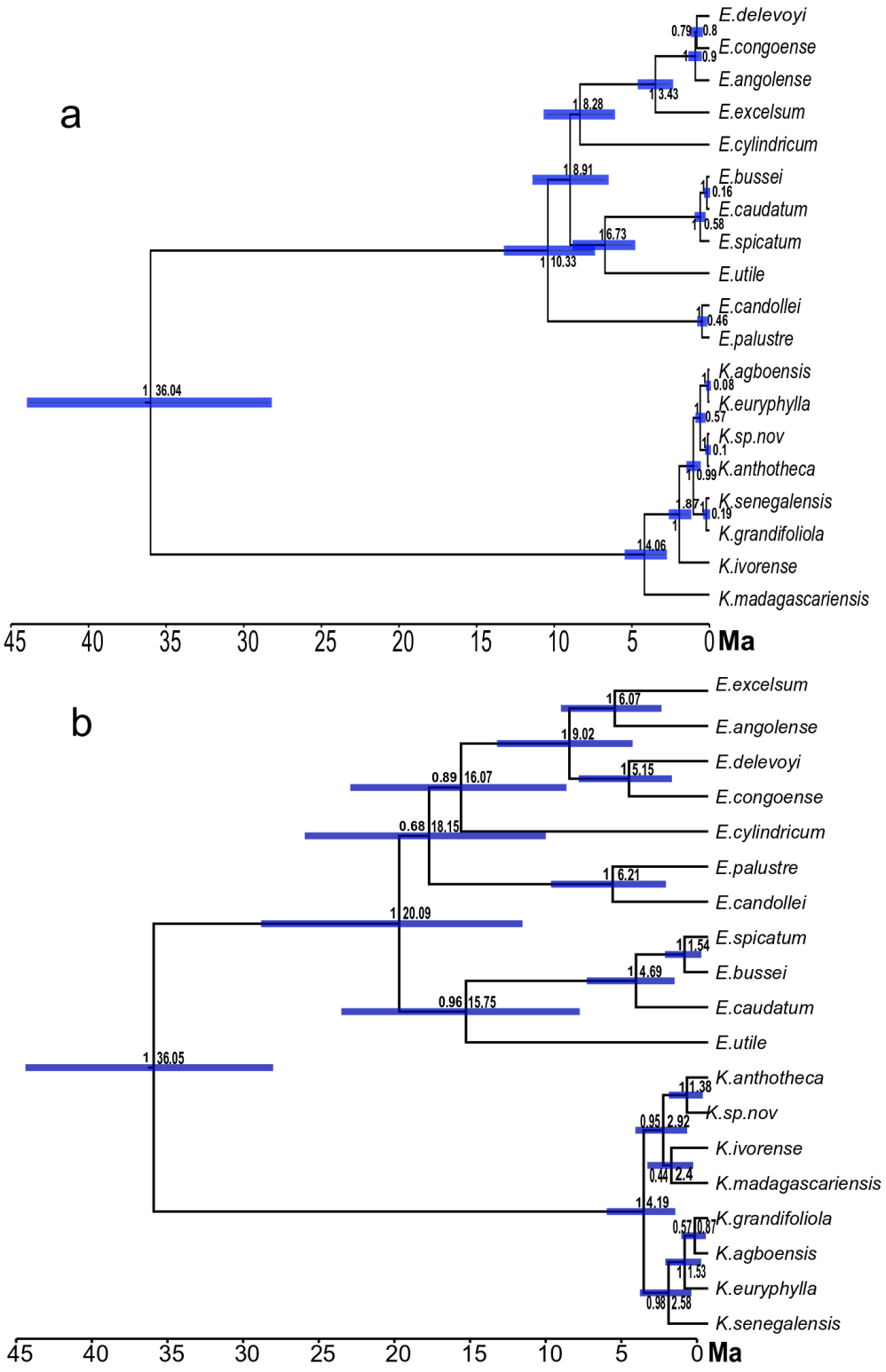

Fig. 3. Divergence time chronograms obtained from the Bayesian maximum clade credibility tree reconstructed with BEAST, retaining one individual per species for the genera *Entandrophragma* (*n* = 11) and *Khaya* (*n* = 8) using the plastome (a) and rDNA sequences (b). The first number on nodes indicates posterior probabilities and the second number the mean divergence time estimate (in Ma). Bars indicate the 95% highest posterior density intervals around node ages.

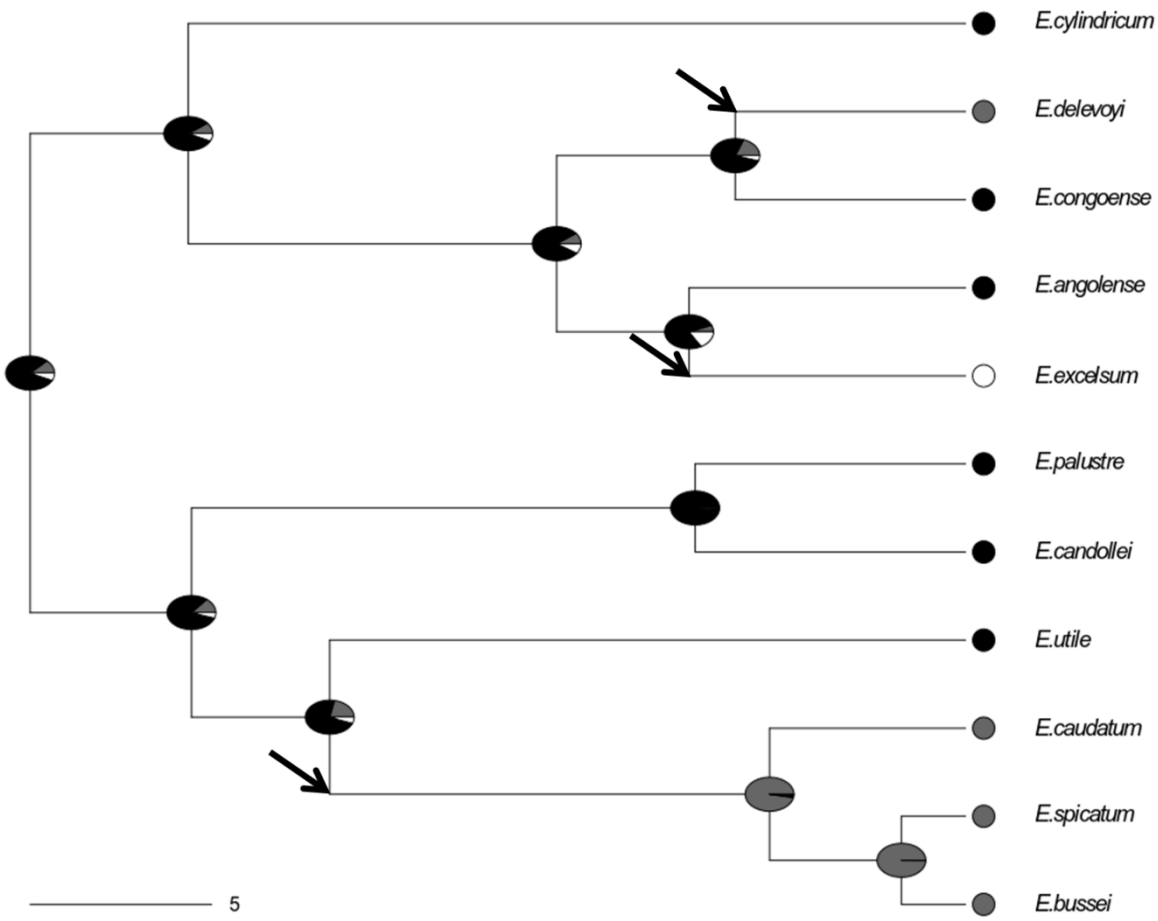

Fig. 4. Chronogram of *Entandrophragma* species with ancestral states reconstructions of preferred biome using a Maximum Likelihood approach implemented in the R package phytools (Revell, 2012). The tip labels indicate the biome inferred from a PCA based on climate data of species occurrences (Fig. S2): black for lowland rain forest species, dark grey for dry forest species, and the white for mountain species. Pie charts on the nodes show probabilities of the corresponding ancestral state reconstructions and the arrows indicates the branches along which biome shifts were inferred.

Taxa	Country and sample codes	Latitude (dd.mm)	Longitude (dd.mm)	Habitat	Vouchers
Entandrophragma angolense	Cameroon (greenH)			Rain forest	
Entandrophragma angolense[$]	DRC (DRC)			Rain forest	BR0000013296377
Entandrophragma bussei	Tanzania (Tan01)			Dry forest	BR0000016993259
Entandrophragma bussei[$]	Tanzania (Tan02)			Dry forest	BR0000016993235
Entandrophragma candollei	Cameroon (greenH)			Rain forest	
Entandrophragma candollei[$]	Cameroon (Cam)	2.45613	12.34828	Rain forest	
Entandrophragma caudatum[*,$]	South-Africa (Afr01)	-25.254498	27.095284	Dry forest	BR0000016993372
Entandrophragma caudatum	South-Africa (Afr02)	-23.806667	31.6408333	Dry forest	WAG1487660
Entandrophragma congoense	DRC (DRC01)	-6.296979	22.68085	Rain forest	BR0000013977511
Entandrophragma congoense[$]	Gabon (Gab)	-0.6833333	11.916666	Rain forest	WAG1096956
Entandrophragma congoense	DRC (DRC02)	-6.296979	22.68085	Rain forest	BR0000013977528
Entandrophragma cylindricum	Cameroon (greenH)			Rain forest	
Entandrophragma cylindricum	DRC (DRC)	0.814988036	24.51104203	Rain forest	
Entandrophragma cylindricum[$]	Ghana (Gha)	6.69366	-2.64457	Rain forest	
Entandrophragma cylindricum	Cameroon (Cam)	3.977554	13.582182	Rain forest	
Entandrophragma delevoyi	DRC (DRC01)	-11.5252283	27.45597444	Dry forest	BR0000013423124
Entandrophragma delevoyi[$]	Zambia (Zam)	-12.9559	28.6844	Dry forest	BR0000013423216
Entandrophragma delevoyi	DRC (DRC02)	-6.05	28.06666667	Dry forest	BR0000013423162
Entandrophragma excelsum[$]	Tanzania (Tan)	-6.829	34.9304	Montane forest	MO-2454114
Entandrophragma palustre[$]	Republic of Congo (DRC)			Dry forest	BRLUC417
Entandrophragma spicatum	Angola (Ang01)	-16.7	14.95	Dry forest	BR0000016993440
Entandrophragma spicatum	Angola (Ang02)	-13	14.3333	Dry forest	WAG1097000
Entandrophragma spicatum[$]	Namibia (Nam)			Dry forest	MO-2454160
Entandrophragma utile	Cameroon (greenH)			Rain forest	
Entandrophragma utile	Cameroon (Cam01)	4.240408	13.435032	Rain forest	WAG1097002
Entandrophragma utile	DRC (DRC)	0.80951	24.47554	Rain forest	
Entandrophragma utile	Cameroon (Cam02)	3.505253	13.610079	Rain forest	
Entandrophragma utile[$]	Bissau-Guinea (GuiB)	7.6352778	-9.25138888	Rain forest	WAG1097008
Khaya sp. nov[$]*.*	DRC ()	0.254731	25.275119	Rain forest	P00790069
Khaya anthotheca[$]	Republic of Congo (ConB)	3.23892	18.25205	Rain forest	BOUKA 2, KHANT_255_1
Khaya agboensis[$]	Ghana (Gha)	6.8239	-2.669017	Rain forest	KHANT_10_1
Khaya euryphylla[$]	Cameroon (Cam)	3.92839	14.88782	Rain forest	Bouka 7, KHANT_218_1
Khaya grandifoliola[$]	Togo (Tog)	6.90804	0.60505	Gallery forest	Segla K2G11, KHGRA_43_1
Khaya ivorensis[$]	Cameroon (Cam)	2.39017	10.71546	Rainforest	Bouka 13, KHIVO_281_1
Khaya madagascariensis[*,$]	Madagascar (Mad)	-12.67055	49.5333333	Rain forest	JFR1500, KHMAD_67_1
Khaya senegalensis	Benin (Ben)	8.391306	1.908306	Savannah	KHSEN_122_1

Khaya senegalensis$^{\$}$	Ghana (Gha)	8.07344	-2.14798	Savannah	KHSEN_658_1

Table. 1. Taxonomy, origin, habitats, and characteristics of samples of *Entandrophragma* and *Khaya* used for pDNA and rDNA phylogenies. * indicate samples used as reference for plastome reconstruction and $^{\$}$ indicate samples used for molecular dating. The letters in voucher identification tags indicate the herbarium in which specimens were collected: BR for Botanic Garden Meise, Mo for the Missouri Botanical Garden, WAG for Wagenigen Herbarium and P for Muséum National d'Histoire Naturelle Paris. The other letters indicate the Dr Olivier Hardy silica data-based collection at Université Libre de Bruxelles.

Highlights:

- Whole plastome and ribosomal DNA sequencing reveal two main diversification periods (Miocene and Pleistocene) of African trees from genus *Entandrophragma* and *Khaya*
- Three biome transitions occurred from humid forest to dry or montane African forests
- Morphologically-based species delimitation is confirmed for *Entandrophragma*

Electronic Supplementary Material

Phylogenetic relationships in two African Cedreloideae genera (Meliaceae) reveal multiple rain/dry forest transitions

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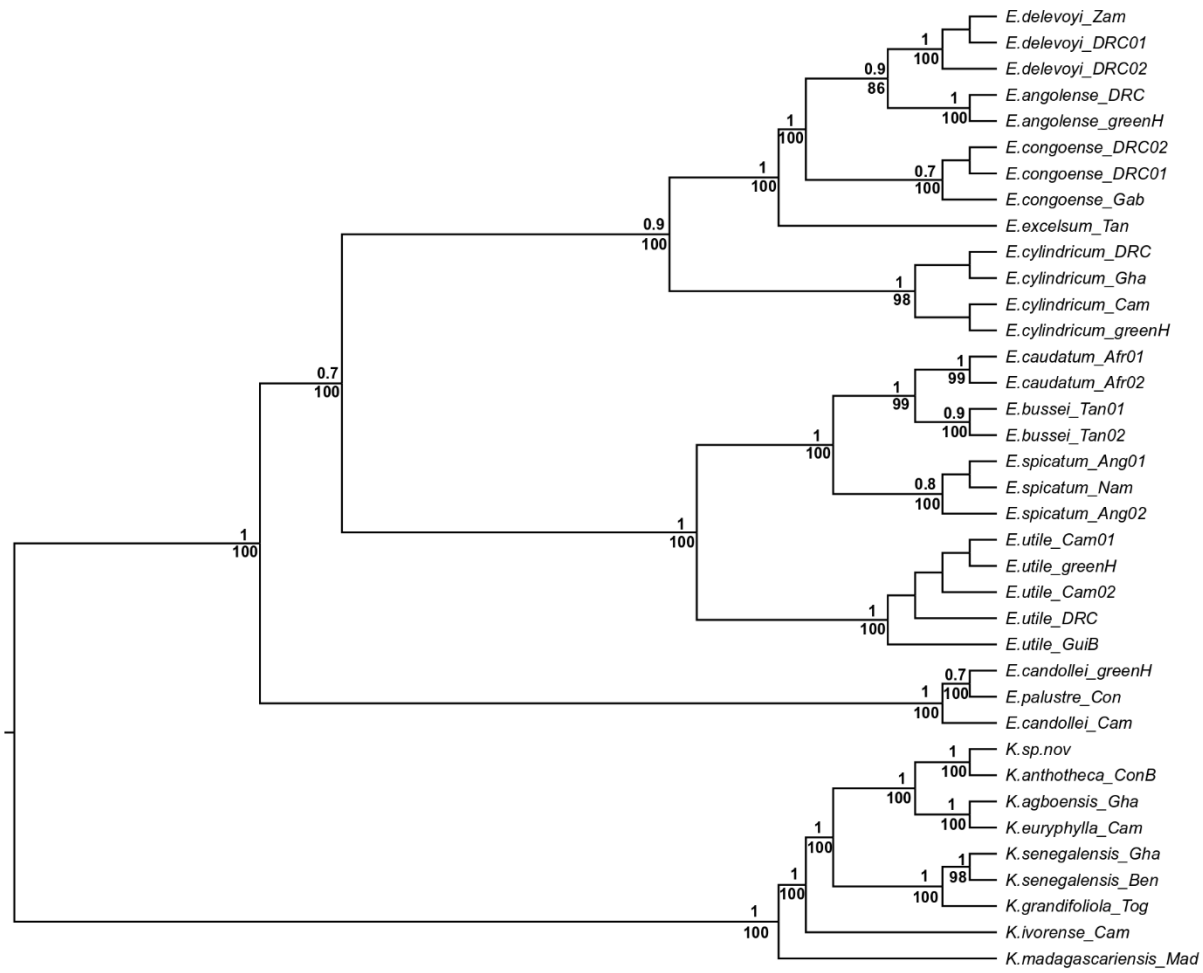

Fig. S1: Bayesian consensus tree of the whole plastome DNA data set of 37 samples of *Entandrophragma* and *Khaya*. Numbers above branches are Bayesian posterior probabilities, and numbers below branches are bootstrap percentages from the ML analysis.

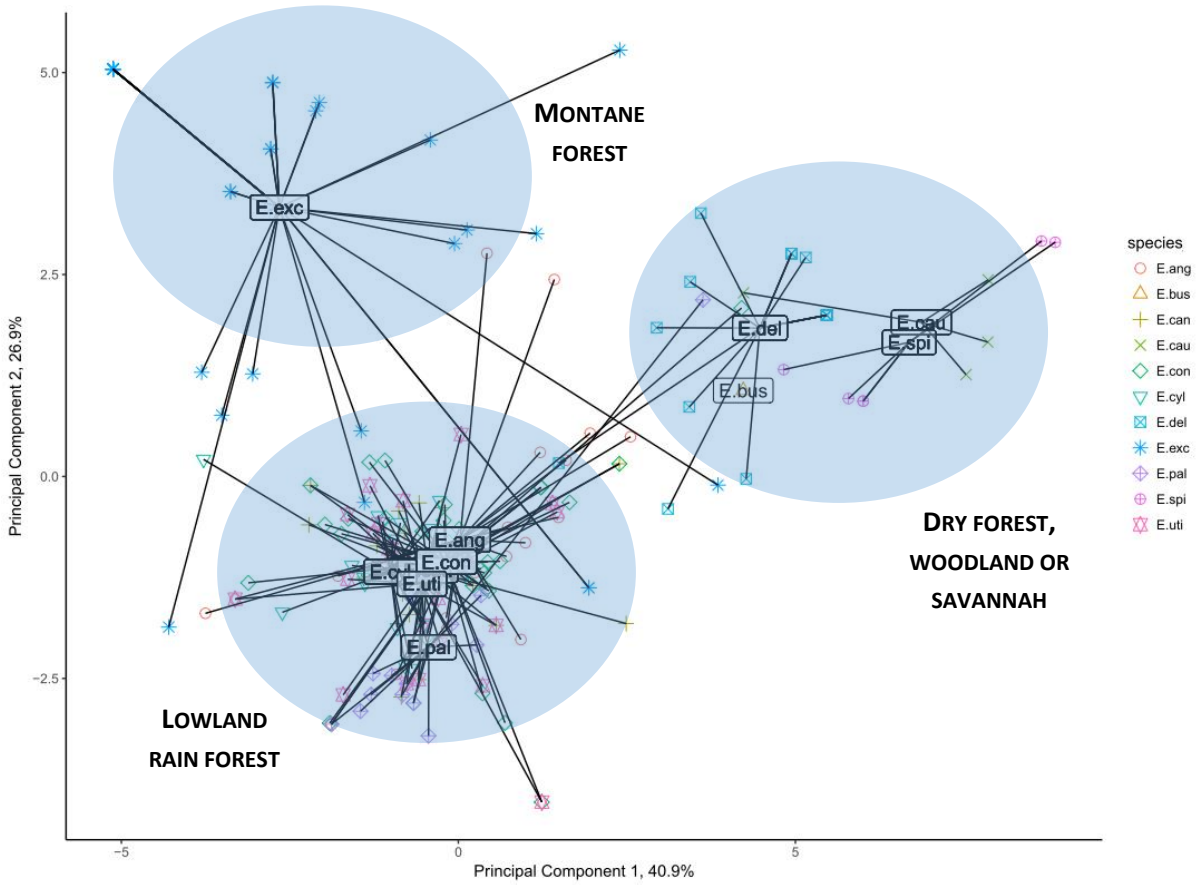

Fig. S2. Principal component analysis (PCA) of bioclimatic variables from occurrence points of 203 individuals of *Entandrophragma* species extracted from the RAINBIO database (Dauby et al., 2016). As sample sizes varied substantially among species in this database, a maximum of 30 individuals per well represented species were randomly chosen to ensure that the PCA reflects the climatic gradients between species. Climate data were extracted from the Wordclim database, retaining all the 19 bioclimatic variables. The centroid of each species is depicted by the abbreviated name of each species. Three climatic niches are shown in blue-grey and correspond to different biomes.