



The impact of rainforest area reduction in the Guineo-Congolian region on the tempo of diversification and habitat shifts in the *Berlinia* clade (Leguminosae)

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Abstract

Aim: The Guineo-Congolian region in Africa constitutes the second largest area of tropical rainforest (TRF) in the world. It covered an estimated 15–22 million km² during the late Miocene (55–11 Ma) and it has experienced since a declining trend, currently reaching 3.4 million km², associated with increasing aridification and the replacement of TRF by savanna habitats. Here, we examine whether rainforest area contraction led to a decrease in net diversification rates linked to increasing extinction, or if it is associated with increasing opportunities for allopatric or ecological speciation during periods of forest fragmentation.

Location: Tropical Africa, Guineo-Congolian region.

Taxon: *Anthonotha*, *Englerodendron*, *Berlinia* clade (Leguminosae).

Methods: We used a target enrichment approach combined with a complete data set representing all genera within the *Berlinia* clade. We combined phylogenomic, dating estimates, habitat reconstruction and diversification rate analyses to infer the effect of change in rainforest area coverage at two taxonomic levels: among genera, and within *Anthonotha* and *Englerodendron*.

Results: We recovered fully resolved and well-supported relationships among all genera and among species within the two genera. Most genera (87.5%) diverged before the Pleistocene, but *Anthonotha* and *Englerodendron* diversified recently, during the most recent cycles of forest contraction and expansion of the Pleistocene.

Main conclusions: Our results suggest that the *Berlinia* clade displays an overall trend of accumulation of species over evolutionary time, suggesting the reduction in TRF area has not decreased net diversification rates. Most habitat shifts to savanna occurred in the Miocene, with no major habitat shifts during the most recent phases of forest expansion–contraction in the Pleistocene. Shifts in habitat from lowland forest to savanna did not trigger diversification rates, but habitat fragmentation might have increased diversification rates through allopatric speciation.

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KEY WORDS

Africa, Detarioideae, Guineo-Congolian region, lowland rain forest, phylogenomics, savanna, target enrichment

1 | INTRODUCTION

Tropical rainforests (TRF) are the most species rich of all terrestrial ecosystems, representing a relatively old biome that has been shaped by geological events and ecological processes (Couvreur, Forest, & Baker, 2011; Davis, Webb, Wurdack, Jaramillo, & Donoghue, 2005; Eiserhardt, Couvreur, & Baker, 2017). There are currently three main regions with TRF: Africa, Southeast Asia and the Neotropical region. In comparison to the latter two, the African rainforest harbours lower tree species diversity (Couvreur, 2015; Linder, 2014), which is estimated to range between 4,500 and 6,000 species (Slik et al., 2015; Sosef et al., 2017). Several characteristics of the African rainforest have been proposed to explain this lower diversity (Couvreur, 2015; Richards, 1973), including a less complex geological history, fewer pronounced environmental gradients (Jacobs, Pan, & Scotese, 2010; Morley, 2000), a low species turnover (as sampling area increases, the number of tree species increases slowly; Plana, 2004; Slik et al., 2015) and the presence of megafauna in both past and present times (Terborgh et al., 2016).

During the Paleogene, TRF in Africa is thought to have extended from West to East Africa in a continuous belt that nearly covered the continent from coast to coast (Morley, 2000), with an estimated area of 15–22 million km² Ma (late Miocene). The area covered by TRF in Africa has experienced an overall decline since its maximum extension in the early Eocene to the Middle Miocene (55–11 Ma), currently covering 3.4 million km². However, palynological data, fossil evidence and palaeoenvironmental reconstructions indicate a very dynamic history of the TRF area coverage in Africa, with events of TRF contraction and expansion of savanna-type ecosystems since the Palaeocene (Jacobs et al., 2010; Morley, 2000; Plana, 2004). Thus, extant tree species diversity has been influenced by both the overall reduction in the TRF area, as well as the repeated cycles of contraction–expansion, which caused periods of isolation and subsequent contacts among these tree lineages (Couvreur, Chatrou, Sosef, & Richardson, 2008; Davis, Bell, Fritsch, & Mathews, 2002). These periods of contraction–expansion and the overall trend in reduction in rainforest area might have impacted diversification in opposite directions. A decline in suitable habitat area is expected to increase extinction rates and/or reduce speciation rates, while recurrent range fragmentation/expansion cycles might increase allopatric speciation by generating new gene flow barriers (Plana, 2004), in accordance with the speciation pump model (Haffer, 1969). The relative significance of these processes on the net diversification rates is still unclear, but it could affect how diversification rates change through time. Diversification rates could have slowed down, as in the “ancient cradle”, accelerated, as in the “recent cradle”, or remained constant, as in the “museum” model (Couvreur, Forest, et al., 2011; Eiserhardt et al., 2017). Speciation can also be triggered by adaptation to new

environmental conditions, potentially causing biome shifts despite the general trend of phylogenetic conservatism of the climatic niche among tree species (Tosso et al., 2018, 2019). However, it is still unclear if ecological speciation processes might have been favoured by periods of faster environmental change. This could have promoted habitat shifts from the TRF habitat to open savanna or woodlands (e.g. miombos) as these environmental habitats became available. Using a mega-phylogeny of angiosperm genera based on two plastid genes, Dagallier et al. (2020) showed that mountainous areas in tropical Africa acted both as a cradle and museum of biodiversity, concentrating both palaeo- and neo-endemics, while TRF acted more as a museum of ancient diversity, characterized by widespread ancient taxa. However, their analysis did not include recent speciation events occurring within genera, hence, the importance of compiling data sets at a finer phylogenetic resolution to improve our understanding of diversification patterns and processes (Eiserhardt et al., 2017).

Contrary to the pattern seen in several plant clades, where higher diversity is found in the neotropical and Southeast Asian rainforests, subfamily Detarioideae (Leguminosae) displays the opposite trend, with most of its species diversity occurring in Africa and Madagascar (de la Estrella et al., 2018). This subfamily is also characterized by entire lineages endemic to the African continent, which originated and diversified within this region. Recent studies at the subfamily level indicate that some genera represent old lineages (Donkpegan et al., 2017; Tosso et al., 2018). This suggests that during the Oligocene and Miocene, when the TRF was larger, higher speciation rates could have been promoted under a larger area and that the overall reduction in the forest area might have caused a reduction in diversification rates within Detarioideae.

Here, we explore the possible effect of area coverage changes in the TRF on the *Berlinia* clade, an endemic lineage within Detarioideae comprising an estimated 179 species and 16 genera. This clade is distributed in the main geographic regions of rainforest in tropical Africa: Upper Guinea, Lower Guinea, Congolia and East Africa, and only seven genera in this group have species widespread across all four regions. We analysed if most of the diversity within the *Berlinia* clade (both at the genus and species levels) originated early in the history of the group (higher diversification rates) and how changes in TRF area might have affected diversification rates in this clade. We test two hypotheses: whether TRF contraction led to a decrease in net diversification rates linked to increasing and higher aridification intensifying extinction (H1; e.g. Kissling et al., 2012) or to an increase in net diversification rates associated with increasing opportunities for allopatric or ecological speciation (H2; Chatrou, Couvreur, & Richardson, 2009; Couvreur et al., 2008). To test these hypotheses, we generated a well-resolved phylogenetic tree using a target enrichment approach (Nicholls et al., 2015; Ojeda et al., 2019)



to sequence 289 genes for all genera in the Berlinia clade. Here, we aim: (a) to estimate the time of divergence of all genera within the Berlinia clade, (b) to determine whether there is evidence of a change in net diversification rates through time associated with a reduction in forest area or an increase associated with a species pump effect and (c) to determine the conditions of forest area and the timing of biome shifts at the genus level in the Berlinia clade.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling and DNA extraction

We sampled 59 specimens representing 56 species and all 16 genera currently recognized within the Berlinia clade. Furthermore, we included a nearly complete representation of all species of *Anthonotha* and *Englerodendron*, except *E. hallei* and *E. sargosii* (de la Estrella et al., 2018; de la Estrella, Wieringa, Breteler, & Ojeda, 2019; Table 1). For the remaining 14 genera within the Berlinia clade, only representative species were included (Table S1). DNA was extracted from leaf tissue material (25–35 mg) obtained from herbarium specimens or silica gel dried samples using a CTAB-modified protocol (Doyle & Doyle, 1987) and the QIAquick PCR Purification Kit (Qiagen).

2.2 | Library preparation, target enrichment and paralogy assessment

Libraries were prepared with a modification of the protocol for plasmid capture (Mariac et al., 2014). Hybrid enrichment was performed on pools of 48 samples per reaction following the MYbaits v2.3.1 protocol, with 23 hr of hybridization, a high stringency post-hybridization

wash and a final amplification involving 15 PCR cycles. We used the Detarioidea v.1 bait previously developed for the entire Detarioidea (Ojeda et al., 2019), which consists of 6,565 probes (120 bp long overlapping baits) targeting 1,021 exons from 289 genes. Paired-end sequencing (2×150 bp) was performed on an Illumina NextSeq with reagent kit V2 at the GIGA platform (Liège, Belgium), assigning approximately 400,000 million reads/sample. We employed the strategy developed by Yang and Smith (2014) to recover regions from the target enrichment. First, we assembled de novo reads for each species using SPAdes ver. 3.9 (Bankevich et al., 2012) and reduced redundancy of the clusters recovered using CD-HIT (Li & Godzik, 2006; 99%, threshold, word size = 10). Then, we performed an all-by-all blast on all the samples and later filtered with a hit fraction cut-off of 0.5. We applied MCL (Van Dongen, 2000) using an inflation value of 1.4 to reduce identified clusters in the samples. We used mafft (Katoh & Kuma, 2002) to align clusters with <1,000 sequences (-genafpair -maxiterate 1,000, and 0.1 minimal column occupancy) and tree inference was generated with RAxML v. 8.2.9 (Stamatakis, 2014). For larger clusters we used PASTA (Mirarab et al., 2015) with minimal column occupancy of 0.01 and trees inferred using fasttree (Price, Dehal, & Arkin, 2009). Finally, orthologues were selected using the strict one-to-one strategy. This final step allows the identification and exclusion of paralog sequences, which is an advantage over other pipelines that only identify these paralog regions (Johnson et al., 2016; Vatanparast, Powell, Doyle, & Egan, 2018).

2.3 | Phylogenomic analyses using gene tree (individual orthologues), supermatrix (concatenation) and species tree estimation

Phylogenetic analyses were performed on each separate orthologue and on the concatenated matrix with maximum likelihood

Genus	Distribution	No. species	No. of samples included	% sampled
<i>Anthonotha</i>	UG, LG, C, EA	17	17	100
<i>Aphanocalyx</i>	UG, LG, C, EA	11	2	18
<i>Berlinia</i>	UG, LG, C, EA	24	2	8
<i>Bikinia</i>	LG, C	10	2	18
<i>Brachystegia</i>	UG, LG, C, EA	26	2	7.6
<i>Didelotia</i>	UG, LG, C	11	2	18
<i>Englerodendron</i>	UG, LG, C, EA	17	15	88
<i>Gilbertiodendron</i>	UG, LG, C	30	2	6.6
<i>Icuria</i>	EA	1	1	100
<i>Isobertlinia</i>	UG, LG, C, EA	5	2	40
<i>Julbernardia</i>	UG, LG, C, EA	11	2	18
<i>Librevillea</i>	LG	1	1	100
<i>Michelsonia</i>	C	1	1	100
<i>Microberlinia</i>	UG, LG, C	1	1	100
<i>Odoniodendron</i>	LG, C	6	2	33
<i>Tetraberlinia</i>	UG, LG, C	7	2	28.5

TABLE 1 Current genera recognized in the Berlinia clade with their distribution in the main regions of rainforest in tropical Africa, Upper Guinea (UG), Lower Guinea (LG), Congolia (C) and East Africa (EA). The total number of species for each genus and the proportion sampled in this study is also indicated



(ML) as implemented in RAxML ver. 8.2 (Stamatakis, 2014) using the GTRCAT model with -f a flags, 1,000 bootstrap and default settings. In addition, we also carried out a Bayesian analysis using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Ronquist et al., 2012). For the Bayesian analyses, we applied four chains, two runs of 50,000,000 generations with the invgamma rate of variation and a sample frequency of 5,000. The performance of these analyses was assessed with Tracer 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). We also performed species tree estimation under the coalescent model using the individual ML gene trees obtained with RAxML to infer a species tree using ASTRAL-II v. 5.5.7 (Mirarab & Warnow, 2015; Sayyari & Mirarab, 2016). Support was calculated using local posterior probability (LPP). *Paramacrolobium coeruleum* was used as outgroup taxon in all analyses.

2.4 | Analysis of discordance among the orthologues recovered

We evaluated the levels of conflict and concordance in the Berlinia clade between species-tree and gene-tree comparisons. For the former we used the concatenated-based species tree obtained with ML with a 68% matrix occupancy and a 100% taxon completeness obtained with the concatenated alignment. We examined gene-tree conflict using the obtained ASTRAL-II tree as the reference species tree. For this analysis we employed the 300 individual gene trees (each corresponding to each cluster we recovered), those recovered with the most complete set of taxa obtained (ML-based) with RAxML and a rapid 200 bootstrap support. Levels of concordance were quantified using the pipeline PhyParts (<https://bitbucket.org/blackrim/phyParts>; Smith, Moore, Brown, & Yang, 2015), which identify clades within each tree as concordant and/or with conflict. We then used ETE3 Python toolkit (Huerta-Cepas, Serra, & Bork, 2016) to visualize the proportion of these clades, as implemented in the script PhyPartsPieCharts (<https://github.com/mossmatters/MJPythonNotebooks>). Both analyses (species-tree and gene-tree concordance) were performed with all branches regardless of their support and also excluding branches with <70% support (using the -s 0.7 filter in the PhyParts; Smith et al., 2015).

2.5 | Dating analyses of the Berlinia clade

To infer the timing of divergence of the lineages within the Berlinia clade, we first used the SortaDate pipeline (Smith, Brown, & Walker, 2018) on the recovered contigs (exons) to estimate the total tree length and the root-to-tip variance. The former is a proxy for sequence variation (level of informativeness), while the latter is used as a proxy for clock-likeness. In addition, we also selected the genes that share at least 30% of nodes with the ML tree (the RAxML tree inferred with the concatenated matrix). We then

chose the top 50 exons (25 with the highest total tree length and 25 with the lowest root-to-tip variance) for dating analyses. Of the 50 genes selected, we found 7 overlapping genes for each category and, as a result, our final selection contained 43 genes that resulted in a concatenated alignment of 22,179 bp. Previous studies have estimated the age of the Berlinia clade between 15.4 Ma (Koenen et al., 2013; Simon et al., 2009) and 48.4 Ma (Bruneau, Mercure, Lewis, & Herendeen, 2008) using seven fossils within Detarioideae (de la Estrella, Forest, Wieringa, Fougère-Danezan, & Bruneau, 2017). Currently, there is only one unequivocal fossil assigned to the Berlinia clade, *Aphanocalyx* leaves from Tanzania (46 Ma; Herendeen & Jacobs, 2000). We ran three separate dating analyses to test the effect of using the two secondary calibrations and the *Aphanocalyx* fossil. We used the two previous estimates (48.4 and 15.4 Ma) as secondary calibrations of the crown age of Berlinia clade, and the fossil *Aphanocalyx* (46 Ma) using Beast v. 1.10.3 (Suchard et al., 2018) under a GTR substitution model with gamma distribution. We first performed an analysis to compare the BD with incomplete sampling and a coalescent constant size as priors using 50 million, GTR and gamma. We then compared the model marginal likelihood obtained between the two models using path sampling and stepping-stone sampling power posteriors and selected the best model (coalescence constant size) to perform the remaining analyses. We performed all the analyses considering a global molecular clock and the Lognormal relaxed clock. Both analyses were run for 50 million generations, sampling trees and parameters every 5,000 generations and a final burn-in of 5 million generations. Tracer 1.7 (Rambaut et al., 2018) was used to assess convergence among the chains as well as to evaluate the ESS parameter (ESS > 200).

2.6 | Ancestral state reconstruction of habitat types

Habitat types were coded at the species level for all representatives in *Anthonotha* (Breteler, 2010) and *Englerodendron* (Breteler, 2006, 2011; van der Burgt, Eyakwe, & Newberry, 2007). A similar approach was used for the monotypic genera *Icuria* (Lubkea, Dolda, Brinkb, Avisc, & Wieringa, 2018), *Michelsonia* (Wieringa, 1999), *Pseudomacrolobium* (INEAC, 1951; Ndayishimye et al., 2012; White, 1979) and *Librevillea* (Aubréville, 1968; Wilks & Issembé, 2000). For *Microberlinia*, we only included one of the two species and coded the habitat type for the species included (Wieringa, 1999). For the remaining genera, the scoring was done to represent the most common habitat reported for all species within each genera (Banak & Breteler, 2004; de la Estrella & Devesa, 2014; Mackinder & Harris, 2006; Mackinder & Pennington, 2011; Wieringa, 1999; Table S2). A total of three habitat types, African montane forest, lowland rainforest and savanna woodland, were recorded for the species included (Table S2). The rooting of the states was determined with the observed states from the outgroup species. Ancestral state reconstructions were performed on the best ML tree recovered using parsimony

and likelihood methods as implemented in Mesquite ver. 2.75 (Maddison & Maddison, 2015).

2.7 | Effect of palaeoenvironmental changes in tropical forest area on diversification rates in the *Berlinia* clade

To assess the effect of changes in forest area on the diversification rates of the *Berlinia* clade, we employed a birth–death (BD) model using maximum likelihood allowing for missing species in the phylogeny (Condamine, Rolland, & Morlon, 2013; Morlon, 2014; Morlon, Parsons, & Plotkin, 2011). We employed the dated phylogeny obtained with the age of 15.4 Ma for the crown age of the *Berlinia* clade. This clade comprises c. 179 species and 16 genera, and our sampling covered all extant genera and 56 (31%) of the species (fraction, $f = 56/179$). We tested three models with the time dependent (no effect of forest area) using (a) constant speciation rate (λ_{con}), (b) exponential variation in speciation rate (λ_{exp}) and (c) linear variation in speciation rate (λ_{lin}). These were analysed considering no extinction (μ_0) and with a constant extinction (μ_{con} ; Morlon et al., 2011). In addition, we also tested the same above models with constant extinction (μ_{con}): constant speciation rate, exponential variation in speciation rate and linear variation of speciation rate but considering the effect of forest area (environmental dependent). The initial

parameters for the speciation function (lam_par_init) were set at 0.009–0.001, and the initial parameters for the mutation function (mu_par_init) at 0.005. As a proxy of forest area change since the Oligocene (30 Ma), we used a compilation of the percentage of land coverage of the TRF estimated from megathermal vegetation in Africa (Kissling et al., 2012). The original megathermal data were used to generate 20,000 points among these megathermal data points in R using a linear method (Table S5). The time-dependent and environmental-dependent diversification models were estimated using the RPANDA ver. 1.7 (Morlon et al., 2016). The effect of the environmental-dependent analysis was assessed from the values obtained from rates of change in speciation rates (α) and the best-fitted model was selected based on the AICc estimates. These models have been recently criticised to provide unreliable estimates of speciation, extinction or net diversification rates, unless additional fossil and/or biological information is used in the interpretation (Louca & Pennell, 2020).

3 | RESULTS

3.1 | Relationships within the *Berlinia* clade

We recovered a mean of 83.77% ($\pm 5\%$) of the target bait, with an average of 34.28% ($\pm 14.14\%$) of the reads mapped to the A.

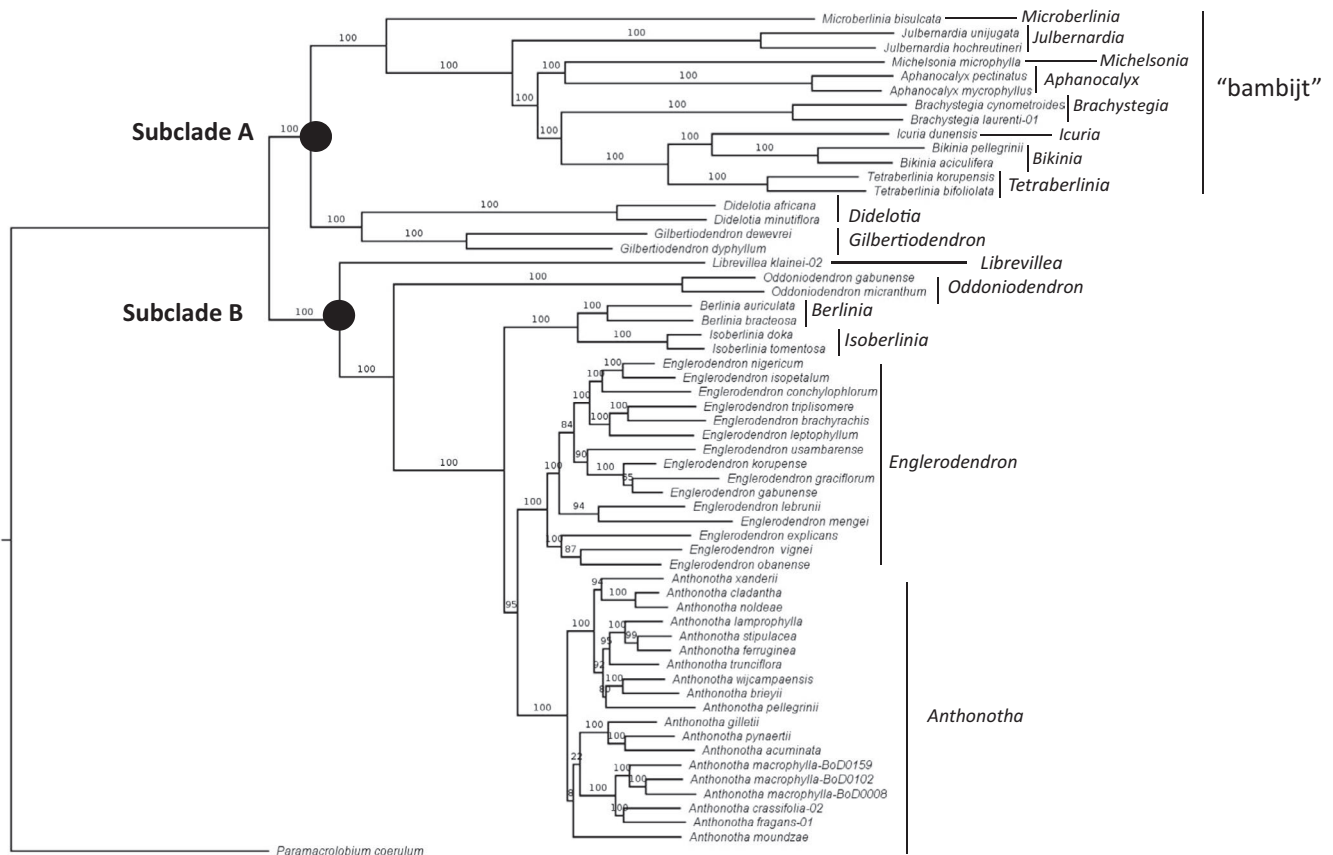


FIGURE 1 Phylogenetic relationships within the *Berlinia* clade obtained with maximum likelihood (ML) as implemented in RAxML using the individual set of cluster genes. Values next to branches represent ML bootstrap values

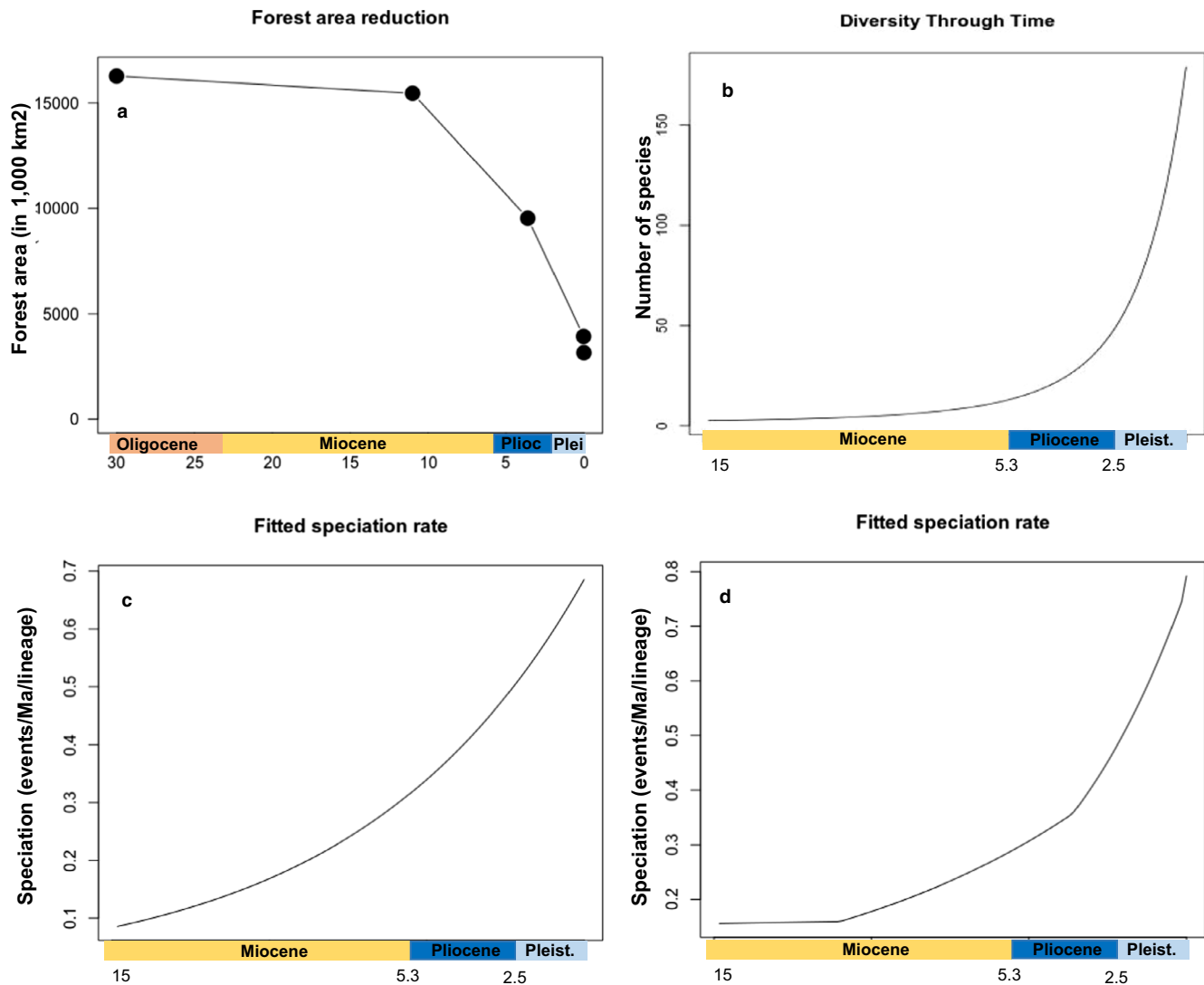


FIGURE 3 Diversification dynamics in the Berlinia clade and potential effect of forest area cover. (a) Estimates of forest area change since the Oligocene (30 Ma) to the present based on biome reconstructions (Kissling et al., 2012), (b) estimated accumulation of species as inferred with the time-dependent BD model, (c) fitted speciation rates as a function of time and (d) Fitted speciation rates as a function of the environmental data (forest area) obtained with the models in RPANDA [Colour figure can be viewed at wileyonlinelibrary.com]

three times older and the divergence of all its genera before the Pleistocene (Figure S4). Our calibration analyses based only on the fossil *Aphanocalyx* resulted in very old age estimates of the Berlinia crown node, exceeding the oldest age estimates recently published for Detarioideae as a whole (>68 Ma; de la Estrella et al., 2017).

3.4 | Ancestral state reconstruction of habitat types

Lowland rainforest was determined to be the ancestral state for the Berlinia clade both using ML (Figure S5) and parsimony analyses (Figure S6). At the genus level, we found three independent shifts to savanna woodland, two of them (*Brachystegia* and *Julbernardia*) occurred in the Miocene, while the most recent (*Isoberlinia*) took place

during the Pliocene. We recovered lowland rainforest as the ancestral state for the Anthonotha clade (*Anthonotha* and *Englerodendron*; Figure 2).

3.5 | Effect of palaeoenvironmental changes in tropical forest area on diversification rates

Estimation of speciation rates as a function of time in the Berlinia clade suggest an overall acceleration of speciation rates throughout the evolutionary history of the clade, with higher accumulation of species since the Pliocene (last 5 Ma; Figure 3a). The model that best fits the data among those tested is the environmental dependency with an exponential increase in speciation rates through the history of the Berlinia clade with no extinction (Table 2). This indicates a



TABLE 2 Results of the models tested with RPANDA. Columns in bold indicate the best-fitted model for the data. LH, log-likelihood; AICc, corrected Akaike Information Criterion; Δ AICc, change in AICc compared with the model with the lowest AICc; λ_0 , speciation parameter at present; α , rate of change in speciation; Models' parameters: λ_{con} , speciation constant; λ_{exp} , speciation exponential; λ_{lin} , speciation linear; μ_0 , no extinction; μ_{con} , constant extinction

Models	LH	AICc	Δ AICc	λ_0	α
Time dependent					
λ_{con} and μ_0	-141.446	284.962	13.60	0.413	—
λ_{exp} and μ_0	-134.210	272.635	1.28	0.685	-0.140
λ_{lin} and μ_0	-135.895	276.005	4.65	0.564	-0.034
λ_{con} and μ_{con}	-141.337	284.744	13.35	0.416	—
λ_{exp} and μ_{con}	-134.210	274.857	3.50	0.685	-0.140
λ_{lin} and μ_{con}	-135.863	275.941	4.58	0.567	-0.034
Environmental dependent					
λ_{con} and μ_0	-141.446	284.962	13.60	0.413	—
λ_{exp} and μ_0	-133.569	271.353	0	1.308	-1.3e-4
λ_{lin} and μ_0	-134.374	272.963	1.61	0.813	-4.316e-05
λ_{con} and μ_{con}	-141.337	284.744	13.35	0.416	—
λ_{exp} and μ_{con}	-133.581	271.377	0.024	1.300	-1.3e-4
λ_{lin} and μ_{con}	-134.374	272.963	1.61	0.813	-4.316e-05

negative correlation between TRF area and speciation rates, suggesting that the overall trend of reduction in tropical forest area in Africa increased, rather than decreased, net diversification rates on this group (Figure 3c).

4 | DISCUSSION

Here, we explored the effect of changes in tropical rainforest (TRF) coverage on the diversification rates on an endemic lineage of legumes in the Guineo-Congolian region. One of our hypotheses was that the rainforest contraction led to a decrease in net diversification rates linked to increasing aridification (Kissling et al., 2012). Our second hypothesis was that rainforest area contraction could have led to an increase in net diversification rates linked to more allopatric or ecological speciation opportunities (Chatrou et al., 2009; Couvreur et al., 2008) during the phases of TRF contraction–expansion. We found that diversification rates in the *Berlinia* clade have increased through time (in parallel with the reduction in TRF area), lending support for our second hypothesis. Our analyses based on the time-dependent diversification models (excluding the effect of forest area changes, Figure 3a) indicate that the *Berlinia* clade has accumulated species at an increasing rate through time (Figure 3b), suggesting an overall increase in speciation rates ($\alpha < 0$) across the clade's evolutionary history (Figure 3c). When considering the effect of rainforest area change through time, we found a negative correlation between diversification rates and TRF area (Figure 3d), which is not supporting the hypothesis that rainforest area reduction decreased diversification rates (H1). Rather, this is consistent with a “speciation pump” hypothesis, whereby the fragmentation/expansion cycles could have fostered allopatric speciation (Chatrou et al., 2009; Haffer, 1969). Additional evidence to support this comes from the current distribution of the most widely distributed species in *Anthonotha* and

TABLE 3 Number of species of *Englerodendron* and *Anthonotha* in the three phylogeographic domains of the Guineo-Congolia region, and their corresponding endemic species

Geographic region	<i>Englerodendron</i>		<i>Anthonotha</i>	
	Total	Endemic	Total	Endemic
Upper guinea (UG)	3	2	4	0
Lower Guinea (LG)	10	6	17	8
Congolia (C), East Africa (EA)	5	1	8	0

Englerodendron. Both genera comprise species with a wide distribution range (Upper Guinea, Lower Guinea and Congolia) and situated on the less derived lineages (Figure 2), while the most recently diverged lineages include species exclusively distributed in Lower Guinea (LG; Table 3). Also, habitat shifts were uncommon and mostly old, not supporting a major role of Plio-Pleistocene climate changes in fostering ecological speciation.

However, we should highlight that the number of species within each genus in the *Berlinia* clade is asymmetrically distributed, with only a few genera comprising most of the species. The two genera we sampled more comprehensively are among the species-rich genera. Thus, the apparent acceleration of diversification rate may be strongly dependent on the choice of genera studied at the species level. More generally, it is possible that forest decline and fragmentation/expansion dynamics lowered diversification in some clades (higher extinction) and increased diversification in others. *Anthonotha* and *Englerodendron* seem to have diversified during the late Pliocene to Pleistocene (3.5–0.5 Ma). Curiously, studies based on plastid sequence data found much older dates for the divergence of lineages within *A. macrophylla*, which resulted in a strong phylogeographic pattern where Upper Guinea diverged from Lower Guinea and Congolia c. 7 Ma ago (Demenou et al., 2020).

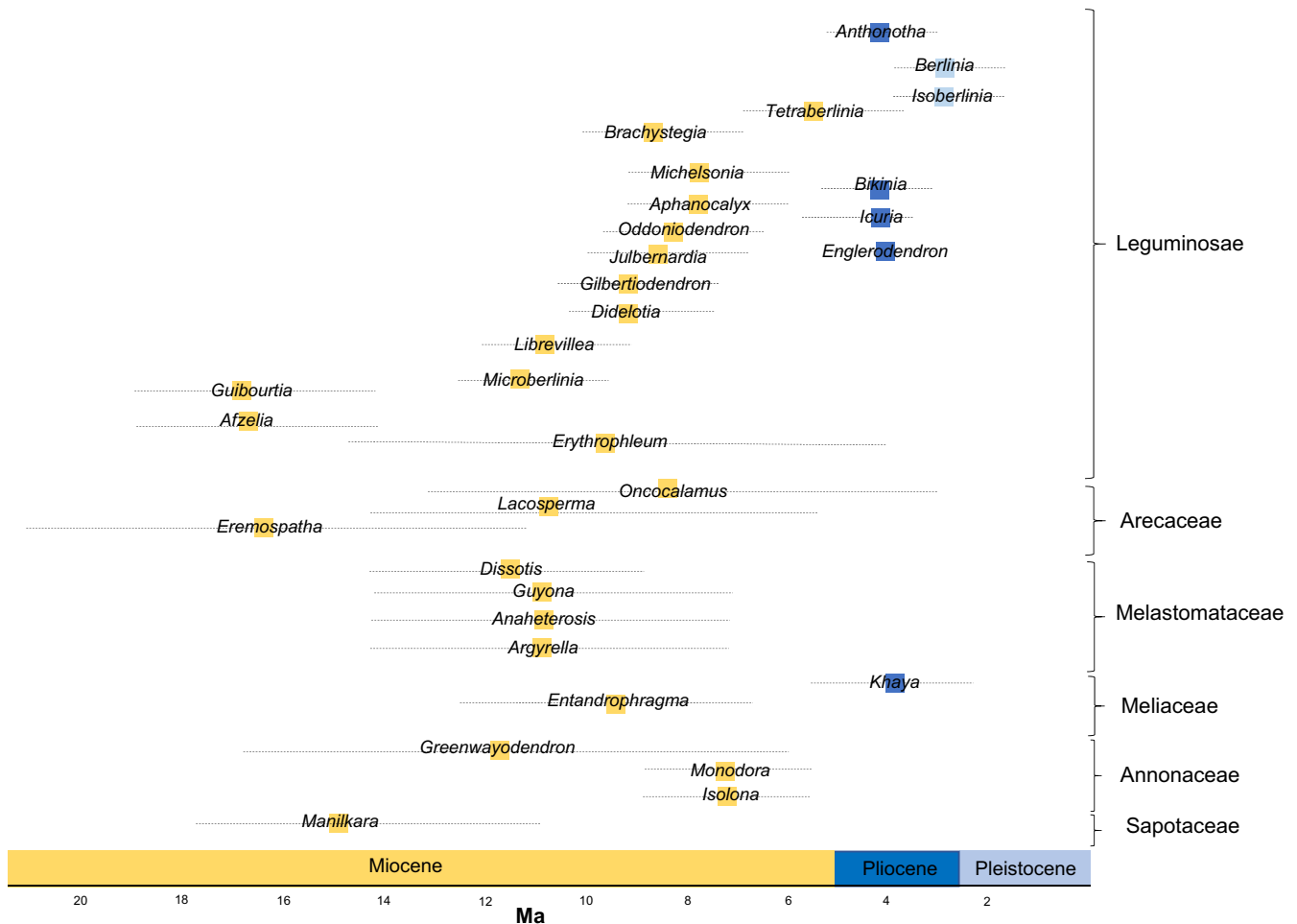


FIGURE 4 Comparison of estimated divergence ages of several genera in the rainforest of tropical Africa recovered in the *Berlinia* clade (this study), other Leguminosae (Donkpegan et al., 2017; Tosso et al., 2018), Arecaceae (Faye et al., 2016), Melastomataceae (Veranso-Libalah et al., 2018), Meliaceae (Monthe et al., 2019), Annonaceae (Couvreur, Porter-Morgan, Wieringa, & Chatrou, 2011; Migliore et al., 2019) and Sapotaceae (Armstrong et al., 2014). Yellow, blue and light blue squares refer to Miocene, Pliocene and Pleistocene, respectively, with the estimated confidence intervals (dashed lines) from the original publications [Colour figure can be viewed at wileyonlinelibrary.com]

By contrast, our nuclear DNA dating analyses estimate the divergence of *A. macrophylla* from different phylogeographic domains to 1.1 Ma (Figure 2). Although these findings seem incompatible, we also observed that different *Anthonotha* species can share the same plastomes (Demenou et al., 2020); therefore, it is possible that hybridization and chloroplast captures among *Anthonotha* species, and may be even among related genera, might explain the apparent discrepancy between dating analyses performed on plastid and nuclear genomes. A similar phenomenon is documented in the genus *Quercus* (Pham, Hipp, Manos, & Cronn, 2017), and seems to appear in *Brachystegia* (A. Boom, pers. comm.), another genus of the *Berlinia* clade. Hence, introgression mechanisms that remain to be elucidated may be involved in the rapid diversification of several genera of the *Berlinia* clade.

The pattern of recent diversification we found here in *Anthonotha* and *Englerodendron* is not commonly observed in tropical African trees (Couvreur et al., 2020), but it has been reported in some tropical trees in the Amazon basin (Dexter et al., 2017). The

Plio-Pleistocene period was characterized by pronounced changes in the extension of TRF, with phases of contraction (during drying and cooling periods), favouring the expansion of grasslands. This phase of more pronounced changes in drying and cooling conditions is associated with the onset of glaciation in the northern hemisphere (3.2, 3.0 and 1.8 Ma), and it has been involved in the overall reduced diversity of modern African TRF species (Morley, 2000). The impact of TRF contraction and expansion as a speciation mechanism have been extensively reported before in Africa, both at continental (Mairal, Pokorny, Aldasoro, Alarcón, & Sanmartín, 2015; Pokorny et al., 2015) as well as regional scales since the Miocene (Couvreur et al., 2008; Davis et al., 2002), but unlike these previous studies, the diversification of *Anthonotha* and *Englerodendron* is inferred to be more recent, and not involving shifts of habitat types (Figure 2; Figures S3 and S4).

Finally, our results suggest that most of the extant genus diversity in the *Berlinia* clade represent old lineages that probably originated under more favourable climatic conditions (larger



extension) of TRF. We found that 87.5% of the generic diversity in the *Berlinia* clade originated at least between the early and middle Miocene (25–15 Ma; Figure 2; Figure S4), before seasonality of rainfall and drier conditions in the middle Miocene promoted the replacement of TRF by open woodland and grassland (Jacobs et al., 2010; Morley, 2000). The early Miocene (25 Ma) is inferred to be characterized with moist climate over most of equatorial Africa and with limited geographic barriers across most of the TRF distribution (before the Neogene uplift and volcanism; Jacobs et al., 2010; Morley, 2000). It is in this context that many genera appeared in other lineages of Detarioideae (Donkpegan et al., 2017; de la Estrella et al., 2017; Koenen, Clarkson, Pennington, & Chatrou, 2015; Tosso et al., 2018), as well as in other tree genera from Annonaceae (Couvreur, Pirie, et al., 2011; Migliore et al., 2019), Arecaeae (Faye et al., 2016), Meliaceae (Monthe et al., 2019), Melastomataceae (Veranso-Libalah, Kadereit, Stone, & Couvreur, 2018) and Sapotaceae (Armstrong et al., 2014; see Couvreur et al., 2020, for a general review; Figure 4).

In conclusion, our results highlight the complex effects the fluctuations of the TRF coverage on the diversification rates and overall diversity of trees in the Guineo-Congolia region. Most of the current diversity at the genus level seems to be of ancient origin, in part promoted by the larger extension of the area of this biome. However, the overall trend in reduction in TRF area does not seem to have caused a reduction on diversification rates on this lineage, and the most recent phases of contraction–expansions could have promoted speciation. Our results also highlight that this effect was not homogenous across the *Berlinia* clade, as some genera could have suffered higher extinction or higher diversification rates perhaps linked to a higher flexibility to shift habitat types. Finally, the sister genera *Anthonotha* and *Englerodendron* appear rather exceptional by their rapid Plio-Pleistocene diversification, which may have also been fostered by extensive introgression mechanisms that remain to be investigated.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The clean sequence reads were deposited at NCBI under BioProject PRJNA472454. The concatenated matrix used in the phylogenomic analyses is deposited in the Dryad Repository (<https://doi.org/10.5061/dryad.tht76hdwj>).

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BIOSKETCHES

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Author contributions: ME, SC, DIO, FF and OJH conceived the idea of the study. ME and SC performed the laboratory experiments. SJ contributed with samples. DIO and SC performed the analyses; DIO prepared the first draft of the manuscript. All authors gave the final approval of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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