

# Menispermaceae and the diversification of tropical rainforests near the Cretaceous–Paleogene boundary

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## Summary

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- Modern tropical rainforests have the highest biodiversity of terrestrial biomes and are restricted to three low-latitude areas. However, the actual timeframe during which tropical rainforests began to appear on a global scale has been intensely disputed. Here, we used the moonseed family (Menispermaceae), an important physiognomic and structural component of tropical rainforests on a worldwide basis, to obtain new insights into the diversification of this biome.
- We integrated phylogenetic, biogeographic and molecular dating methods to analyse temporal and spatial patterns of global diversification in Menispermaceae.
- Importantly, a burst of moonseed diversification occurred in a narrow window of time, which coincides with the Cretaceous–Paleogene (K–Pg) boundary. Our data also suggest multiple independent migrations from a putative ancestral area of Indo-Malay into other tropical regions.
- Our data for Menispermaceae suggest that modern tropical rainforests may have appeared almost synchronously throughout the three major tropical land areas close to, or immediately following, the K–Pg mass extinction.

## Introduction

Modern tropical rainforests are the most species-rich and productive of the Earth's terrestrial biomes. They are restricted to the tropical zones of three land areas – Indo-Malay/Australasia, Afro-tropics and Neotropics – which all have high rainfall and equable temperature (Richards, 1996). Following the definition of Burnham & Johnson (2004) we use the phrase modern tropical rainforest to include several key features: high diversity and abundance of angiosperm trees and lianas; high proportions of entire-margined and large leaves; high abundance of leaf drip tips; large fruit and seed size, and abundant epiphytes. However, the actual timeframe during which modern tropical rainforests began to appear remains contentious. Some fossil floras from the mid-Cretaceous have been suggested as reminiscent of tropical rainforests (Upchurch & Wolfe, 1987; Wolfe & Upchurch, 1987; Morley, 2000). Recent dated phylogenies of important components of tropical rainforests (e.g. Malpighiales and Palmae) have also bolstered support for a mid-Cretaceous origin of this biome (Davis *et al.*, 2005; Couvreur *et al.*, 2011a).

Terrestrial plant ecosystems were undoubtedly altered dramatically at the Cretaceous–Paleogene (K–Pg) boundary 65.5 million yr ago (Ma) (Wolfe & Upchurch, 1986; Vajda *et al.*, 2001; McElwain & Punyasena, 2007; Nichols & Johnson, 2008; Schulte *et al.*, 2010). The prevailing view is that modern tropical rainforests began to appear after the K–Pg boundary (e.g. Upchurch & Wolfe, 1987; Wing & Boucher, 1998; Morley, 2000). Among the lines of evidence supporting this view are the paucity of fossils of large stems (Wheeler & Baas, 1991; Wing & Boucher, 1998) and large seeds (Tiffney, 1984; Sims, 2010) of angiosperms in the Cretaceous, which some consider a requirement for germination in low light (e.g. tropical understorey). Epiphytic ferns, characteristic of tropical rainforests (Burnham & Johnson, 2004), exhibit a shift of diversification rates near the K–Pg boundary (Schuettpelz & Pryer, 2009), in agreement with the hypothesis that modern rainforests began to appear after the K–Pg boundary. However, some families apparently were not affected during this K–Pg period. For example, three relatively ancient lineages with members in tropical rainforests, that is, the liverwort family Lejeunaceae (Wilson *et al.*, 2007) and the angiosperm families Annonaceae (Couvreur *et al.*, 2011b) and Palmae

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(Couvreur *et al.*, 2011a), have constant diversification rates over time.

The plant macrofossil evidence for angiosperm tropical rainforest in Africa comes from the late Eocene to late Oligocene of Cameroon (*c.* 39–26 Ma; Jacobs, 2004). The earliest record of Neotropical rainforest comes from the middle–late Paleocene of Colombia (*c.* 58 Ma; Jaramillo *et al.*, 2006; Wing *et al.*, 2009). In North America the fossil record suggests that forests similar to modern rainforest were established in the early Paleocene (64.1 Ma; Johnson & Ellis, 2002). Although floristic and vegetational changes in Southeast Asia remain poorly understood (Morley, 2000), these aforementioned fossil observations lead us to conclude that the establishment of modern tropical rainforests in different tropical areas was not synchronous.

Lianas afford important opportunities for the investigation of rainforests in that they are largely dependent on the presence of a developed rainforest biome, in which they reach their highest diversity. Lianas constitute 15–25% of the woody stem density and species diversity in modern rainforests (Gentry, 1991) and contribute up to 40% of forest leaf area and leaf productivity (Hegarty & Caballé, 1991). They are therefore an important physiognomic and structural component of modern tropical rainforests (Gentry, 1991; Schnitzer & Bongers, 2002). Additionally, lianas also play a crucial role in many aspects of rainforest dynamics, including suppressing tree regeneration, increasing tree mortality, indirectly promoting pioneer tree growth, and providing essential food and much-needed structural components of the habitat to many forest animals (Emmons & Gentry, 1983; Schnitzer & Bongers, 2002). Thus, lianas are regarded as a key indicator of modern tropical rainforests (Upchurch & Wolfe, 1987; Gentry, 1991; Richards, 1996).

In this paper we test the hypothesis that the establishment of modern tropical rainforests in different tropical areas was not synchronous by examining the historical diversification and biogeography of the angiosperm family Menispermaceae (moonseed family). Menispermaceae are a very important representative of liana families in tropical rainforests (Upchurch & Wolfe, 1987; Gentry, 1991; Richards, 1996) and therefore offer a remarkable opportunity for studying the diversification of tropical rainforests on a worldwide basis. Menispermaceae are one of the 10 most dominant liana families in tropical rainforests (Supporting Information Fig. S1; also see Fig. 2 in Nabe-Nielsen, 2001), which contribute substantially to the diversity and abundance of woody plants (Gentry, 1991; Nabe-Nielsen, 2001). Only two genera of the family, *Antizoma* (three species) and *Menispermum* (two species), are not distributed in tropical rainforests at all, whereas the remaining 70 genera are entirely or mostly distributed in tropical rainforests (Supporting Information Table S1). Stem anatomical features indicate that moonseed plants are well adapted to warm humid habitats (Carlquist, 2007). Additionally, the swollen regions at the base of the petioles are also an adaptation to tropical rainforests, with the function of turning the lamina to face the maximum light (Forman, 1986). Both the fossil record and molecular dating studies indicate that Menispermaceae are an ancient angiosperm lineage. The oldest putative fossil endocarp of the family comes from the Turonian of

central Europe (91 Ma). Some fossil leaves found in North America and Asia (but unconvincingly determined as Menispermaceae) extend back to the Early Cretaceous (Doria *et al.*, 2008). Recent molecular clock estimates suggest a stem age of 121.8 Ma for the family (115.6–125.0; Jacques *et al.*, 2011).

Here, we first reconstruct a robust phylogenetic framework for Menispermaceae using five chloroplast DNA regions with more extensive sampling at the generic level than in any previous study. By integrating phylogenetic, biogeographic and molecular dating methods, we then investigate the temporal and spatial diversification of Menispermaceae on a global basis. Finally, we use the moonseed family as an indicator to explore the diversification of tropical rainforests worldwide. That is, we attempt to assess whether the establishment of modern tropical rainforests in different tropical areas was synchronous or asynchronous.

## Materials and Methods

### Taxon and gene sampling

We sampled 90 species from 59 of the 72 extant genera of Menispermaceae to represent the geographic and taxonomic diversity of the family. Outgroups included 12 species from the other five families of Ranunculales (Wang *et al.*, 2009b).

We sequenced *c.* 7600 bp of chloroplast DNA, including *rbcl*, *atpB*, *matK*, and *ndhF* genes, and the *trnL-F* regions (*trnL* intron, and *trnL* (UAA) 3' exon-*trnF* (GAA) intergenic spacer). All terminal taxa represent single species and include five DNA markers except for *Cissampelos owariensis* (lacking *matK* and *trnL-F*) and *Tiliacora gabonensis* (lacking *atpB*). Voucher information and GenBank accession numbers are listed in Table S2. The primers used in this study are listed in Table S3. See Supporting Information Notes S1 for more details.

### Phylogenetic analysis

Phylogenetic analyses for the combined dataset were carried out using parsimony (PAUP v4.0b10; Swofford, 2003), Bayesian inference (BI; MrBayes v3.1.2; Ronquist & Huelsenbeck, 2003), and maximum likelihood (ML; RAxML v7.0.4; Stamatakis, 2006). Parsimony heuristic searches were performed with 1000 random sequence addition replicates, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. Internal branch support under MP was estimated by using 1000 bootstrap (BS) replicates. For BI analyses, we partitioned data *a priori* on the basis of gene identity and general biochemical or evolutionary constraints (Table S4). The Akaike Information Criterion (AIC), computed via Modeltest v3.06 (Posada & Crandall, 1998), was used to determine the best-fit model for each partition. Based on Bayes factors, partitioning strategy P<sub>10</sub> (codon pos1 + 2, pos3, intron, and spacer) was identified as optimal for our data and was applied in all subsequent Bayesian and ML analyses. Initial analyses providing data for comparison of the different partition strategies were run for 1 500 000 generations, and analyses applying the final best-fit model were run for 4 000 000 generations. Runs were started from a random

**Fig. 1** Combined chronogram and biogeographic analysis of Menispermaceae (a). Lineage-through-time (LTT) plots for Menispermaceae (b). Dating analysis was performed using r8s software. The topology corresponds to the majority rule consensus tree of the Bayesian stationary sample. Numbers in red near branches indicate the node number, as referred to Table 1 and Fig. S3. Large pie charts show the relative probabilities of alternative ancestral distributions obtained by Statistical Dispersal-Vicariance Analysis (S-DIVA) optimizations over the 5000 Bayesian trees (white > red); areas (frequencies < 0.1) are collectively given with black colour. Subfamilies based on Wang *et al.* (2009b). Other labelling follows that of Ortiz *et al.* (2007). Plots summarize the results of penalized likelihood (PL) analyses of 1000 Bayesian trees. The line in bold corresponds to the maximum credibility tree from the PL analysis. The shaded panel on the LTT plots highlights the time window in which a dramatic increase in diversification rates occurred.

tree sampled every 1000 generations of the MCMC chain, with default priors and the option *prset ratepr* set as variable. Majority-rule (> 50%) consensus trees were constructed after removing the 'burn-in period' samples (the first 25% of sampled trees). Finally, RAxML was run on the above optimal 10 partitions, each partition with a GTR +  $\Gamma$  model and all model parameters estimated, executing 1000 rapid bootstrap inferences before a thorough ML search. A robust phylogenetic framework for Menispermaceae is presented in Fig. S2.

### Divergence-time estimates

We used the penalized likelihood (PL) (r8s v1.71; <http://loco.biosci.arizona.edu/r8s/>) and Bayesian relaxed clock (BRC) (MULTIDIVTIME; <http://statgen.ncsu.edu/thorne/multidivtime.html>) approaches to date divergence times. Here, we selected 14 fossils that were confidently placed in our tree based on morphological evaluations to use as minimum-age constraints (Table S5). Additionally, a maximum-age constraint of 130 Ma was independently enforced on the basal node of the tree based on the crown age of *Eudicotyledoneae* (Bell *et al.*, 2010).

### Biogeographic analyses

In order to minimize the uncertainties associated with phylogenetic inference, the recently developed Statistical Dispersal-Vicariance Analysis (S-DIVA; Nylander *et al.*, 2008; Yu *et al.*, 2010) approach was used to infer ancestral distributions on the phylogeny of Menispermaceae. We scored the five main regions: Indo-Malayan (extending across most of South and Southeast Asia and into the southern parts of East Asia), Afrotropical, Australasian, Neotropical, and Holarctic. We randomly sampled 5000 trees from the MCMC output as a 'trees file' and used the majority-rule consensus tree derived from the MCMC stationary sample as a final representative tree.

The DIVA approach can be highly biased owing to incomplete taxon sampling, especially if the missing taxa occupy a basal position in the phylogeny (Barber & Bellwood, 2005). To test the effects of 13 missing genera on our DIVA optimizations, we first identified the diagnostic morphological characters for different clades, and then placed these missing genera in the corresponding clades (Fig. S3). We optimized selected characters onto the majority-rule consensus tree from the Bayesian analysis based on the combined dataset using MacClade v4.06. Two genera, *Dialythea* and *Ungulipetalum*, have several missing characters (Supporting Information Notes S2); we therefore placed them according to previous studies (Troupin, 1962; Barneby & Krukoff, 1971). The DIVA analyses were re-run with the 13 genera included. The biogeographic scenario presented here (Fig. 1a) was not

significantly altered by the addition of missing taxa, assuming our inferences of their relationships are accurate (Fig. S4).

### Diversification rate analyses

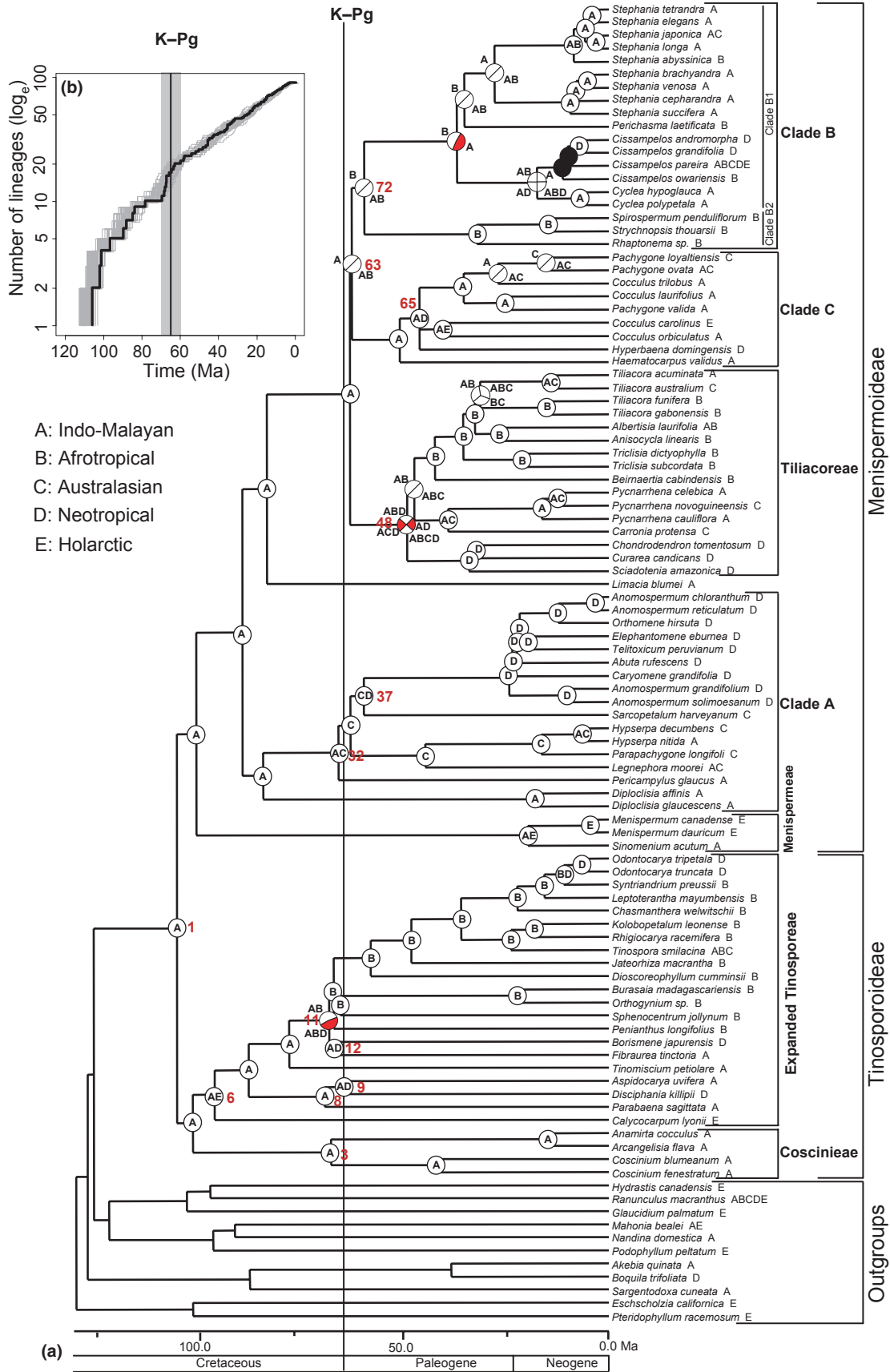
The temporal dynamics of diversification in Menispermaceae were first visualized with lineage-through-time (LTT) plots by using the R package APE (Paradis *et al.*, 2004). The ultrametric trees obtained from the PL analyses were used to generate semi-logarithmic LTT plots calculated for 1001 dated phylogenies. We determined the time point at which incomplete taxon sampling would begin to have a significant effect on the LTT plot following Couvreur *et al.* (2011a). We found that at 38 Ma, a dramatic increase of missing taxa occurred (19% rising to 31%). We therefore performed subsequent diversification analyses for Menispermaceae that were terminated at 38 Ma.

We used a maximum likelihood method to assess homogeneity of diversification rates across lineages with the R package LASER v2.2 (Rabosky, 2006). In rejecting a time-homogeneous rate, we wished to identify the lineages in which significant rate changes occurred around the K-Pg boundary. The absolute net diversification rates were calculated by using the PL chronogram under two extremes of the relative extinction rate (speciation rate/extinction rate = 0 and 0.9) following the whole-clade method (Magallón & Sanderson, 2001). We derived a 95% credibility interval on the expected diversity of the family through time. Using the age estimate for the stem group, standing diversities for the clades were compared with these sets of critical values, and those exceeding the upper values were considered unexpectedly species-rich, given the estimated overall diversification rate for the family. Calculations were performed using GEIGER v1.3-1 (Harmon *et al.*, 2008).

Additionally, we also used a sliding window analysis to visually examine the diversification rate change over time following Meredith *et al.* (2011). Sliding window analyses were carried out with two (PL and BRC) timetrees that were augmented by grafting additional lineages that diverged before 38 Ma. The time span from 38 to 110 Ma was divided into 7-million-yr sliding windows, every 2 million yr.

### Ancestral vegetation type

The ancestral vegetation type of Menispermaceae was reconstructed under a maximum likelihood approach using the Markov k-state one-parameter model implemented in Mesquite v2.74 (<http://mesquiteproject.org/mesquite/mesquite.html>). Four vegetation types were categorized: (1) tropical rainforests; (2) other tropical vegetation; (3) 1 + 2; and (4) temperate forests. We used the genus as an OTU except for *Cocculus* and *Pachygone*, neither of which is monophyletic.



## Results and Discussion

### Phylogeny of Menispermaceae

Phylogenetic analyses of the combined five-region DNA dataset generated a highly-resolved and well-supported evolutionary framework (87% of ingroup internodes received > 70% support from MP and ML methods; Fig. S2). Two major clades are identified within Menispermaceae, which correspond to Menispermoidae and Tinosporoideae (*sensu* Wang *et al.*, 2009b). Within Menispermoidae, the *Sinomenium*–*Menispermum* clade is sister to all other Menispermoidae with strong support, in agreement with the results of Ortiz *et al.* (2007). However, Hoot *et al.* (2009) and Jacques *et al.* (2011) suggest the *Sinomenium*–*Menispermum* clade as the earliest-diverging lineage in the family with weak to moderate support based on *rbcl* and *atpB* sequences. Our analyses place *Diploclisia* within clade A with moderate to strong support, while Hoot *et al.* (2009) and Jacques *et al.* (2011) found poor support for *Diploclisia* as sister to Tiliaceae, clades A, B and C. The clade of Madagascan *Rhaptonea*, *Strychnopsis* and *Spirospermum* (clade B2) is the sister group of clade B1, which was also recovered by Ortiz *et al.* (2007), while Jacques *et al.* (2011) placed clade B2 (sampling *Rhaptonea* and *Strychnopsis*) within clade C with poor support. In agreement with Hoot *et al.* (2009) and Jacques *et al.* (2011), we recovered the southeastern Asian genus *Tinomiscium* in the expanded Tinosporae, while Ortiz *et al.* (2007) found this genus to be sister to all other Menispermaceae. We have also confirmed that the *ndhF* sequence from *T. petiolare* by Ortiz *et al.* (2007) is a PCR-based artifact (Wang *et al.*, 2009b).

### Laurasian origin of major rainforest plant families

Based on our time estimates, Menispermaceae differentiated in the mid-Cretaceous (node 1: 106.3–109.1 Ma; Fig. 1a,

Table 1), which corresponds to the initial rise to dominance of angiosperms (Upchurch & Wolfe, 1987; Feild *et al.*, 2011) and the initial assembly of the tropical rainforest biome (Davis *et al.*, 2005; Couvreur *et al.*, 2011a). Our biogeographic reconstruction shows that the most recent common ancestor of Menispermaceae was likely present in the Indo-Malayan region of Laurasia (Fig. 1a). The oldest moonseed fossils have all been found in Europe and North America (Doria *et al.*, 2008). Our vegetation-type analysis demonstrates that tropical rainforest is inferred as the ancestral biome for Menispermaceae (LH = 1.0; Fig. S7). Our results therefore support the proposition that moonseeds originated and became differentiated in a rainforest biome of Laurasia during the mid-Cretaceous. A similar situation is also found in other rainforest groups, such as Palmae (Couvreur *et al.*, 2011a), Annonaceae (Takahashi *et al.*, 2008) and Zingiberales (Kress & Specht, 2006). Thus, our data for Menispermaceae provide additional evidence for a Laurasian origin of some major plant families from tropical rainforests.

### Burst of diversification of tropical rainforests

Our time estimates indicate that extant Menispermaceae date to the mid-Cretaceous (Fig. 1a, Table 1), but after the initial origin of the family, migrations did not occur until the end of the Cretaceous or the early Paleocene with the exception of one dispersal event into North America (*c.* 96 Ma; see Notes S1). Multiple colonization events occurred independently from the Indo-Malayan region into the other tropical regions: there were at least three dispersal events into the Afrotropics (nodes 11, 48 and 72), at least two such movements into Australasia (nodes 32 and 48) and at least four into the Neotropics (nodes 9, 12, 48 and 65) (Figs 1a, S4; Table 1). Additionally, there is one dispersal event from Australasia into the Neotropics (node 37). Most of these major migration events occurred in the timeframe of 60–70 Ma, close to the K–Pg boundary (Fig. 1a; see nodes 9, 11, 12, 32, 37

**Table 1** Estimated ages (million yr ago, Ma) for the nodes of interest in the Menispermaceae using Penalized Likelihood (r8s) and Bayesian Relaxed Clock (Multidivtime) methods with multiple calibrations

Nodes	r8s		Multidivtime	
	Age	LHPD-UPHD	Age	LHPD-UPHD
1 – Crown group of Menispermaceae	106.3	101.7–107.9	109.1	102.2–115.2
3 – Beginning diversification of Indo-Malayan Coscinieae	68.4	63.4–78.2	71.7	60.8–82.2
6 – Migration into North America	96.9	91.9–100.0	95.6	88.5–102.3
8 – Appearance of SE Asian <i>Parabaena</i>	69.9	58.0–79.5	69.4	56.9–80.0
9 – Migration into Neotropics	65.1	53.7–75.2	65.5	52.1–76.4
11 – Migration into Afrotropics	68.8	62.2–73.0	70.1	63.0–78.4
12 – Migration into Neotropics	67.8	62.3–74.4	69.3	61.9–76.9
13 – Diversification in Afrotropics	67.6	63.8–71.2	68.0	60.6–75.3
32 – Migration into Australasia	66.3	53.1–69.1	67.5	56.6–78.9
33 – Diversification in Australasia	62.4	48.7–65.3	63.9	52.9–75.6
37 – Migration into Neotropics	60.3	45.3–61.8	61.8	50.6–73.8
48 – Migration into Neotropics, Australasia, and Afrotropics	49.3	34.9–55.7	49.4	37.5–61.3
63 – Separation of clade B and C	63.3	56.7–70.0	62.1	52.6–72.0
65 – Migration into Neotropics	47.1	33.5–54.7	48.7	37.4–60.1
72 – Migration into Afrotropics	60.2	53.5–64.7	59.7	50.2–69.8

The node numbers correspond to those in Figs 1, S3. UHPD and LHPD are the upper and lower Highest Posterior Densities, respectively.

and 72). Some endocarp and leaf fossils of Menispermaceae have been reported from the Paleocene of the Neotropics, such as the Cerrejón Formation of Colombia (58–60 Ma) (Doria *et al.*, 2008; Wing *et al.*, 2009; Herrera *et al.*, 2011) and the Salamanca Formation of Patagonia (*c.* 61.7 Ma) (Iglesias *et al.*, 2007). Moonseed endocarp, pollen and leaf fossils have also been reported from the Paleocene of Europe, Asia and North America (Doria *et al.*, 2008; Jacques, 2009; Herrera *et al.*, 2011).

Given the inferred credibility intervals of the estimated times of divergence, the majority of the above colonization events seem to have occurred within a 10-million-yr time window (*c.* 60–70 Ma), and at least six genera or lineages of Menispermaceae, such as *Anamirta*–*Arcangelisia*, *Cosciniium*, *Parabaena*, *Aspidocarya*, *Fibraurea* and *Pericampylus*, occurred in the Indo-Malayan region within the same short timeframe (Fig. 1a, Table 1). These results indicate that the diversification of most modern moonseeds occurred near the K–Pg boundary. Further support for a burst of diversification of the Menispermaceae during this time window comes from our diversification analyses. The LTT plots for Menispermaceae are anti-sigmoid curves (Fig. 1b); that is, they rise steeply at first, curve over to a plateau, then rise steeply again between 70 and 60 Ma. Sliding window analyses generated a similar result (Fig. S5). Our diversification rate estimates also indicate that certain lineages near the K–Pg boundary have an elevated diversification rate relative to Menispermaceae overall, such as nodes 63, 72 and 48 (above the upper), nodes 13, 33, 37 and 65 (near the upper) (Table S6; Fig. S6). Menispermaceae, as an ancient lineage with members in tropical rainforests, has a shift of diversification rates near the K–Pg boundary, as seen in epiphytic ferns (Schuettpezel & Pryer, 2009). This pattern contrasts with studies of Lejeunaceae (Wilson *et al.*, 2007), Palmae (Couvreur *et al.*, 2011a) and Annonaceae (Couvreur *et al.*, 2011b), whose net diversification rates are constant over time.

This 10 million yr time span, 60–70 Ma, during which Menispermaceae rapidly diversified and migrated into the other major tropical landmasses, coincides with a tumultuous period of the environment on Earth (Upchurch & Wolfe, 1987; Miller *et al.*, 2010). The K–Pg boundary, *c.* 65.5 Ma, is famous for global vegetational upheaval and rapid ecosystem failure (Wolfe & Upchurch, 1986; Vajda *et al.*, 2001; McElwain & Punyasena, 2007; Nichols & Johnson, 2008; Schulte *et al.*, 2010). At the end of the Cretaceous, fossil pollen data suggest that up to 57% of all plant species in North America disappeared (Wilf & Johnson, 2004), 40% disappeared in Central Africa and New Zealand (Morley, 2000), and 70% disappeared in tropical South America (De la Parra *et al.*, 2007). The massive destruction of plant life produced vacant niches. Moonseed fruits are easily detached and presumably dispersed by mammals and birds (Forman, 1986), which underwent a rapid diversification near the K–Pg boundary (Feduccia, 1995; Bloch *et al.*, 2007; Wible *et al.*, 2007). These dispersers may have facilitated seed dispersal of Menispermaceae and thereby promoted moonseed plants to occupy new niches during this period.

Both direct (Upchurch & Wolfe, 1987; Morley, 2000) and indirect (Davis *et al.*, 2005; Couvreur *et al.*, 2011a; this study) evidence suggests that tropical rainforests were present in the

mid-Cretaceous. However, at that time this biome was located at middle paleolatitudes (Morley, 2000; Couvreur *et al.*, 2011a), rather than to the low latitudes of today (Richards, 1996; Morley, 2000). Our study shows that a burst of diversification of Menispermaceae occurred around the K–Pg boundary (Figs 1, S5, S7). This timeframe is similar to estimates for epiphytic ferns, which have a niche entirely dependent on the presence of a developed rainforest biome and which diversified most markedly in the Cenozoic (Schuettpezel & Pryer, 2009). Several key angiosperm elements of modern tropical rainforests, such as Sapindales (Wang *et al.*, 2009a), Fabaceae (Lavin *et al.*, 2005) and Rubiaceae (Antonelli *et al.*, 2009), also appear to have diverged or radiated at or near the K–Pg boundary. Recent study indicates that the escalation of angiosperm vein densities occurred in the mid-Cretaceous (*c.* 100 Ma) and near the K–Pg boundary (68–58 Ma); however, it is only during the latter that angiosperm vein density values represent the earliest known densities comparable to the vein densities found across extant megathermal rainforests (Feild *et al.*, 2011). Evidence from palaeofloras indicates that only during the Early Cenozoic do flowering plants seem to have attained a level of ecological prominence comparable to that of today (Crane & Herendeen, 2006). Additionally, Sims (2010) found a striking increase in within-flora seed size following the K–Pg boundary. Thus, ‘archetypal’ tropical rainforests (*sensu* Burnham & Johnson, 2004) might not have developed until near the K–Pg boundary.

Our study shows that multiple Afrotropical, Australasian and Neotropical moonseeds evolved independently within a narrow time window (*c.* 60–70 Ma), and multiple lineages of the Indo-Malayan moonseeds also originated during the same period (Fig. 1a). Given that lianas are a key indicator of modern tropical rainforests (Upchurch & Wolfe, 1987; Gentry, 1991; Richards, 1996), we therefore suggest that modernization of tropical rainforests may have begun nearly synchronously in all three tropical areas. Based on fossil data, however, the appearance of modern tropical rainforests in all three tropical areas seems to be asynchronous (Johnson & Ellis, 2002; Jacobs, 2004; Wing *et al.*, 2009). Fossil assemblages containing many species often represent quasi-mature or mature floras. For example, fossil evidence of well-developed Neotropical rainforests comes from the Eocene (Burnham & Johnson, 2004), but Wing *et al.* (2009) reported Paleocene pollen and leaf diversity at a remarkable 60–80% of modern levels, and Jaramillo *et al.* (2006) showed that middle Eocene pollen floras exceeded modern rainforest floras in their diversity. Additionally, different groups could have had different recovery abilities after the K–Pg extinction event (Briggs, 1991). It may thus be that the rates of large-scale recovery or establishment in each region and the opportunity for fossil formation differed among tropical regions.

## Conclusion

Molecular dating, together with phylogenetic and biogeographic analyses, shows that the rapid rise of the tropical family Menispermaceae occurred on a pantropical scale within a short

timeframe (60–70 Ma), which seems to coincide with the K–Pg extinction event. Whereas molecular dating studies indicate a near-simultaneous migration of Menispermaceae from the Indo-Malayan region into the other tropical regions following the K–Pg extinction event, the evidence for this in the fossil record is less certain and requires further study. Here, we have used the widespread tropical family Menispermaceae as an exemplar for investigating the diversification of tropical rainforests. Our data support the hypothesis that the modern tropical rainforests began to form almost synchronously throughout all tropical areas around the K–Pg boundary. Yet, Menispermaceae represent only a moderate-sized angiosperm family of *c.* 520 species. The hypothesis remains to be further tested by studying other important components of tropical rainforests, especially liana families, through integration of phylogenetic, biogeographic, fossil and molecular dating methods.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Number of liana species in the 10 most dominant families based on the inventories.

**Fig. S2** Fifty percent consensus rule cladogram of trees resulting from Bayesian analysis.

**Fig. S3** Placements of 13 missing genera.

**Fig. S4** Inferred ancestral areas based on DIVA with the addition of 13 genera not sampled in our molecular analyses.

**Fig. S5** Sliding window analysis of the net diversification rate.

**Fig. S6** Confidence intervals of expected clade diversity (log scale) according to age of stem group.

**Fig. S7** Ancestral vegetation type reconstruction for Menispermaceae.

**Table S1** Genera of Menispermaceae indicating the number of recognized and sampled species, geographic distributions, and vegetation types

**Table S2** Taxa, voucher and GenBank accession numbers for the sequences used in this study

**Table S3** Primers used for amplification and sequencing in this study



**Table S4** Partitioning strategies used in this study

**Table S5** Fossil calibration points used in this study as minimum age constraints

**Table S6** Age and diversification rates estimated for the nodes of interest in the Menispermaceae

**Notes S1** Expanded Materials and Methods, and Results and Discussion.

**Notes S2** Data matrix of 14 morphological characters and character states used in this study.

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