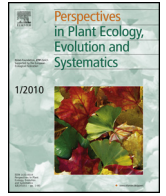




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Research article

The historical origins of palaeotropical intercontinental disjunctions in the pantropical flowering plant family Annonaceae



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ABSTRACT

Geographic isolation of sister taxa in the African and Asian tropics (palaeotropical intercontinental disjunction; PID) is a major biogeographic pattern explained by four competing hypotheses: rafting on the Indian tectonic plate ('Gondwanan vicariance hypothesis'); migration facilitated by a northern mid-latitude corridor of frost-free climates during the Eocene ('boreotropical migration hypothesis'); overland dispersal across Arabia associated with the Miocene Climatic Optimum; and transoceanic dispersal. The explanatory challenges posed by PIDs are addressed here using the pantropical flowering plant family Annonaceae as a study system. Molecular dating and ancestral area reconstructions were undertaken using plastid DNA sequence data (ca. 6 kb) derived from an extensive taxon sampling, incorporating ca. 75% of all genera, with phylogenetically informed sampling of species within genera that are distributed across the African and Asian tropics. Statistical dispersal–vicariance analysis and likelihood reconstructions indicated 12 intercontinental dispersal events between Africa and Asia. All but one of these dispersals were from Africa to Asia. Between 10 and 12 vicariance events were inferred, ranging from the late Palaeocene to the late Miocene, with mean divergence times of seven events in the Miocene. Although migration through the Eocene boreotropics has previously been highlighted as the predominant process underlying intercontinental disjunctions in Annonaceae, our results indicate that post-boreotropical processes have also had a major impact on shaping PIDs. Palaeogeographic reconstructions and the fossil record from the Arabian Peninsula support the plausibility of a hypothesized window of overland dispersal opportunity for lowland tropical forest taxa prior to climate deterioration commencing in the late Middle Miocene, providing an alternative to transoceanic dispersal. The patterns observed underscore the hypothesis that intercontinental floristic exchange, facilitated by both the Eocene boreotropics and the erosion of oceanic and climatic biogeographic barriers between Africa and Asia in the Miocene, had a substantial impact on the assembly of palaeotropical forest floras.

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Introduction

The African and Asian tropics, collectively referred to as the Old World tropics or Palaeotropics, comprise about 50% of the world's tropical rain forests (Morley, 2000) and harbour some of the hottest biodiversity hotspots on the planet (Myers et al., 2000). Tropical forest biomes in Africa and Asia are separated by vast expanses of ocean and climatically unsuitable terrain. Associated with this geographical gap in the distribution of tropical forests is the major

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biogeographic pattern of palaeotropical intercontinental disjunction (PID) of tropical forest taxa, i.e. geographical isolation of sister taxa in the African and Asian tropics.

The close floristic relationship between the African and Asian tropics, with numerous disjunct palaeotropical vascular plant taxa, has long been recognized (e.g. [Brenan, 1978](#); [Thorne, 1972](#)). [Thorne \(1972\)](#) identified hundreds of genera (e.g. *Canarium*, *Claoxylon*, *Mansonia*, *Nepenthes*, *Syzygium*, *Wrightia*) and several flowering plant families (e.g. Anacardiaceae, Dipterocarpaceae, Pandaceae, and Pandanaceae) which show discontinuous ranges between the African (Africa and Madagascar) and the Indian, Malesian or Australasian-Pacific tropics. Molecular phylogenetic analyses have shown that some traditionally recognized taxa showing presumed PIDs are para- or polyphyletic (e.g. [Saunders et al., 2011](#); [Sirichamorn et al., 2012](#); [Wang et al., 2012](#)), but many other PIDs at the infrageneric and deeper levels have been shown to represent reciprocally monophyletic groups on either continent. These intercontinental tropical disjunctions are of considerable biogeographic interest as they pose an explanatory challenge, and may provide insights into the formation and erosion of biogeographic barriers and assembly of tropical floras in the past ([Givnish and Renner, 2004](#); [Milne, 2006](#); [Thorne, 2004](#)). Four major competing hypotheses on the origin of PIDs can be differentiated ([Fig. 1](#)).

The 'Gondwanan vicariance' or 'Indian raft' hypothesis ([Fig. 1A](#))

Distributional disjunction between tropical Africa and Asia has been explained by the sequential break-up of the Gondwanan supercontinent, rafting of biota on the Indian plate, and facilitation of biotic exchange between India and Southeast Asia by convergence and collision of the Indian and Eurasian plates ([Conti et al., 2002](#); [McKenna, 1973](#); [Morley, 1998, 2000, 2003](#); [Raven and Axelrod, 1974](#)). Gondwana began to fragment in the Middle Jurassic, with India separating from Madagascar in the Late Cretaceous, 90–85 Ma, and drifting north-eastwards: the centre of the Indian craton moved ca. 6000 km from ca. 30°S in the Late Cretaceous to its current position, 23.5°N ([Ali and Aitchison, 2008](#)). The exact timing and mode of collision of India with continental Asia are disputed. Geodynamic models by [van Hinsbergen et al. \(2012\)](#) indicate a collision of an extended microcontinental fragment and continental Asia at approximately 50 Ma, followed by 'hard' continent to continent collision ca. 25 Ma, whereas previous models have indicated collision of India and continental Asia at ca. 35 Ma ([Ali and Aitchison, 2008](#)). Dispersal and establishment of floristic elements of Indian origin in Southeast Asia during the middle Eocene, 50–39 Ma, has been hypothesized based on palynological data ([Morley, 2003](#)).

Subsequent to the separation of Africa, Madagascar and India, and prior to the collision of India and Asia, some biotic exchange was likely between these areas due to dispersal. Palynological data suggest that dispersal between Africa and Madagascar occurred frequently until the mid-Maastrichtian, ca. 68 Ma ([Morley and Dick, 2003](#)). Prior to collision, India may have made a glancing contact with Sumatra, potentially facilitating biotic exchange with Southeast Asia in the late Palaeocene from ca. 57 Ma onwards ([Ali and Aitchison, 2008](#)).

The 'Gondwanan vicariance' hypothesis has been advanced for a variety of tropical vascular plant taxa based on macrofossil evidence ([Bande, 1992](#); [Bande and Prakash, 1986](#); [Srivastava and Mehrotra, 2013](#)), palynological data ([Dutta et al., 2011](#); [Morley, 1998, 2000](#)), fossil resin chemistry ([Dutta et al., 2011](#)) and molecular divergence time estimates or phylogenetic relationships ([Conti et al., 2002](#); [Dayanandan et al., 1999](#); [Ducouso et al., 2004](#); [Rutschmann et al., 2004](#)).

The 'boreotropical migration' hypothesis ([Fig. 1B](#))

Micro- and macrofossil evidence from numerous localities including the early Eocene London Clay ([Collinson, 1983](#); [Reid and Chandler, 1933](#)) and the middle Eocene Clarno Formation in north-central Oregon ([Manchester, 1994](#)) indicates the presence of an extensive frost-free and humid climate belt in the northern mid-latitudes, the boreotropics ([Wolfe, 1975](#)), during a warm phase peaking in the Late Palaeocene-Early Eocene thermal maximum, ca. 52 Ma ([Zachos et al., 2001](#)). The boreotropics harboured closed tropical aspect rain forests comprising various plant lineages which have been characterized as megathermal, i.e. frost-intolerant and restricted to tropical climates ([Morley, 2000](#); [van Steenis, 1962](#)). Climate deterioration in the late Eocene, and a drastic temperature drop at the Eocene-Oligocene boundary, ca. 34 Ma, resulted in expansion of vegetation adapted to drier and colder climates in large parts of Eurasia and Northern America, disrupting the northern megathermal forest belt ([Collinson, 1992](#); [Wolfe, 1992](#)). It has been hypothesized that the boreotropics facilitated intercontinental exchange of tropical biota in the Palaeocene and Eocene, not only across northern mid-latitudes, facilitated by land bridges that connected Laurasian fragments ([Brikiatis, 2014](#)), but also between the northern mid-latitude and equatorial megathermal forests ([Davis et al., 2002](#); [Morley, 2000, 2003, 2007](#)). In the late Eocene and Oligocene, when the boreotropics were disrupted and climates suitable for megathermal vegetation receded to equatorial regions, boreotropical taxa were driven to extinction or retreated towards the equator, forming isolated megathermal forest pockets in southern North America and Europe, and finding refuge in megathermal forests of Southeast Asia ([Kubitzki and Krutsch, 1996](#); [Mai, 1995](#); [Morley, 2000, 2003, 2007](#)).

Based on fossil evidence and temporally congruent molecular divergence time estimates, several authors have suggested that the boreotropics and its disruption in the late Eocene-early Oligocene played an important role in shaping current tropical disjunction patterns in several vascular plant lineages by vicariance, i.e. geographic isolation of previously connected populations (e.g. [Baker and Couvreur, 2013](#); [Chanderbali et al., 2001](#); [Couvreur et al., 2011a](#); [Davis et al., 2002](#); [Erkens et al., 2009](#); [Muellner et al., 2006](#); [Renner et al., 2001](#); [Richardson et al., 2004](#)).

The 'Miocene geodispersal' hypothesis ([Fig. 1C](#))

Micro- and macrofossil evidence indicates that megathermal elements almost completely disappeared from the northern mid-latitudes after the end of the Eocene cooling event ([Morley, 2000, 2007](#)). There is evidence for the persistence of pockets of rainforest and monsoonal forests in Europe and North America, but these were separated from each other and palaeoequatorial rainforests and monsoonal forests in Africa, America and Asia by vast stretches of climatically unsuitable terrain and marine gaps ([Morley, 2007](#)). Some studies, however, have suggested that dispersal from Africa to Asia via Arabia may have been feasible for tropical forest taxa during the early to middle Miocene, ca. 23–12 Ma ([Cruaud et al., 2011](#); [van Welzen et al., 2014](#); [Zhou et al., 2012](#)). Land connections formed between Africa and Southwest Asia due to the collision of the Afro-Arabian plate with the Iranian and Anatolian plates during this time ([Popov et al., 2004](#); [Rögl, 1998](#)), coinciding with a warming phase peaking in the Middle Miocene Climatic Optimum (MMCO), 17–15 Ma ([Zachos et al., 2001](#)). Extensive biotic exchange between Asia and Afro-Arabia, i.e. range expansion of independent clades (geodispersal *sensu* [Lieberman, 2000](#)), particularly well documented for fossil mammal faunas including primates, has been linked to the formation of these land bridges ([Bernor et al., 1987](#); [Kappelman et al., 2003](#)). During the MMCO pockets of subtropical

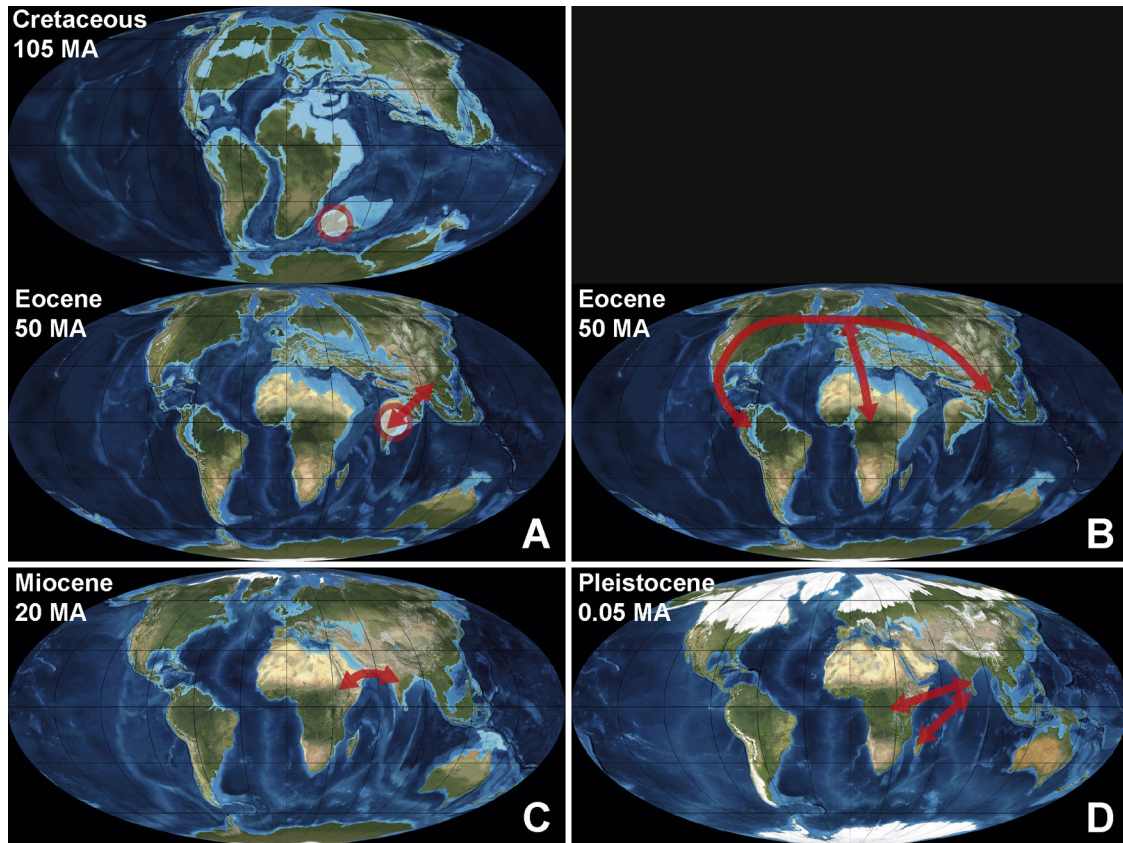


Fig. 1. Graphical representation of hypothesized historical biotic exchange between the African and Asian tropics. (A) Gondwanan vicariance hypothesis: rafting of biota on the Indian plate and biotic exchange facilitated by convergence and collision of the Indian and Eurasian plates in the Eocene, ca. 50 Ma. Note that the mode and timing of plate collision are disputed (see Section “Introduction”). (B) Boreotropical migration hypothesis: biotic exchange facilitated by an extensive frost-free and humid climate belt in the northern mid-latitudes, the boreotropics, during a warm phase peaking in the Late Palaeocene–Early Eocene, ca. 52 Ma. (C) Miocene geodispersal hypothesis: window of overland dispersal opportunity across Arabia in the early to middle Miocene associated with land bridge formation between Africa and Southwest Asia and a warm phase peaking in the Middle Miocene Climatic Optimum, 17–15 Ma. (D) Long-distance dispersal hypothesis: transoceanic dispersal between the African–Madagascan tropics and the Southeast Asian tropics. Red arrows indicate directionality of biotic exchange. Paleobasemap source: Mollewide Plate Tectonic Maps (<http://cpgeosystems.com/mollglobe.html>).

forests in the northern mid-latitudes (Morley, 2007) as well as equatorial rain forests expanded and Asian tropical forests are documented from the northwest of the Indian subcontinent (Pound et al., 2012). The associated putative window of overland dispersal opportunity for tropical forest taxa would have been of very limited duration, however, as the climate became distinctly cooler, drier and more seasonal from the late Middle Miocene onwards, resulting in the expansion of drought-adapted savanna and xerophytic shrubland vegetation (Jacobs, 2004; Kürschner, 1998; Pound et al., 2012; Zachos et al., 2001).

The ‘long distance dispersal’ hypothesis (Fig. 1D)

Dispersal of propagules across large marine barriers between continents (transoceanic or long-distance dispersal: LDD) and subsequent establishment offers an alternative mechanism to explain intercontinental disjunctions. This explanation, on first sight, seems to lack biological plausibility given the limited desiccation tolerance, limited preadaptation to brackish or salt water, and limited average dispersal distances of propagules exhibited by the vast majority of vascular plants, even when considering factors facilitating LDD such as animal vectors, prevailing wind and oceanic currents or island-hopping routes. However, LDD may show little correlation with morphological diaspore syndromes and standard means of dispersal, and may rather be facilitated by non-standard

means such as rare rafting events on vegetation floats, diaspores in the mud adhering to the feet of birds, or extreme meteorological events (Higgins et al., 2003; Nathan, 2006). Successful establishment of a plant subsequent to very rare LDD events, even in the frequency of a single event every tens of millions of years, and subsequent diversification may have an important biogeographical impact. The historical assembly of tropical forest biomes at the continental scale is highly complex, but molecular divergence time estimates, in combination with fossil data, indicate that LDD has indeed been an important factor in the assembly of tropical forest biomes (e.g. Renner, 2004; Pennington et al., 2006).

In most studies in which Miocene or Pleio-Pleistocene divergence of African and Asian species or clades has been inferred (e.g. de Wilde et al., 2011; Li et al., 2009; Liu et al., 2013; Sirichamorn et al., 2014; Renner, 2004; van Welzen et al., 2014), in which the disjunction is therefore temporally incongruent with both the ‘Gondwanan vicariance’ and the ‘boreotropical migration’ hypotheses, authors typically invoke transoceanic dispersal of propagules. In contrast to vicariance and geodispersal, which can be correlated with tectonic or climatic events, LDD events cannot be easily linked to specific abiotic events. Moreover, as LDD events are associated with the long tail of dispersal probabilities they are very rare, but considering stochasticity they could have occurred at any point in time in the evolution of a plant taxon. Hypothesized historical LDD is inherently impossible to falsify, but in the context of

the present study focusing on the historical biogeography of tropical forest taxa, it is important to note that for splits of African and Asian species or clades which likely occurred subsequent to climate deterioration in the late middle Miocene, temporally incongruent with the three other hypotheses on processes underlying PIDs, long-distance dispersal may provide the most feasible explanation.

Study system Annonaceae

Annonaceae, a pantropical flowering plant family (108 genera; 2292 species; Chatrou et al., 2012) of trees, shrubs and lianas (Fig. 2), provide an excellent opportunity to inject new data into the explanatory challenge posed by PIDs. Annonaceae are widely distributed in the tropics and subtropics, and they are a characteristic element in lowland tropical forests of both the Old and the New World (Keßler, 1993). Based on their extant and fossil distribution, the family has been characterized as megathermal (frost-intolerant and restricted to tropical climates: Morley, 2000; van Steenis, 1962), and significant positive correlations of Annonaceae species diversity and abundance with rainfall and temperature, respectively, have been identified (Punyasena et al., 2008). These characteristics indicate that Annonaceae may have shown a high degree of climate niche conservatism in their evolution, and that they may be good trackers of past tropical forest distributions (Couvreur et al., 2011a,b). Intergeneric phylogenetic relationships in the Annonaceae have been extensively studied using plastid DNA sequence data with dense generic sampling (Chatrou et al., 2012), and several PIDs have been identified and corroborated by molecular data at the infrageneric level (Chaowasku et al., 2012; Stull et al., 2011; Thongpaiboj, 2008; Zhou et al., 2012) and deeper nodes (Couvreur et al., 2011a; Richardson et al., 2004; Su and Saunders, 2009). Hypotheses to explain these PIDs largely represent the spectrum of hypotheses advanced to explain this major biogeographic pattern in flowering plants in general. Doyle and Le Thomas (1997), prior to the availability of molecular evidence for estimating divergence dates, hypothesized a complex biogeographical history of the Annonaceae crown group involving Gondwanan breakup, boreotropical migration and some subsequent dispersal as processes underlying PIDs. The importance of boreotropical migrations underlying current intercontinental disjunctions has been highlighted in most recent analyses incorporating molecular data (Couvreur et al., 2011a; Erkens et al., 2009; Pirie et al., 2006; Richardson et al., 2004; Surveswaran et al., 2010), while Zhou et al. (2012) stressed that overland dispersal across Arabia is the biologically most plausible explanation for the African-Asian split in the palaeotropical genus *Uvaria*.

Annonaceae are the largest family in the early-divergent Magnoliids (Massoni et al., 2014), and the oldest evidence for crown group Annonaceae, a fossilized flower described as *Futabanthus* (Takahashi et al., 2008), indicates an origin of the crown group prior to the early Coniacian (ca. 89 Ma). The relative antiquity of the Annonaceae crown group in combination with multiple identified splits between western (Africa, Madagascar) and eastern (Asia, Australia-Pacific) Palaeotropical clades, as well as the apparently high degree of climate niche conservatism make Annonaceae an excellent study system to investigate tropical forest evolution and assembly in the Palaeotropics across a broad time-scale (Couvreur and Baker, 2013; Couvreur et al., 2011a).

Study aims

The primary objective of the present study is to provide a framework to identify vicariance and dispersal events that underlie PIDs in the Annonaceae, to identify the directionality of biotic intercontinental exchange in the Palaeotropics, and estimate the timing

of these events, enabling an evaluation of competing hypotheses on the origins of PIDs. To achieve this we use a chloroplast DNA sequence data matrix (ca. 6 kb), integrating wide generic sampling (ca. 75% of the family) with phylogenetically informed sampling of the palaeotropical genera *Artabotrys*, *Hubera*, *Sphaerocoryne*, and *Uvaria*, and the only pantropical Annonaceae genus, *Xylopia*. Relaxed molecular clock analyses and ancestral area reconstructions are used to identify the directionality and timing of dispersal events and the timing of vicariance between the western (Africa and Madagascar) and eastern (India, Southeast Asia and Australia) Palaeotropics.

Materials and methods

Taxon sampling

The data matrix comprised five outgroup species representing four families in the Magnoliales (Magnoliaceae, Degeneriaceae, Himantandraceae and Eupomatiaceae), and an ingroup of 142 Annonaceae species representing the four Annonaceae subfamilies, all tribes except Dendrokingstonieae, and ca. 75% of genera in the family (Chatrou et al., 2012). The taxon sampling was designed to allow molecular divergence time estimates of both intergeneric and infrageneric African-Asian splits across a broad time-scale and in a single framework. For most genera single representatives were included, but multiple species were sampled for genera that are distributed across the African and Asian tropics (*Artabotrys*, *Hubera*, *Sphaerocoryne*, *Uvaria* and the only pantropical genus in the Annonaceae, *Xylopia*). Sampling of *Artabotrys*, *Hubera*, *Uvaria* and *Xylopia* was phylogenetically informed, i.e. it included early-divergent species and additional species representing the geographic range of all major clades identified in recent studies, and bracketing their crown nodes (Chaowasku et al., 2012; Stull et al., 2011; Thongpaiboj, 2008; Zhou et al., 2012; see Appendix 1 for species numbers in the genera and species coverage in the cited studies and present analyses). One African and two Asian species are currently accepted in *Sphaerocoryne*, and accessions of these three species were included in the analyses. For *Friesodielsia*, previously shown to be polyphyletic (Wang et al., 2012), species of both the African and Asian clades were selected.

DNA region sampling, amplification and sequencing

Five chloroplast DNA regions (*rbcl*, *matK*, *ndhF*, *trnL-F* including the *trnL-F* spacer and the *trnL* intron, and the *psbA-trnH* spacer), which have frequently been used in the Annonaceae phylogenetics, were downloaded from GenBank or newly amplified (215 sequences were newly generated for this study, see Appendix 2 for voucher information and GenBank accession numbers). Total genomic DNA was extracted from living, silica gel-dried, or herbarium material using the Innuprep Plant DNA Kit (Analytik Jena, Jena, Germany) according to the manufacturer's protocol. For amplification, each 25 µl PCR contained 12.55 µl ddH₂O, 5 µl 10× reaction buffer, 3 µl MgCl₂ (25 mM), 0.5 µl dNTPs (10 mM each), 0.75 µl of each forward and reverse primer (10 µM), 1.25 µl bovine serum albumin (BSA, 10 mg/ml), 0.2 µl Flexitaq DNA polymerase (Promega, Madison, WI, U.S.A.) and 1 µl of DNA template. Amplification and sequencing primers were the same as in Wang et al. (2012) except that primer pair *ndhF*-1216F/1621R, which performed poorly in the amplification of DNA from several taxa, was substituted by the newly designed primers *ndhF*-s8bF [3'-TGTTGGTATCCACCCTTGC-5'] and *s8bR* [3'-TCCTATGTACCCGACGAACAAGT-5']. Amplifications using DNA extracted from herbarium specimens frequently required several

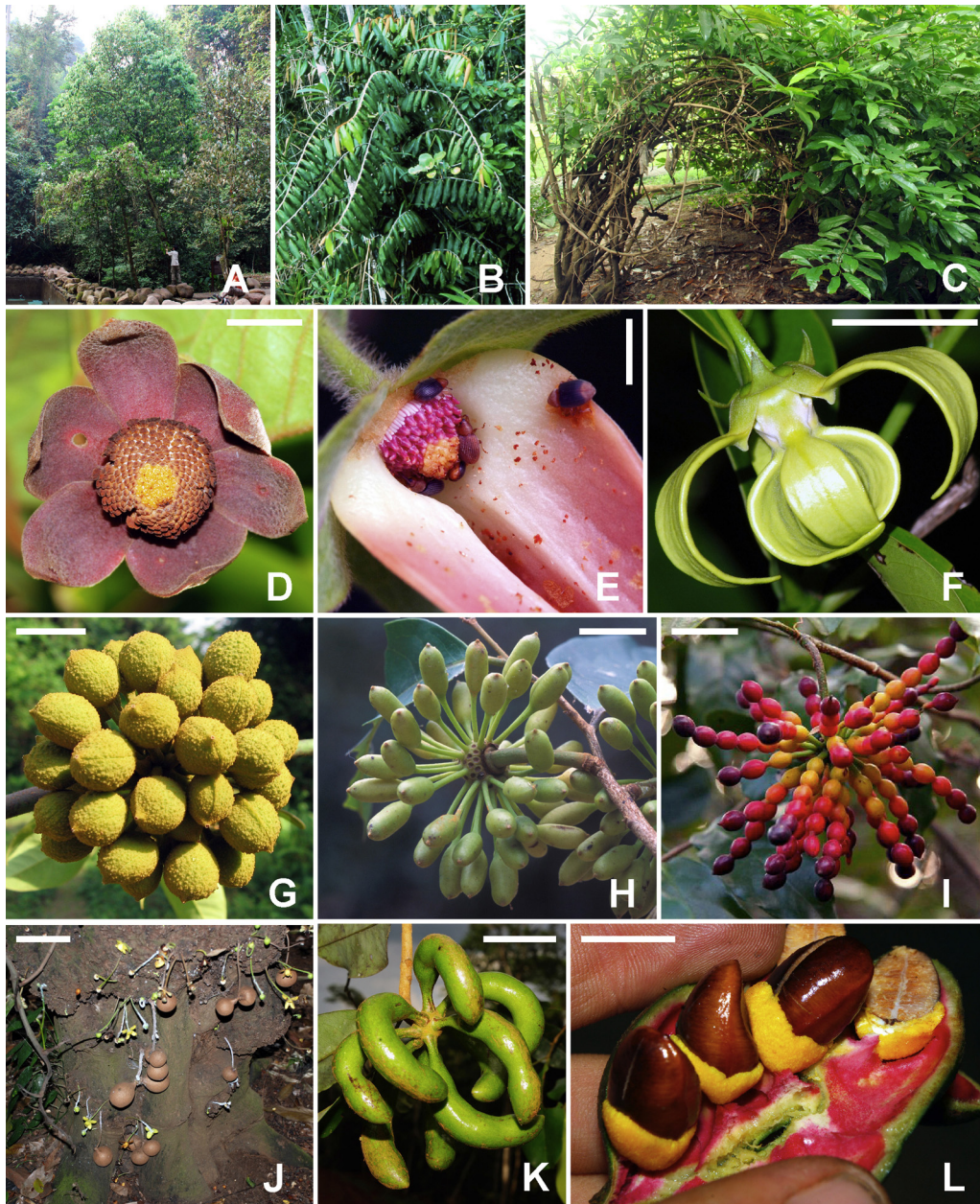


Fig. 2. Annonaceae: growth habits and flower and fruit morphology. (A) *Mitrephora wangii*, tree growth habit. (B) *Xylopia involucrata*, small tree growth habit. (C) *Artabotrys hexapetalus*, shrubby-lianescent growth habit. (D) *Uvaria littoralis*, flower, scale bar = 1 cm. (E) *Dasymploche dasymaschlum*, flower, with proximal petal removed to show pollination chamber and beetle pollinators (*Endaeidius* sp., Curculionidae, Coleoptera), scale bar = 5 mm. (F) *Artabotrys hexapetalus*, basal part of inner petals forming pollination chamber, scale bar = 2.5 cm. (G) *Uvaria muricata*, fruit composed of numerous monocarps (fruitlets derived from individual carpels after fertilization), scale bar = 15 mm. (H) *Hubera jenkinsii*, fruit composed of several monocarps, scale bar = 1 cm. (I) *Desmos chinensis*, fruit composed of numerous, multi-seeded, moniliform monocarps, scale bar = 2.5 cm. (J) *Stelechocarpus burahol*, flowers and fruits borne directly on the trunk, scale bar = 10 cm. (K) *Xylopia staudtii*, fruit composed of several monocarps, scale bar = 2.5 cm. (L) *Xylopia staudtii*, open monocarp showing arillate seeds, scale bar = 1 cm. Photo credits: A, D, F, J: Daniel C. Thomas; B: David M. Johnson; C: Xue Bine; E, I: Pang Chun Chiu; G, K, L: Thomas Couvreur; H: Simon Gardner; G, K, L, and H were made available via the World Annonaceae scratchpad (Couvreur and Annonaceae community, 2014).

internal primer pairs, and for some samples amplification failed or only partial sequences were generated (see Appendix 2 for percentages of missing data). Amplification products were visualized under UV light after electrophoretic separation on a 1.5% agarose TBE gel stained with SYBR Safe gel stain (Invitrogen, Carlsbad, CA, U.S.A.). BGI (Hong Kong, P.R. China) performed PCR product purification, amplification and sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.), as well as sequencing using an AB 3730 DNA Analyser (Applied Biosystems).

Alignment

Sequences were assembled and edited using Geneious v.6 (Drummond et al., 2010). The sequences were pre-aligned using the MAFFT (Katoh et al., 2009) plugin in Geneious and subsequently manually checked and optimized. Two highly homoplasious inversions in the *psbA-trnH* spacer (11 bp in 28 species of various tribes in the subfamily Annonoideae, as well as 14 bp in 15 species of tribe Miliuseae and one species of tribe Fenerivieae in subfamily Malmeoideae, respectively) were reverse-complemented, thereby

retaining substitution information in the fragments (see Pirie et al., 2006; Thomas et al., 2012). Two mutational hotspots in the *psbA-trnH* spacer (up to 32 bp and 46 bp, respectively) were excluded from the analyses because of difficult homology assessment.

Divergence time estimation

Two fossils, *Endressinia brasiliana* and *Futabanthus asamigawaensis*, were used to calibrate the phylogeny (for a review of the utility of different Magnoliales fossils for calibration see Pirie and Doyle, 2012). *E. brasiliana*, a fossilized flowering shoot from the late Aptian (Early Cretaceous, ca. 112 Ma ago) of Brazil (Mohr and Bernardes de Oliveira, 2004), is related to the magnolialean clade consisting of *Degeneria*, *Galbulimima*, *Eupomatia*, and Annonaceae (Doyle and Endress, 2010). *F. asamigawaensis*, a fossilized flower from the early Coniacian (Late Cretaceous, ca. 89 Ma) of Japan, shows features consistent with a placement in the Annonaceae crown group (Takahashi et al., 2008), as it lacks internal stamino-odes that are present in *Degeneria*, *Galbulimima*, *Eupomatia*, and in *Anaxagorea*, which is sister to the remaining Annonaceae.

Three calibration schemes were used. The first scheme followed Pirie and Doyle (2012) by fixing the root node to 112 Ma, based on the age of *Endressinia*, and by assigning a uniform calibration prior distribution from 89 Ma to 112 Ma to the Annonaceae crown node using the age of *Futabanthus* as hard lower bound and the age of *Endressinia* as upper bound, respectively. These calibrations provide minimal divergence time estimates. In the second scheme the root was fixed to 136.4 Ma, and a uniform calibration prior distribution from 89 Ma to 136.4 Ma was assigned to the Annonaceae crown node using the age of *Futabanthus* as hard lower bound and the earliest occurrence of unequivocal angiosperm crown group pollen grain fossils from the Hauterivian (Early Cretaceous, 136.4–130 Ma ago; Friis et al., 2010; Hughes, 1994) as a provisional hard upper bound. The latter scheme biases the estimates towards considerably older ages than the occurrence of the single fossilized structures of *Endressinia* and *Futabanthus*, and includes a provisional maximum age constraint. The third calibration scheme employed lognormal calibration prior distributions. The root node was calibrated using the age of the *Endressinia* fossil as offset and the shape of the lognormal distribution was defined with a median of 121.1 and a 95% probability interval between 112 and 136.4 Ma using the Hauterivian pollen grains as soft upper bound (offset: 112, mean: 10.9, log(Stdev): 0.6). The Annonaceae crown node was constrained with a lognormal prior distribution using the age of the *Futabanthus* as offset and defining the distribution shape with a median at ca. 98 Ma and a 95% probability interval between 89 and 113 Ma covering the Albian (offset: 89; mean 10.7; log(Stdev): 0.6). This calibration scheme assigned the highest probabilities to substantially older ages than the fossil ages accounting for errors associated with the incompleteness and limited knowledge of the Magnoliales fossil record.

Bayesian divergence time estimation was performed using BEAST v.1.8 (Drummond and Rambaut, 2007). The analyses were run using an uncorrelated rates relaxed molecular clock model assuming a lognormal distribution of rates (UCLD). Five data partitions were defined a priori based on DNA region identity. To avoid erroneous root recovery of unconstrained analyses (see Pirie and Doyle, 2012), Magnoliaceae taxa (*Magnolia kobus*, *Liriodendron tulipifera*) were implicitly selected as outgroups by putting a monophyly constraint on all other taxa. The random starting tree option was selected, and starting values for the root node of 112 and 136.4, and 89 for the Annonaceae crown node were selected to ensure that the starting tree was compatible with the selected hard calibration priors. Best-fitting nucleotide substitution models for the concatenated matrix and each nucleotide sequence

partition were determined with MrModeltest v.2 (Nylander, 2004) using the Akaike information criterion (AIC). The Yule process was selected as tree prior and a single overall UCLD model was applied for all partitions. An uninformative uniform prior (lower bound: 0, upper bound: 0.1) was selected for ucl.mean. Four MCMC analyses were run, each with 100 million generations and sampling every 10,000th generation. Time-series plots of all parameters were analysed in Tracer v.1.5 (Rambaut and Drummond, 2009) to check for adequate effective sample sizes (ESS > 200) and convergence of the model likelihood and parameters between each run. Trees were combined in LogCombiner v.1.8 (Drummond and Rambaut, 2007), setting the burn-in to 25% of the initial samples of each MCMC run. Post-burn-in samples were summarized using the maximum clade credibility tree (MCC) option in TreeAnnotator v.1.8 (Drummond and Rambaut, 2007).

Pirie and Doyle (2012) hypothesized that the assumptions of current molecular dating methods including uncorrelated rates relaxed molecular clock methods assuming lognormal distributions of rates as implemented in BEAST, do not well fit abrupt substitution rate changes. To avoid problems associated with abrupt rate changes in the calculation of divergence times for and within Annonaceae subfam. Malmeoideae, we ran additional BEAST analyses excluding taxa of Annonaceae subfam. Annonoideae (see Thomas et al., 2012), which shows a distinctly higher substitution rate than subfam. Malmeoideae (Pirie and Doyle, 2012).

Ancestral area reconstructions

Seven areas were delimited based on current distribution data and palaeogeographical reconstructions following Couvreur et al. (2011a): a, North/Central America; b, South America; c, Africa; d, Madagascar; e, India; f, Continental Asia and western Malasia (west of Wallace's Line); g, Southeast Asia east of Wallace's Line, northern Australia and Pacific islands (Fig. 3A).

Distributions were assigned based on taxonomic sources such as monographs, revisions and regional floras (see Erkens et al., 2012). For most genera which were represented by a single accession generic ranges were scored. For some genera that occur in more than one of the seven areas, and for which previous ancestral area analyses were available, respective inferred ancestral areas were coded, viz. *Anaxagorea* (Scharaschkin and Doyle, 2005); *Gutteria* (Erkens et al., 2009); *Isolona* (Couvreur et al., 2008); *Pseuduvaria* (Su and Saunders, 2009). For genera represented by multiple accessions (*Artabotrys*, *Hubera*, *Sphaerocoryne*, *Uvaria*, *Xylopia*) species distributions were scored.

Ancestral areas at internal nodes within the MCC trees of the BEAST analysis were inferred using two methods: (1) a Bayesian approach to dispersal–vicariance analysis (DIVA) (Ronquist, 1997) implemented in S-DIVA/RASP (Yu et al., 2010); and (2) a likelihood approach using the dispersal–extinction–cladogenesis model (L-DEC) implemented in Lagrange (Ree and Smith, 2008). For both analyses the maximum number of ancestral areas was constrained to two, reflecting the assumption that the ranges of ancestral Annonaceae species were similar to those of their extant descendants.

For the S-DIVA analyses, the ancestral areas were reconstructed on the post burn-in trees of the BEAST analyses. Relative frequencies of ancestral areas reconstructed for each node were recorded and plotted onto the MCC trees of the BEAST analysis constraining the root node to 112 Ma.

Constraints on rates of dispersal between areas in the L-DEC analyses were implemented in five time slices. Scaling factor matrices for dispersal rates within these time slices (see Appendix 3) were selected based on geographic distance, palaeoclimatic data and hypothesized tropical forest habitat continuity and biotic

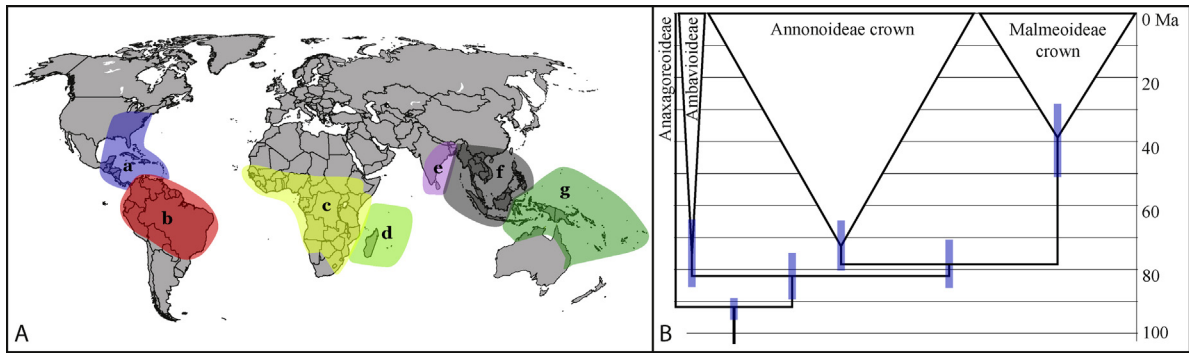


Fig. 3. (A) Area units used in the ancestral area reconstructions: a, North/Central America; b, South America; c, Africa; d, Madagascar; e, India; f, Continental Asia and western Malesia (west of Wallace's Line); g, Southeast Asia east of Wallace's Line, northern Australia and Pacific islands. (B) Chronogram (root constraint 112 Ma): Overview with Annonaceae subfamilies collapsed and outgroup pruned. Blue bars indicate 95% HPDs.

connectivity for tropical forest taxa (modified from the matrices used by Couvreur et al., 2011a). Analyses were subsequently performed using the MCC trees from both BEAST analyses using uniform calibration prior distribution (root node settings 112 and 136.4) as input trees. To test the impact of including age estimates for subfam. Malmeoideae based on analyses excluding subfam. Annonoideae (see Section "Molecular divergence time estimation") analyses were also run using manipulated MCC trees (root node settings 112 and 136.4) featuring divergence age estimates of all nodes of the Malmeoideae crown group based on the analyses excluding the Annonoideae taxa.

Results

Phylogenetic relationships

The maximum clade credibility tree of the BEAST analysis fixing the root node at 112 Ma is presented in Figs. 3B, 4 and 5. While relationships in subfam. Annonoideae are mainly well supported (Fig. 4), relationships in subfam. Malmeoideae are only poorly supported (Fig. 5). Nine strongly supported splits of clades of which one includes extant species restricted to the western Palaeotropics (Africa, Madagascar) while the other includes species restricted to the eastern Palaeotropics (Asia, Australia, western Pacific islands) can be identified (Figs. 4 and 5). These include: two splits in subfam. Ambavioideae (nodes A2 and B3 in Fig. 4); six splits in subfam. Annonoideae, including three splits in tribe Xylopieae (one within *Artabotrys*, node C3; two within *Xylopia*, nodes D2 and E2; Fig. 4) and three splits in tribe Uvarieae (within *Uvaria* and *Sphaerocoryne*, nodes H2 and J2, respectively; one split in the *Desmos-Dasymaschalon-Friesodielsia-Monanthotaxis* clade, node I2; Fig. 4); and one split in subfam. Malmeoideae (within *Hubera*, node L2; Fig. 5).

Ancestral area reconstructions

The reconstructions of the S-DIVA are presented in Figs. 4 and 5. The ancestral areas inferred by S-DIVA, and the results of the L-DEC analyses for selected nodes are given in Table 1. Fig. 6 provides an overview of inferred dispersal and vicariance events and divergence time estimates at nodes of interest.

Eight dispersal events between the African and Asian Palaeotropics (nodes A1/2, E1/2, F1/2, H1/2, I1/2, J1/2, K1/2, L1/2) were reconstructed in all analyses (S-DIVA, L-DEC, using root constraints of 112 Ma and 134.6 Ma). Additional dispersal events were inferred between nodes B2/3, C1/2, C2/3, D1/2, G1/2, and G2/3, but

not in all analyses (see Fig. 6). Moreover, S-DIVA indicated dispersal from a wide ancestral area in Africa and South America to Asia (nodes B1/2). Inferred directionality of dispersal events between the African and Asian Palaeotropics was from Africa to Asia, except for the dispersal event inferred in the *Hubera* clade (between nodes L1/2) for which dispersal from Asia to Africa received the highest support. An ancestral distribution in the African and Asian Palaeotropics and subsequent vicariance was supported at 10 nodes in all analyses (A2, B3, C3, E2, F2, H2, I2, J2, K2, L2). Additionally, vicariance was inferred at node D2 except in the L-DEC analyses under the root constraint of 136.4 Ma, and at node G2 in the S-DIVA analyses.

Molecular divergence time estimates

Mean divergence age estimates and 95% highest posterior density intervals (HPDs) from the analyses using a uniform calibration prior distribution and a root constraint at 112 Ma are presented in Figs. 4 and 5. Divergence time estimates of nodes of interest from all analyses (uniform and lognormal calibration prior distributions) are given in Table 1. Mean ages of the nodes for which vicariance between the western (Africa, Madagascar) and eastern Palaeotropics (Asia, Australia) has been inferred, cover a range from 49 to 7 Ma (95% HPDs from 58 to 4 Ma) in the analyses using a lognormal calibration prior distribution; from 47 to 6 Ma (95% HPDs from 55 to 3 Ma) in the analyses using a uniform calibration prior distribution and constraining the root node to 112 Ma; and from 54 to 7 Ma (95% HPDs from 65 to 4 Ma) in the analyses using a uniform calibration prior distribution and constraining the root node to 136.4 Ma. In all analyses, mean ages of seven vicariance events are in the Miocene (23–5.3 Ma) (Fig. 6). Exclusion of taxa of the Annonoideae resulted in distinctly older age estimates of and within the Malmeoideae crown group including the infrageneric African-Asian split in *Hubera* (node L2; Table 1 and Fig. 6).

Discussion

The present study strongly indicates that dispersal and vicariance in the Miocene, temporally incongruent with both Gondwanan vicariance and boreotropical migration, has played an important role in establishing current palaeotropical distribution patterns in Annonaceae. Seven of the ten to twelve inferred vicariance events between the western and eastern Palaeotropics fall into a Miocene timeframe. This contrasts with previous studies focusing on intergeneric relationships, which highlighted that the family's fossil record and the timing of most inferred intercontinental

Table 1
Clade support, divergence time estimates and ancestral area reconstructions. Node labels and ancestral area units refer to Figs. 4 and 5. L-DEC reconstructions that fall within two log-likelihood units of the optimal reconstructions and show a relative probability of ≥ 0.1 are indicated. Dispersal and vicariance events based on optimal reconstructions in L-DEC and S-DIVA are indicated. Events which are only recovered in specific reconstructions (L-DEC or S-DIVA) are indicated by the respective reconstruction method in brackets. Abbreviations: DAs: dispersal to Asia between this and the subsequent shallower node; DAF: dispersal to Africa between this and the subsequent shallower node; DEC: likelihood reconstructions under the dispersal-extinction-cladogenesis model; DIVA: Statistical Dispersal-Vicariance Analysis; HPD: highest posterior density date range; lognorm: lognormal prior distribution; lognorm-exAn: lognormal prior distribution, Annonoideae excluded; Ma: million years ago; MP: marginal probability; NA: not available; PP: posterior probability; RP: relative probability; uni112: uniform prior distribution, root node constraint of 112 Ma; uni112-exAn: uniform prior distribution, root node constraint 112 Ma, Annonoideae excluded; uni136: uniform prior distribution, root node constraint of 136 Ma; uni136-exAn: uniform prior distribution, root node constraint of 136 Ma, Annonoideae excluded; V: vicariance between Africa and Asia.

Node	Analysis	Clade support (PP)	Divergence time mean (95% HPD) (Ma)	S-DIVA reconstructions (area/MP)	L-DEC reconstructions (area/RP)	Dispersal/vicariance events
A1	lognorm	1	45.1 (31.9–59.5)	NA	NA	NA
A1	uni112	1	42.8 (30.5–56.3)	c/1	ac/1	DAs
A1	uni136	1	49.9 (34.3–66.1)	c/1	ac/0.43, c/0.28, cf/0.23, cd/0.06	DAs
A2	lognorm	1	29.2 (17.1–41.5)	NA	NA	NA
A2	uni112	1	27.6 (16.3–39.5)	cf/0.5, cg/0.5	cf/1	V
A2	uni136	1	32.3 (18.9–46.6)	cf/0.5, cg/0.5	cf/0.79, ce/0.21	V
B1	lognorm	1	31.5 (20.5–44)	NA	NA	NA
B1	uni112	1	29.7 (18.5–41.1)	ac/1	ac/1	DAs (DIVA)
B1	uni136	1	34.7 (21.7–49)	ac/1	ac/0.81, c/0.19	DAs (DIVA)
B2	lognorm	1	18.6 (10.3–27.4)	NA	NA	NA
B2	uni112	1	18.3 (9.4–25.2)	cf/0.56, cd/0.44	cd/0.7, cf/0.3	DAs (DECc)
B2	uni136	1	20.3 (11–30.3)	cf/0.56, cd/0.44	cd/0.59, cf/0.28, c/0.13	DAs (DECc)
B3	lognorm	0.75	15.8 (7.8–24.1)	NA	NA	NA
B3	uni112	0.81	14.7 (7.7–22.8)	df/1	df/0.47, d/0.33, f/0.11, cd/0.09	V
B3	uni136	0.8	17.3 (8.4–26.9)	df/1	df/0.37, d/0.32, f/0.12	V
C1	lognorm	1	57.5 (46–68.3)	NA	NA	NA
C1	uni112	1	54.6 (43.9–65.1)	c/1	c/1	
C1	uni136	1	63.2 (49.9–77)	c/1	c/1	
C2	lognorm	1	22.4 (13–32.5)	NA	NA	NA
C2	uni112	1	21.3 (12.8–31.6)	c/1	cf/0.51, c/0.49	
C2	uni136	1	25 (14.5–37.2)	c/1	c/0.5, cf/0.5	DAs
C3	lognorm	1	11.5 (7.5–16.1)	NA	NA	NA
C3	uni112	1	10.9 (7.1–15)	cf/0.93, cg/0.07	cf/1	V
C3	uni136	1	12.7 (8.3–17.7)	cf/0.93, cg/0.07	cf/1	V
D1	lognorm	1	31.4 (23.5–40.3)	NA	NA	NA
D1	uni112	1	29.8 (22.2–37.7)	c/0.99	c/0.56, cf/0.3, bc/0.11	DAs
D1	uni136	1	34.9 (26.1–44.6)	c/0.98	c/0.67, cf/0.33	
D2	lognorm	0.96	27.2 (17.4–37)	NA	NA	NA
D2	uni112	1	25.6 (15.9–35.5)	cf/1	cf/0.54, c/0.46	V
D2	uni136	1	30.1 (18.3–41.3)	cf/1	c/0.51, cf/0.49	V (DIVA)
E1	lognorm	1	20.5 (14.9–26.2)	NA	NA	NA
E1	uni112	1	19.4 (14–24.3)	c/1	c/0.81, cf/0.18	DAs
E1	uni136	1	22.7 (16.3–30)	c/1	c/0.82, cf/0.18	DAs
E2	lognorm	1	16.6 (12.2–21.4)	NA	NA	NA
E2	uni112	0.96	15.7 (11.4–20.3)	cf/0.53, cg/0.47	cf/1	V
E2	uni136	0.96	18.35 (13.3–23.7)	cf/0.54, cg/0.46	cf/1	V
F1	lognorm	1	55.9 (47.8–64.6)	NA	NA	NA
F1	uni112	1	53.6 (46.1–61.3)	c/1	c/0.72, cf/0.17, bc/0.11	DAs
F1	uni136	1	62.2 (51.4–73)	c/1	c/0.84, cf/0.16	DAs
F2	lognorm	1	48.7 (39.7–57.7)	NA	NA	NA
F2	uni112	1	46.8 (38.2–55.3)	cf/1	cf/0.73, c/0.15, bc/0.12	V
F2	uni136	1	54.3 (42.9–65.2)	cf/1	cf/0.60, c/0.22, bc/0.18	V
G1	lognorm	NA	NA	NA	NA	NA
G1	uni112	1	36.2 (29.4–43.3)	c/0.89, cf/0.11	c/0.56, cf/0.44	
G1	uni136	1	42.3 (33.9–51.2)	c/0.9, cf/0.1	c/0.75, cf/0.25	
G2	lognorm	NA	NA	NA	NA	NA
G2	uni112	0.35	35	cf/1	c/0.56, cf/0.44	DAs (DEC), V (DIVA)
G2	uni136	0.35	40.5	cf/1	c/0.61, cf/0.39	DAs (DEC)
G3	lognorm	1	22.3 (13.9–31.2)	NA	NA	NA
G3	uni112	1	20.8 (12.7–29)	f/1	f/0.64, cf/0.36	
G3	uni136	1	24.3 (15–34.5)	f/1	f/0.62, cf/0.38	
H1	lognorm	1	15.6 (10.3–20.6)	NA	NA	NA
H1	uni112	1	14.8 (9.9–19.8)	c/1	c/1	DAs
H1	uni136	1	17.2 (11.6–23.1)	c/1	c/1	DAs
H2	lognorm	1	12.4 (7.9–17.5)	NA	NA	NA
H2	uni112	1	11.7 (7.5–16.6)	cf/0.99	cf/0.77, ce/0.23	
H2	uni136	1	13.6 (8.6–19.5)	cf/0.99	cf/0.78, ce/0.22	
I1	lognorm	0.9	26.6 (19.8–34.1)	NA	NA	NA
I1	uni112	0.9	25.3 (20.8–34.3)	c/1	c/1	DAs
I1	uni136	0.9	29.5 (21.4–38)	c/1	c/1	DAs
I2	lognorm	1	15.9 (10.7–21.3)	NA	NA	NA
I2	uni112	1	15 (10.2–20.1)	cf/1	cf/0.86, ce/0.14	
I2	uni136	1	17.4 (11.7–23.4)	cf/1	cf/1	

Table 1 (Continued)

Node	Analysis	Clade support (PP)	Divergence time mean (95% HPD) (Ma)	S-DIVA reconstructions (area/MP)	L-DEC reconstructions (area/RP)	Dispersal/vicariance events
J1	lognorm	1	17.2 (10.3–24.6)	NA	NA	NA
J1	uni112	1	16.2 (9.5–23.5)	c/1	c/0.73, cf/0.27	DAs
J1	uni136	1	18.9 (10.8–27.4)	c/1	c/0.74, cf/0.26	DAs
J2	lognorm	1	7.5 (3.5–12)	NA	NA	NA
J2	uni112	1	7.1 (3.4–11.4)	cf/1	cf/1	
J2	uni136	1	8.2 (3.9–13.1)	cf/1	cf/0.86, ce/0.14	
K1	lognorm	1	82.6 (73.9–91.5)	NA	NA	NA
K1	lognorm-exAn	1	79.3 (67.7–90.6)	NA	NA	NA
K1	uni112	1	78.5 (70.8–85.7)	c/1	c/0.80, ac/0.20	DAs
K1	uni112-exAn	1	73.8 (62.5–85.1)	NA	c/0.58, ac/0.42	DAs
K1	uni136	1	90.5 (78.8–102.9)	c/1	c/0.57, ac/0.43	DAs
K1	uni136-exAn	1	89 (80.5–104.9)	NA	c/0.57, ac/0.43	DAs
K2	lognorm	1	40.7 (29.2–54.5)	NA	NA	NA
K2	lognorm-exAn	1	56.5 (42.8–70.9)	NA	NA	NA
K2	uni112	1	38.8 (28.3–51.1)	cf/0.87, ac/0.08, cd/0.05	cf/0.51, ac/0.49	V
K2	uni112-exAn	1	50.6 (37.5–63.7)	NA	cf/0.88, ac/0.12	V
K2	uni136	1	45.7 (32.6–60)	cf/0.88, ac/0.07, cd/0.05	cf/1	V
K2	uni136-exAn	1	57.4 (43.3–70.1)	NA	cf/1	V
L1	lognorm	1	9 (5.7–12.5)	NA	NA	NA
L1	lognorm-exAn	1	13.5 (8.2–19.2)	NA	NA	NA
L1	uni112	1	8.5 (5.5–11.9)	ef/0.4, df/0.39, cf/0.21	ef/1	DAf
L1	uni112-exAn	1	11.9 (7.1–16.8)	NA	ef/1	DAf
L1	uni136	1	10 (6.3–13.8)	ef/0.4, df/0.39, cf/0.21	ef/1	DAf
L1	uni136-exAn	1	13.7 (8.5–19.3)	NA	ef/1	DAf
L2	lognorm	1	6.5 (3.7–9.6)	NA	NA	NA
L2	lognorm-exAn	1	9.6 (5.1–14.4)	NA	NA	NA
L2	uni112	0.99	6.2 (3.4–9.2)	de/0.68, ce/0.32	ce/0.67, de/0.33	V
L2	uni112-exAn	0.99	8.6 (4.6–13)	NA	ce/0.5, de/0.5	V
L2	uni136	0.99	7.3 (4.2–10.8)	de/0.68, ce/0.32	de/0.8, ce/0.2	V
L2	uni136-exAn	0.99	9.8 (5.5–14.7)	NA	de/0.5, ce/0.5	V

dispersal events are consistent with pathways through the boreotropics in the Eocene (Couvreur et al., 2011a; Erkens et al., 2009; Pirie et al., 2006; Richardson et al., 2004; Surveswaran et al., 2010). The results of the present study evoke biogeographic scenarios involving both boreotropical dispersal from Africa to Asia, and post-boreotropical processes such as relatively frequent transoceanic long-distance dispersal or geodispersal across Arabia and western Asia.

The 'Gondwanan vicariance' hypothesis and the origin of Malmeoideae

Annonaceous fossil seeds (Bonde, 1993) and wood (Bande, 1973; Guleria and Mehrotra, 1998; Mehrotra, 1990) have been described from the Deccan Intertrappean beds of Central India dating to the Maastrichtian-Danian, ca. 72–62 Ma. These fossils indicate the presence of Annonaceae on the Indian fragment prior to collision with the Eurasian plate at ca. 50 Ma (van Hinsbergen et al., 2012) or 35 Ma (Ali and Aitchison, 2008) depending on different reconstructions, and a potential role of Indian rafting and Gondwanan vicariance in the origin of PIDs in the family.

Divergence estimates of African-Asian splits in the present analysis postdate the break-up of the Gondwanan supercontinent and the separation of the India and Madagascar fragments in the Late Cretaceous, 90–85 Ma, by a considerable margin. Prior to large-scale molecular divergence time estimation for the family, rafting on the Indian tectonic plate had been proposed to explain the disjunct distributions of the palaeotropical Annonaceae genera *Artabotrys* and *Uvaria*, as well as of the only pantropical genus in the family, *Xylopi*a (Doyle and Le Thomas, 1997; Le Thomas and Doyle, 1996). The results of the present study, however, indicate that five of six infrageneric African-Asian splits in Annonaceae are of Miocene origin, while one vicariance event in *Xylopi*a is inferred in the Oligocene. In addition to this temporal incongruence, splits in the inferred tree topologies do not mirror the sequence of

Gondwanan break-up. Sampling of Madagascan and Indian taxa is limited, but the available data indicate that: (i) Madagascan species of *Artabotrys*, *Uvaria* and *Xylopi*a are nested within clades dominated by species from mainland Africa (Zhou et al., 2012; present study), and are not sister to Indian or Southeast Asian clades, and Madagascan species of *Hubera* are nested within a clade for which an Asian origin has been inferred (Chaowasku et al., 2012; present study) and (ii) Indian Annonaceae taxa or clades are often nested within Asian clades, and not sister to Southeast Asian clades (e.g. in *Artabotrys*, present study; *Goniothalamus*, Tang, 2014; *Hubera*, Chaowasku et al., 2012, present study; *Meiogyne*, Thomas et al., 2012; *Uvaria*, Zhou et al., 2012).

The only divergence time estimates which may be reconciled with the 'Gondwanan vicariance' hypothesis are those of subfam. Malmeoideae. Dispersal from Africa to Asia has been inferred in ancestral area reconstructions between the stem and crown nodes of the Malmeoideae, with mean divergence time estimates indicating a timeframe of 89–43 Ma. Given these divergence time estimates in combination with the Indian fossil record and hypotheses of frequent dispersal between Africa and India until the middle Maastrichtian (ca. 71–65 Ma) on the basis of palynological data (Morley and Dick, 2003), rafting of the ancestors of Malmeoideae on India seems plausible (see also Su and Saunders, 2009), but the inferred divergence time estimates are also consistent with boreotropical migration.

The competing 'boreotropics' hypothesis was supported over Indian rafting as the best-fitting hypothesis in likelihood ancestral area reconstructions by Couvreur et al. (2011a), in which temporal dispersal constraints based on tectonic, palaeogeographic and palaeoclimatic reconstructions were used. Couvreur et al. (2011a) argued that Annonaceae present on the Indian fragment may have been driven to extinction as the Indian fragment rafted northwards and was exposed to substantial climate changes. Couvreur et al. (2011a) argued, moreover, that the lamellate rumination type of the seeds found in the Deccan Intertrappean beds differs from the

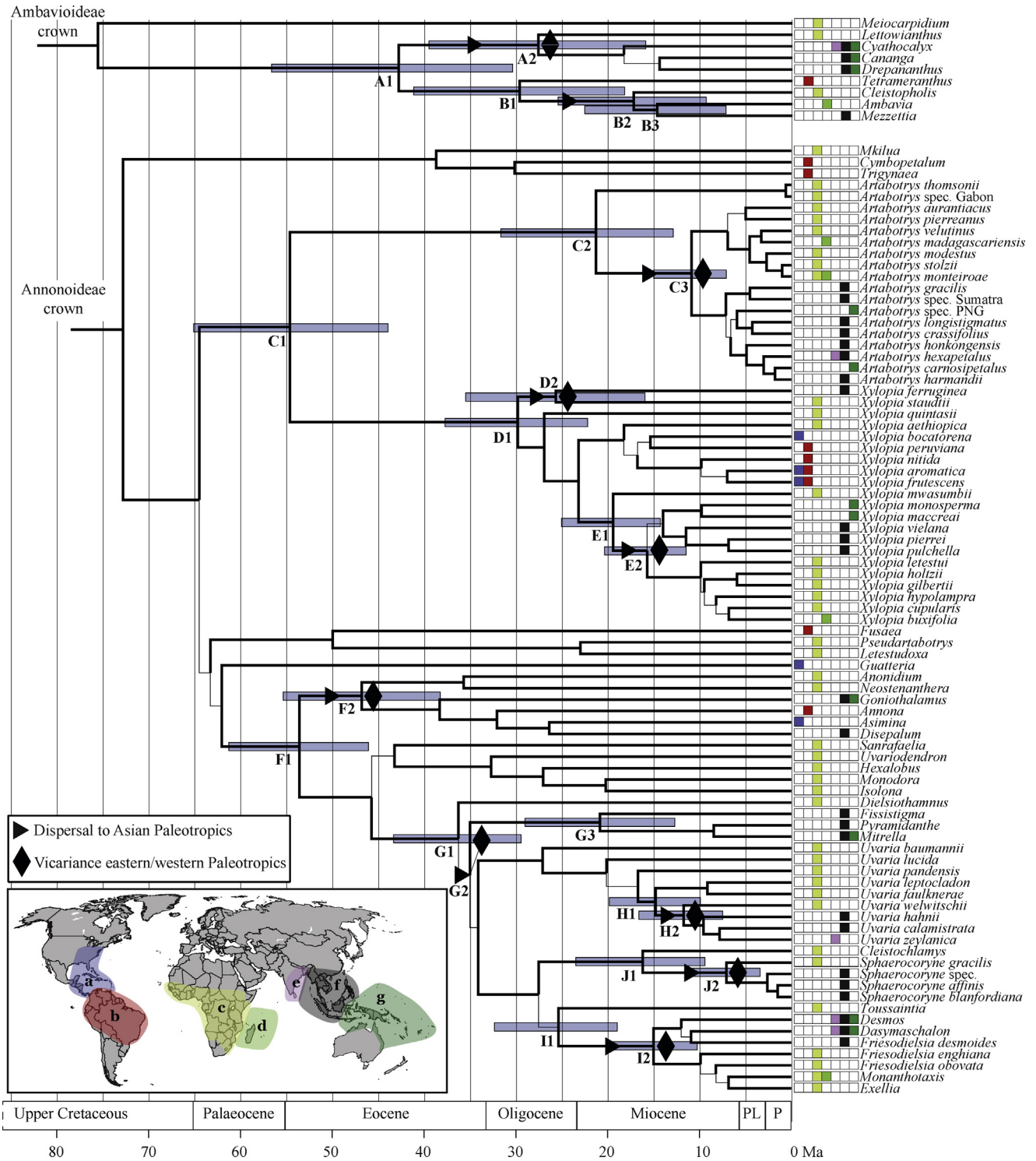


Fig. 4. Chronogram (root constraint 112 Ma): Ambavioideae and Annonoideae crown groups. For details of node support, divergence times and ancestral area reconstruction results at nodes of interest (A–I) see Table 1. Scale bars indicate 95% HPDs. Thin branches lead to nodes with posterior clade probabilities of <0.96. Dispersal and vicariance events inferred in the S-DIVA analyses are mapped on the chronogram. Coloured squares next to the terminal names indicate distributions corresponding to area units shown in Fig. 3A: red, South America; blue, North/Central America; yellow, Africa; light green, Madagascar; lavender, India; black, Continental Asia and western Malesia (west of Wallace's Line); dark green, Southeast Asia east of Wallace's Line, northern Australia and Pacific islands.

spiniform ruminations inferred as ancestral for the Malmeoideae crown group (although ancestral character state reconstruction is equivocal at the stem node: Pirie and Doyle, 2012) and may represent a different lineage altogether. Most importantly, in their review paper on fossil calibration in Annonaceae, Pirie and Doyle

(2012) emphasize that seeds with spiniform ruminations, which are highly indicative of Malmeoideae as this rumination type is only very rarely found in other Annonaceae taxa (Cyathocalyx in the Ambavioideae), were present in the early Eocene London Clay flora (Reid and Chandler, 1933). However, while this provides some

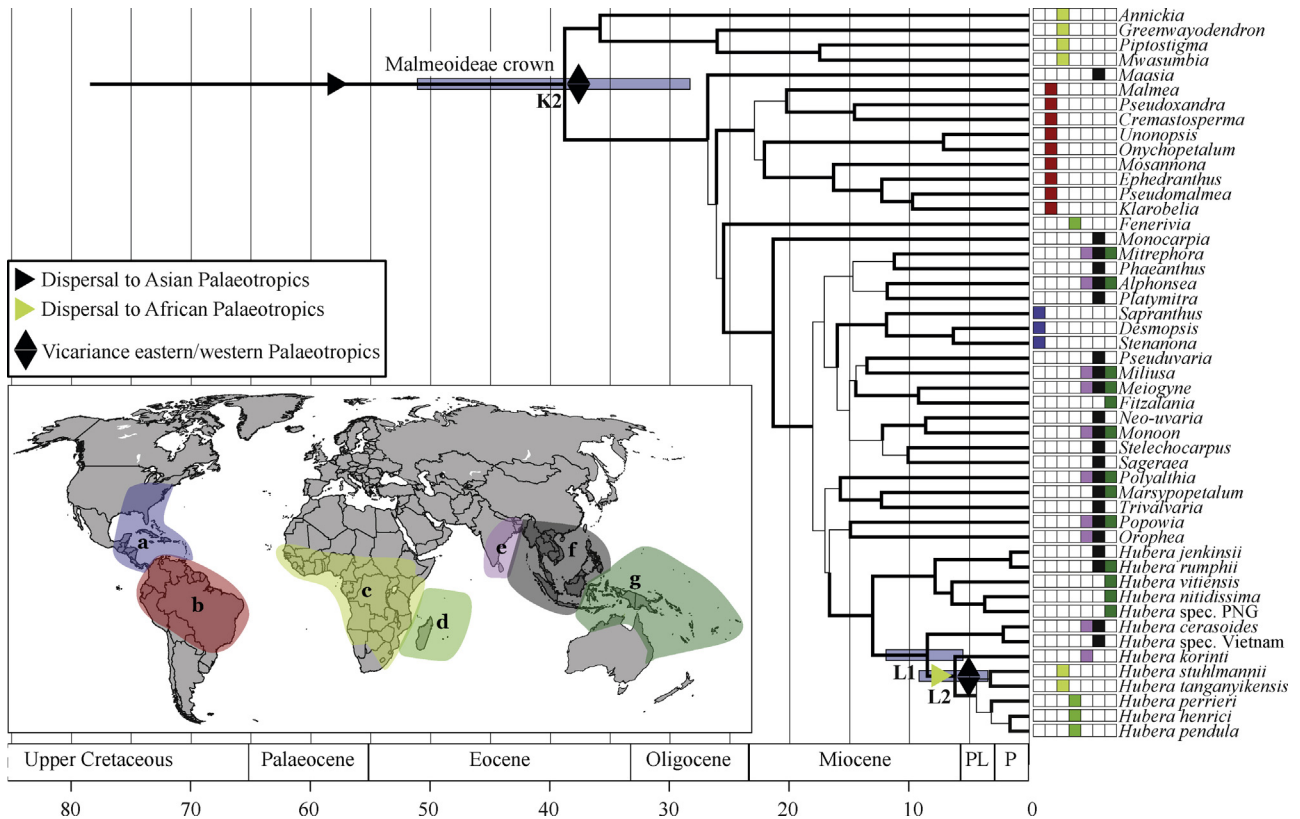


Fig. 5. Chronogram (root constraint 112 Ma): Malmeoideae crown group. For details of node support, divergence times and ancestral area reconstruction results at nodes of interest (K, L) see Table 1. Scale bars indicate 95% HPDs. Thin branches lead to nodes with posterior clade probabilities of <0.96. Dispersal and vicariance events inferred in the S-DIVA analyses are mapped on the chronogram. Coloured squares next to the terminal names indicate distributions corresponding to area units (a–g) shown in Fig. 3A: red, South America; blue, North/Central America; yellow, Africa; light green, Madagascar; lavender, India; black, Continental Asia and western Malesia (west of Wallace's Line); dark green, Southeast Asia east of Wallace's Line, northern Australia and Pacific islands.

support for the boreotropics hypothesis, a considerable degree of uncertainty about the origins of the Malmeoideae remains because of the poorly supported backbone of the malmeoid phylogeny, uncertainty in ancestral area reconstructions, and the necessarily crude modelling of dispersal constraints in uniform time slices in the reconstructions under the DEC model, as well as a very limited number of Annonaceae fossils from India and the uncertainty of their affinities. Additional 'hard' fossil data are required to further investigate the historical biogeography of the group.

The 'boreotropical migration' hypothesis

Records of numerous fossilized Annonaceae seeds from the early Eocene London Clay flora (Collinson, 1983; Reid and Chandler, 1933), together with records from the middle Eocene of Northern America (Manchester, 1994), provide evidence for a wide distribution of Annonaceae in the Northern Hemisphere during the early Palaeogene. This fossil evidence, in combination with temporally congruent molecular divergence time estimates for several predominantly tropical flowering plant lineages (e.g. Antonelli et al., 2009; Baker and Couvreur, 2013; Davis et al., 2002; Muellner et al., 2006; Renner et al., 2001) including Annonaceae (Couvreur et al., 2011a; Erkens et al., 2009; Pirie et al., 2006; Richardson et al., 2004; Surveswaran et al., 2010), provides strong support for wider boreotropical distributions and the 'boreotropical migration' hypothesis, i.e. intercontinental biotic exchange through a northern mid-latitude tropical vegetation and climate corridor in the Eocene. Couvreur et al. (2011a), based on analyses of a dataset

with dense generic sampling, emphasized that most intercontinental splits in the largest Annonaceae subfamily, Annonoideae, are likely to have originated in the Eocene.

The present analyses also indicate a number of dispersal and vicariance events such as the earliest African-Asian splits in the Ambavioideae and Annonoideae, which are temporally congruent with a scenario involving dispersal from Africa to the boreotropics, eastwards dispersal to the Asian boreotropics, and subsequent vicariance. However, several dispersal events between Africa and Asia are inferred in the Oligocene to Miocene or Miocene, when climates suitable for megathermal vegetation had receded to equatorial regions and Eurasia no longer offered a conduit for overland dispersal between Africa and Asia for tropical forest taxa (Collinson, 1992; Morley, 2000, 2003, 2007; Wolfe, 1992). Mean divergence times indicate that the majority of inferred vicariance events are clustered in the Miocene, i.e. they are inferred to have occurred much more recently than the drastic temperature drop at Eocene-Oligocene boundary (Zachos et al., 2001), which resulted in the almost complete disappearance of tropical forest from the northern mid-latitudes. Additional hypotheses are required to explain these patterns.

Feasibility of the 'Miocene geodispersal' hypothesis

Overland dispersal from Africa, across Arabia, to Asia during the Miocene has sometimes been invoked to explain PIDs and splits between African and Asian angiosperm clades (Cruaud et al., 2011; van Welzen et al., 2014; Zhou et al., 2012). The main arguments

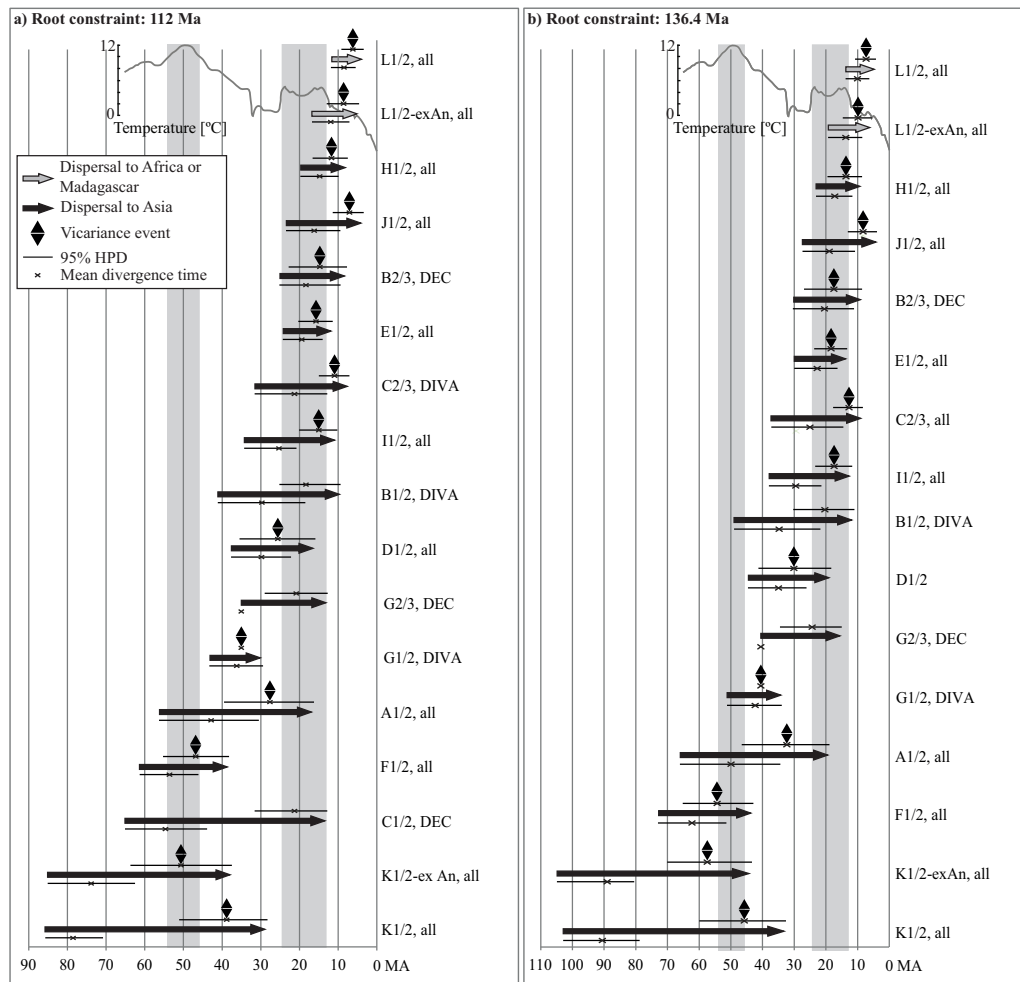


Fig. 6. Overview of divergence time estimates (analyses using uniform prior distributions and root constraints of 112 Ma and 136.4 Ma, respectively), and inferred dispersal and vicariance events at nodes of interest. Node names (A–K) correspond to node labels in Figs. 4 and 5 and Table 1. The horizontal time axis indicates millions of years ago (Ma). Labels to the right indicate the respective nodes (A–L) and respective analyses (see Section “Materials and methods”): all, all performed ancestral area reconstructions; DEC, likelihood reconstructions under the DEC model; DIVA, S-DIVA analysis; exAn, analyses using divergence times for Malmeoideae based on divergence time estimation excluding Annonoideae taxa. The inserted temperature curve represents estimated mean deep-sea temperature change based on oxygen isotope data (Zachos et al., 2001). The two vertical grey blocks represent the Early Eocene Climatic Optimum and the timeframe from the Late Oligocene Warming to the end of the Mid-Miocene Climatic Optimum, respectively.

for this hypothesis are: (i) the availability of land connections during this time (Popov et al., 2004; Rögl, 1998); (ii) a warm phase in the Miocene peaking in the Middle Miocene Climatic Optimum, 17–15 Ma (Zachos et al., 2001), potentially providing suitable climate and vegetation corridors; and (iii) biological plausibility, e.g. in the case of primate-dispersed African Annonaceae genus *Uvaria*, the lack of adaptations facilitating successful long-distance transoceanic dispersal (Zhou et al., 2012).

Arabian palaeoenvironments are difficult to reconstruct because of the paucity of fossil records in the region, but fossil assemblages of some sites clearly indicate open vegetation: Kingston and Hill (1999), in a review of late Miocene palaeoenvironments in Arabia, concluded that Arabia seems to have been dominated by open woodland or bushland ecosystems in the early Miocene. There is, however, also some micro- and macrofossil evidence for the presence of some deciduous broad-leafed tropical forest or woodland, vegetation-fringed rivers and streams, and mangrove and fresh water marshland of humid, tropical or subtropical climate in Arabia from early to middle Miocene strata (Kürschner, 1998; Otero and Gayet, 2001; Pound et al., 2012; Privé-Gill et al., 1999; Whybrow and McClure, 1980). The limited

palynological evidence suggests that flowering plant families predominantly associated with humid tropical climates such as Arecaceae, Combretaceae, Myrtaceae and Sapotaceae, which are currently rare or absent on the Arabian Peninsula, were present at some Miocene localities (Mandaville, 1984; Whybrow and McClure, 1980). In contrast to the hypothesis of dominance of open woodland or bushland ecosystems in Arabia in the early Miocene (Kingston and Hill, 1999), analyses of Arabian fossil vertebrate faunas using molar crown height as proxy for humidity indicate that early and middle Miocene Arabian mammal faunas were predominantly adapted to closed vegetation types (Ataabadi, 2010).

Although there is no clear indication for extensive tropical forest, the sparse fossil record indicates that the climate and vegetation of the Arabian Peninsula may have been a filter for, rather than a barrier to, overland dispersal of tropical forest taxa in the early to middle Miocene. Most extant Annonaceae are restricted to lowland tropical rain forest habitats, but species of several genera, including the palaeotropical genera *Artabotrys*, *Hubera*, *Sphaerocoryne*, *Uvaria* and the pantropical genus *Xylopia*, are also found in regions characterized by monsoonal climate including pronounced dry seasons in Asia and Australia (Jessup, 2007; Keßler, 1993), dry

deciduous forests and open woodland in Africa (Verdcourt, 1971) and savanna vegetation in South America (Maas et al., 2001). It has not been investigated, however, whether adaptations to seasonally drier climates are derived or ancestral characters in the respective genera, and ancestral niche reconstructions on the basis of the climate niche parameters of extant species and near species-level phylogenies could be used to explore this. Annonaceae seed fossils have been identified in fossil assemblages indicative of seasonally dry tropical forest or woodland vegetation in Ethiopia in the middle Miocene (Tiffney et al., 1994; Wheeler et al., 2007). Based on the fossil record and habitats occupied by extant species, it can be hypothesized that for some tropical forest taxa which were pre-adapted to seasonally drier conditions or associated with vegetation fringing rivers and streams there was a window of opportunity for overland dispersal across Arabia in the early to middle Miocene prior to climate deterioration in the late middle Miocene (Jacobs, 2004; Kürschner, 1998; Pound et al., 2012; Zachos et al., 2001).

Several African-Asian vicariance events in the Annonaceae such as infrageneric splits in *Artabotrys*, *Sphaerocoryne*, *Uvaria* and *Xylopi*a, as well as the split in the *Desmos-Dasymaschalon-Friesodielsia-Monanthotaxis* clade in the tribe Uvarieae, were inferred to have occurred in the Miocene. This is temporally congruent with biogeographical scenarios involving overland dispersal from Africa through Arabia to western Asia and subsequent vicariance due to climate deterioration and habitat fragmentation.

Long-distance dispersal and African-Asian disjunction in *Hubera*

The genus *Hubera* shows biogeographical patterns which are exceptional within Annonaceae in several aspects. The genus shows one of the widest generic distributions in the family, second only to *Xylopi*a, the only pantropical genus in the family. *Hubera* species occur in Eastern Africa, Madagascar, India, continental Southeast Asia, Malesia and some western Pacific islands (Chaowasku et al., 2012). In the present analyses, dispersal from an ancestral area in India and Southeast Asia to Africa or Madagascar has been inferred within *Hubera*, in contrast with the other dispersal events in which a west-to-east pattern has been inferred. Mean divergence times indicate that dispersal and vicariance in *Hubera* occurred subsequent to the middle Miocene, which, in combination with the wide distribution of the genus including Madagascar and some isolated Pacific islands, indicates that long-distance dispersal across water bodies likely played an important role in shaping this distribution pattern. *Hubera* is characterized by relatively small, one-seeded 'monocarps' (fruitlets derived from individual carpels after fertilization) with a fleshy, green or red pericarp (Fig. 2H), and frugivory by primates (*Hubera rumphii*; Kanamori et al., 2010), fruit bats (*Hubera pendula*; Tang et al., 2007) and birds (*Hubera korintii*; Ratnayake et al., 2006) has been observed. Long-distance dispersal across the Indian Ocean may have been facilitated by seed dispersal by volant animals, but given relatively short seed retention times of potential avian dispersal vectors (see discussion in Johnson et al., 2014), other factors need to be considered to explain the wide distribution and potential transoceanic dispersal in *Hubera*. These factors include a relatively wide habitat range including not only lowland rain forests but also seasonally dry forests and woodland, as well as coastal shrub (e.g. *Hubera stuhlmannii*, *Hubera tanganyikensis*; see IUCN, 2013), wind currents seasonally changing from east to west (winter monsoon) and the formation of island chains between the Madagascar region and India providing an avenue for dispersal by island-hopping (Warren et al., 2010), as well as 'non-standard' mechanisms such as extreme climatic events responsible for long-distance dispersal (Nathan, 2006).

It should be noted, however, that the molecular divergence time analyses excluding Annonoideae species resulted in 95% HPDs that include the Middle Miocene Climatic Optimum, and which are therefore also temporally consistent with Miocene overland dispersal.

Methodological considerations

An additional hypothesis explaining the inferred clustering of African-Asian splits in the Miocene is that they represent a methodological artefact related to under-sampling. The current study uses a single framework to investigate all identified African-Asian splits in the Annonaceae. This has the major advantage of clear comparability of clade ages, which is otherwise often highly problematic when comparing multiple studies using different taxon sets, calibration priors and methods for divergence time analysis. Two problematic aspects of the approach adopted in the present study, however, are incomplete and uneven taxon sampling. The phylogenetically informed infrageneric sampling used in the present study was based on previous molecular phylogenetic studies including only ca. 30–65% of the species diversity in the respective genera (*Artabotrys*: Thongpaiboj, 2008; *Hubera*, Chaowasku et al., 2012; *Uvaria*: Zhou et al., 2012; *Xylopi*a, Stull et al., 2011; see Appendix 1), and such under-sampling can result in under-estimates of divergence times (Linder et al., 2005). The uneven taxon sampling, which is primarily the result of limited material and molecular data availability, as well as the lack of integration of fossil distributions in the biogeographical analyses (see, e.g. Nauheimer et al., 2012), because of unknown placement of relevant fossils within the Annonaceae crown group, reduces the reliability of the biogeographical analyses. Near-complete species sampling would be needed to produce more reliable divergence time estimates, particularly for the infrageneric splits, and the impact of incomplete and uneven taxon sampling on the biogeographical analyses clearly requires further investigation.

Conclusions

The results of the analyses in combination with the biogeographic information described above indicate complex and varied origins of PIDs in Annonaceae. PIDs are broadly distributed in time, ranging from the late Palaeocene to the late Miocene, with the majority of inferred dispersal events occurring in the Miocene. While previous studies focusing on intergeneric relationships in Annonaceae highlighted that the family's fossil record and the timing of most inferred intercontinental dispersals are consistent with pathways through the boreotropics in the Eocene (Couvreur et al., 2011a; Erkens et al., 2009; Pirie et al., 2006; Richardson et al., 2004; Surveswaran et al., 2010), the results of the present study indicate not only the important role of boreotropical migration, but also post-boreotropical transoceanic or overland dispersals in the Miocene in shaping PIDs. This underscores the hypothesis that Tertiary overland migration and long-distance dispersal subsequent to the break-up of the Gondwanan supercontinent had a major impact on the assembly of tropical forest biomes (e.g. Couvreur et al., 2011a; Davis et al., 2002; Givnish and Renner, 2004; Nie et al., 2013; Pennington et al., 2006; Richardson et al., 2004; Warren et al., 2010). Palaeogeographic reconstructions and the sparse fossil record from the Arabian Peninsula, moreover, indicate the erosion and formation of biogeographic barriers and support the plausibility of a window of opportunity for overland dispersal of tropical lowland forest taxa across Arabia prior to climate deterioration commencing in the late Middle Miocene. This does not falsify the hypothesis of frequent long-distance dispersal across

the Indian Ocean as a process underlying PIDs that originate in the Miocene, but it clearly negates the need to invoke transoceanic dispersal for these events 'by default'. Densely sampled phylogenies of palaeotropical taxa and, most importantly, additional fossil evidence from Arabia and adjacent areas are needed to evaluate whether there was congruent range expansion of independent clades of tropical lowland forest taxa across Arabia, consistent with the hypothesized window of opportunity for overland dispersal in the Miocene.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ppees.2014.11.001>.

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