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# Inferring geographic range evolution of a pantropical tribe in the coffee family (Lasiantheae, Rubiaceae) in the face of topological uncertainty

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ABSTRACT

In this study we explore what historical biogeographic events are responsible for the wide and disjunct distribution of extant species in Lasiantheae, a pantropical group of trees and shrubs in the coffee family. Three of the genera in the group, *Lasianthus*, *Saldinia*, and *Trichostachys*, are found to be monophyletic, while there are indications that the fourth, *Ronabea*, is paraphyletic. We also address how the uncertainty in topology and divergence times affects the level of confidence in the biogeographic reconstruction. A data set consisting of chloroplast and nuclear ribosomal DNA data was analyzed using a Bayesian relaxed molecular clock approach to estimate phylogenetic relationships and divergence times, and the dispersal–extinction–cladogenesis (DEC) method to reconstruct geographic range evolution. Our results show that the Lasiantheae stem lineage originated in the neotropics, and the group expanded its range to the palaeotropics during the Eocene, either by continental migration through the boreotropics or by transatlantic long-distance dispersal. Two cases of Oligocene/Miocene over water-dispersal were also inferred, once from the paleotropics to the neotropics within *Lasianthus*, and once to Madagascar, concurrent with the origin of *Saldinia*. A lot of the diversification within *Lasianthus* took place during the Miocene and may have been influenced by climatic factors such as a period of markedly warm and moist climate in Asia and the aridification of the interior of the African continent. When biogeographic reconstructions were averaged over a random sample of 1000 dated phylogenies, the confidence in the biogeographic reconstruction decreased for most nodes, compared to when a single topology was used. A good understanding of phylogenetic relationships is necessary to understand the biogeographic history of a group, bit since the phylogeny is rarely completely known it is important to include phylogenetic uncertainty in biogeographic analysis. For nodes where the resolution is uncertain, the use of a single "best" topology as a basis for biogeographic analysis will result in inflated confidence in a biogeographic reconstruction which may be just one of several possible reconstructions.



## 1. Introduction

Lasiantheae is a group in the coffee family comprising more than 260 species of mainly shrubs and small trees. It consists of four genera, three of which have restricted but widely separated geographic ranges; *Ronabea* in tropical America, *Trichostachys* in West Africa, and *Saldinia* in Madagascar; and one which is pantropical, *Lasianthus*. Species in Lasiantheae occur nearly exclusively in the understory of tropical rainforests. Understanding the biogeographic history of this group may therefore aid in the search for shared patterns among different organismal groups that can explain how the world's most species-rich ecosystems, the tropical rainforests (Morley, 2000), have been assembled.

Based on available evidence, it seems unlikely that Lasiantheae should be old enough for the pantropical distribution pattern of extant members of the group (Fig. 1) to be a remnant of a Gondwanan distribution. Had this been the case, we might have expected Lasiantheae to have been represented in the fossil record from the Cretaceous of the former Gondwanan continents. On the contrary, the oldest fossil that can be assigned to Rubiaceae, *Paleorubiaceophyllum eocenicum*, was found in North America and appears in strata from the Eocene (Roth and Dilcher, 1979). A previous molecular dating analysis indicated that the Rubiaceae crown group is 86.6 Myr (95% HPD 73–101 Myr, Bremer and Eriksson, 2009), which is too young for any splits between sister groups occurring in the old and new worlds to be explained by Gondwanan vicariance, as the connection between the African and South American plates was broken 96 Ma (Morley, 2003). Studies of the

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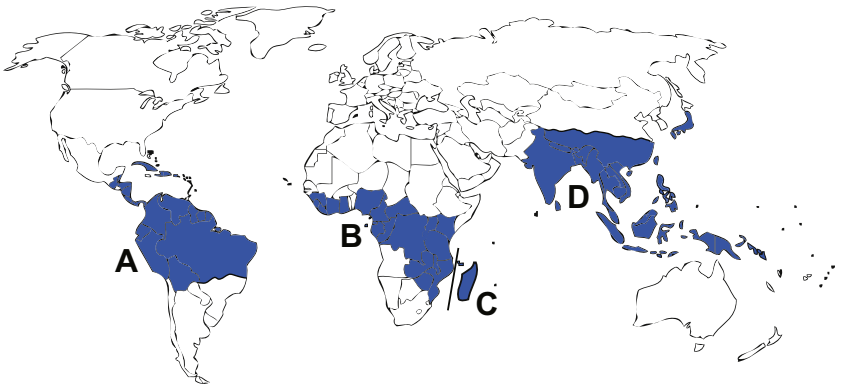


**Fig. 1.** Geographic distribution of Lasiantheae. Areas used in the biogeographic analysis are indicated by capital letters; tropical America (A), Africa (B), Madagascar (C), and Asia (including temperate and tropical Asia as well as Australasia) (D).

biogeographic history of several tropical angiosperm families have shown that they are too young to have acquired their trans-oceanic distributions from a Gondwanan ancestry (for example, Davis et al., 2002; Givnish et al., 2000; Renner and Meyer, 2001; Yuan et al., 2003) but in a few early diverging angiosperm groups, or groups occurring in temperate climate, vicariance caused by the breakup of Gondwana still holds as an explanation (Manos, 1997; Swenson et al., 2001; Chanderbali et al., 2001; Vinnersten and Bremer, 2001; Bremer, 2002).

An alternative explanation of the distribution pattern of extant Lasiantheae involves continental migration through the Northern Hemispere boreotropics (Wolfe, 1975) in the early Paleogene. During this time, global climate was very warm (Zachos et al., 2001) and tropical vegetation was present at much higher latitudes than today (Wolfe, 1975; Tiffney, 1985a). The existence of a connection between the Eurasian and North American continental plates, the north Atlantic land bridge, allowed boreotropical taxa to expand their ranges over both continents (Tiffney, 1985b; Morley, 2003). At the end of the Eocene, when temperatures declined markedly (Zachos et al., 2001), many taxa were forced to recede towards the equator (Wolfe, 1978, 1980; Collinson et al., 1999) and previously continuous distributions were fragmented. Today, representatives from such lineages are found primarily in refugia in China, Southeast Asia, Central America, especially in Mexico, and in Macaronesia (Morley, 2000). Fossil evidence has shown that numerous groups of tropical angiosperms occurred in the northern hemisphere in the early Eocene (Morley, 2000), coinciding with the early Eocene climatic optimum. For several angiosperm groups with tropical amphi-pacific or amphi-atlantic distributions, including clades in Rubiaceae, phylogenetic, biogeographic, and molecular dating analyses have indicated that the disjunct distribution probably has resulted from the disruption of the boreotropical flora (Smedmark and Anderberg, 2007; Antonelli et al., 2009; Smedmark et al., 2010; Couvreur et al., 2011; Manns et al., 2012). The latest that any tropical taxon has been proposed to have used this route of migration is 32 Mya (Davis et al., 2004).

A third explanation is long-distance dispersal, which has been shown to have been the cause of transatlantic disjunctions in a number of groups of angiosperms (Renner, 2004). Possible modes of trans-oceanic dispersal of species in Lasiantheae could be via birds eating the relatively small, fleshy fruits or, perhaps more likely, by rafting of entire plants. For the genus *Lasianthus,* recent intercontinental dispersal has been suggested to be responsible for the pantropical distribution of the group (Xiao and Zhu, 2007). For tropical groups of plants, divergences between sister clades occurring on both sides of the Atlantic estimated to be younger than 32 Ma, after which no land connection has been available to tropical taxa, can only be explained by long-distance dispersal.

A biogeographic question of special interest is the colonization of Madagascar, which has been identified as a biodiversity hotspot (Myers et al., 2000); however, the mechanisms that have generated this high diversity are poorly understood (Agnarsson and Kuntner, 2012). A review of phylogenetic studies of a wide range of Malagasy organismal groups indicated that the predominant biogeographic force has been transoceanic dispersal during the Cenozoic (Yoder and Nowak, 2006). The same study also pointed to the African mainland as a major source of the biodiversity in Madagascar.

In this study, we use the dispersal–extinction–cladogenesis (DEC) method (Ree et al., 2005; Ree and Smith, 2008) in the computer software Lagrange (ver. 20110117, Ree and Smith, 2008) to reconstruct geographic range evolution within Lasiantheae. This method is based on explicit models that allow dispersal rates between any two areas to vary through time. Models may incorporate information about, for example, paleogeographic changes that have caused areas to be variously connected or disconnected during different time periods, or changes in climate, wind directions, or water currents, which can be expected to have affected the likelihood of range expansion between areas. The DEC method accounts for any uncertainty in the inferred geographic range evolution by evaluating alternative scenarios in a probabilistic framework. However, if there is topological uncertainty involving alternative resolutions among groups distributed in different geographic areas, each resolution is likely to imply a different biogeographic scenario. Despite this, topological uncertainty is rarely taken into account in historical biogeography. Typically, a biogeographic analysis is performed on a single phylogenetic tree. Recently, a method was developed that extends the parsimony-based dispersal-vicariance analysis (DIVA, Ronquist, 1997), to account for phylogenetic uncertainty: Bayes-DIVA (Nylander et al., 2008). Only rarely has the same approach been used with parametric methods (Smith, 2009; Smedmark et al., 2010). In these studies, DEC reconstructions were averaged over a sample of dated phylogenies from the posterior distribution from a Bayesian divergence-time analysis, thereby accounting for the uncertainty in phylogenetic relationships and divergence times.

Morphologically, species in Lasiantheae are more or less woody, ranging from sub-shrubs to small trees, usually with axillary inflorescences and characteristically blue drupes. Each drupe has two to many locules, either with a single bilocular pyrene or two to many unilocular pyrenes. The pyrene usually has a germination slit in the form of a ventral lid (Piesschaert et al., 2000). Synapomorphic

features for Lasiantheae are the presence of a single ovule attached at the base of each locule, developing into a seed with a soft oily endosperm and a large embryo. The largest of the four genera in the group is the pantropical *Lasianthus*, with 225 species (Govaerts et al., 2012), a majority of which are found in tropical and subtropical Asia. Of the remaining species, 13 are found in mainland Africa and three in the neotropics. A synapomorphy for *Lasianthus* is a gynoecium with numerous locules. The Malagasy genus *Saldinia* includes 22 described species (Govaerts et al., 2012) characterized by drupes with two locules, of which one is aborted, and a bilocular pyrene that lacks a germination slit. *Saldinia* has functionally unisexual flowers and a foetid odor of its vegetative parts. The West African *Trichostachys* is a genus consisting of 14 species (Govaerts et al., 2012). They have terminal inflorescences and pyrenes that are either uni- or bilocular. The fourth genus in the group, the neotropical *Ronabea*, consists of three species of herbs or small shrubs with purple to black drupes (Taylor, 2004).

The first phylogenetic study to include a representative of this clade (Bremer, 1996) showed that *Lasianthus* does not belong in Psychotrieae, where it was placed traditionally (Bentham and Hooker, 1873; Schumann, 1891), but among the earliest diverging lineages within Rubioideae. Subsequent molecular phylogenetic studies have gradually led to an improved understanding of the phylogenetic relationships of *Lasianthus*. Somewhat surprisingly from a morphological perspective, *Lasianthus*, with its woody habit and drupaceous fruits, was shown to be the sister group of the herbaceous *Perama,* which has capsular fruits (Andersson and Rova, 1999). Bremer and Manen (2000) suggested the new tribe Lasiantheae, including *Lasianthus* and *Trichostachys*, and Piesschaert et al. (2000) showed that *Saldinia* and *Ronabea* belong to the same clade as these two genera. The current understanding of phylogenetic relationships is that *Trichostachys*, *Saldinia* and *Ronabea* constitute the sister group of *Lasianthus* (Piesschaert et al., 2000). These genera together constitute the tribe Lasiantheae (Bremer and Manen, 2000; Robbrecht and Manen, 2006).

Several non-Psychotrieae genera that previously were proposed to be closely related to *Lasianthus* (Bentham and Hooker, 1873) have been shown to belong elsewhere in Rubiaceae, or to be nes*ted w*ithin *Lasianthus*. For example, three species of *Saprosma* Blume (*S. foetens*, *S. fruticosum*, and *S. ternatum*) have been shown to be the sister group of Paederieae, and therefore the genus has been included in this group (Rydin et al., 2009). One species of *Saprosma*, *S. crassipes*, has instead been shown to be nested inside *Lasianthus* (Xiao and Zhu, 2007). Xiao and Zhu (2007) suggested that *Saprosma* species with ovaries that have two locules, developing into drupes with two pyrenes, should be transferred to *Lasianthus*. No type species has been designated for *Saprosma*, but since two species, *S. arboreum* and *S. fruticosum*, are mentioned in the protologue (Blume, 1826), either of these would be suitable to select as type of the genus. Regardless of what future studies will show, *Lasianthus* will have priority over *Saprosma* since *Lasianthus* is an earlier valid name.

Two other genera that were considered to be closely related to *Lasianthus* by Hooker (1873) are *Metabolos* Blume and *Litosanthes* Blume (Puff and Igersheim, 1994). *Metabolos* has recently been shown to be an ingroup in *Hedyotis* L. (Wikström et al., 2013). *Litosanthes* was described as a monotypic genus but has also been treated in a wide sense, including a number of Asian *Lasianthus* species (Deb and Gangopadhyay, 1989). The genus is now included in *Lasianthus* (Gangopadhyay and Chakrabarty, 1992; Xiao and Zhu, 2007). Another genus currently treated as a synonym of *Lasianthus* is the monotypic neotropical *Dressleriopsis* Dwyer, which has been shown to belong in *Lasianthus* based on morphological characters (Robbrecht, 1982).

There are three main aims of this study. The first is to find out where Lasiantheae originated, and by what processes it acquired its present intertropical disjunct distribution. We use maximum likelihood estimation of geographic range evolution and molecular dating analysis to obtain age estimates of key biogeographic events in the history of the group. The large uncertainty intervals typically associated with age estimates in molecular dating analyses often make explicit hypothesis testing challenging. Ideally, the inferred age of a disjunction between the neotropics and paleotropics will provide support for one biogeographic scenario over other possible biogeographic scenarios. Disjunctions in the last 31 Myr could only be explained by long-distance dispersal, since there have been no climatically available land connections between the neotropics, Africa and Asia for tropical plant taxa in this period. Any neotropical–paleotropical disjunctions estimated in the interval from 60 to 32 Ma would be consistent with Boreotropical range expansion between continents, followed by isolation due to the cooling climate and disruption of the Boreotropical flora. Prior to 60 Ma but subsequent to 95 Ma, disjunctions would again be best explained by long-distance dispersal, while any older age estimates (before 96 Ma) could be explained by vicariance caused by the breakup of Gondwana.

The second aim is to explore the impact of topological uncertainty, as well as the uncertainty in divergence time estimates, on the level of confidence in the biogeographic reconstructions. We compare the results obtained when running the biogeographic analysis on a single chronogram, the maximum clade credibility tree from the BEAST analysis, to the results obtained when using 1000 phylogenetic trees sampled at random from the posterior distribution.

The third aim is to use phylogeny to test the classification of the group; the circumscription of the tribe and the monophyly of genera. A genus of special interest in this respect is *Ronabea.* Piesschaert (2001) noted that two of the species in *Ronabea*, *R. latifolia* and *R. emetica*, differ distinctly in the dorsal ornamentation of their pyrenes. Also, Taylor (2004) reported that an unpublished phylogenetic study by Andersson, based on *rps16* data, placed *Ronabea latifolia* and *R. emetica* as a basal grade on another lineage, although it is unclear whether this result received significant support.

## 2. Materials and methods

### 2.1. Taxon sampling

A total of 80 terminals representing putatively different species were included in the study. Of these, 63 belong to Lasiantheae, and were selected to represent all previously identified major lineages and all genera classified in this group. Seventeen species in genera outside Lasiantheae were also included in the data sets to obtain a good representation from the remainder of Rubioideae, and especially taxa that have been shown to be closely related to Lasiantheae (Bremer and Eriksson, 2009).

The taxa sampled in this study represent 24% of the total number of species in Lasiantheae. Like in studies of character evolution, it is very important to have an adequate taxon sample when reconstructing the biogeographic history of a group. If a taxon with a specific character state, or geographic distribution, differing from that of closely related species, is left out of the study, the optimization in that part of the tree will not be correct. In the present study, there are a couple of genera for which it is unlikely that a proportion of missing species would have any effect on the biogeographic reconstruction. That is the case for *Saldinia* and *Trichostachys*, which are both restricted to a single one of the geographic areas used in the biogeographic analysis (Madagascar and mainland Africa, respectively), and for neither of which there is any reason to believe that the group should be non-monophyletic. In the case of

*Ronabea*, the one missing species occurs in the neotropics, just like the other two species in the genus, but since there are indications that *Ronabea* is not monophyletic (Piesschaert, 2001; Taylor, 2004) it is possible that the inclusion of the third species could affect the biogeographic reconstruction in that part of the tree. *Lasianthus* has a wide geographic distribution, and the species that we were not able to include in the present study occur in all the areas where the group is found, 173 species in Asia, 8 in Africa, and 2 in the neotropics. Whether the inclusion of these species would affect the biogeographic results depends on if there is a clear geographic structure in the phylogenetic results. If all Asian species form a group it is less likely that including additional species from this area will affect the outcome of the biogeographic analysis.

### 2.2. DNA extraction, PCR, and sequencing

Leaf material was sampled from herbarium specimens in most cases, but for a few species silica gel dried material was used. Voucher specimens are listed in the appendix. DNA extractions were carried out using the cetyl trimethyl ammonium bromide (CTAB) extraction method (Doyle and Doyle, 1990) and amplifications followed standard polymerase chain reaction (PCR) procedures. The *rps16* intron was amplified using the rpsF and rpsR2 primers (Oxelman et al., 1997), and sequenced using the same two primers. The *trnTF*-region was amplified with the a1F (Razafimandimbison and Bremer, 2002) and 2670R primers (Rydin et al., 2008) and for sequencing four additional primers, 820F, 940R, 1880F (Rydin et al., 2008), and d (Taberlet et al., 1991), were also used. The internal transcribed spacer (ITS) region was amplified using the primers P17 (Popp and Oxelman, 2001) and P25R (Oxelman, 1996) and sequenced with the same two primers. In some cases, when readings were poor, ITS2 and ITS3 (White et al., 1990) were also used for sequencing. For amplification and sequencing of external transcribed spacer (ETS) the primers Erit-F (Negrón-Ortiz and Watson, 2002) and 18S-E (Baldwin and Markos, 1998) were used. Sequences were assembled and edited using the Phred (Green and Ewing, 2002) and Phrap (Green, 1999) modules in Pregap4 and Gap4 (Staden et al., 1998). All new sequences have been submitted to EMBL and accession numbers are presented in the appendix. Sequence alignments were performed by eye, in the sequence alignment editor Se-Al (Rambaut, 1996).

### 2.3. Model selection

The data set was divided into two partitions, nrDNA (the ITS and ETS regions) and cpDNA (*rps16* and the *trnTF* region). For each partition, the best-fitting evolutionary model was identified under the Akaike information criterion (AIC; Akaike, 1973), calculated with MrAIC (ver. 1.4.4, Nylander, 2004), which tests substitution models in combination with different ways of modeling rate variation among sites. For the cpDNA data set, AIC favored the General time reversible (GTR; Tavaré, 1986) substitution model, with gamma distributed rate variation among sites (+Γ), and for the nrDNA data set, the Hasegawa-Kishino-Yano (HKY; Hasegawa et al., 1985) model with gamma distributed rate variation and a proportion of invariable sites (+Γ+I), was the favored evolutionary model.

### 2.4. Estimation of phylogeny and divergence times

We used a Bayesian approach to phylogenetic inference and estimation of divergence times, as implemented in the BEAST software package (version 1.6.2, Drummond and Rambaut, 2007). The molecular data was divided into two partitions, cpDNA and nrDNA, each analyzed with its own model of molecular evolution, including separate substitution models (see Section 2.3), models of rate variation across sites, and relaxed molecular clocks. We used a randomly generated start tree and a Yule model, with a constant speciation rate per lineage, as the tree prior. To account for lineage-specific rate-heterogeneity we used an uncorrelated log-normal clock, which implies that evolutionary change along a branch is proportional to branch length (Drummond et al., 2006). Two types of calibration data were used in the analysis, one fossil and one age estimate from a previous molecular dating analysis. The fossil is pollen of *Faramea* from the late Eocene found in Puerto Rico (Graham, 1985). It is of a biporate form that is present in some extant *Faramea* species and more or less unique among extant angiosperms (Erdtman, 1966). For further discussion about this fossil, its morphology and use for calibration see Bremer and Eriksson (2009). We used the estimated age of the *Faramea* pollen to set a minimum age of the *Faramea* stem node. For this calibration point, a uniform prior with a minimum bound at 37 Ma was used. The second calibration point is the age of the Rubioideae crown group, which was set to 77.9 Ma based on a molecular dating analysis by Bremer and Eriksson (2009). For this node (i.e., the tree model root height), a prior with a normal distribution and a standard deviation of 7 Myr, was used. This corresponds to the 95% highest posterior density (HPD) interval (65–91 Ma) obtained in the previous analysis (Bremer and Eriksson, 2009). Both nodes used for calibration were constrained to be monophyletic. To make sure that the selected priors do not interfere with one another in an unforeseen way, and that they do not have a strong influence on the result, a BEAST analysis was initially run without any data (Heled and Drummond, 2012). The joint prior distribution was checked to make sure that the effective priors were what they were intended to be, and the results were compared to the results obtained with the data to verify that the priors were updated by the data.

The Markov chain Monte Carlo (MCMC) was run for 100 million generations, sampling hypotheses every 5000 generations. At this point, an effective sample size of more than 200 samples had been obtained for all parameters. The output was visualized using Tracer (Rambaut and Drummond, 2003), making sure that parameter values were fluctuating at stable levels. The analysis was repeated three times to ensure that the chain had really reached the posterior distribution. Based on these results, the first 25% of the trees were discarded as burn-in and the remaining 15 000 samples summarized as a maximum clade credibility tree with posterior probabilities of nodes, mean divergence times, and HPD intervals of age estimates in TreeAnnotator (Drummond and Rambaut, 2007).

### 2.5. Analysis of biogeographic history

Distribution data for the species in the study was taken from Govaerts et al. (2012). The definition of areas for biogeographic analysis was based on the focus of the study, which is on large-scale biogeographic patterns, such as range expansion between continents, but also with a special interest in the colonization of Madagascar. We also wished to keep the number of areas to a minimum, since a large number of areas severely will affect computational feasibility in the software we used for the biogeographic analysis, Lagrange (ver. 20110117, Ree and Smith, 2008). The four geographic areas used in the analyses were: tropical America (A), Africa (B), Madagascar (C), and Asia (including temperate and tropical Asia as well as Australasia) (D), see Fig. 1. For each cladogenetic event in the tree, Lagrange calculates the geographic ranges of the resulting daughter lineages with the highest likelihood. First, an analysis using an unconstrained model, where all transitions between areas were equally likely at all points in time, was performed using the Maximum clade credibility tree from the Beast analysis. Then, to explore the impact of topological uncertainty on the confidence in biogeographic reconstruction, a random

**Table 1**
Amount of phylogenetic information in each of the molecular data sets. For each region, the number of terminals, the number of aligned DNA characters, the range in sequence length, and the number of variable characters, is given.

| DNA region | Terminals | Aligned DNA characters | Sequence length | Variable characters, entire data set | Variable characters, ingroup |
|---|---|---|---|---|---|
| *rps16* | 79 | 1372 | 760–851 | 457 | 167 |
| *trnTF* | 72 | 3072 | 448–2047 | 1271 | 632 |
| ITS | 74 | 839 | 348–767 | 502 | 435 |
| ETS | 67 | 540 | 289–458 | 403 | 316 |

ITS, internal transcribed spacer region; ETS, external transcribed spacer region.

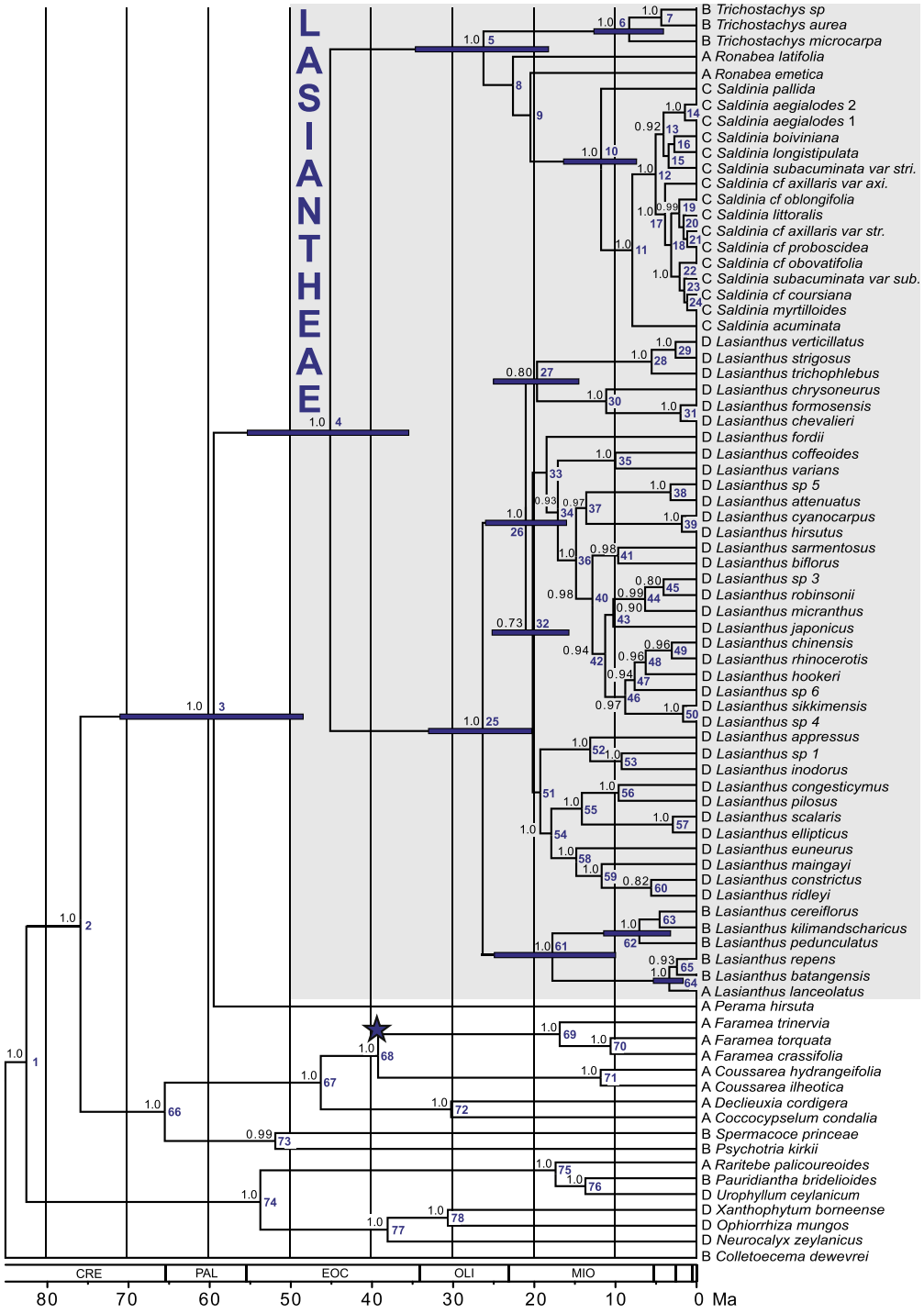


**Fig. 2.** Maximum clade credibility tree from the Beast analysis based on the combined data analyzed under the two-partition model. The tree is a chronogram with branches drawn proportional to time. Posterior probabilities above 0.70 are indicated above branches and 95% HPDs of age estimates are indicated for selected nodes. All nodes in the tree are numbered for identification. Posterior probabilities, estimated ages, and 95% HPDs of age estimates for all nodes are found in the electronic supplementary Appendix 2. Time scale from the International Commission on Stratigraphy (http://www.stratigraphy.org).

**Table 2**
Estimated ages and 95% highest posterior density intervals (HPD) of clades in Figs. 2 and 3.

| Clade | | Age estimate (Ma) | 95% HPD (Ma) |
|---|---|---|---|
| 3 | *Perama* stem node/Lasianteae stem node | 59 | 71–48 |
| 4 | Lasianteae crown group | 45 | 55–36 |
| 5 | Ronabea-Saldinia-Trichostachys-clade | 26 | 35–18 |
| 6 | Trichostachys crown group | 8 | 13–4 |
| 10 | *Saldinia* crown group | 12 | 16–7 |
| 25 | *Lasianthus* crown group | 26 | 33–20 |
| 26 | Asiatic clade in *Lasianthus* | 21 | 26–16 |
| 61 | African and Neotropical clade in *Lasianthus* | 18 | 25/10/13 |

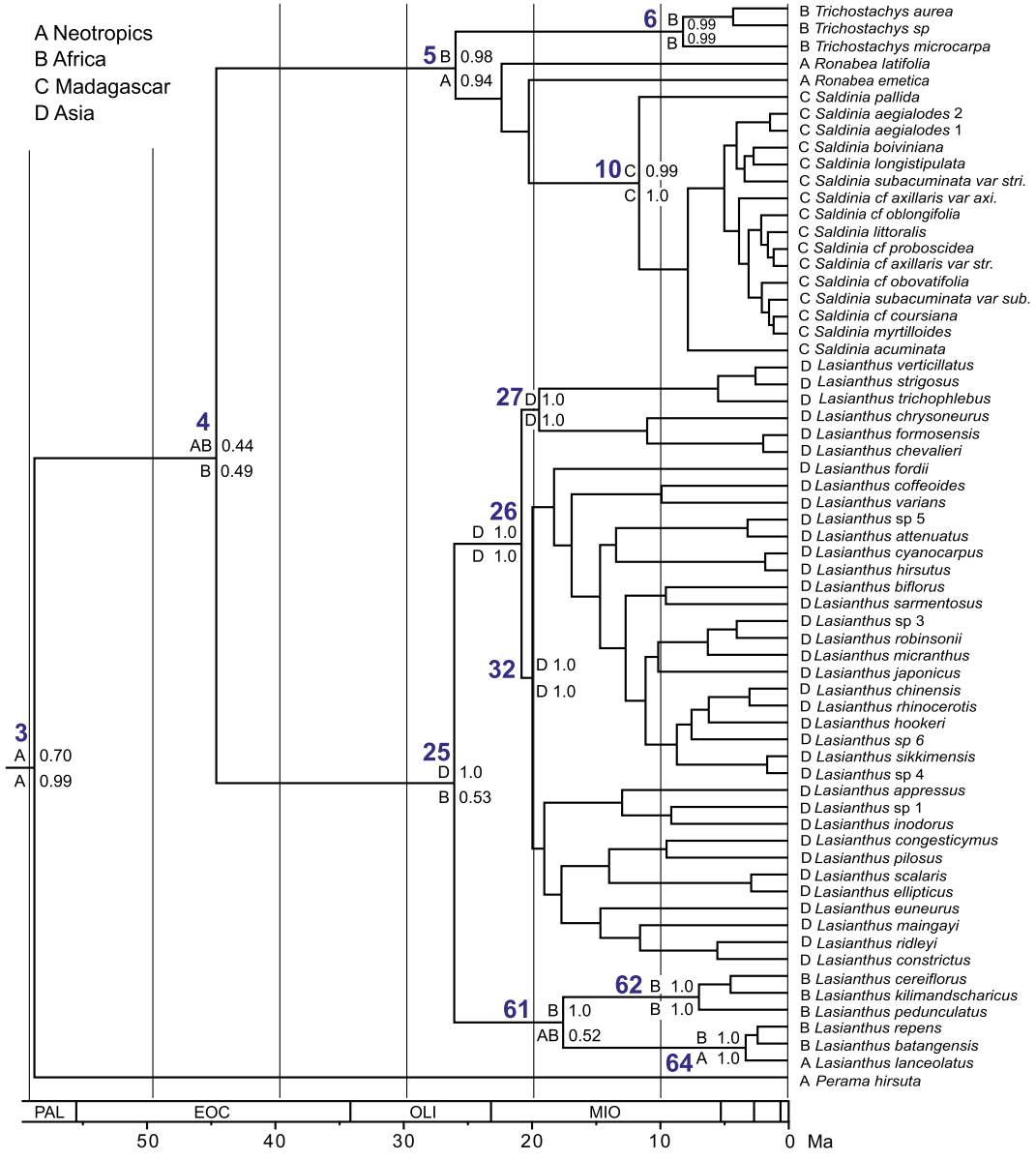


**Fig. 3.** A section of the maximum clade credibility tree from the Beast analysis (Fig. 2) showing the reconstruction of geographic range evolution with the highest likelihood in Lasiantheae. The geographic range inherited by each of the two daughter lineages resulting from a cladogenetic event is indicated by capital letters. For all biogeographic reconstructions, relative probabilities, based on analyses of 1000 trees sampled at random from the posterior distribution, are shown. Relative probabilities of reconstructions implying the same geographic area as the ML-reconstruction were summed for each daughter lineage. Clades discussed in the text are marked with numbers. Time scale from the International Commission on Stratigraphy (http://www.stratigraphy.org).

sample of 1000 chronograms was drawn from the posterior distribution from the Beast analysis. A Lagrange analysis was run for each of the trees and the reconstructions from all analyses summarized for a number of nodes of interest.

## 3. Results

In this study, 46 new *rps*16 DNA sequences, 52 new sequences from the *trnTF* region, 49 from the ITS region, and 54 ETS sequences

**Table 3**
Reconstructions of geographic range evolution for selected nodes. Likelihood values of reconstructions were calculated based on the maximum clade credibility tree. Relative probabilities were calculated both based on analyses of 1000 trees sampled at random after burnin and based on the maximum clade credibility tree alone. For each node, results for the two reconstructions with the highest likelihood are shown. The four geographic areas used in the analyses were: tropical America (A), Africa (B), Madagascar (C), and Asia (including temperate and tropical Asia as well as Australasia) (D), see Fig. 1.

| Node | Reconstruction 1 | lnL | Rel. prob. | Rel. prob. 1 tree | Reconstruction 2 | lnL | Rel. prob. |
|---|---|---|---|---|---|---|---|
| 3 | A\|A | −50.75 | 0.70 | 0.70 | AB\|A | −51.83 | 0.19 |
| 4 | B\|AB | −51.03 | 0.44 | 0.52 | A\|A | −51.66 | 0.34 |
| 5 | B\|A[a] | −50.39 | 0.94 | 0.99 | – | – | – |
| 6 | B\|B | −50.38 | 0.99 | 1.0 | B\|AB | – | 0.001 |
| 10 | C\|C | −50.38 | 0.99 | 1.0 | AC\|C | – | 0.004 |
| 25 | D\|B | −50.88 | 0.53 | 0.61 | D\|A | −51.31 | 0.39 |
| 26 | D\|D | −50.38 | 1.0 | 1.0 | – | – | – |
| 61 | B\|AB | −51.01 | 0.52 | 0.60 | B\|B | −51.16 | 0.48 |

[a] Relative probabilities given the topology in Figs. 2 and 3. See also Fig. 4.

were produced. All EMBL/GenBank accession numbers of sequences are shown in Appendix 1. Information content of each amplified DNA region is given in Table 1. The combined data set included 80 terminals, of which 63 represent species in Lasiantheae.

Trees from the separate analyses of cpDNA (*trnTF* and *rps16*) and nrDNA (ITS and ETS) were congruent concerning the relationships among major lineages within Lasiantheae (results not shown). There were, however, some supported contradictions regarding species-level relationships. In *Lasianthus*, *L. ridleyi* is the sister of *L. maingayi* in the nrDNA tree and of *L. constrictus* in the cpDNA tree. Within *Saldinia* both data sets resolve *S. pallida* and *S. acuminata* as the two first diverging lineages in the group. The remainder of the genus forms a clade within which there are extensive contradictions regarding the relationships of individual species. Since these conflicts are restricted to species nested inside *Lasianthus* and *Saldinia*, and this paper does not address relationships or biogeographic patterns within genera, we still chose to combine the data sets.

The maximum clade credibility tree, the tree in the Bayesian sample with the highest sum of posterior probabilities, from the BEAST analysis of the combined data is shown in Fig. 2. Posterior probabilities, age estimates, and 95% highest posterior density intervals (HPDs) for all nodes can be found in Appendix 2. *Perama* is strongly supported to be the sister of a likewise strongly supported Lasiantheae (Fig. 2, nodes a and b). Within Lasiantheae, the three genera *Lasianthus* (node 25), *Saldinia* (node 10), and *Trichostachys* (node 6) are all strongly supported to be monophyletic. *Ronabea* is found in the same clade as *Saldinia* and *Trichostachys* (node 5) and is not supported to be monophyletic. There is substantial uncertainty regarding the relationships among the four lineages in clade f; *Saldinia*, *Trichostachys*, and the two *Ronabea* species (see Fig. 4 and below).

The Lasiantheae crown group (node 4) is estimated to have begun to diversify around 45 Ma (Table 2), some time in the late Paleocene to early Eocene (95% HPD 55–36 Ma). The crown groups of the two major lineages within Lasiantheae, *Lasianthus* (node 25) and the *Ronabea-Saldinia-Trichostachys*-clade (node 5), are both estimated to have begun to diversify around 26 Ma, in the Oligocene or late Miocene (95% HPD 33–20 Ma for node 25 and 35–18 Ma for node 5).

Especially for the node of the Lasiantheae crown group (Fig. 3, node 4), there is quite a lot of uncertainty in the biogeographic reconstruction, especially when 1000 trees are considered (Table 3). One obvious reason for the low relative probability values are the fact that the branches surrounding this node are very long. The internodes below and above node 4, are both estimated to span more than 10 My. Since all possible biogeographic scenarios will be evaluated in a likelihood reconstruction, and the long time spans allow for more complicated sequences of biogeographic events to take place, the relative probability of any one reconstruction will generally be lower for a long internode than for a short one.

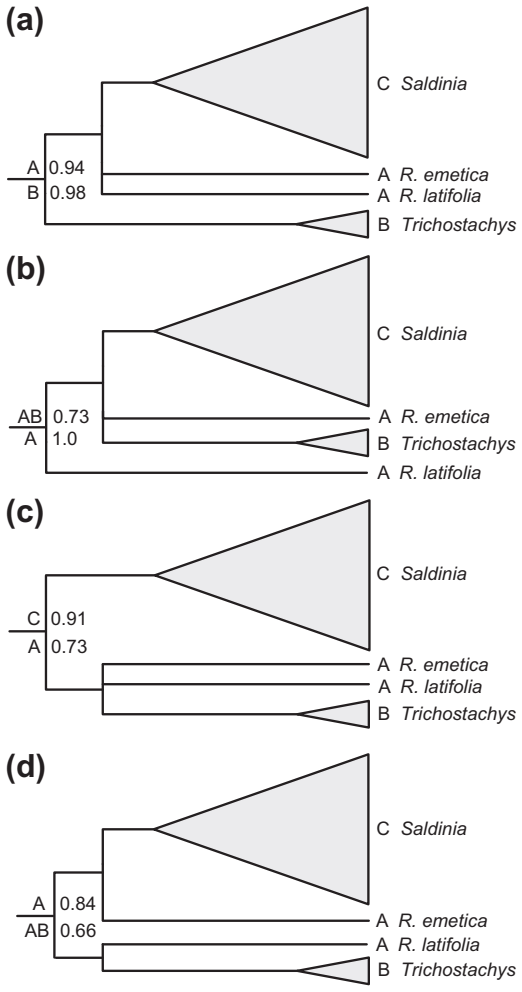


**Fig. 4.** The four most frequent topologies of the *Ronabea-Saldinia-Trichostachys*-clade (Fig. 2, clade 5) in order of decreasing frequency in the posterior tree sample from the Beast analysis. For each topology, the biogeographic reconstruction for node 5 with the highest likelihood is shown. The geographic range inherited by each of the two daughter lineages is indicated by capital letters. The relative probabilitiy, based on analyses of 1000 trees, for each daughter lineage represents the sum of relative probabilities of all reconstructions implying the same geographic area as that of the ML-reconstruction. Relative probabilities represent the likelihood of the reconstruction given the topology.

The stem lineage of Lasiantheae is inferred to originally have occurred in the neotropics but to have expanded its range to include

Africa before the divergence of the two major lineages in Lasiantheae at around 45 Ma (node 4, 95% HPD 55–36 Ma). The *Lasianthus* lineage evolved in Africa, while its sister group, the *Trichostachys-Ronabea-Saldinia* lineage, continued to inhabit a wide geographic range encompassing both Africa and the neotropics. The *Lasianthus* lineage then expanded its range to Asia some time in the interval from 45 to 26 Ma (95% HPD 55–20 Ma), and at node 25 (Fig. 3) the range was split by allopatric cladogenesis, resulting in an Asian (Fig. 3, node 61) and an African (Fig. 3, node 61) daughter lineage. Within *Lasianthus*, in clade 61, a dispersal event from Africa to the neotropics is inferred to have taken place some time between 18 and 3 Ma (95% HPD 25–2 Ma). There is considerable topological uncertainty within the *Trichostachys-Ronabea-Saldinia* (node 5), which makes it difficult to get a good picture of the biogeographic history within this group. In the topology that occurs most frequently in the posterior sample (Fig. 3 node 5 and Fig. 4a), *Trichostachys* is the sister of the remainder of the clade and *Ronabea* is paraphyletic with respect to *Saldinia*. For this topology, the reconstruction with the highest relative probability implies that the transatlantic range inferred for the group's ancestral lineage was split by allopatric cladogenesis around 26 Ma (Fig. 3, node 5, 95% HPD 35–18 Ma). *Trichostachys* then diversified in Africa and *Ronabea* in tropical America, and the ancestral lineage of *Saldinia* dispersed to Madagascar some time between 20 and 12 Ma (95% HPD 28–7 Ma). The other topologies found in the posterior distribution, and the reconstruction of geographic range evolution with the highest relative probability for node 5 associated with each of these, are shown in Fig. 4, in order of decreasing frequency in the tree sample. In the second most common topology (Fig. 4b), *Ronabea* is paraphyletic, with *R. latifolia* sister to a clade comprising the other three lineages. The other two resolutions are; *Saldinia* sister to a clade consisting of *Ronabea* and *Trichostachys* (Fig. 4c), and *R. latifolia* sister to *Trichostachys* and *R. emetica* sister to *Saldinia* (Fig. 4d).

## 4. Discussion

All included representatives of *Lasianthus*, *Saldinia*, *Trichostachys*, and *Ronabea* in this study form a clade, validating the opinion that these four genera belong in Lasiantheae (Robbrecht and Manen, 2006). While *Lasianthus*, *Saldinia*, and *Trichostachys* are found to be monophyletic, this does not seem to be the case with *Ronabea*. Although phylogenetic relationships within the clade where *Ronabea* is found are not resolved with good support, there are strong indications that *Ronabea* is paraphyletic, either with respect to *Saldinia* alone, or to both *Saldinia* and *Trichostachys* (Fig. 4). Therefore, *Ronabea* remains enigmatic and in need of further study. This study also confirms that *Perama* is the sister of Lasiantheae, despite the fact that it shares no obvious morphological synapomorphies with members of Lasiantheae (Piesschaert et al., 2000).

Historical biogeographical and molecular dating analyses indicate that the Lasiantheae lineage originated in the neotropics and dispersed to the paleotropics before the origin of the Lasiantheae crown group. This range expansion is estimated to have happened some time between 59 and 45 Ma (95% HPD 71–36 Ma). This corresponds well with the timing of the expansion of the boreotropical forests, which peaked around the early Eocene climatic optimum, ca 50 Ma (Morley, 2000). The ancestral area inferred for the Lasiantheae crown group (Fig. 3, node 4) – the neotropics and Africa – was not geographically contiguous during the period from before 45 Ma to 26 Ma when the group is estimated to have occupied this area (Figs. 3 and 4 – 5 internode). A possible interpretation is that Lasiantheae entered Eurasia via the north Atlantic land bridge, and that the split between the two major Lasiantheae lineages (Fig. 3, node 4) took place in the northern hemisphere boreotropical forests. *Lasianthus* would then have disappeared from Eurasia as the climate cooled at the end of the Eocene (Zachos et al., 2001). This hypothesis implies that the actual colonization of Africa took place later, perhaps not until Africa connected with Eurasia in the early Miocene (Morley, 2000). The inferred diversification of extant *Lasianthus* in Africa around this time (Fig. 3, node 61) is consistent with this scenario. This scenario also implies that the range expansion into Asia, inferred to have taken place along the stem lineage of *Lasianthus* (Figs. 3 and 4 – 25 internode), occurred across the Eurasian continent, rather than over the Indian ocean from Africa to Asia. There is, however, no fossil evidence from Lasiantheae to confirm that the group actually occurred in the northern hemisphere in the Eocene. The only Rubiaceae taxon for which there are reliable fossils from the Eocene of Europe is *Cephalanthus* (see discussion in Antonelli et al., 2009). *Cephalanthus* belongs in Cinchonoideae, and biogeographic and molecular dating analyses have indicated that other lineages within this clade have indeed used the boreotropic route of migration (Antonelli et al., 2009; Manns et al., 2012).

Long-distance dispersal can never be ruled out entirely as an explanation of range expansion, and in this case the age estimates of nodes 3 and 4 (Fig. 2) do not preclude that dispersal could have taken place across the Atlantic before geodispersal between continents was made possible by the coincidence of the warm and wet Boreotropical climate, and the availability of the north Atlantic land bridge, from around 60 Ma. A distribution in the northern hemisphere boreotropics is, however, the only explanation we can find for the inferred ancestral range spanning the paleo- and neotropics in the Eocene and early Oligocene (Figs. 3 and 4 – 5 internode).

A striking aspect of the results from the dating and biogeographic analyses (Fig. 3) is the two coinciding cases of vicariance inferred to have taken place at 26 Ma. One is in *Lasianthus* (node 25), where vicariance is inferred to have taken place between Africa and Asia, and the other in the *Trichostachys-Ronabea-Saldinia*-clade (node 5), where vicariance between the neotropics and Africa is the most likely reconstruction. For both of these nodes, the estimated ages are too young to invoke the break up of the boreotropical flora to explain this result. The 95% HPDs (33–20 Ma for node 25 and 35–18 for node 5) do, however, not entirely preclude this explanation, since a tropical taxon has been shown to have used this route of migration as late as 32 Ma (Davis et al., 2004). We have not been able to find any other plausible causes of vicariance.

Within *Lasianthus*, there is one large strictly Asian clade (Fig. 3, node 26) and one smaller African/Neotropical clade (Fig. 3, node 61). Diversification within the Asian clade is estimated to have begun around 21 Ma. During the Oligocene and earliest Miocene, climates were drier and cooler in the Sunda region, but at 21 Ma a major climatic change occurred (Morley and Flenley, 1987). During a period of about 10 Myr, climate was warm and wet throughout a large part of southeast and east Asia, leading to a substantial expansion of tropical forests in the region (Morley, 1998). Our results indicate that the extant diversity of *Lasianthus* in Asia (187 spp.) began to form during this time of markedly warm and moist climate.

The African/Neotropical clade (Fig. 3, node 61) is divided into an eastern lineage, comprising the three east African *Lasianthus* species in the study (*L. cereiflorus*, *L. kilimandscharicus*, and *L. pedunculatus*), and a western lineage, comprising two West African species (*L. repens* and *L. batangensis*) and one species occurring in the Caribbean (*L. lanceolatus*). The split between the western and eastern lineages is estimated at approximately 18 Ma. In the earliest Miocene (c 23–20 Ma) climates were moist over most of equatorial Africa and rain forests extended more or less from coast to coast (Morley, 2000). With the general global

cooling that began at the start of the Miocene, a slow and progressive aridification commenced on the African continent (Zachos et al., 2001, 2008) leading to a change in vegetation. Rain forests became greatly reduced and were replaced by open woodland and grassland. Palynological data shows that grasses increased from 21 Ma and that extinctions were frequent in the period from 16 to 10 Ma (Morley, 2000). Thus the split into an eastern and a western lineage in clade 61 (Fig. 3) is inferred to have been concurrent with the fragmentation of rain forests on the African continent. It is possible that the appearance of a barrier of drier climate in the interior of Africa was the cause of the formation of these two separate lineages.

With the exception of the neotropical *L. lanceolatus*, the western and eastern clades correspond to the sections Membranacei and Succulenti, respectively, proposed for African *Lasianthus* by Jannerup (2006). This infrageneric division was based mainly on the texture of the stipules and the number of pyrenes per fruit. It is interesting that Jannerup (2006) noted that *L. panamensis*, with a distribution from Costa Rica to Colombia, morphologically is very similar to *L. batangensis* of sect. Membranacei, since our results imply that a member of the West African lineage dispersed to the neotropics. It has been proposed that members of *Lasianthus* dispersed twice from the paleo- to the neotropics (Robbrecht, 1982), an issue that cannot be addressed by the present study, since we have only been able to include one of the three species of *Lasianthus* known to occur in the neotropics, *L. lanceolatus*. The inferred long-distance dispersal from Africa to the Caribbean in clade 61 (Fig. 3) is estimated to have taken place some time between 18 and 3 Ma (Table 2).

Phylogenetic support for the interrelationships among *Trichostachys*, *Saldinia* and the two species of *Ronabea* is poor, which makes it difficult to get a clear picture of the biogeographic history of this clade (Fig. 2, node 5). The reconstruction of biogeographic events with the highest likelihood within this group involves a dispersal event from the neotropics to Madagascar some time between 20 and 12 Ma (Fig. 3). This is not an expected pattern, since previous studies have shown that a majority of the angiosperm flora on Madagascar originated in mainland Africa (Yoder and Nowak, 2006; Agnarsson and Kuntner, 2012). This has also been shown to be the case in other groups within Rubiaceae (Wikström et al., 2010). In the case of *Saldinia*, such a biogeographic scenario does not receive strong support by our data. Reconstructions compatible with an African origin for the ancestor of *Saldinia* are found in topologies that are less frequent in the posterior sample (Fig. 4c, and possibly b). Dispersal from South America to Madagascar is, however, mentioned as a secondary route of dispersal in a review of recent literature (Agnarsson and Kuntner, 2012), where 11 such cases are reported, albeit with no suggestion as to how these dispersals are supposed to have taken place. A better understanding of phylogenetic relationships within the *Trichostachys-Ronabea-Saldinia*-clade will clearly be needed to understand the biogeographic history of the clade.

Two ways that a member of Lasiantheae could disperse long distances is either by rafting, or as seeds dispersed by birds. In a review of phylogenetic studies of angiosperm groups that show evidence of dispersal across the tropical Atlantic, Renner (2004) showed that sea currents have been responsible for dispersal in both directions, while wind dispersal primarily concerned dispersals from South America to Africa. Fruits in Lasiantheae are fleshy, drupaceous, and most commonly blue in colour. At maturity, they reach a size of up to 10 mm. They seem to be adapted for bird dispersal, but while different species of birds have been reported to eat Rubiaceae fruits (Blake and Loiselle, 1992; Fleming and Kress, 2011) we are not aware of data explicitly showing that this includes fruits of Lasiantheae. Many groups of frugivorous birds evolved in the Oligocene/Miocene (c. 34–10 Ma, Flemming and Kress, 2011). This is a period when a lot of diversification took place within Lasiantheae, and many of the main lineages evolved. Thus, the radiation of extant Lasiantheae may be correlated in time with the suggested timing of the evolution of modern groups of frugivorous birds.

To summarize the biogeographic results from this study, the Lasiantheae lineage is inferred to have originated in the neotropics and to have expanded its range to the palaeotropics during the Eocene. There is, however, considerable uncertainty in the reconstruction of geographic range evolution for the early nodes in the group, making it more difficult to get a clear picture of biogeographic events. The long internodes in this part of the tree are likely to contribute to the low probability values, since a likelihood based method like DEC will take into account the increased chance of additional biogeographic events occurring on a long branch. The relative probability of any one reconstruction will therefore be low for a node surrounded by long branches. The inferred range expansion could have taken place either by continental migration through the boreotropics or by transatlantic long-distance dispersal. Evidence in favour of the former hypothesis is the estimated timing, the transatlantic range inferred to have persisted up to 30 My (Fig. 3, 3–4–5 internodes), and the two instances of vicariance (Fig. 3, nodes 5 and 25) that could perhaps have been caused by the breakup of the boreotropical flora. In addition, two instances of long-distance dispersal are inferred to have taken place in the Oligocene/Miocene, once from Africa to the neotropics within *Lasianthus,* and once to Madagascar in association with the origin of *Saldinia*.

When a random sample of 1000 trees from the posterior distribution was used in the biogeographic analysis, the confidence in the biogeographic reconstruction decreased for most nodes, compared to when a single topology was used. This gives an indication that there is uncertainty in the topology, branch lengths or estimated divergence times that influences the reconstruction of geographic range evolution. When looking at the biogeographic reconstruction based on the maximum clade credibility tree one could, for example, easily have been convinced by the reconstruction for the crown node of the *Trichostachys-Ronabea-Saldinia*-clade (Fig. 3, node 5). This node is supported by a posterior probability of 1.0, and the relative probability of the reconstruction of geographic range evolution is 0.99 (Table 3). When the result was averaged over 1000 trees, this decreased to 0.94, and an examination of the topologies found in the tree sample showed that there were four types of resolution present. For each of these, quite different biogeographic scenarios are supported (Fig. 4) and we have to conclude that based on the present data it is not possible to say much about the biogeographic events for this node, other than that all reconstructions involve a split between a neotropical lineage and a lineage that is at least partly palaeotropical. It is obvious that a good understanding of phylogenetic relationships is essential in order to understand the biogeographic history of a group. Usually, the phylogeny is not completely known, and in such cases it is important not to overlook phylogenetic uncertainty in biogeographic analysis in order to avoid reporting misleading results. For nodes where the resolution is uncertain, the use of a single "best" topology as a basis for biogeographic analysis will result in inflated confidence in a biogeographic reconstruction which is in fact just one of several possible reconstructions.

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## Appendix A

See Appendixes 1 and 2.

**Appendix 1**
List of taxa included in the study. For each species there is information about geographic origin and voucher, as well as EMBL/Genbank numbers of DNA sequences.

| Species | Origin | Voucher | Rps16 | TrnTF | ITS | ETS |
|---|---|---|---|---|---|---|
| Coccocypselum condalia Pers. | Brazil | Pirani & Bremer 4891 (SPF) | EU145499[8] | EU145547[8] | EU145358[8] | HM042509[1] |
| Colletoecema dewevrei (De Wild.) E.M.A.Petit | Zaire | Lisowski 47195 (K) | KF704874 | EU145532[8] | EU145353[8] | – |
| Coussarea hydrangeifolia (Benth.) Benth. & Hook.f. ex Müll.Arg. | Bolivia | Fuentes 5504 (GB) | EU145501[8] | EU145549[8] | EU145360[8] | – |
| Coussarea ilheotica Müll.Arg. | Brazil | De Carvalho et al. 4081 (K) | AM9005972 | HM042586[1] | HM042454[1] | HM042510[1] |
| Declieuxia cordigera Mart. & Zucc. ex Schult. & Schult.f. | Brazil | Pirani & Bremer 4893 (SPF) | AM117298[6] | EU145551[8] | EU145361[8] | – |
| Faramea crassifolia Benth., Hooker's | Guyana | Jansen Jacobs et al. 3882 (GB) | HM042567[1] | HM042587[1] | HM042463[1] | HM042510[1] |
| Faramea torquata Müll.Arg. | Ecuador | Ståhl 3021 (GB) | HM042568[1] | HM042588[1] | HM042455[1] | HM042512[1] |
| Faramea trinervia K.Schum. & Donn.Sm. | Costa Rica | Gomez-Laurito 8374 (CR) | AM900598[2] | HM042589[1] | – | HM042513[1] |
| Lasianthus appressus Hook.f. | Thailand | Larsen 46093 (AAU) | KF704875 | KF704918 | KF704969 | KF704821 |
| Lasianthus attenuatus Jack | Vietnam | Averyanov et al. VH1218 (AAU) | KF704876 | – | KF704970 | KF704822 |
| Lasianthus batangensis K.Schum. | Gabon | Andersson & Nilsson 2284 (GB) | AY538439[4] | – | – | – |
| Lasianthus batangensis K.Schum. | Liberia | Adam 20063 (UPS) | – | KF704919 | KF704971 | KF704823 |
| Lasianthus biflorus (Blume) M.G.Gangop. & Chakrab. | China | Zhu 2655 (?) | DQ282649[3] | – | – | – |
| Lasianthus biflorus (Blume) M.G.Gangop. & Chakrab. | Thailand | Kerr 15221 (AAU) | – | – | KF704972 | – |
| Lasianthus cereiflorus E.A.Bruce | Tanzania | B. Bremer 3092 (UPS) | KF704877 | – | – | – |
| Lasianthus cereiflorus E.A.Bruce | Tanzania | Manktelov et al. 89214 (UPS) | – | KF704920 | KF704973 | KF704824 |
| Lasianthus chevalieri Pit. | Vietnam | Averyanov et al VH2673 (AAU) | AM900596[2] | – | HM042453 | HM0425089[1] |
| Lasianthus chinensis (Champ.) Benth. | China | Xiao 04010 (?) | DQ282641[3] | – | – | – |
| Lasianthus chrysoneurus (Korth.) Miq. | China | Zhu 03159 (?) | DQ282642[3] | – | – | – |
| Lasianthus chrysoneurus (Korth.) Miq. | Thailand | Larsen et al. 43311 (AAU) | – | KF704921 | KF704974 | KF704825 |
| Lasianthus coffeoides Fyson | India | Klackenberg & Lundin 80 (S) | AF004061[4] | KF704922 | KF704975 | KF704826 |
| Lasianthus congesticymus H.Zhu | Thailand | Larsen et al. 45522 (AAU) | KF704878 | KF704923 | KF704976 | KF704827 |
| Lasianthus constrictus Wight | Thailand | Larsen et al. 45643 (AAU) | KF704879 | KF704924 | – | – |
| Lasianthus cyanocarpus Jack | (?) | Ridsdale 11/8 XVII 11.8.17.136 (?) | KF704880 | KF704925 | KF704977 | KF704828 |
| Lasianthus ellipticus Wight | Thailand | Larsen et al. 45620 (AAU) | KF704881 | KF704926 | KF704978 | KF704829 |
| Lasianthus euneurus Stapf | Borneo | Beaman 9088 (S) | KF704882 | KF704927 | KF704979 | KF704830 |
| Lasianthus fordii Hance | Japan | Togasi 1435 (GB) | KF704883 | – | KF704980 | – |
| Lasianthus formosensis Matsum. | Vietnam | Averyanov & Binh VH3772 (AAU) | KF704884 | KF704928 | KF704981 | KF704831 |
| Lasianthus hirsutus (Roxb.) Merr. | Vietnam | Gong 04298 (?) | DQ282637[3] | – | – | – |
| Lasianthus hirsutus (Roxb.) Merr. | Thailand | Larsen et al. 45047 (AAU) | – | KF704929 | KF704982 | – |
| Lasianthus hookeri C.B.Clarke ex Hook.f. | China | Zhu 03157 (?) | DQ282643[3] | – | – | – |
| Lasianthus hookeri C.B.Clarke ex Hook.f. | Thailand | Larsen et al. 46728 (AAU) | – | KF704930 | KF704983 | KF704832 |
| Lasianthus inodorus Blume | Borneo | Beaman 7194 (S) | KF704885 | KF704931 | – | KF704833 |
| Lasianthus japonicus Miq. | Japan | Chevi 139 (GB) | KF704886 | KF704932 | KF704984 | KF704834 |
| Lasianthus kilimandscharicus K.Schum. | Malawi | Lantz 119 (UPS) | AM117327[6] | KF704933 | EU145366 | – |
| Lasianthus lanceolatus (Griseb.) Urb. | Hispaniola | Fuertes 350 (S) | + | – | – | – |
| Lasianthus lanceolatus (Griseb.) Urb. | Puerto Rico | Taylor CM 11719 (MO) | – | EU145554 | EU145367 | KF704835 |
| Lasianthus maingayi Hook.f. | Thailand | Larsen et al. 43312 (AAU) | KF704887 | KF704934 | KF704985 | KF704836 |
| Lasianthus micranthus Hook.f. | Vietnam | Tirvengadum et al. 3287 (AAU) | KF704888 | KF704935 | – | KF704837 |
| Lasianthus pedunculatus E.A.Bruce | Tanzania | Andreasen 71 (UPS) | EU145504 | KF704936 | EU145368 | KF704838 |
| Lasianthus pilosus Wight | Thailand | Larsen et al. 43154 (AAU) | KF704889 | KF704937 | KF704986 | KF704839 |
| Lasianthus cf repens Hepper | Gabon | Andersson & Nilsson 2307 (GB) | KF704890 | KF704938 | KF704987 | KF704840 |
| Lasianthus rhinocerotis Blume | Malaysia | Zhu 03123 (?) | DQ282639[3] | – | – | – |
| Lasianthus rhinocerotis Blume | Thailand | Larsen et al. 2013 (AAU) | – | KF704939 | KF704988 | KF704841 |
| Lasianthus ridleyi King & Gamble | Thailand | Larsen 46088 (AAU) | KF704891 | KF704940 | KF704989 | KF704842 |

(*continued on next page*)

**Appendix 1** (*continued*)

| Species | Origin | Voucher | Rps16 | TrnTF | ITS | ETS |
|---|---|---|---|---|---|---|
| Lasianthus robinsonii Ridl. | Brunei | Bygrave 35 (K) | KF704892 | KF704941 | KF704990 | – |
| Lasianthus sarmentosus Craib | Thailand | Niyomdham 4488 (AAU) | KF704893 | KF704942 | KF704991 | – |
| Lasianthus scalaris Craib | Thailand | Larsen et al 44161 (AAU) | KF704894 | KF704943 | KF704992 | KF704843 |
| Lasianthus sikkimensis Hook.f. | China | Zhu 03155 (?) | DQ282644[3] | – | – | – |
| Lasianthus sikkimensis Hook.f. | Thailand | Larsen et al. 46316 (AAU) | – | KF704944 | KF704993 | KF704844 |
| Lasianthus sp 1 | Borneo | Nielsen & Balslev 1086 (AAU) | KF704895 | KF704945 | KF704994 | KF704845 |
| Lasianthus sp 3 | Vietnam | Kainulainen et al. 17 (S) | KF704896 | KF704946 | KF704995 | KF704846 |
| Lasianthus sp 4 | Vietnam | Kainulainen et al. 57 (S) | KF704897 | KF704947 | KF704996 | KF704847 |
| Lasianthus sp 5 | Vietnam | Krüger et al. 31 (S) | KF704898 | KF704948 | KF704997 | KF704848 |
| Lasianthus sp 6 | Vietnam | Krüger et al. 4 (S) | KF704899 | KF704949 | KF704998 | KF704849 |
| Lasianthus strigosus Wight | Australia | B & K Bremer 3902 (UPS) | EU145505 | EU145556 | EU145369 | KF704850 |
| Lasianthus trichophlebus Hemsl. ex F.B. Forbes & Hemsl. | Thailand | Larsen 45393 (AAU) | KF704900 | KF704950 | KF704999 | KF704851 |
| Lasianthus cf varians (Thwaites) Thwaites | Sri Lanka | Klackenberg 189 (S) | KF704901 | KF704951 | KF705000 | KF704852 |
| Lasianthus verticillatus (Lour.) Merr. | China | Zhu 03156 (?) | DQ282640[3] | – | – | – |
| Lasianthus verticillatus (Lour.) Merr. | Thailand | Larsen et al. 43012 (AAU) | – | – | KF705001 | KF704853 |
| Neurocalyx zeylanicus Hook | Sri Lanka | B & K Bremer 937 (S) | AM900594[2] | EU145562 | HM042457[1] | KF704854 |
| Ophiorrhiza mungos L. | Cult. UPS | Bremer 3301 (UPS) | AF004035[5] | KF704952 | EU145377 | HM042514[1] |
| Pauridiantha bridelioides Verdc. | Tanzania | Bridson 584 (BR) | HM042573[1] | HM042616[1] | HM0424861 | HM0425431 |
| Perama hirsuta Aubl. | French Guiana | Andersson et al 1990 (S) | KF704902 | KF704953 | KF705002 | KF704855 |
| Psychotria kirkii Hiern | Tanzania | Bremer 3066 (UPS) | AF410728 | KF704954 | FJ208592 | KF704856 |
| Raritebe palicoureoides Wernham | Ecuador | Jaramillo & Rivea 195 (NY) | AF004075[5] | – | – | – |
| Raritebe palicoureoides Wernham | Panama | Antonio T 1697 (AAU) | – | HM042593[1] | HM0424811 | HM042520[1] |
| Ronabea emetica (L.f.) A.Rich. | Bolivia | Steinbach 434 (S) | KF704903 | KF704955 | KF705003 | KF704857 |
| Ronabea latifolia Aubl. | Guatemala | Contreras 9152 (S) | KF704904 | KF704956 | KF705004 | – |
| Saldinia acuminata Bremek. | Madagascar | Razafimandimbison et al. 605 (S) | KF704905 | KF704957 | KF705005 | KF704858 |
| Saldinia aegialodes 1 Bremek. | Madagascar | Razafimandimbison 506 (UPS) | KF704906 | KF704958 | KF705006 | KF704859 |
| Saldinia aegialodes 2 Bremek. | Madagascar | Razafimandimbison 516 (UPS) | + | + | – | + |
| Saldinia cf axillaris (Lam. ex Poir.) Bremek. var. axillaris | Madagascar | Razafimandimbison et al. 1004 (S) | KF704907 | KF704959 | KF705007 | KF704860 |
| Saldinia cf axillaris (Lam. ex Poir.) var. strigosa Bremek. | Madagascar | Kainulainen et al. 139 (S) | KF704908 | – | KF705008 | KF704861 |
| Saldinia boiviniana Bremek. | Mayotte | Mouly 590 (P) | – | KF704960 | KF705009 | KF704862 |
| Saldinia cf coursiana Bremek. | Madagascar | Kårehed et al. 286 (UPS) | KF704909 | KF704961 | KF705010 | KF704863 |
| Saldinia littoralis Bremek. | Madagascar | Schatz & Lowry 1307 (K) | KF704910 | KF704962 | KF705011 | KF704864 |
| Saldinia longistipulata Bremek. | Madagascar | Kårehed et al. 257 (UPS) | KF704911 | EU145558 | EU145371 | KF704865 |
| Saldinia myrtilloides Bremek. | Madagascar | Kårehed et al. 281 (UPS) | – | KF704963 | KF705012 | KF704866 |
| Saldinia cf oblongifolia Bremek. | Madagascar | Kainulainen et al. 106 (S) | KF704912 | – | KF705013 | – |
| Saldinia cf obovatifolia Bremek. | Madagascar | Razafimandimbison et al. 981 (S) | KF704913 | KF704964 | KF705014 | KF704867 |
| Saldinia pallida Bremek. | Madagascar | Bremer et al 4038-B38 (UPS) | KF704914 | KF704965 | KF705015 | KF704868 |
| Saldinia cf proboscidea Bremek. | Madagascar | Kainulainen et al. 64 (S) | KF704915 | KF704966 | KF705016 | KF704869 |
| Saldinia subacuminata var. strigosa Bremek. | Madagascar | Kårehed et al. 202 (UPS) | KF704916 | KF704967 | KF705017 | KF704870 |
| Saldinia subacuminata var. subacuminata Bremek. | Madagascar | Razafimandimbison et al. 606 (UPS) | KF704917 | KF704968 | – | KF704871 |
| Spermacoce princeae (K.Schum.)Verdc. | Kenya | Luke & Luke 8371 (?) | HM042566[1] | HM042585[1] | HM042452[1] | HM042507[1] |
| Trichostachys aff aurea Hiern | Gabon | Andersson & Nilsson 2304 (GB) | EU145507 | EU145559 | EU145372[8] | KF704872 |
| Trichostachys microcarpa K.Schum. | Dem.Rep. of Congo | Lisowski 47098 (BR) | AF191491[7] | – | – | KF704873 |
| Trichostachys sp | Cameroon | Sonké 1725 (UPS) | AM900595[2] | EU145560 | EU145373[8] | – |
| Urophyllum ceylanicum (Wight) Thwaites | Sri Lanka | Klackenberg 214 (S) | AM900620[2] | HM042598[1] | HM042465[1] | HM0425221 |
| Xanthophytum borneense (Valeton) Axelius | Borneo | Axelius 316 (S) | EU145513 | EU145567 | EU145381 | HM042515[1] |

[1] Smedmark et al. (2010).
[2] Smedmark et al. (2008).
[3] Xiao and Zhu (2007).
[4] Andersson and Antonelli (2005).
[5] Andersson and Rova (1999).
[6] Bremer and Eriksson (2009).
[7] Piesschaert et al. (2000).
[8] Rydin et al. (2008).

**Appendix 2**
Posterior probabilities, estimated ages, and 95% highest posterior densities of all nodes in Fig. 2.

| Node | PP | Age estimate (Ma) | 95% HPD (Ma) |
|---|---|---|---|
| 1 | 1.0 | 82.4 | 93.5–71.1 |
| 2 | 1.0 | 75.7 | 86.9–65.2 |
| 3 | 1.0 | 59.3 | 70.9–48.3 |
| 4 | 1.0 | 45.0 | 55.2–35.5 |
| 5 | 1.0 | 26.2 | 34.5–18.2 |
| 6 | 1.0 | 8.2 | 12.6–4.0 |
| 7 | 1.0 | 4.3 | 7.1–1.9 |
| 8 | 0.36 | 22.5 | 29.9–15.0 |
| 9 | 0.52 | 20.4 | 27.4–14.2 |
| 10 | 1.0 | 11.7 | 16.3–7.4 |
| 11 | 1.0 | 7.9 | 11.1–5.0 |
| 12 | 1.0 | 5.0 | 6.8–3.4 |
| 13 | 0.92 | 4.0 | 5.7–2.5 |
| 14 | 1.0 | 1.4 | 2.8–0.2 |
| 15 | 0.31 | 3.4 | 4.9–1.9 |
| 16 | 0.57 | 2.7 | 4.2–1.3 |
| 17 | 1.0 | 3.8 | 5.0–2.6 |
| 18 | 0.67 | 3.1 | 4.3–2.0 |
| 19 | 0.99 | 2.1 | 3.3–1.3 |
| 20 | 0.32 | 1.6 | 2.4–0.8 |
| 21 | 0.56 | 1.1 | 1.9–0.5 |
| 22 | 1.0 | 2.0 | 3.1–1.1 |
| 23 | 0.52 | 1.5 | 2.3–0.7 |
| 24 | 0.53 | 1.1 | 1.9–0.4 |
| 25 | 1.0 | 26.3 | 32.9–20.2 |
| 26 | 1.0 | 21.0 | 25.9–16.0 |
| 27 | 0.8 | 19.6 | 24.9–14.4 |
| 28 | 1.0 | 5.5 | 8.7–2.5 |
| 29 | 1.0 | 2.5 | 4.6–0.9 |
| 30 | 1.0 | 11.1 | 16.5–6.1 |
| 31 | 1.0 | 1.9 | 3.6–0.6 |
| 32 | 0.73 | 20.1 | 25.1–15.7 |
| 33 | 0.74 | 18.4 | 23.0–13.8 |
| 34 | 1.0 | 17.0 | 21.4–12.5 |
| 35 | 0.93 | 10.0 | 14.5–5.4 |
| 36 | 1.0 | 14.8 | 18.9–10.9 |
| 37 | 0.97 | 13.5 | 17.7–9.4 |
| 38 | 1.0 | 3.2 | 5.6–1.1 |
| 39 | 1.0 | 1.8 | 3.3–0.5 |
| 40 | 0.98 | 12.8 | 16.5–9.3 |
| 41 | 0.98 | 9.6 | 13.9–5.4 |
| 42 | 0.94 | 11.2 | 14.6–8.1 |
| 43 | 0.9 | 10.2 | 13.4–7.0 |
| 44 | 0.99 | 6.3 | 9.4–3.3 |
| 45 | 0.8 | 4.0 | 6.5–1.8 |
| 46 | 0.97 | 8.7 | 11.8–5.8 |
| 47 | 0.94 | 7.6 | 10.3–4.7 |
| 48 | 0.96 | 6.2 | 8.9–3.5 |
| 49 | 0.96 | 3.0 | 6.1–0.2 |
| 50 | 1.0 | 1.6 | 3.0–0.5 |
| 51 | 0.67 | 19.2 | 24.1–14.6 |
| 52 | 1.0 | 13.0 | 17.8–8.1 |
| 53 | 1.0 | 9.2 | 13.8–4.9 |
| 54 | 1.0 | 17.8 | 22.6–13.3 |
| 55 | 1.0 | 14.1 | 18.6–9.5 |
| 56 | 1.0 | 9.5 | 14.0–5.4 |
| 57 | 1.0 | 2.9 | 5.0–1.0 |
| 58 | 1.0 | 14.8 | 19.5–10.3 |
| 59 | 1.0 | 11.6 | 16.3–7.3 |
| 60 | 0.82 | 5.5 | 11.6–0.5 |
| 61 | 1.0 | 17.7 | 24.8–9.9 |
| 62 | 1.0 | 7.0 | 11.4–3.2 |
| 63 | 0.54 | 4.5 | 8.0–1.7 |
| 64 | 1.0 | 3.3 | 5.3–1.6 |
| 65 | 0.93 | 2.4 | 4.0–1.0 |
| 66 | 1.0 | 65.5 | 76.1–55.1 |
| 67 | 1.0 | 46.2 | 52.8–40.3 |
| 68 | 1.0 | 39.1 | 43.2–37.4 |
| 69 | 1.0 | 16.8 | 24.4–9.5 |
| 70 | 1.0 | 10.5 | 16.7–5.3 |
| 71 | 1.0 | 11.8 | 19.2–5.3 |
| 72 | 1.0 | 30.1 | 41.2–18.1 |
| 73 | 0.99 | 51.8 | 66.1–37.6 |
| 74 | 1.0 | 53.6 | 74.1–34.5 |
| 75 | 1.0 | 17.3 | 25.9–9.7 |
| 76 | 1.0 | 13.6 | 21.7–6.4 |
| 77 | 1.0 | 38.0 | 55.1–22.9 |
| 78 | 1.0 | 30.5 | 46.1–16.2 |

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