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HISTORICAL BIOGEOGRAPHY OF THE PREDOMINANTLY NEOTROPICAL SUBFAMILY CINCHONOIDEAE (RUBIACEAE): INTO OR OUT OF AMERICA?

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The Rubiaceae is the fifth largest plant family and is found on all continents, mostly in (sub-)tropical regions. Despite a large representation of Rubiaceae in the Paleotropics, the subfamily Cinchonoideae has its primary distribution in the Neotropics. Within the Cinchonoideae only two tribes, Naucleeae and Hymenodictyeae, have Paleotropical centers of distribution. In this study, we used information from five chloroplast DNA markers and fossil data to investigate when the subfamily was established in the Neotropics as well as major subsequent dispersal events within and out of the Neotropics and within the Paleotropics. Our results show that the ancestor of Cinchonoideae and its sister, Ixoroideae, was present in South America during the Late Cretaceous. Dispersal to Central America was estimated to occur during the Early Paleocene, and subsequent dispersals to the Caribbean islands occurred during the Oligocene–Miocene. The ancestor of Naucleeae and Hymenodictyeae dispersed to the Paleotropics no later than the Eocene, and the findings of fossils of *Cephalanthus* in Europe and western Siberia support its presence in the boreotropical forests. Long-distance, trans-Pacific dispersal during the Miocene was indicated for the remaining Paleotropical Cinchonoideae.

Keywords: biogeography, Cinchonoideae, dating, Neotropics, Paleotropics, Rubiaceae.

Online enhancements: appendixes.

Introduction

A large number of subtropical and tropical plant families have disjunct distributions between the Paleotropics and the Neotropics (Raven and Axelrod 1974), most commonly between tropical America and tropical Africa. Such disjunct distributions have often been attributed to a presence on the Gondwana continent and then subsequent vicariance following the break up of this large landmass (Raven and Axelrod 1974; Gentry 1982; Renner 1993; Chanderbali et al. 2001). Recent molecular dating analyses, however, have shown that most plant families are too young to have originated before the break up of Gondwana (Wikström et al. 2001; Bremer et al. 2004), which is in agreement with studies integrating phylogenies and fossil data (Magallón et al. 1999; Magallón and Sanderson 2001).

Today, other hypotheses are often employed to explain disjunct distributions between the Paleotropics and the Neotropics. Such explanations involve long-distance dispersal by wind, water, rafting, or avian transport (Renner et al. 2001; Dick et al. 2003, 2007; Givnish et al. 2004; Lavin et al. 2004; Pennington and Dick 2004; Schaefer et al. 2009), as well as dispersal via land bridges connecting the two areas at various geological times (Lavin and Luckow 1993; Lavin et al. 2000; Chanderbali et al. 2001; Renner et al. 2001;

Davis et al. 2002, 2004; Zerega et al. 2005; Muellner et al. 2006). The North Atlantic Land Bridge (NALB), which was established some time in the Late Paleocene or Early Eocene when boreotropical forests existed in the northern hemisphere, and the Bering Land Bridge, which connected present-day Siberia with Alaska during the Pleistocene (Tiffney 1985; Tiffney and Manchester 2001), are commonly invoked routes of exchange between the Old World and the New World (Lavin and Luckow 1993; Lavin et al. 2000; Chanderbali et al. 2001; Renner et al. 2001; Davis et al. 2002, 2004; Zerega et al. 2005; Muellner et al. 2006), but temporary island chains between Africa and South America have also been considered (Morley 2003; Pennington and Dick 2004).

Members of the family Rubiaceae occur on all continents of the world, but their major distributions are in subtropical or tropical regions. The majority of species is found in the Paleotropics; however, taxa with a Neotropical distribution are found within all three subfamilies (i.e., Rubioideae, Ixoroideae, and Cinchonoideae; Bremer et al. 1995, 1999). On the basis of its present distribution, Rubiaceae were among the Gondwana-derived taxa Raven and Axelrod (1974) proposed to have migrated between Africa and South America during or before the Paleocene, with only limited exchange between the continents from the Eocene and later. The migration between Africa and South America was suggested to have been facilitated through dispersal over a narrow Atlantic Ocean or via island chains stretching across it (Raven and Axelrod 1974, p. 601). Morley (2003) proposed that trans-Atlantic island chains were present along the Walvis Ridge and the Sierra Leone Rise until the Early Tertiary.

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Raven and Axelrod (1974) further stated that most Central American and Caribbean Rubiaceae taxa originated from South America during the Late Cenozoic or later. This view was shared by Gentry (1982), who placed Neotropical Rubiaceae in a group of Andean-centered taxa. Andean-centered taxa have their main distribution within the northern and southern Andes but are also well represented in Central America, which constitutes a second center of speciation for this group (Gentry 1982). More recently, in a study of Neotropical Rubiaceae, Antonelli et al. (2009) suggested an Early Paleocene origin for the family and a subsequent dispersal to the Neotropics via the NALB during the Late Paleocene or the Early Eocene.

Magallón et al. (1999) integrated knowledge of phylogenetic relationships between major angiosperm lineages with the fossil record and estimated divergence times of sister clades on the basis of the oldest fossil assigned to either clade. The divergences of Gentianales (to which the Rubiaceae belong) and its sister, Lamiales s.l. (Olmstead et al. 1993), were estimated to have occurred in the Early Eocene (53.2 million years ago [Mya]; Magallón et al. 1999). Wikström et al. (2001) used divergences in molecular data to estimate divergence times for a large number of plant families. In their study, the Gentianales crown group was estimated to 61–64 million years (Myr), whereas Bremer et al. (2004), using molecular data and fossil calibrations to investigate divergence times among the Asterids, reported an estimated age of 78 Myr for the divergence of Gentianales.

Although the Rubiaceae is one of the largest plant families (>13,000 species), there are limited fossil data for the group, and no known Rubiaceae fossil predates the Eocene. Middle Eocene material of *Paleorubiaceophyllum eocenicum* (Berry) Roth & Dilcher, which is suggested to represent the oldest Rubiaceae fossil (Bremer and Eriksson 2009), was recently reported to include stems with alternated leaves; its affinity is, therefore, uncertain (Graham 2009). Among more confidently identified fossils (Graham 2009), two have been placed in the Eocene: infructescences and fruits of *Emmenopterys* (Manchester 1994; Wehr and Manchester 1996) and pollen of *Faramea* (Graham 1985).

Recent dating analyses of Rubiaceae (Antonelli et al. 2009; Bremer and Eriksson 2009) have resulted in divergence-time estimates of Rubiaceae that were older than those indicated by the large-scale dating analysis of Wikström et al. (2001). Antonelli et al. (2009) estimated the divergence time of the Rubiaceae crown group to be 66.1 Myr, and Bremer and Eriksson (2009) estimated it to be 86.6 Myr. These divergence-time estimates indicate that Rubiaceae originated well after the existence of any direct land connection between Africa and South America (~96 Mya; Morley 2003) and when the distance between the two continents was 800 km or more(85 Mya; Burnham and Graham 1999). In each of their studies, Antonelli et al. (2009) and Bremer and Eriksson (2009) estimated that the three subfamilies, Cinchonoideae, Ixoroideae, and Rubioideae, diversified 51.3 and 38.7 Mya, 48.1 and 59.6 Mya, and 47.9 and 77.9 Mya, respectively.

The Cinchonoideae is essentially Neotropical, with its distribution centered in Central America, South America, and the Caribbean. Within Cinchonoideae only two tribes, Naucleeae and Hymenodictyeae, are predominantly distributed in the Paleotropics, although members of Naucleeae are also found in North and South America; additionally Chiococceae ranges to the islands of the western Pacific, with the highest diversity found in the Greater Antilles. In contrast to Cinchonoideae, Rubioideae and Ixoroideae have their major distributions in the Paleotropics, with only a smaller portion of genera distributed in the Neotropics. However, in a recent study of Ixoroideae, Kainulainen et al. (2009) found support for two Neotropical clades as early-diverging lineages within Ixoroideae, whereas Paleotropical members of the subfamily formed a large sister group to one of the two Neotropical lineages. A sister-group relationship between Cinchonoideae and Ixoroideae has been established in several studies (Rova et al. 2002; Bremer and Eriksson 2009; Rydin et al. 2009).

The knowledge of the close sister-group relationship between Cinchonoideae and Ixoroideae, in combination with an increased amount of sequence data for their taxa and an understanding of distribution ranges, fossil data, and paleogeograpical scenarios, provide a good platform to address some biogeographical hypotheses for the group. In this article, we use molecular dating analysis and multiple dispersal-vicariance analyses (DIVA) with molecular and fossil data to reconstruct the biogeographical history of Cinchonoideae, in particular with a focus on (1) when the subfamily was established in the Neotropics and (2) major subsequent dispersal events within the subfamily.

Material and Methods

Taxon Sampling

The focus of this study was primarily the Cinchonoideae. Sampling within the subfamily was almost identical to that of Manns and Bremer (2010), which included most genera of all recognized tribes. Several genera, especially those with a distribution in several geographical areas, were represented by more than one taxon. In total, 171 Cinchonoideae taxa were investigated.

Sampling within Ixoroideae was less extensive; a total of 32 Ixoroideae taxa were included in the study. Our sampling included representatives from all of our distribution areas (see "Analyses" and "Selection of Distribution Areas") of the tribes labeled as early-diverging lineages by Kainulainen et al. (2009). Sampling from the large Paleotropical clade did not cover all tribes, although several Paleotropical taxa from this clade were included so as not to bias the sampling.

In addition, 19 taxa from the third subfamily, Rubioideae, were included, along with two taxa from Luculieae and two taxa from Coptosapelteae. Representatives of non-Rubiaceae Gentianales taxa, which were used to root the trees, were *Alstonia scholaris* (Apocynaceae), *Gelsemium sempervirens* (Gelsemiaceae), and *Exacum affine* (Gentianaceae), all of which have previously been placed within the sister group of Rubiaceae (Backlund et al. 2000). With the broad sampling outside the ingroup, we aimed to reduce the number of inferred ancestral areas for the nodes of special interest, as DIVA tends to suggest many equally parsimonious distributions, especially toward the root node (Ronquist 1996).

Five different chloroplast markers were used in the analyses: the *rbc*L, *ndh*F, and *trn*T-L-F regions, the *atp*B-*rbc*L

spacer, and the *rps*16 intron. New sequences were generated for *Randia aculeata* (voucher: *Taylor* DT-307 [MICH]), using extraction and amplification protocols described by Manns and Bremer (2010). All other sequences were previously published by B.B.'s plant molecular lab at the Bergius Foundation (Manns and Bremer 2010) or were downloaded from GenBank (accession numbers and references are presented in app. A). The sequences were aligned manually using the alignment editor Se-Al, version 2.0a11 (Rambaut 1996).

Analyses

A combined data set of all five markers was used to generate a posterior distribution of trees using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best suitable evolutionary model for each chloroplast marker was selected by mrAIC (Nylander 2004), using the AICc criterion (Posada and Buckley 2004). Each MrBayes analysis used two separate Markov chain Monte Carlo (MCMC) runs with four chains each, for 25,000,000 generations, and the temperature was set to 0.15 to increase mixing. Every 1,000th tree topology was sampled in a tree file. After removal of burn-in topologies (first 20,000 in each run), the remaining trees from the tree files were pooled to produce a majority-rule consensus tree.

To test whether amplified sequence data had evolved in a clocklike manner (under rate constancy), we performed a likelihood-ratio (LR) test (Felsenstein 1981) using PAUP*, version 4.0b10 (Swofford 2003), and three randomly selected phylogenies from the tree files produced in the MrBayes analyses.

Divergence-Time Analyses

To estimate tree topology and divergence times, an analysis was run in a Bayesian framework, using the BEAST 1.6.1 package (Drummond and Rambaut 2007) with the uncorrelated lognormal clock model and the molecular evolutionary model GTR + Γ, as suggested by mrAIC (Nylander 2004) for the combined matrix. To decrease the number of generations in the MCMC chain, a random tree of the post-burn-in topologies generated in MrBayes was used as a starting tree in the analysis.

The BEAST analysis was conducted using four fossil constraints (listed in "Prior and Age Calibration Points") in combination with a maximum age constraint for the tree height and 26,000,000 generations. Estimated mean ages for a selected number of nodes, corresponding to larger clades, was investigated using Tracer, and a maximum clade credibility tree was constructed using TreeAnnotator (supplied in the BEAST package; Drummond and Rambaut 2007). The maximum clade credibility tree topology was compared with the majority-rule consensus tree from the MrBayes analysis.

Prior and Age Calibration Points

Tree height was constrained using a uniform prior, with the upper limit set to 88 Myr on the basis of the age estimate for the Gentianales split and the uncertainty of this estimate (78 ± 10 Myr; Bremer et al. 2004). In addition, four Rubia-

ceae fossils were used to constrain ages of nodes within the phylogeny by specifying prior information. These priors were set as uniform priors, with minimum ages set on the basis of the fossil information and maximum ages set to 145 Myr. The upper limit is based on the first occurrences of triaperturate pollen in the fossil record (Crane et al. 1995). All fossil age constraints were set to the divergence of the smallest strongly supported clade (posterior probability [pp] = 1.0) in the majority-rule consensus tree to which the fossil was assigned.

Considering the many members of Rubiaceae, the limited knowledge of detailed pollen morphology in many Rubiaceae genera, and the similarity of Rubiaceae pollen to pollen found in other members of Gentianales, pollen fossils must be selected carefully. We found two pollen fossils that were reliably assigned to specific genera within Rubiaceae.

Faramea pollen from the Late Eocene Gatuncillo flora near Alcalde Diaz, Panama, shows a distinct biporate form (Graham 1985) that is also present in some extant *Faramea* species and is considered to be more or less unique among extant angiosperms (Erdtman 1966). The age of the *Faramea* pollen of the Gatuncillo flora was estimated to be 37 Myr; this value was used as a minimum age constraint for the *Faramea* and *Coussarea* split.

Although the *Scyphiphora* pollen, found in the Early Miocene layers of Marshall Islands in the West Pacific, is of the rather common tricolporate Rubiaceae type, its distinct pores with protruding, papilla-like rims caused Leopold (1969) to assign it to *Scyphiphora*. The identification of this fossil is further strengthened by the fossil's affiliation to a fossil mangrove flora, which exist in a habitat in which extant *Scyphiphora* is found. The age of the *Scyphiphora* pollen fossil was estimated to be ~23 Myr by Saenger (1998). This age was used as a minimum age constraint for the *Scyphiphora-Canthium* clade, as the sister relationship of *Scypohiphora* and *Ixora* is only weakly supported.

Knowledge of fruit and flower morphology is often very useful when determining the family and genus affinity of any fossil, since classification of extant taxa is primarily based on fertile parts. Among the different fruit fossils assigned to Rubiaceae, *Emmenopterys* constitutes the oldest and most confidently identified (Graham 2009). On the basis of infructescence and fruit fossils of the Middle Eocene Clarno flora (Oregon, U.S.A.), Manchester (1994) described *Emmenopterys dilcheri* and compared it with extant *Emmenopterys henryi* Oliv. Two years later, Wehr and Manchester (1996) reported on *Emmenopterys* fruit fossils from the Middle Eocene Republic flora (Washington, U.S.A.). We used the estimated age of *E. dilcheri* (44 Myr; Manchester 1994) as a minimum age prior for the Condamineeae crown node (sensu Kainulainen et al. 2010), since resolution within that clade was not supported.

Finally, several *Cephalanthus* fruit fossils have been reported from different localities in Europe and western Siberia and dated to the Late Eocene–Early Oligocene (Mai and Walther 1978, 1985) and the Middle and Late Miocene (Raniecka-Bobowska 1959; Dorofeev 1960, 1963; Friis 1985). An additional *Cephalanthus* fruit fossil from Eocene layers in the Pipe-Clay Series (Dorset, England) was acknowledged by Mai and Walther (1978), although it was originally

listed as unidentified by Chandler (1962). These fruits are characterized as schizocarps with two one-seeded, obovoid, slightly dorsiventrally flattened mericarps, and the seeds are strophiolate. We took *Cephalanthus kireevskianus* (Dorof.) Dorofeev from the Late Eocene and Early Oligocene (Mai and Walther 1978, 1985) to be the calibration point for the split between *Cephalanthus* and the rest of Naucleeae and assigned its minimum age to 34 Myr, representing the Eocene-Oligocene transition.

Biogeographic Analysis

Ancestral-area reconstruction was conducted using DIVA (Ronquist 1996, 1997). In DIVA, the most parsimonious reconstruction of ancestral ranges is inferred on a given phylogeny by minimizing the numbers of dispersal and extinction events that are needed to explain the present distribution (Ronquist 1997). We used a Bayes-DIVA approach, as presented by Nylander et al. (2008), to account for phylogenetic uncertainty in the biogeographic analysis, and analyses were run on 15,000 trees from the posterior distribution of trees obtained in the BEAST analysis.

The results of the DIVA analyses were added together in a majority-rule consensus tree. Marginal posterior distribution of ancestral areas are presented at each node, representing the uncertainty in the ancestral-area reconstruction as well as in the topology.

Biogeographic Data and Selection of Distribution Areas

Species distribution was mainly compiled from the World Checklist of Rubiaceae (Govaerts et al. 2008) and is supplied in table 1. For outgroup taxa, the information on distribution was compiled from the literature (Mabberley 1997; Thulin 2001; Zhen and Jianhua 2007). Several genera within Cinchonoideae tribes that have a wide distribution are probably polyphyletic or paraphyletic (McDowell et al. 2003; Achille et al. 2006; Rova et al. 2009; Manns and Bremer 2010). Distributions based on genus level would, therefore, not provide a correct distribution pattern, and we chose to code terminals using species distributions. In most cases, the geographical distribution of the sampled species of a genus corresponded to the total geographical distribution of the genus. However, for some genera, the coding of terminals on the basis of species distribution may influence the ancestral-area reconstruction; these cases are addressed in "Discussion."

Selection of Distribution Areas

The aim of this study was to study the large-scale biogeography of Cinchonoideae, in particular, with a focus on when the subfamily was established in the New World and subsequent distributional changes. Seven geographical areas of distribution were circumscribed on the basis of present and past separation of major landmasses:

A. Southern North America and Central America north of the Panamanian Isthmus; more or less isolated from South America until the uplift of the Panamanian Isthmus (~3.5 Mya; Saito 1976; Iturralde-Vinent and MacPhee 1999).

B. Caribbean islands and southern Florida, excluding Trinidad and Tobago.

C. Tropical South America, including Trinidad and Tobago.

D. Mainland Africa, including the Arabian Peninsula.

E. Madagascar, the Comoros, the Mascarenes, and the Seychelles.

F. Eurasia: Europe and Asia, including the Sunda Islands and the Philippines and excluding the Arabian Peninsula.

G. The Pacific islands, including New Guinea, New Caledonia, Fiji, Hawaii, and Australia and the smaller islands of Melanesia, Micronesia, and Polynesia.

Results

Phylogenetic Analysis

In the Bayesian inference majority-rule consensus tree (fig. 1), Cinchonoideae (1.0 pp) and Ixoroideae (1.0 pp) were recovered as sister groups (1.0 pp) and Colletoecemateae was placed within Rubioideae (1.0 pp). Luculieae (1.0 pp) and Coptosapelteae (1.0 pp) were placed outside the three subfamilies. Resolutions between Rubioideae, Luculieae, and Coptosapelteae received low pp values.

Maximum pp values (1.0) were found for all tribes within Cinchonoideae and for a number of sister-tribe relationships (i.e., Guettardeae-Rondeletieae, Naucleeae-Hymenodictyeae, Chiococceae-Hillieae-Hamelieae-*Chione/Colleteria*, and Cinchoneae-Isertieae), while early splits within the subfamily were unresolved or received only low pp values. As in Cinchonoideae, early splits within Ixoroideae had low pp values while tribes and some intertribal relationships had high pp values. Detailed information on topology and support values is presented in appendix B, available in the online edition of the *International Journal of Plant Sciences*.

Likelihood Ratio Test

The likelihood ratio statistic (D) was expected to be distributed as χ^2, with the number of degrees of freedom corresponding to the number of taxa minus 2 (Page and Holmes 1998). The distance between the likelihood of the tree with or without an enforced molecular clock, multiplied by 2 ($D = 2$ ($\log L_{no\ clock} - \log L_{clock}$)), was compared with the χ^2 distribution: using the LR test, $D = 2[-88,140.69 - (-89,399.29)] = 2517.2$, $D = 2[-88,131.22 - (-89,376.07)] = 2489.7$, and $D = 2[-88,133.96 - (-89,365.32)] = 2462.72$; df $= 226$, $P = 0.05$, $\chi^2 = 262.07$. The LR test was found to strongly reject the molecular clock.

Divergence-Time Analysis

Apart from a few weakly supported nodes, the topology of the maximum clade credibility tree from the BEAST analysis (figs. 2–4) was congruent with the majority-rule consensus tree from the MrBayes analysis. Rubiaceae were estimated to have originated in the Late Cretaceous (fig. 2A). Before the end of the Cretaceous the Cinchonoideae had diverged from Ixoroideae, and in the Paleocene both Cinchonoideae and Ixoroideae diversified further. More detailed information on

estimated divergence times and confidence intervals for larger clades is found in table 2.

Biogeographical Analysis

The most favored ancestral-area reconstruction indicates that Rubiaceae originated in Asia (F) and South America (C; fig. 2B). However, the reconstruction is ambiguous and several alternative—although less likely—reconstructions are also presented. Members of one descendant lineage including Luculieae, Coptosapelteae, and Rubioideae diversified within Asia, with subsequent dispersal events to other areas reconstructed for Rubioideae. These are shown in figure 2B.

South America (C) is the most likely ancestral area for the other descendant lineage of Rubiaceae, which includes the subfamilies Ixoroideae and Cinchonoideae. Ancestral-area reconstruction of Ixoroideae involves South America (C), solely or together with Africa (+D). One descendant lineage radiates in South America and later disperses to Central America, the Caribbean, and Asia. Reconstruction of ancestral areas for early splits in the other descendant lineage are ambiguous and involve Africa (D) or South America (C), alone or in combination, and possibly Asia (+F). Further ancestral-area reconstructions within Ixoroideae are shown in figure 2B.

From a South American source area, the ancestor of Cinchonoideae (node 24) dispersed to Central America (+A) and/or possibly Africa (+D), but the reconstruction is ambiguous (fig. 3B). The tribes Cinchoneae and Isertieae diversified in South America, and for the remaining Cinchonoideae the ancestor (node 26) was present in Central America (A) and Africa (D) or, almost as likely, only in Central America (A). The most favored reconstructions for the subsequent nodes indicate that two descendant lineages including Guettardeae, Rondeletieae, Hamelieae, Hillieae, *Chione + Colleteria*, and Chiococceae diversified in Central America, while a third descendant lineage including Naucleeae and Hymenodictyeae diversified in Africa.

Ancestral Hymenodictyeae dispersed from Africa (D) to Asia (+F) or to Asia and Madagascar (+E), with subsequent vicariance between Asia and Africa + Madagascar. In its sister tribe, the most favored reconstruction indicates that Naucleeae diversified in Africa (D) before the transoceanic back dispersal of *Cephalanthus* from Africa to Central America (+A). In the other descendant lineage, dispersals to Asia (+F) and/or Madagascar (+E) are ambiguously reconstructed. The *Pausinystalia-Burttdavya* lineage radiated in Africa, with later dispersals to Asia (+F) and the Pacific (+G) and also to the Neotropics (+A/+C). Ancestral reconstruction for the *Janotia-Breonadia* lineage favors either Madagascar alone (E) or Madagascar and Africa (+D), while the *Neonauclea-Adina* lineage is reconstructed to Asia (F) and experienced several later dispersal events to the Pacific (+G).

The ancestor of the Hamelia, Hillieae, and *Chione + Colleteria* lineage remained in Central America (A) or dispersed to the Caribbean (+B; fig. 4B). Ancestral reconstruction of *Chione + Colleteria* is ambiguous and includes all Neotropical areas, while the Hamelieae and Hillieae lineage originated in Central America (A), with several more recent dispersals to the Caribbean (+B) and South America (+C) within the tribes.

Chiococceae s.l. most likely originated in Central America (A) and later dispersed to the Caribbean (+B). Restricted Caribbean (B) ancestral distributions are reconstructed for a small *Exostema* lineage (*Exostema spinosum–Exostema lineatum*) and for the large *Exostema acuminata–Asemnantha* lineage. Within this large lineage, back dispersal to Central America (+A) is reconstructed for the ancestor of *Osa* and *Nernstia*, as are several dispersals to South America (+C) and two parallel transoceanic dispersals to the Pacific (+G).

The Guettardeae and Rondeletieae lineage most likely originated in Central America (A; fig. 5B), and Central America is also the most favored ancestral reconstruction for Rondeletieae. In Rondeletieae, parallel dispersals to the Caribbean (+B) are reconstructed for the two descendant lineages, followed by vicariance between the two areas in both of the lineages and a recent back dispersal to Central America (+A) from a restricted Caribbean distribution in the *Rondeletia pitreana–Acrosynanthus* lineage.

Ancestral reconstruction of Guettardeae includes Central America alone (A) or, less likely, Central America and South America (+C). *Rogiera* most likely radiated in Central America and later dispersed to South America (+C). For the remaining Guettardeae, ancestral area for a number of consecutive nodes is ambiguously reconstructed. The reconstructions include Central America and/or South America and, less frequently, the Caribbean, and the sequence of dispersal events is not clear. Most reconstructions for ancestral *Machaonia* include Central America (A), alone or in combination with South America (+C) and the Caribbean (+B). The *Arachnothryx-Gonzalagunia* lineage most likely originated in Central America (A), and from this distribution early dispersal to South America (+C) is indicated. For the *Malanea-Antirhea* lineage, South America (C) is the most favored ancestral area, possibly also involving Central America (+A). Within this lineage, several transoceanic dispersals (+F/+G/+E) from the Neotropics are reconstructed.

Discussion

Topology of the Phylogenetic Tree

Our majority-rule consensus tree (fig. 1) shows an overall congruence with other recent large-scale molecular phylogenetic analyses of Rubiaceae (Rova et al. 2002; Bremer and Eriksson 2009; Rydin et al. 2009) that have all resulted in a general topology of the family. This topology resolves three subfamilies (i.e., Cinchonoideae, Ixorioideae, and Rubioideae) and two clades placed outside of the three subfamilies (i.e., Luculieae and Coptosapelteae). The relationships between the three subfamilies, Luculieae, and Coptosapelteae are more or less unresolved or are poorly supported, except for a sister-group relationship between Cinchonoideae and Ixoroideae.

There is also a general congruence between our study and previous studies (Rova et al. 2002; Bremer and Eriksson 2009; Kainulainen et al. 2009; Rydin et al. 2009) regarding relationships within Rubioideae and Ixoroideae. In Cinchonoideae, however, resolution in the backbone topology is generally weakly supported (Rova et al. 2002; Antonelli et al. 2009; Bremer and Eriksson 2009; Rydin et al. 2009; Manns and Bremer 2010) in earlier studies. Our study resolves Cin-

Table 1

Taxa Included in This Study, with Information of Total Number of Species within the Genus, Distribution Area, Classification, and GenBank Accession Numbers

Taxon	Species	Genus distribution	Species distribution	Classification
Acrosynanthus latifolius Standl.	5	B	B	RON
Acrosynanthus minor Urb.			B	RON
Acunaeanthus tinifolius (Griseb.) Borhidi	1	B	B	RON
Adina pilulifera (Lam.) Franch. ex Drake	3	F	F	NAU
Adina rubella Hance			F	NAU
Adinauclea fagifolia (Teijsm. & Binn. ex Havil.) Ridsdale	1	F	F	NAU
Antirhea inconspicua (Seem.) Christoph.	~40	E–G	G	GUE
Antirhea madagascariensis Chaw			E	GUE
Antirhea megacarpa Merr. & L.M.Perry			G	GUE
Arachnothryx buddleioides (Benth.) Planch.	~108	A, C	A	GUE
Arachnothryx chimboracensis (Standl.) Steyerm.			C	GUE
Arachnothryx hondurensis (Donn.Sm.) Lorence			A	GUE
Arachnothryx leucophylla (Kunth) Planch.			A	GUE
Arachnothryx spectabilis (Steyerm.) Rova, Delprete & B.Bremer			C	GUE
Asemnantha pubescens Hook.f.	1	A	A	CHI
Badusa corymbifera (G.Forst.) A.Gray	3	F, G	G	CHI
Balmea stormae Martínez	1	A	A	HIL
Bikkia artensis (Montrouz.) Guillaumin	~20	F, G	G	CHI
Bikkia macrophylla (Brongn.) K.Schum.			G	CHI
Bikkia tetrandra (L.f.) A.Rich.			G	CHI
Blepharidium guatemalense Standl.	1	A	A	RON
Bobea gaudichaudii (Cham. & Schltdl.) H.St.John & Herbst	4	G	G	GUE
Breonadia salicina (Vahl) Hepper & J.R.I.Wood	1	D, E	D, E	NAU
Breonia chinensis (Lam.) Capuron, Adansonia	~20	E	E	NAU
Burttdavya nyasica Hoyle	1	D	D	NAU
Catesbaea holacantha C.Wright ex Griseb.	~17	B	B	CHI
Catesbaea parviflora Sw.			B	CHI
Cephalanthus natalensis Oliv.	6	A–D, F	D	NAU
Cephalanthus occidentalis L.			A, B	NAU
Cephalanthus salicifolius Humb. & Bonpl.			A	NAU
Ceratopyxis verbenacea (Griseb.) Hook.f.	1	B	B	CHI
Chiococca alba (L.) Hitchc.	~24	A–C	A–C	CHI
Chione venosa (Sw.) Urb.	1	A–C	A–C	Unplaced
Chomelia angustifolia Benth.	~77	A–C	C	GUE
Chomelia spinosa Jacq.			A, C	GUE
Chomelia tenuiflora Benth.			A, C	GUE
Ciliosemina pedunculata (H.Karst.) Antonelli	2	C	C	CIN
Cinchona calisaya Wedd.	~24	A, C	C	CIN
Cinchona officinalis L.			C	CIN
Cinchona pubescens Vahl			A, C	CIN
Cinchonopsis amazonica (Standl.) L.Andersson	1	C	C	CIN
Colleteria seminervis (Urb. & Ekman) David W. Taylor	2	B	B	Unplaced
Corynanthe mayumbensis (R.D.Good) N.Hallé	4	D	D	NAU
Corynanthe paniculata Welw.	3	D	D	NAU
Cosmibuena grandiflora (Ruiz & Pav.) Rusby	4	A, C	A, C	HIL
Cosmibuena valerioi (Standl.) C.M.Taylor			A	HIL
Coutaportla ghiesbreghtiana (Baill.) Urb.	2	A	A	CHI
Coutarea hexandra (Jacq.) K.Schum.	5	A, C	A, C	CHI
Cubanola daphnoides (Graham) Aiello	2	B	B	CHI
Deppea blumenaviensis (K.Schum.) Lorence	~29	A, C	C	HAM
Deppea erythrorhiza Schltdl. & Cham.			A	HAM
Deppea grandiflora Schltdl.			A	HAM
Deppea splendens Breedlove & Lorence			A	HAM
Eosanthe cubensis Urb.	1	B	B	CHI
Erithalis fruticosa L.	8	A–C	A–C	CHI
Erithalis harrisii Urb.			B	CHI
Exostema acuminatum Urb.	~45	A–C	B	CHI
Exostema caribaeum (Jacq.) Schult. in J.J.Roemer & J.A.Schultes			A–C	CHI
Exostema lineatum (Vahl) Schult.			B	CHI

Table 1

(*Continued*)

Taxon	Species	Genus distribution	Species distribution	Classification
Exostema longiflorum (Lamb.) Schult. in J.J.Roemer & J.A.Schultes			B	CHI
Exostema spinosum (Le Vavass.) Krug & Urb.			B	CHI
Gonzalagunia affinis Standl. ex Steyerm.	~40	A–C	C	GUE
Gonzalagunia cornifolia (Kunth.) Standl.			C	GUE
Gonzalagunia dependens Ruiz & Pav.			C	GUE
Gonzalagunia rosea Standl.			A, C	GUE
Guettarda boliviana Standl.	~158	A–G	C	GUE
Guettarda crispiflora Vahl			A–C	GUE
Guettarda ferruginea Griseb.			B	GUE
Guettarda speciosa L.			D–G	GUE
Guettarda tournefortiopsis Standl.			A, C	GUE
Guettarda uruguensis Cham. & Schltdl.			C	GUE
Gyrostipula comorensis J.-F.Leroy	3	E	E	NAU
Gyrostipula foveolata (Capuron) J.-F.Leroy			E	NAU
Haldina cordifolia (Roxb.) Ridsdale	1	F	F	NAU
Hamelia cuprea Griseb.	~17	A–C	B	HAM
Hamelia papillosa Urb.			B	HAM
Hamelia patens Jacq.			A–C	HAM
Hillia illustris (Vell.) K.Schum.	~23	A–C	C	HIL
Hillia parasitica Jacq.			B, C	HIL
Hillia triflora (Oerst.) C.M.Taylor			A, C	HIL
Hintonia latiflora (Sessé & Moç. ex DC.) Bullock	3	A	A	CHI
Hodgkinsonia ovatiflora F.Muell.	2	G	G	GUE
Hoffmannia ghiesbreghtii (Lem.) Hemsl.	~115	A–C	A	HAM
Hymenodictyon floribundum (Hochst. & Steud.) B.L.Rob.	~23	D–F	D	HYM
Hymenodictyon orixense (Roxb.) Mabb.			F	HYM
Isertia coccinea (Aubl.) J.F.Gmel.	~14	A–C	C	ISE
Isertia laevis (Triana) Boom			A, C	ISE
Isertia parviflora Vahl			C	ISE
Isertia pittieri (Standl.) Standl.			C	ISE
Isidorea pedicellaris Urb. & Ekman	~17	B	B	CHI
Janotia macrostipula (Capuron) J.-F.Leroy	1	E	E	NAU
Joosia umbellifera H.Karst.	~11	A, C	A, C	CIN
Keriantera preclara J.H.Kirkbr.	1	C	C	ISE
Ladenbergia amazonensis Ducke	~34	A, C	C	CIN
Ladenbergia oblongifolia (Humb. ex Mutis) L.Andersson			C	CIN
Ladenbergia pavonii (Lamb.) Standl.			C	CIN
Lorencea guatemalensis (Standl.) Borhidi	1	A	A	CHI
Ludekia borneensis Ridsdale	2	F	F	NAU
Machaonia acuminata Humb. & Bonpl.	~32	A–C	A, C	GUE
Machaonia erythrocarpa (Standl.) Borhidi			A	GUE
Machaonia portoricensis Baill.			B	GUE
Machaonia williamsii Standl.			C	GUE
Malanea forsteronioides Müll.Arg.	~40	A–C	C	GUE
Mazaea phialanthoides (Griseb.) Krug & Urb.	2	B	B	RON
Metadina trichotoma (Zoll. & Moritzi) Bakh.f.	1	F, G	F, G	NAU
Mitragyna inermis (Willd.) Kuntze	7	D, F, G	D	NAU
Mitragyna rotundifolia (Roxb.) Kuntze			F	NAU
Mitragyna rubrostipulata (K.Schum.) Havil.			D	NAU
Mitragyna stipulosa (DC.) Kuntze			D	NAU
Morierina montana Vieill.	2	G	G	CHI
Myrmeconauclea strigosa (Korth.) Merr.	4	F	F	NAU
Nauclea diderrichii (De Wild.) Merr.	~10	D, F, G	D	NAU
Nauclea orientalis (L.) L.			F, G	NAU
Neolamarckia cadamba (Roxb.) Bosser	2	F, G	F, G	NAU
Neonauclea brassii S.Moore	~68	F, G	F, G	NAU
Neonauclea clemensiae Merr. & L.M.Perry			G	NAU
Nernstia mexicana (Zucc. & Mart. ex DC.) Urb.	1	A	A	CHI
Ochreinauclea maingayi (Hook.f.) Ridsdale	2	F	F	NAU
Osa pulchra (D.R.Simpson) Aiello	1	A	A	CHI

Table 1

(*Continued*)

Taxon	Species	Genus distribution	Species distribution	Classification
Ottoschmidtia microphylla (Griseb.) Urb.	1	B	B	GUE
Paracorynanthe antankarana Capuron ex J.-F.Leroy	2	E	E	HYM
Pausinystalia johimbe (K.Schum.) Pierre ex Beille	5	D	D	NAU
Pausinystalia macroceras (K.Schum.) Pierre ex Beille			D	NAU
Pertusadina malaccensis Ridsdale	4	F, G	F	NAU
Phialanthus ellipticus Urb.	~21	B	B	CHI
Phyllacanthus grisebachianus Hook.f.	1	B	B	CHI
Phyllomelia coronata Griseb.	1	B	B	RON
Pinarophyllon bullatum Standl.	2	A	A	HAM
Plocaniophyllon flavum Brandegee	1	A	A	HAM
Portlandia platantha Hook.f.	6	B	B	CHI
Pseudomiltemia filisepala (Standl.) Borhidi	1	A	A	HAM
Remijia chelomaphylla G.A.Sullivan	~45	C	C	CIN
Remijia macrocnemia (Mart.) Wedd.			C	CIN
Rhachicallis americana (Jacq.) Hitchc.	1	A, B	A, B	RON
Rogiera amoena Planch.	15	A, C	A, C	GUE
Rogiera cordata (Benth.) Planch.			A	GUE
Roigella correifolia (Griseb.) Borhidi & M.Fernández Zeq.	1	B	B	RON
Rondeletia deamii (Donn.Sm.) Standl.	~120	A–C	A	RON
Rondeletia hameliifolia Dwyer & M.V.Hayden			A	RON
Rondeletia intermixta Britton			B	RON
Rondeletia nipensis Urb.			B	RON
Rondeletia odorata Jacq.			B	RON
Rondeletia pitreana Urb. & Ekman			B	RON
Rondeletia poitaei Grisb.			B	RON
Rondeletia portoricensis Krug & Urb.			B	RON
Rovaeanthus suffrutescens (Brandegee) Borhidi	2	A	A	RON
Salzmannia nitida DC.	1	C	C	CHI
Sarcocephalus latifolius (Sm.) E.A.Bruce	2	D	D	NAU
Schmidtottia sessilifolia (Britton) Urb.	~15	B	B	CHI
Scolosanthus lucidus Britton	~27	B	B	CHI
Siemensia pendula (C.Wright ex Griseb.) Urb.	1	B	B	CHI
Sinoadina racemosa (Siebold & Zucc.) Ridsdale	1	F	F	NAU
Solenandra ixoroides Hook.f.	~12	A–C	B	CHI
Solenandra mexicana (A.Gray) Borhidi			A	CHI
Solenandra parviflora (A.Rich. ex Humb. & Bonpl.) Borhidi			B	CHI
Stenostomum acreanum (K.Krause) C.M Taylor	~46	A–C	C	GUE
Stenostomum acutatum DC.			B	GUE
Stenostomum lucidum (Sw.) C.F.Gaertn.			A, B	GUE
Stenostomum resinosum (Vahl) Griseb.	5	A–C	B	GUE
Stilpnophyllum grandifolium L.Andersson	4	C	C	CIN
Strumpfia maritima Jacq.	1	A–C	A–C	CHI
Suberanthus neriifolius (A.Rich.) Borhidi & M.Fernández Zeq.	9	B	B	RON
Syringantha coulteri (Hook.f.) T.McDowell	1	A	A	HAM
Timonius celebicus Koord.	~170	E–G	F	GUE
Timonius timon (Spreng.) Merr.			F, G	GUE
Tinadendron noumeanum (Baill.) Achille	2	G	G	GUE
Uncaria guianensis (Aubl.) J.F.Gmel.	~39	A, C–G	C	NAU
Uncaria rhynchophylla (Miq.) Miq. ex Havil.			F	NAU
Uncaria tomentosa (Willd. ex Schult.) DC.			A, C	NAU
Acranthera siamensis (Kerr) Bremek.	~39	F	F	COP
Coptosapelta diffusa (Champ.) Steenis	~16	F–G	F	COP
Coptosapelta flavescens Korth.			F	COP
Luculia grandifolia Ghose	4	F	F	LUC
Luculia pinceana Hook.			F	LUC
Alberta magna E.Mey.	6	D	D	IXOR
Augusta rivalis (Benth.) J.H.Kirkbr.	4	A, C, F	A, C	IXOR
Calycophyllum candidissiumum (Vahl) DC.		A–C	A, C	IXOR
Canthium inerme (L.f.) Kuntze		D–G	D	IXOR
Coffea arabica L.	~102	D, E	D	IXOR

Table 1

(*Continued*)

Taxon	Species	Genus distribution	Species distribution	Classification
Condaminea corymbosa (Ruiz & Pav.) DC.	4	A, C	A, C	IXOR
Coptosperma neurophyllum (S.Moore) Degreef		D–G	D	IXOR
Cremaspora triflora (Thonn.) K.Schum.	2	D, E	D, E	IXOR
Crossopteryx febrifuga (Afzel. Ex G.Don) Benth.		D	D	IXOR
Emmenopterys henryi Oliv.	2	F	F	IXOR
Ferdinandusa speciosa Pohl	~24	A, C	C	IXOR
Heinsia crinita (Afzel.) G.Taylor	5	D	D	IXOR
Ixora coccinea L.	~540	A–G	F	IXOR
Ixora finlaysoniana Wall. ex G.Don			F	IXOR
Kraussia floribunda Harv.		D	D	IXOR
Mussaenda erythrophylla Schumach. & Thonn.		D–G	D	IXOR
Mussaenda scratchleyi Wernham			G	IXOR
Pavetta lanceolata Eckl.		D, F, G	D	IXOR
Pinckneya bracteata (Bartram) Raf.		B[a]	B	IXOR
Posoqueria latifolia (Rudge) Schult.		A–C	A, C	IXOR
Psilanthus manni Hook.f.		D, F, G	D	IXOR
Randia aculeata L.		A–C	A–C	IXOR
Retiniphyllum pilosum (Spruce ex Benth.) Müll.	~20	C	C	IXOR
Sabicea diversifolia Pers.	~120	A–E	E	IXOR
Sabicea villosa Willd. ex Schult.			A–C	IXOR
Scyphiphora hydrophyllacea C.F.Gaertn.	1	E–G	E–G	IXOR
Sipanea hispida Benth. ex Wernham	~19	C	C	IXOR
Steenisia pleurocarpa (Airy Shaw) Bakh.f.	5	F	F	IXOR
Tricalysia cryptocalyx Baker		D–E	E	IXOR
Vangueria madagascariensis J.F.Gmel.		D, E	D, E	IXOR
Virectaria major (K.Schum.) Verdc.		D	D	IXOR
Warszewiczia coccinea (Vahl) Klotzsch	8	A, C	A, C	IXOR
Amphidasya ambigua (Standl.) Standl.		A, C	A, C	RUBI
Argostemma hookeri King		D, F, G	F	RUBI
Bouvardia ternifolia (Cav.) Schltdl.		A	A	RUBI
Colletoecema dewevrei (De Wild) E.M.A.Petit		D	D	RUBI
Coussarea hydrangeifolia (Benth.) Benth. & Hook.f. ex Müll.Arg.		A–C	C	RUBI
Faramea multiflora A.Rich.		A–C	A, C	RUBI
Knoxia platycarpa Arn.		D, F, G	F	RUBI
Lasianthus pedunculatus E.A.Bruce		A–D, F, G	D	RUBI
Lasianthus strigosus Wight			F	RUBI
Lerchea bracteata Valeton		F	F	RUBI
Morinda citrifolia L.		A–G	F, G	RUBI
Ophiorrhiza mungos L.		F, G	F	RUBI
Palicourea crocea (Sw.) Schult.		A–C	A–C	RUBI
Pauridiantha paucinervis (Hiern) Bremek.		D, E	D, E	RUBI
Pravinaria leucocarpa Bremek.		F	F	RUBI
Psychotria kirkii Hiern		A–G	D	RUBI
Spermacoce hispida L.		A–G	F, G	RUBI
Trichostachys aurea Hiern in D.Oliver & auct. suc. (eds.)		D	D	RUBI
Urophyllum ellipticum (Wight) Twaites		F, G	F	RUBI
Exacum affine Balf.f.	~65	D–G	D	GENT
Alstonia scholaris (L.) R.Br.	~43	A, D, F, G	F, G	APOC
Gelsemium sempervirens (L.) J.St.-Hil.	3	A, F	A[b]	GELS

Note. If a genus is represented by more than one species, the total number of species within the genus and the genus distribution are supplied only at the first species listed. Distribution abbreviations are as follows: A = Central America, B = the Caribbean islands, C = South America, D = Africa, E = Madagascar, F = Asia, and G = the Pacific islands. Classification abbreviations (three letters refer to tribe, four letters to family or subfamily) are as follows: APOC = Apocynaceae, CIN = Cinchoneae, CHI = Chiococceae, COP = Coptosapelteae, GELS = Gelsemiaceae, GENT = Gentianaceae, GUE = Guettardeae, HAM = Hamelieae, HIL = Hillieae, HYM = Hymenodictyeae, ISE = Isertieae, IXOR = Ixoroideae, LUC = Luculieae, NAU = Naucleeae, RON = Rondeletieae, RUBI = Rubioideae. Distribution areas and number of species within each genus were retrieved from the World Checklist of Rubiaceae and studies by Mabberley (1997), Thulin (2001), Zhen and Jianhua (2007), and Rova et al. (2009).

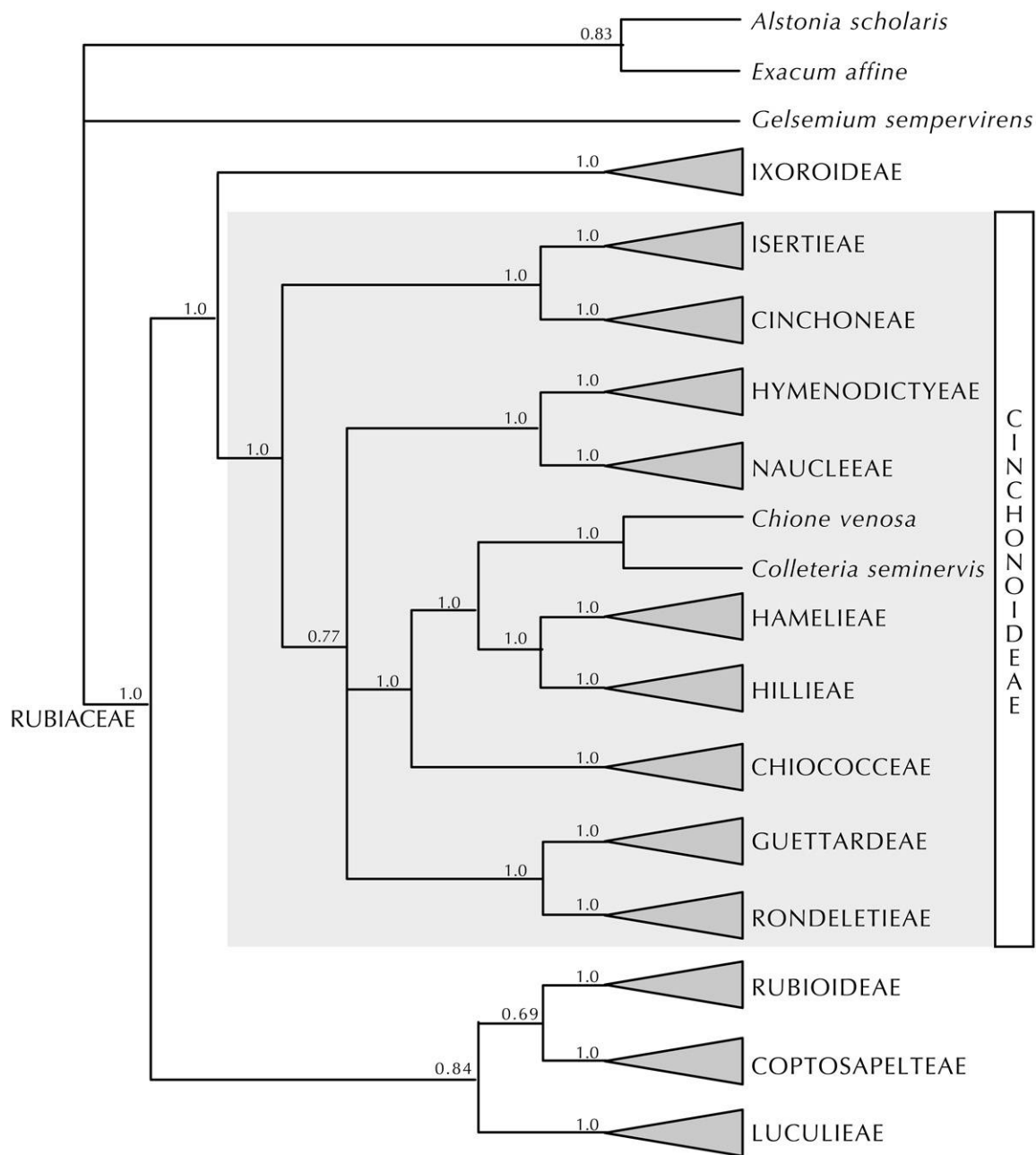[a] In the southeastern USA.

[b] In North America.

Fig. 1 Simplified 50% majority-rule consensus tree from the Bayesian phylogenetic analysis of Rubiaceae, with special emphasis on the subfamily Cinchonoideae. Clade credibilities are indicated above the branches. The complete tree is supplied in appendix B, available in the online edition of the *International Journal of Plant Sciences*.

choneae and Isertieae in a sister-group relationship with all other Cinchonoideae (fig. 1). This result is congruent with the results of Manns and Bremer (2010) but is in conflict with those of Antonelli et al. (2009) and Rydin et al. (2009).

Evaluation of Divergence-Time Estimates

Selection of taxa is crucial in divergence-time analyses, and it is important to use acquired knowledge in the sampling process. If only more recently derived taxa within a large clade are sampled, we will get an unrealistically young age estimate for the diversification of the clade. Recent phylogenetic analyses in Rubiaceae have, however, provided a good knowledge of the relationships within the family, and therefore we can be rather confident in our sampling. In this article, estimated divergence times refer to when the different clades diversified (i.e., crown node ages).

Two recent articles have dealt with dating of Rubiaceae: that of Antonelli et al. (2009) and that of Bremer and Eriksson (2009). Our mean divergence-time estimates are, generally,

older than those of Antonelli et al. (2009), with the exception of nodes placed close to their single-fossil calibration point (i.e., Cinchonoideae tribes Hymenodictyeae, Naucleeae, Isertieae, and Cinchoneae). The sampling of Antonelli et al. (2009), however, was more limited than ours: they constrained the tree height to a maximum of 78 Myr (compared with 88 Myr in our analysis), which may partly explain our comparatively older estimates for nodes that are distant to their single calibration point.

As compared with Bremer and Eriksson (2009), our study gave younger mean divergence-time estimates for Rubiaceae, Rubioideae, and Coptosapelteae, while the mean divergence-time estimates for the split between Cinchonoideae and Ixoroideae and diversification of nodes within these subfamilies were generally older in our study. The largest deviation was found in the estimate for Cinchonoideae, for which our analysis provided a mean divergence time of 57.4 Myr compared with 38.7 Myr in their study. The differences in our studies probably resulted from the use of different fossils and tree height priors. Bremer and Eriksson (2009) did not constrain tree height, and in addition to two fossils that we also examined (i.e., *Faramea* and *Scyphiphora*), they used a younger *Cephalanthus* fossil estimated to 14 Mya and did not use the *Emmenopterys* fossil, which was the oldest fossil included in our study.

Biogeography

Neotropical Rubiaceae. On the basis of its present distribution, Raven and Axelrod (1974) listed Rubiaceae among the taxa that were believed to have dispersed from Africa to the Neotropics during the Late Cretaceous, after the break up of Gondwana. Island chains stretching over a (at the time) narrow Atlantic Ocean were suggested to have facilitated this dispersal. Gentry (1982) shared this view of Neotropical Rubiaceae as a Gondwana-derived lineage and suggested that Rubiaceae belong to a group of taxa (i.e., Andean-centered taxa) that often exhibit two centers of diversification, one in the Andes and one in Central America. An alternative to a Gondwanan origin was presented by Antonelli et al. (2009), who suggested that Rubiaceae originated in the Laurasian boreotropical forests and used the NALB to reach the Neotropics in the Late Paleocene or the Early Eocene.

The Late Cretaceous origin of Rubiaceae (fig. 2*A*) contradicts a presence on the Gondwanan continent, and the early split between an Asian and a South American lineage that is indicated in our analysis (fig. 2*B*) does not support a dispersal from Africa, as suggested by Raven and Axelrod (1974). Instead, ancestral Rubiaceae are reconstructed here to have been distributed in Asia and South America followed by a vicariance event leading to two geographically separated lineages. The Asian lineage includes Luculieae, Coptosapelteae, and Rubioideae, and the South American lineage includes Cinchonoideae and Ixoroideae.

The suggested presence of Rubiaceae in South America predates the existence of the NALB, and it is unlikely that ancestral Rubiaceae used this route to reach the Neotropics. However, early ancestors of Rubiaceae might have been represented in the Eurasian boreotropical forests, which were already present across the middle latitudes north of the Tethys during Cenomanian-Turonian times (Morley 2003), and

long-distance dispersal across a narrow Atlantic Ocean could explain its ancestral distribution in Asia and South America. This route was recently suggested for Cucurbitaceae (Schaefer et al. 2009).

Although sampling within Ixoroideae is limited, certain biogeographical patterns are paralleled in this subfamily and in Cinchonoideae. First, a restricted South American ancestral distribution is suggested for the first diverging lineage in both subfamilies; second, although reconstructions are ambiguous, the possibility of dispersal to the Paleotropics (Africa) during the Paleocene is indicated for both subfamilies, although not until the Eocene was a Paleotropical distribution more likely (figs. 2*B*, 3*B*).

The South American ancestral lineage of Ixoroideae has a present-day distribution that is primarily in South America, Central America, and the Caribbean islands. A few genera are, however, distributed in the Paleotropics, among them *Emmenopterys*. Fruit fossils of *Emmenopterys* constitute the oldest confidently identified remains of Rubiaceae (Graham 2009) and were found in layers from the Eocene in western North America (Manchester 1994; Wehr and Manchester 1996), supporting a prior presence (of this genus at least) on the North American continent. The seeds of *Emmenopterys* are winged.

At least one additional dispersal event from the Neotropics to the Paleotropics has occurred within the South American Ixoroideae lineage: the three Paleotropical taxa *Dolicholobium*, *Mastixiodendron*, and *Mussaendopsis* that form a clade within Condamineeae (Kainulainen et al. 2010). Our analysis did not include any of these genera, but they most likely represent a separate dispersal event since they are only distantly related to *Emmenopterys* (Kainulainen et al. 2010). None of these have winged seeds like *Emmenopterys*, but the fruits of *Mastixiodendron* are fleshy drupes (Kainulainen et al. 2010).

The present-day land connection between the South American and North American continents, the Isthmus of Panama, was established during the Pliocene (~3.5 Mya; Saito 1976). However, the possibility of earlier land connections between South America and North America (during the Late Cretaceous–Tertiary) and tectonic changes in the Caribbean region have been widely debated during recent decades (Pindell 1988; Briggs 1994; Iturralde-Vinent and MacPhee 1999). Pindell et al. (1988) suggested that a volcanic arc was formed between South America and North America during the Late Cretaceous. This arc then moved (north-)eastward in relation to South America and North America until the Eocene–Oligocene, when it is thought to have formed a landmass termed Gaarlandia (33–35 Mya; Iturralde-Vinent and McPhee 1999). This landmass was later subdivided into the present-day Greater Antilles (Iturralde-Vinent and MacPhee 1999). It is not known to what extent this arc constituted exposed land area(s), but exchange of biota between South America and North America has occurred since the Campanian (Morley 2003). Extant terrestrial (animal) lineages of the Caribbean are, however, not older than ~40 Myr (Briggs 1994; Iturralde-Vinent and MacPhee 1999), probably because of transgression, subsidence, and the K/T (Cretaceous-Tertiary) bolide impact (Iturralde-Vinent and MacPhee 1999).

Following the split from Ixoroideae, the ancestral Cinchonoideae is suggested to have dispersed northward from South
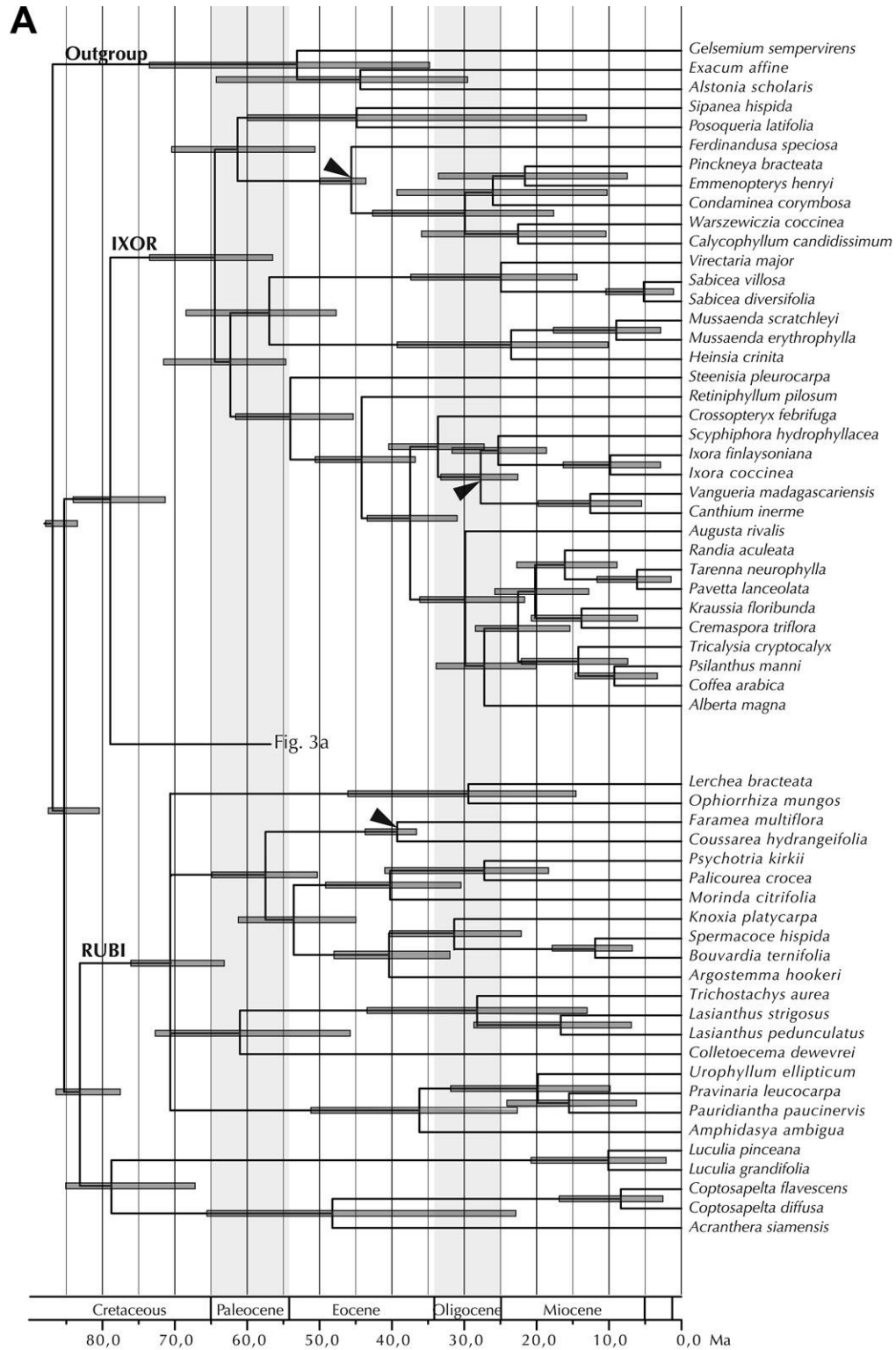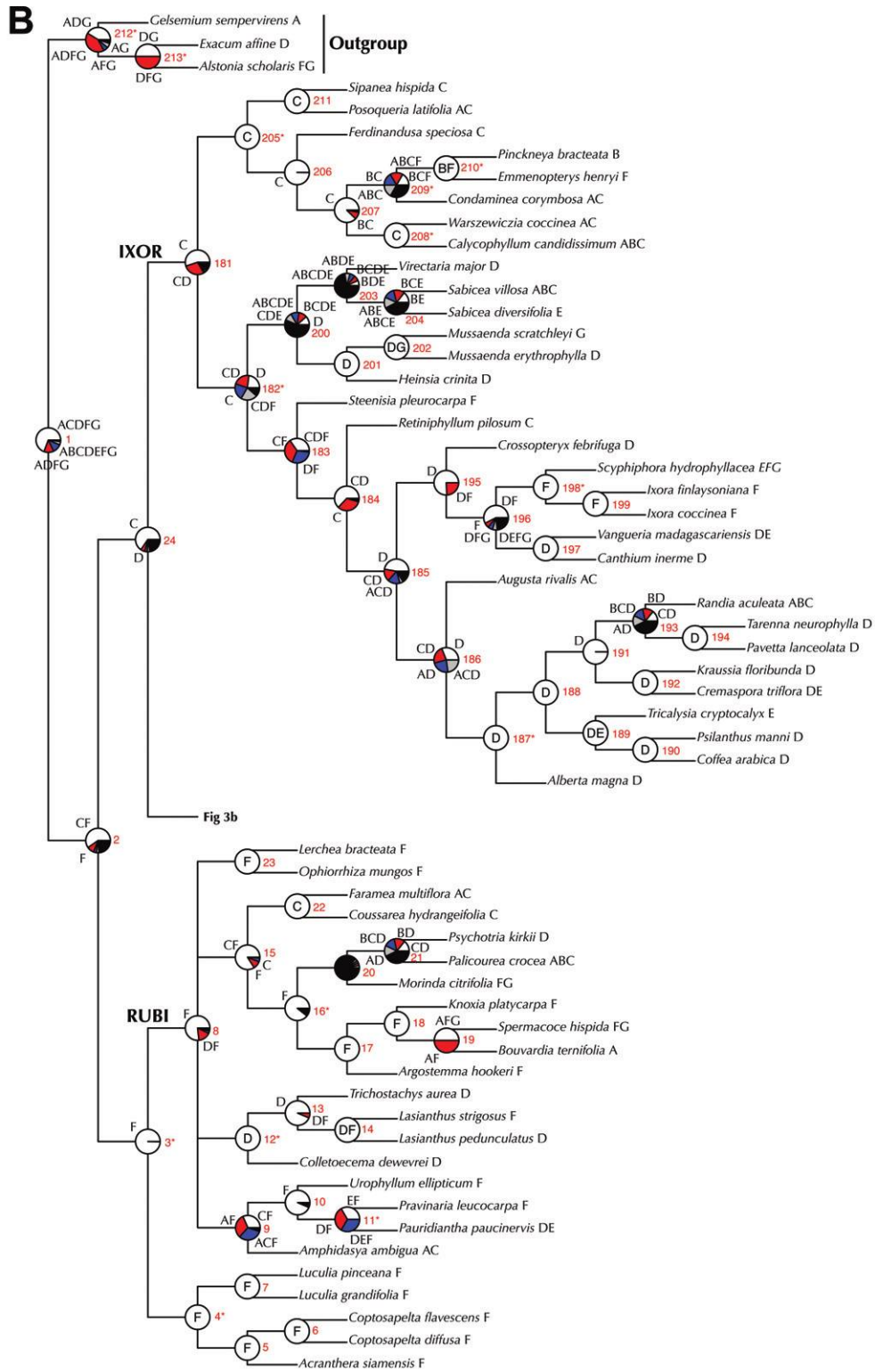
A

Outgroup

IXOR

Fig. 3a

RUBI

Gelsemium sempervirens
Exacum affine
Alstonia scholaris
Sipanea hispida
Posoqueria latifolia
Ferdinandusa speciosa
Pinckneya bracteata
Emmenopterys henryi
Condaminea corymbosa
Warszewiczia coccinea
Calycophyllum candidissimum
Virectaria major
Sabicea villosa
Sabicea diversifolia
Mussaenda scratchleyi
Mussaenda erythrophylla
Heinsia crinita
Steenisia pleurocarpa
Retiniphyllum pilosum
Crossopteryx febrifuga
Scyphiphora hydrophyllacea
Ixora finlaysoniana
Ixora coccinea
Vangueria madagascariensis
Canthium inerme
Augusta rivalis
Randia aculeata
Tarenna neurophylla
Pavetta lanceolata
Kraussia floribunda
Cremaspora triflora
Tricalysia cryptocalyx
Psilanthus manni
Coffea arabica
Alberta magna

Lerchea bracteata
Ophiorrhiza mungos
Faramea multiflora
Coussarea hydrangeifolia
Psychotria kirkii
Palicourea crocea
Morinda citrifolia
Knoxia platycarpa
Spermacoce hispida
Bouvardia ternifolia
Argostemma hookeri
Trichostachys aurea
Lasianthus strigosus
Lasianthus pedunculatus
Colletoecema dewevrei
Urophyllum ellipticum
Pravinaria leucocarpa
Pauridiantha paucinervis
Amphidasya ambigua
Luculia pinceana
Luculia grandifolia
Coptosapelta flavescens
Coptosapelta diffusa
Acranthera siamensis

Cretaceous	Paleocene	Eocene	Oligocene	Miocene

80,0   70,0   60,0   50,0   40,0   30,0   20,0   10,0   0,0 Ma

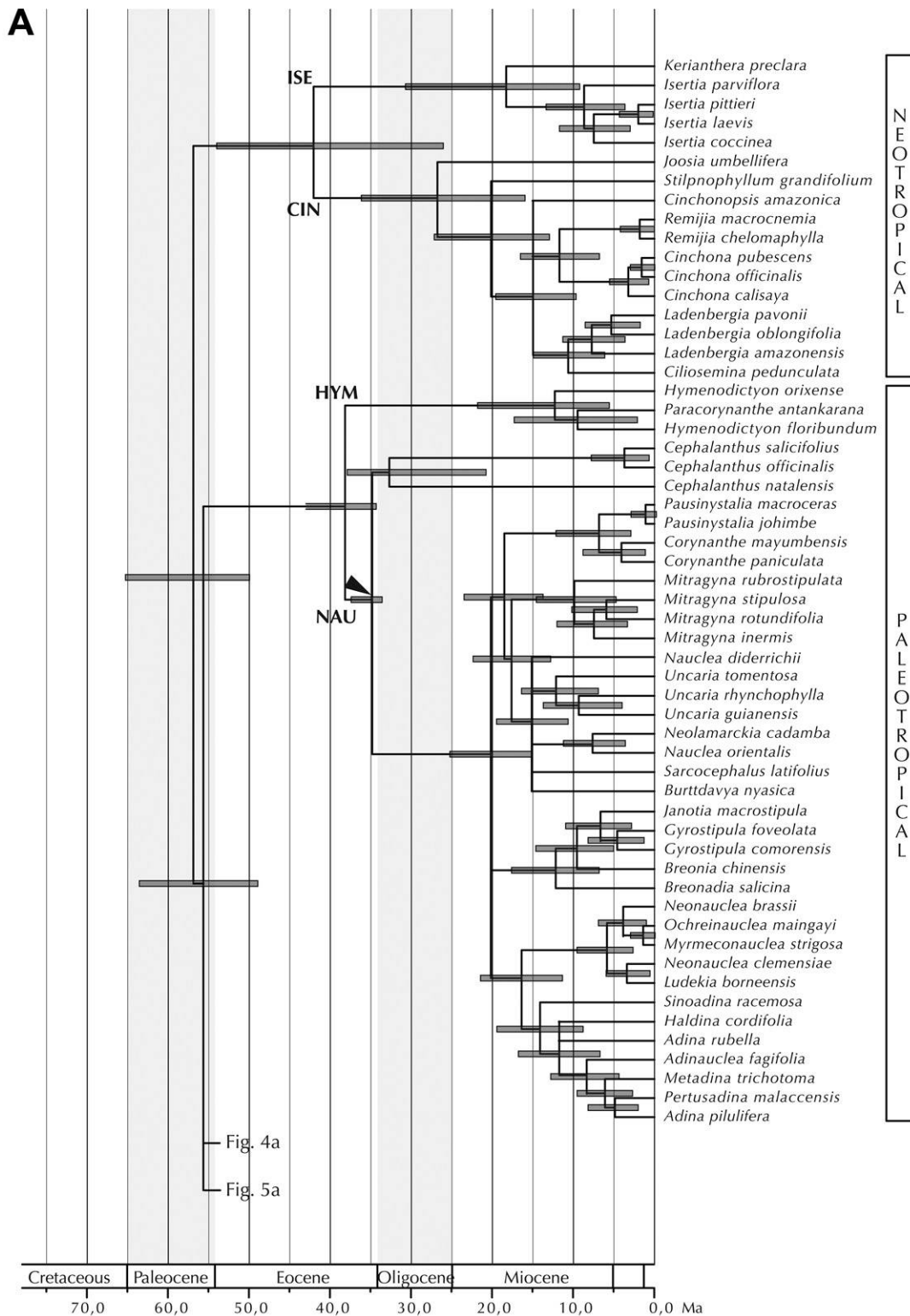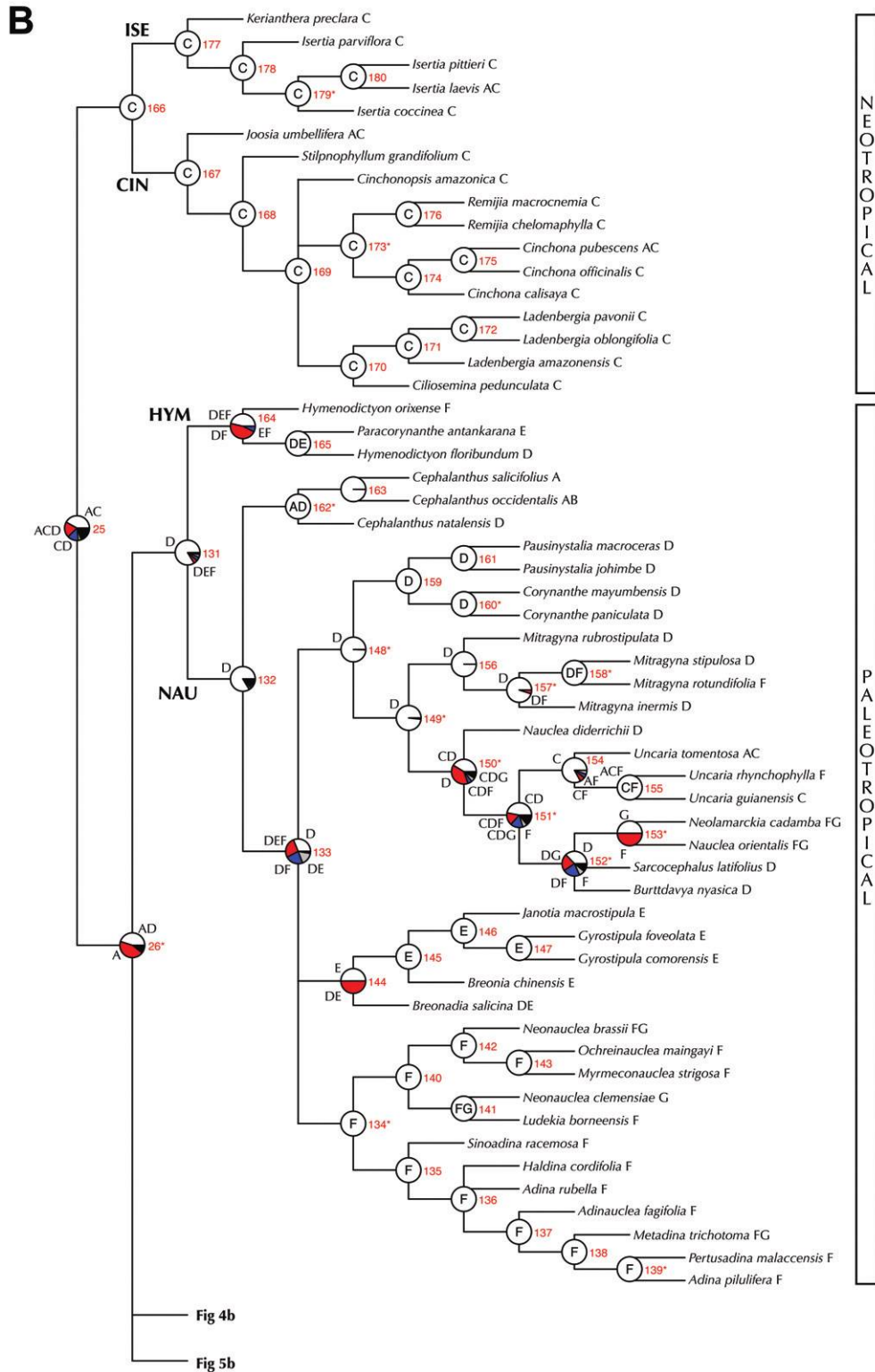**Fig. 2** *A*, Chronogram from the BEAST analysis showing estimated median divergence times and 95% confidence intervals for the root portion of the tree, with outgroup and early splits within Rubiaceae, Rubioideae (RUBI), and Ixoroideae (IXOR). Arrowheads indicate position of a fossil prior: *Faramea* (the *Faramea-Coussarea* clade), 37 Myr; *Emmenopterys* (the *Ferdinandusa-Calycophyllum* clade), 44 Myr; *Scyphiphora* (the *Scyphiphora-Canthium* clade) 27 Myr. *B*, Summary of the dispersal-vicariance analyses presented in a 50% majority-rule consensus tree based on 15,000 topologies generated in the BEAST analysis. The tree shows outgroup and early splits within Rubiaceae, RUBI, and IXOR. Pie charts at individual nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal-vicariance analysis (DIVA) while integrating over tree topologies, using the Markov chain Monte Carlo method. The marginal probabilities are a product of the phylogenetic

uncertainty in the rest of the tree and the biogeographic uncertainty at each node (conditional on the node occurring). In the pie charts, the first four areas with the highest probability are colored according to relative probability in the following order: white > red > blue > gray. Any remaining areas are collectively shown in black. Node numbers are given for each node, and the details of the reconstructed distributions are supplied in appendix C, available in the online edition of the *International Journal of Plant Sciences*. An asterisk in association with the node number indicates that the node is not well supported in the phylogenetic analyses (<95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names, where A = Central America, B = the Caribbean islands, C = South America, D = Africa, E = Madagascar, F = Asia, and G = the Pacific islands.
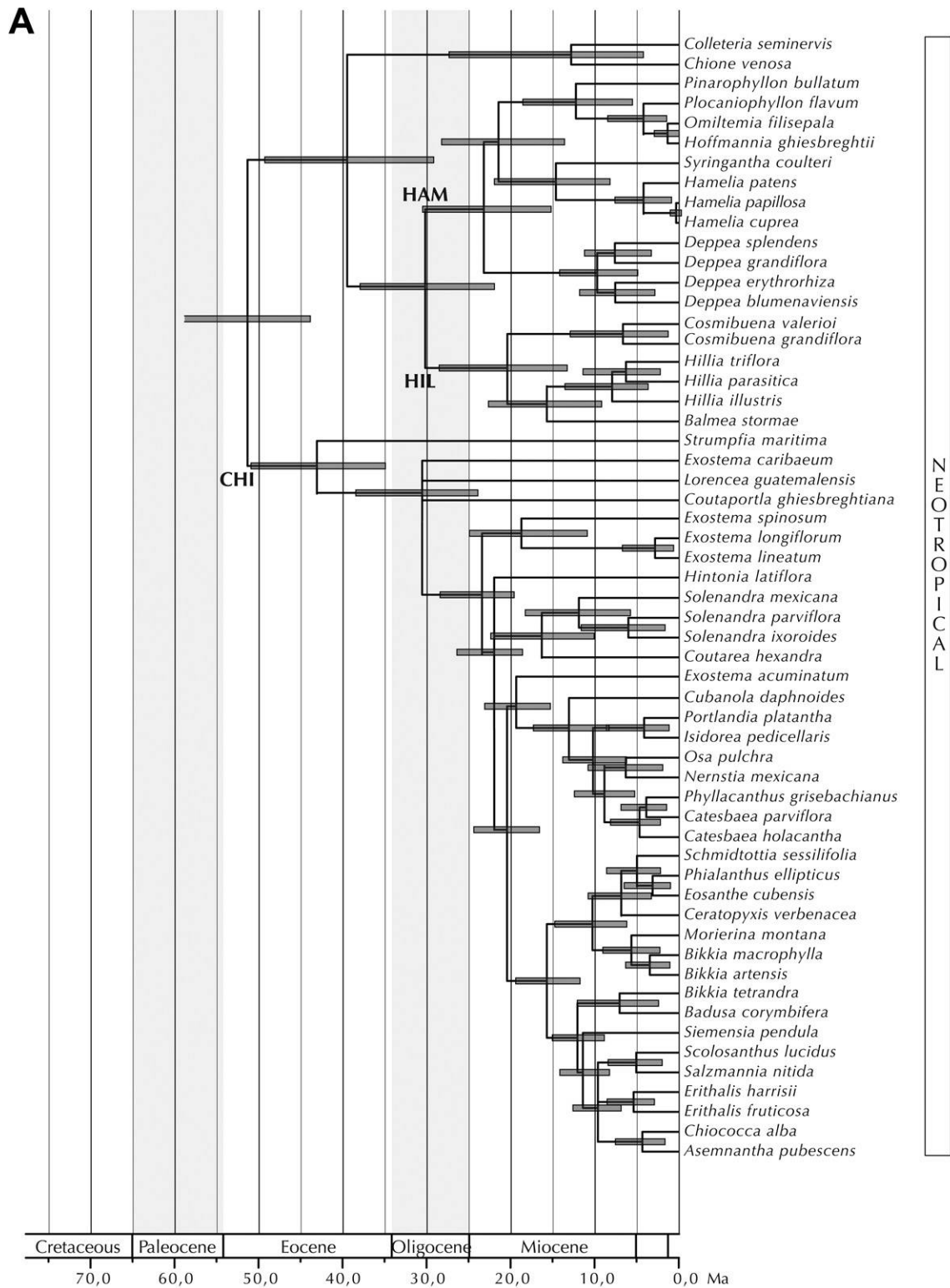
Fig. 3 *A,* Chronogram from the BEAST analysis showing estimated median divergence times and 95% confidence intervals for Cinchoneae (CIN), Isertieae (ISE), Naucleeae (NAU), and Hymendictyeae (HYM). Arrowhead indicates the position of the *Cephalanthus* (Naucleeae clade) fossil prior, 34 Myr. *B,* Summary of the dispersal-vicariance analyses presented in a 50% majority-rule consensus tree based on 15,000 topologies generated in the BEAST analysis, showing CIN, ISE, NAU, and HYM. Pie charts at individual nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal-vicariance analysis (DIVA) while integrating over tree topologies, using the Markov chain Monte Carlo method. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty at

each node (conditional on the node occurring). In the pie charts, the first four areas with the highest probability are colored according to relative probability in the following order: white > red > blue > gray. Any remaining areas are collectively shown in black. Node numbers are given for each node, and the details of the reconstructed distributions are supplied in appendix C, available in the online edition of the *International Journal of Plant Sciences*. An asterisk in association with the node number indicates that the node is not well supported in the phylogenetic analyses (<95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names, where A = Central America, B = the Caribbean islands, C = South America, D = Africa, E = Madagascar, F = Asia, and G = the Pacific islands.
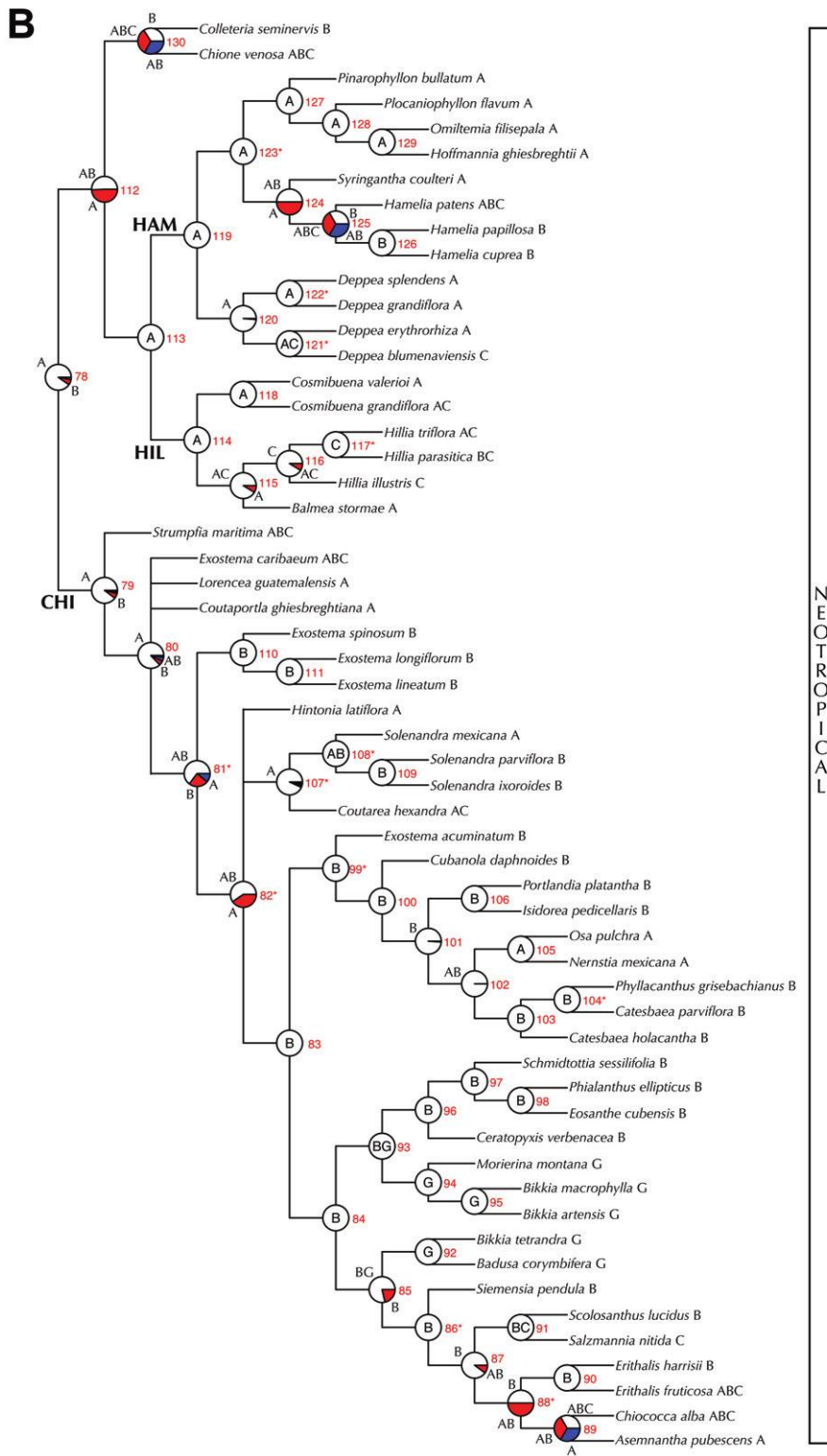
Fig. 4 *A*, Chronogram from the BEAST analysis showing estimated median divergence times and 95% confidence intervals for Chiococceae (CHI), Hamelieae (HAM), Hillieae (HIL), and *Chione + Colleteria*. *B*, Summary of the dispersal-vicariance analyses presented in a 50% majority-rule consensus tree based on 15,000 topologies generated in the BEAST analysis, showing CHI, HAM, HIL, and *Chione + Colleteria*. Pie charts at individual nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal-vicariance analysis (DIVA) while integrating over tree topologies, using the Markov chain Monte Carlo method. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty at each node (conditional on the node occurring). In the pie charts, the first four areas with

the highest probability are colored according to relative probability in the following order: white > red > blue > gray. Any remaining areas are collectively shown in black. Node numbers are given for each node, and details of the reconstructed distributions are supplied in appendix C, available in the online edition of the *International Journal of Plant Sciences*. An asterisk in association with the node number indicates that the node is not well supported in the phylogenetic analyses (<95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names, where A = Central America, B = the Caribbean islands, C = South America, D = Africa, E = Madagascar, F = Asia, and G = the Pacific islands.

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Table 2

Divergence-Time Estimates (Myr) with 95% Highest Probability Density (HPD) Intervals for Larger Clades within Rubiaceae

Clade/taxon	Mean age (95% HPD interval) in this study	Mean age (95% HPD interval)[a]	Median age (95% credibility interval)[b]
Rubiaceae	84.9 (80.8–87.9)	86.6 (72.9–100.8)	66.1 (63.0–68.8)
Rubioideae	70.4 (63.6–76.5)	77.9 (65.3–90.7)	47.9 (43.4–52.5)
Luculieae	10.8 (2.5–21.2)	. . .	. . .
Coptosapelteae	47.3 (23.3–65.9)	51.2 (26.2–76.0)	. . .
Ixoroideae/Cinchonoideae	78.5 (71.7–84.5)	73.1 (58.4–88.7)	62.7 (59.7–66.2)
Ixoroideae	64.7 (56.9–73.9)	59.6 (45.7–73.7)	48.1 (43.8–52.4)
Cinchonoideae	57.4 (50.3–65.6)	38.7 (28.1–52.5)	51.3 (47.8–54.6)
Cinchoneae/Isertieae	41.5 (26.5–54.4)	. . .	45.2 (39.7–50.0)
Cinchoneae	27.2 (16.4–36.6)	15.6 (5.3–28.6)	28.6 (22.9–35.1)
Isertieae	19.4 (9.6–31.1)	. . .	25.0 (19.9–31.4)
Naucleeae/Hymenodictyeae	38.6 (34.7–43.2)	19.7 (14.9–25.3)	36.3 (34.8–38.1)
Naucleeae	35.2 (34.0–37.8)[c]	16.0 (14.0–19.6)	33.9[d]
Hymenodictyeae	12.9 (6.0–22.2)	3.6 (.1–9.0)	. . .
Chiococceae /Hamelieae/Hillieae/ *Chione + Colleteria*	51.4 (44.2–59.1)	. . .	47.3 (42.5–52.1)
Chiococceae	43.1 (35.2–51.2)	27.6 (15.4–40.5)	19.2 (16.1–23.2)
Hamelieae	23.2 (15.1–30.8)	13.5 (7.4–20.8)	. . .
Hillieae	20.9 (13.6–28.8)	11.7 (5.1–18.8)	. . .
Chione + Colleteria	14.2 (4.5–27.7)	. . .	. . .
Rondeletieae/Guettardeae	43.2 (34.8–51.1)	27.5 (18.3–37.3)	35.3 (29.9–40.8)
Rondeletieae	35.8 (28.9–42.1)	22.4 (12.1–32.0)	. . .
Guettardeae	35.9 (26.1–44.1)	23.0 (14.5–31.2)	. . .

Note.   Divergence-time estimates retrieved from the BEAST analysis in this study and from previous studies of Rubiaceae (Antonelli et al. 2009; Bremer and Eriksson 2009). An ellipse indicates that information is not available.

[a] From Bremer and Eriksson (2009).
[b] From Antonelli et al. (2009).
[c] Fossil prior set to 34 Myr.
[d] Fossil constraint.

America during the Late Cretaceous or Paleocene, extending distribution to southern North America and northern Central America and possibly also Africa (fig. 3). The dispersal northward coincided with the possible existence of a volcanic arc between South America and North America (Pindell et al. 1988; Iturralde-Vinent and McPhee 1999); however, whether this arc allowed overland migration is unclear, and long-distance dispersal is not excluded. A dispersal route from South America to North America during the Paleocene has been suggested for Malpighiaceae (Davis et al. 2002, 2004).

Cinchoneae and Isertieae diversified within South America (fig. 3B). The biogeographical history of this lineage and its correlation with the Andean orogeny were recently presented by Antonelli et al. (2009); our results are mainly congruent with their findings.

Remaining Neotropical Cinchonoideae (Chiococceae, Guettardeae, Hamelieae, Hillieae, Rondeletieae, *Chione + Colleteria*) are found in two lineages that are indicated to have had a Central American ancestral distribution (figs. 4B, 5B). A sister-group relationship between these two lineages was strongly supported by Manns and Bremer (2010), although it was not in this study. From their Central American ranges, further dispersals within the Neotropics have been frequent (figs. 4B, 5B), and together with the South American lineage these Central American lineages support the view

of two Neotropical centers of diversification suggested by Gentry (1982).

Starting in the Late Eocene or possibly in the Early Eocene (*Chione* and *Colleteria*), several dispersals from Central America to the Caribbean islands—and also in reverse—are indicated for Hamelieae, Hillieae, Chiococceae, Guettardeae, and Rondeletieae (figs. 4, 5). These dispersals coincide with or postdate the hypothesized existence of Gaarlandia (33–35 Mya) and support a continuous presence of exposed land areas in the Caribbean region since the Eocene, although the majority of the dispersals are estimated to the Miocene. It is also clear that Central American and Caribbean Cinchonoideae diverged in these two areas well before the Late Cenozoic. Late Cenozoic or more recent divergence of Neotropical Rubiaceae has been suggested by Raven and Axelrod (1974).

The sister taxa *Chione* and *Colleteria* (fig. 4B) share an extant distribution in the Caribbean islands. *Chione* is present in Central America as well as in South America. They both have fleshy fruits; the fruits of *Chione* are eaten by birds, and its dry fruits float on (tap-)water for at least 9 d (Taylor 2003). Together with Hamelieae, Hillieae, and Chiococceae, they are placed within one of the Central American lineages. From our reconstruction it is not clear when the dispersal from Central America to the Caribbean islands occurred; it might have been before the split of *Chione + Colleteria* from

Hamelieae and Hillieae, although later dispersal seems more likely considering the marine transgression that is suggested to have occurred in the Caribbean in the Early to Middle Eocene (Iturralde-Vinent and McPhee 1999).

Hamelieae has to a large extent remained in Central America, although some dispersals within the Neotropics can be inferred. Our results indicate that dispersal to the Caribbean islands occurred during the Miocene, before the split between *Syringantha* and *Hamelia* (fig. 4). These two genera are present in Central America, and *Hamelia* is also found in the Caribbean islands and South America. However, little is known of the phylogenetic relationships within *Hamelia*, and the estimated time of dispersal may be the result of sampling only Caribbean taxa and the widespread *Hamelia patens*. Denser sampling would probably give a better estimate of the time of dispersal, not only to the Caribbean islands but also to South America.

Hoffmannia is primarily distributed at higher elevations in Central America, but two species (i.e., *Hoffmannia pedunculata* Sw. and *Hoffmannia tubiflora* Griseb.) are present in the Caribbean islands. As in *Hamelia*, relationships within *Hoffmannia* are poorly known, and because our study included only one Central American representative, any dispersal to the Caribbean islands is not reconstructed here. However, since *Hamelia* and *Hoffmannia* are placed in two different clades and as sisters to Central American taxa (fig. 4B), the dispersals from Central America to the Caribbean islands most likely represent two separate events. Both *Hamelia* and *Hoffmannia* have fleshy fruits that are eaten by birds (Bremer 1987; Levey 1987), while other members of Hamelieae with a more restricted distribution have dry fruits; this may explain the presence of *Hamelia* and *Hoffmannia* in the Caribbean islands.

Our reconstruction also suggests a dispersal event from Central America to South America in the ancestor of *Deppea blumenaviensis* and *Deppea erythrorhiza* (fig. 4B). This dispersal refers to the South American distribution of *D. blumenaviensis* in an otherwise restricted Central American genus.

Extant distribution of Hillieae is essentially in Central America and South America, and our results indicate that the tribe originated in Central America, where it dispersed to South America during the Miocene (fig. 4). Two *Hillia* species (*Hillia parasitica* and *Hillia tetrandra* Sw.) have spread to the Caribbean islands. *Hillia tetrandra* has not been included in molecular studies; however, morphological studies of *Hillia* (Taylor 1994) did not support *H. tetrandra* and *H. parasitica* as being closely related, and their presences in the Caribbean islands are probably not correlated. Seeds of *Hillia* have a tuft of trichome-like processes or filaments and are wind dispersed (Taylor 1994). A second dispersal from Central America to South America is seen in *Cosmibuena* (*Cosmibuena grandiflora*; fig. 4B), a genus of four species; of these, two are present in Central America and South America, while the remaining two are restricted to Central America. Seeds of *Cosmibuena* are also winged, with irregular margins (Taylor 1994).

Chiococceae s.l. is predominantly Caribbean in its distribution (~70% of all species). Central America and South America have ~30 species (~15%), and almost as many are distributed in the Pacific (Motley et al. 2005). Still, Central

America is reconstructed as the most likely ancestral distribution area of Chiococceae (fig. 4B).

The monotypic *Strumpfia maritima*, sister to all other Chiococceae, has fleshy, indehiscent fruits and is estimated to have diverged from the remaining Chiococceae before the time of Gaarlandia. *Strumpfia* is widely distributed on limestone substrates near seacoasts in the Caribbean basin, including on many of the Caribbean islands, the Caribbean coasts of southern Mexico and northern Central America, southern Florida, and northern Venezuela.

The remaining Chiococceae are suggested to have spread from Central America to the Caribbean in the Oligocene–Early Miocene, and by the Early Miocene two lineages (i.e., *Exostema spinosum–Exostema lineatum* and *Erithalis acuminatum–Erithalis fruticosa*; fig. 4) had a restricted Caribbean distribution. During the Late Oligocene, general subsidence and higher sea levels resulted in diminished exposed land area in the Caribbean region (Iturralde-Vinent and McPhee 1999). If Chiococceae was present in the Caribbean region during this time, extinctions and rapid diversification due to changing environments could explain the difficulties in resolving relationships in this part of the phylogeny. A Miocene or even earlier presence of *Exostema* in the Caribbean region has been suggested by McDowell et al. (2003). The seeds of *Exostema* are generally small, winged, and wind dispersed; however, McDowell et al. (2003) stated that ecological constraints rather than seed dispersal limited the distribution of *Exostema*.

A later Late Miocene dispersal from Central America to the Caribbean is reconstructed for the ancestor of *Solenandra*, a Neotropical genus with small, wind-dispersed seeds found in Cuba, Hispaniola, and Mexico. *Solenandra* shares a Central American ancestor with *Coutarea*, a primarily South American genus with one widespread species, *Coutarea hexandra*, found throughout Central and South America. *Coutarea* was placed as sister to South American *Exostema corymbosum* (Ruiz & Pav.) Spreng. and *Exostema maynense* Poepp. (not included in this study) by McDowell et al. (2003), indicating a dispersal in this lineage from Central America to South America.

Our analysis suggests that the Central American distribution of the ancestor of *Osa pulchra* and *Nernstia mexicana* stems from a more recent (Late Miocene) back dispersal from the Caribbean to Central America, followed by a vicariance event. This dispersal is to be paralleled by a dispersal leading to a Central American distribution of the shared ancestor of *Asemnantha* and *Chiococca*.

Rondeletieae has its major distribution in the Caribbean islands, with most species restricted to single islands and more limited numbers of taxa in Central America and South America. Members of Rondeletieae have capsular fruits and generally unwinged seeds. *Rondeletia* is by far the largest genus and comprises ~120 species, of which some are Central American and a few others are South American but to the majority are Caribbean. Of the non-Caribbean taxa, two Central American species (i.e., *Rondeletia deamii* and *Rondeletia hameliifolia*) were included, while no South American taxa were represented in our study.

Following dispersals from Central America to the Caribbean estimated to the Late Eocene–Early Oligocene, two par-
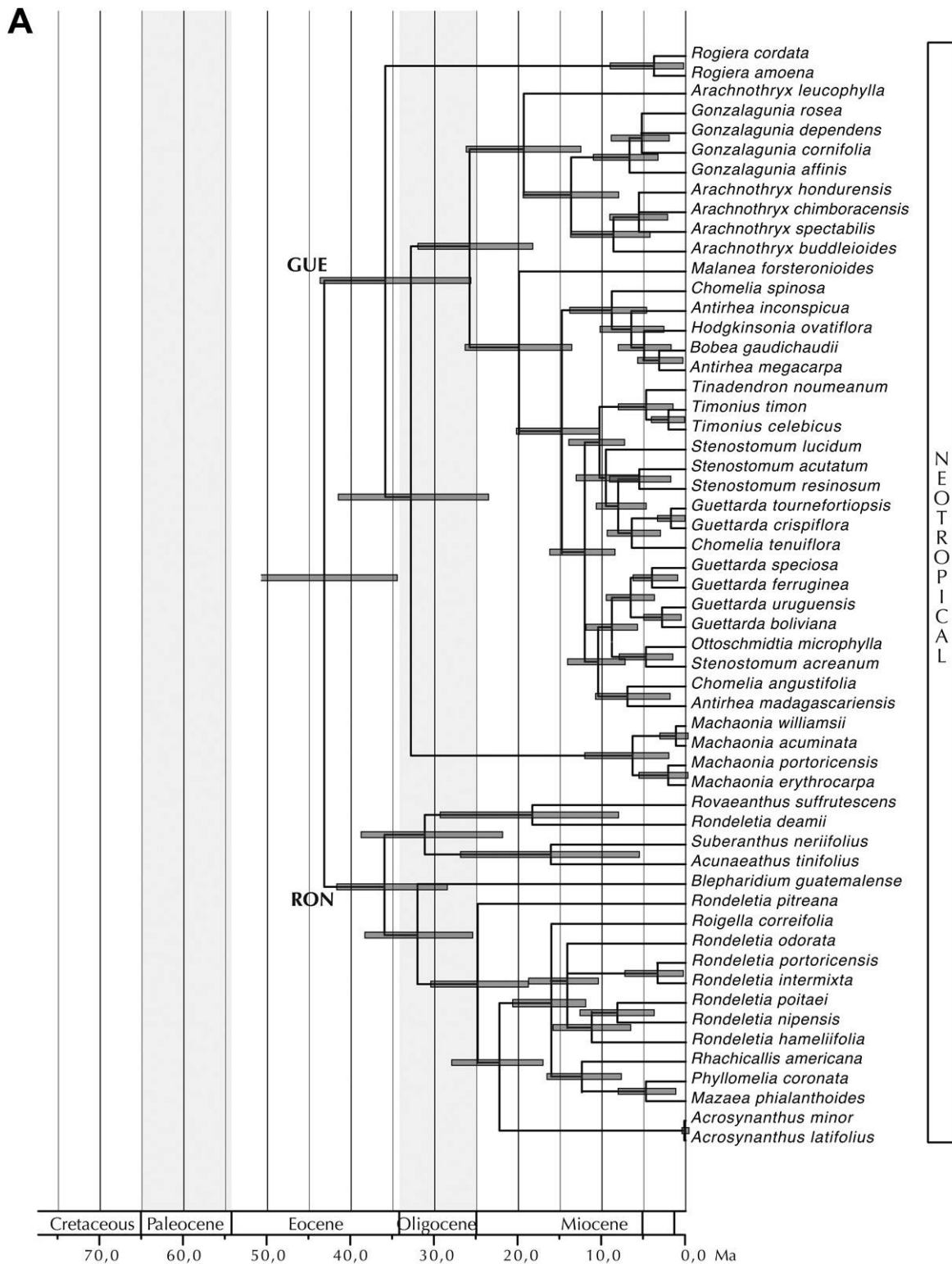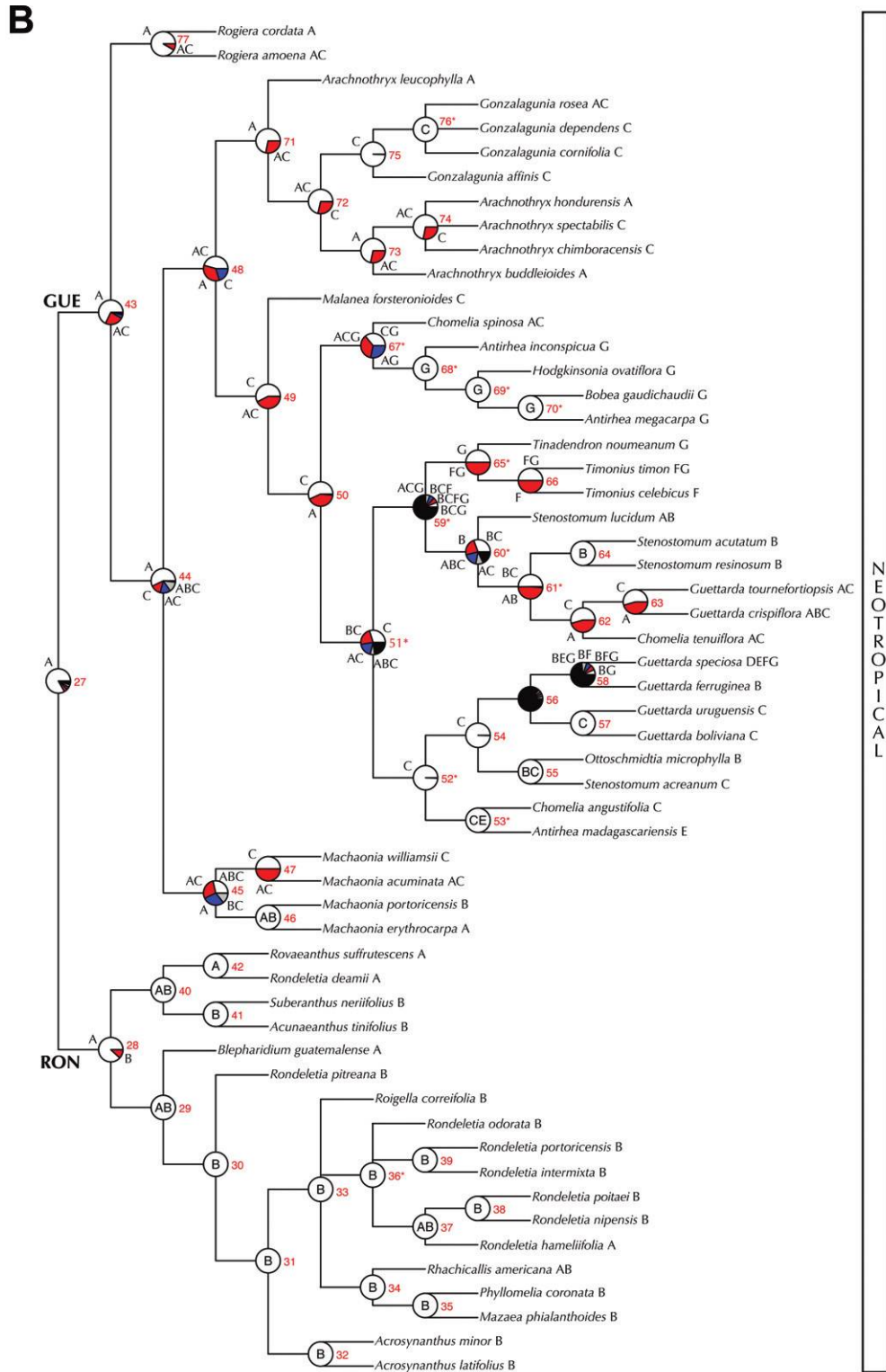
Fig. 5 *A*, Chronogram from the BEAST analysis showing estimated median divergence times and 95% confidence intervals for Rondeletieae (RON) and Guettardeae (GUE). *B*, Summary of the dispersal-vicariance analyses presented in a 50% majority-rule consensus tree based on 15,000 topologies generated in the BEAST analysis, showing RON and GUE. Pie charts at individual nodes represent marginal probabilities for each alternative ancestral area derived by using dispersal-vicariance analysis (DIVA) while integrating over tree topologies, using the Markov chain Monte Carlo method. These probabilities are a product of the phylogenetic uncertainty in the rest of the tree and the biogeographic uncertainty at each node (conditional on the node occurring). In the pie charts, the first four areas with the highest probability are colored according to relative

B

probability in the following order: white > red > blue > gray. Any remaining areas are collectively shown in black. Node numbers are given for each node, and details of the reconstructed distributions are supplied in appendix C, available in the online edition of the *International Journal of Plant Sciences*. An asterisk in association with the node number indicates that the node is not well supported in the phylogenetic analyses (<95% Bayesian posterior probability). Distributions for the terminal taxa are given next to the taxon names, where A = Central America, B = the Caribbean islands, C = South America, D = Africa, E = Madagascar, F = Asia, and G = the Pacific islands.

allel vicariance events are reconstructed in Rondeletieae (fig. 5). *Rondeletia deamii*, which is placed outside core *Rondeletia*, and *Rovaeanthus* both diversified in Central America, while the sister group of *Roveanthus*, *Suberanthus*, and *Acunaeanthus* diversified in the Caribbean islands. The remaining species of *Rondeletia* are found in a large Caribbean clade together with *Acrosynanthus*, *Mazaea*, *Phyllomelia*, *Rachicallis*, and *Roigella*, forming a sister group to the Central American *Blepharidium*.

Within the large Caribbean clade, at least two back dispersals to Central America are suggested to have occurred (fig. 5B). The first back dispersal, estimated in the Miocene, is reconstructed for the ancestor of the Central American *Rondeletia hameliifolia* and the Caribbean *Rondeletia nipensis* and *Rondeletia poitaei*. Rova et al. (2009) found *R. hameliifolia* to be closely related to the South American *Rondeletia purdiei* Hook.f., which grows in low-altitude (<1000 m) areas of northern Colombia and Venezuela (Andersson 1992), indicating further dispersal to South America or possibly the other way around. The second back dispersal is that of *Rachicallis*, which has an extant distribution in the Caribbean basin, widely in the islands and along the coast of northern Central America and southern Mexico.

Our analysis indicates that Guettardeae most likely had a Central American ancestral distribution (fig. 5B). *Rogiera*, sister to all other Guettardeae, is almost entirely Central American, with *Rogiera amoena* also extending into Colombia. *Machaonia*, *Arachnothryx*, *Gonzalagunia*, and *Malanea*, which were placed in early, successively diverging lineages in the remaining Guettardeae, are distributed in all of the Neotropical regions defined here. They are all rather species-rich genera with largely unknown phylogenies. *Machaonia* and *Arachnothryx* have capsular fruits, while *Gonzalagunia* and *Malanea* have generally fleshy fruits. Our reconstruction suggests a Central American and/or South American and to some extent Caribbean ancestral distribution for the corresponding nodes (fig. 5B). These ambiguous reconstructions may reflect our poor knowledge of phylogenetic relationships within the genera, limited sampling, and an absence of Caribbean representatives in the sampling. The wide extant distribution of all four genera may, however, support a wide ancestral distribution in the Neotropics during the Oligocene, as indicated by our results.

Dispersals To and Within the Paleotropics

Naucleeae and Hymenodictyeae. The two sister clades Naucleeae and Hymenodictyeae are different from the other tribes of Cinchonoideae in that their major distributions in the Paleotropics (i.e., Africa, Madagascar and the Mascarenes, Asia, and the Pacific), with only a few Naucleeae taxa present in the Neotropics. Antonelli et al. (2009) placed Naucleeae and Hymenodictyeae as a sister group to the remaining Cinchonoideae and proposed that Rubiaceae used the NALB as a pathway from the Old World to South America in the Late Paleocene–Early Eocene. The *Cephalanthus* fossils documented in different layers from the Eocene to Oligocene in Europe were considered to strengthen their hypothesis (Antonelli et al. 2009).

Our ancestral-area reconstructions and divergence-time estimates point in another direction. A Neotropical presence of the ancestor of Cinchonoideae and the divergence-time estimate for the split of Naucleeae and Hymenodictyeae indicate that the lineage dispersed to the Old World at the latest by the end of the Eocene (fig. 3). This dispersal could have been trans-Atlantic or along the NALB. So far, no fossils have been found in North America to support a migration route over the continent for this lineage. However, the *Cephalanthus* fossils from different localities in Europe (Raniecka-Bobowska 1959; Dorofeev 1960, 1963; Mai and Walther 1978, 1985; Friis 1985) clearly indicate that the lineage was present in Eurasia at the end of the boreotropical era and had a long-lasting presence in the region despite decreasing temperatures from the Late Eocene and Oligocene (Tiffney and Manchester 2001; Zachos et al. 2001). Extant *Cephalanthus* species have a pan-subtropical distribution and grow in swamps, wet areas, and/or near water. Three species are found in the New World, and of these one species, *Cephalanthus occidentalis*, grows in warm temperate regions of North America, consistent with a tolerance to cooler conditions. The back dispersal of *Cephalanthus* to the New World is estimated to the Early Oligocene, coinciding with its presence in Eurasia.

Furthermore, ancestral distribution areas reconstructed for Hymenodictyeae and early splits within Naucleeae, as well as an extant distribution of *Cephalanthus* (fig. 3B), indicate a boreotropical presence that was interrupted by the thermal cooling that occurred from the Middle Eocene to the Oligocene (Tiffney and Manchester 2001; Zachos et al. 2001). This cooling forced frost-sensitive boreotropical taxa to migrate south (or to become extinct), and remant taxa are today found in tropical regions in the Paleotropics as well as the Neotropics (Morley 2003). In this study, therefore, Eurasia is considered as part of these distribution areas during the Paleocene-Oligocene. Ancestral-area reconstructions for all Naucleeae except *Cephalanthus* suggest a presence in Africa exclusively or together with Asia and/or Madagascar, whereafter three geographically separated lineages are resolved, one African, one Malagasy, and one Asian (fig. 3B), consistent with a previous boreotropical distribution. The same ancestral areas are reconstructed for Hymenodictyeae (fig. 3B).

Morley (2003) considered dispersal of boreotropical taxa to Africa to be rare because of latitudinal barriers such as the Sahara Desert, the Mediterranean Sea, and the uplifts of the Alps. Therefore, hypotheses involving migration southward to tropical Africa may seem unlikely. However, the arid climate present in the Sahara today was not established until ~5 Mya; since the split of Africa from South America, the climate in the Sahara region as well as in other parts of northern Africa have supported periods of Mediterranean vegetation or tropical forests (Le Houérou 1997). Furthermore, following the movement of the African continent toward Eurasia, the Tethys closed in the Early Miocene (20–16 Mya) and the two continents became connected (Rögl 1999). The African lineage of Naucleeae is estimated to have diversified during the Middle to Late Miocene. Dispersal of boreotropical Malpighiaceae to Africa and subsequently to Madagascar due to a cooling climate in the Oligocene has been suggested by Davis et al. (2004).

The African Naucleeae lineage is not well supported and consists of three commonly recognized clades (*Pausinystalia-Corynanthe*, *Mitragyna*, and *Nauclea-Burttdavya*) with unresolved or only weakly supported interrelationships in previous studies (Razafimandimbison and Bremer 2002; Manns and Bremer 2010). While the *Pausinystalia-Corynanthe* clade has retained its African distribution, several dispersal events are reconstructed within the other two (fig. 3B). During the Late Miocene, at least three different dispersals out of Africa are suggested. The first dispersal refers to *Mitragyna* with a present distribution in Africa and Asia (+ New Guinea), and the Asian species is shown as derived. The seeds of *Mitragyna* are completely surrounded by wings (Razafimandimbison and Bremer 2002).

The second dispersal from Africa led to an Asian or Pacific distribution of the ancestor of *Nauclea orientalis* and *Neolamarckia*. Relationships within the *Nauclea-Burttdavya* clade are, however, only weakly supported and are not congruent in the different analyses, and ancestral-area reconstructions are ambiguous. The placement of the African *Nauclea didderrichii* as sister to all other species of the group is inconsistent with the results of previous studies (Razafimandimbison and Bremer 2002; Manns and Bremer 2010), in which *Nauclea* was monophyletic and *Neolamarckia* was placed outside *Nauclea*, *Burttdavya*, and *Sarcocephalus*. This alternative topology indicates two parallel dispersals from Africa to Asia or the Pacific. Both *Nauclea* and *Neolamarckia* have indehiscent fruits.

The third dispersal from Africa of the African Naucleeae lineage is indicated as being to the Neotropics and was followed by a later dispersal to Asia in *Uncaria* (fig. 3B). The pantropical genus *Uncaria* has its largest species diversity in Asia (29 out of the 34 species; Ridsdale 1978). The genus is represented in our analysis by two Neotropical and one Asian species, while African taxa were not available. Our sampling is thus too limited to say whether the Asian taxa form a monophyletic group, which would support a single dispersal to the region, and whether the African taxa have a basal or derived position in the genus. The fruits of *Uncaria* are capsular and bipolar, with fusiform wings at both ends.

The ancestral distribution of the Malagasy Naucleeae lineage possibly also included Africa, and the lineage diversified during the Late Miocene (fig. 3A). Dispersal to Madagascar was probably through long-distance dispersal, and the source area seems to involve Asia (however, the support is weak; fig. 3B). The seeds of *Breonadia* and *Breonia* are unwinged, although rudimentary wings are sometimes observed in species of *Breonia*, and the fruit of *Breonia* can function as a dispersal unit that floats on water (Razafimandimbison 2002). Apart from *Breonadia*, which is sister to the remaining taxa and has an African (+ Saudi Arabian and Yemeni) and Malagasy distribution, the lineage has an extant distribution that is restricted to Madagascar and the Comoros. Although not shown here, dispersal to the Comoros was probably from Madagascar (Wikström et al. 2010).

Neonauclea is a large, paraphyletic genus distributed in Asia and the Pacific islands (Razafimandimbison and Bremer 2002). It is here represented by two species found in Sulawesi, Western New Guinea, and Papau New Guinea that are

reconstructed as two parallel dispersals from Asia to the Pacific (fig. 3B). However, a combination of poor sampling, limited knowledge of relationships within *Neonauclea*, and the unclear relationship to *Ludekia*, *Myrmeconauclea*, and *Ochreinauclea* make this result uncertain.

Other Neotropical-Paleotropical Dispersal Events

Parallel dispersals in the Miocene from the Caribbean to the Pacific and Asia are suggested to have occurred in Chiococceae (fig. 4B). Representatives of the nonmonophyletic Pacific genus *Bikkia* are found in two separate clades: the colorfully flowered "*Thiollierea-Bikkia*" and *Morierina*, which occur in New Caledonia, form a sister group to one Caribbean clade, while the white-flowered *Bikkia* and *Badusa*, which are present on New Guinea, the Philippines, the Moluccas, Micronesia, Fiji, Tonga, and Niue to the Wallis Islands (Motley et al. 2005), form a sister group to another Caribbean clade. The absence of these genera on mainland Asia indicates a long-distance dispersal across the Pacific Ocean.

Our results are congruent with those of Motley et al. (2005), who discussed the biogeography of Chiococceae s.l. and suggested an origin in the Neotropics and one or two long-distance dispersals to the South Pacific. They concluded that because the Pacific taxa of Chiococceae are all wind dispersed, anemochory is a successful strategy with which to cross from the Neotropics to the Pacific islands, while other fruit types are successful on a more local scale.

As in Chiococceae, several dispersals from the Neotropics to the Paleotropics are reconstructed in Guettardeae and are estimated to the Miocene. The Paleotropical distribution within Guettardeae, however, cannot be explained by anemochory. Although Guettardeae is essentially Neotropical, a number of Guettardeae genera placed within a large clade of taxa possessing fleshy fruits have a distribution in the Pacific islands, Asia, and the Indian Ocean. Of these genera, *Guettarda* is also found in the Neotropics, although the genus was shown to be polyphyletic, with nonrelated clades corresponding to different geographical regions (Achille et al. 2006).

Lack of resolution and weakly supported nodes make inferences regarding number of dispersal events from the Neotropics to the Paleotropics difficult, although at least three dispersals followed by vicariance may be inferred (fig. 5B). Our results indicate that the ancestor of *Hodgkinsonia*, *Bobea*, *Antirhea megacarpa*, and *Antirhea inconspicua* was present in the Pacific islands, and the clade is sister to the Neotropical *Chomelia spinosa*. A parallel scenario is suggested for *Tinadendron* + *Timonius* and a Neotropical clade consisting of *Chomelia tenuiflora*, *Guettarda crispiflora*, *Guettarda tournefortiopsis*, and *Stenostomum*. A third Neotropical-Paleotropical dispersal is reconstructed for the ancestor of the Malagasy *Antirhea madagascariensis* and the South American *Chomelia angustifolia*.

However, in a study by Manns and Bremer (2010), *Antirhea madagascariensis* was detected as sister taxon to the Malagasy/Mascarenian *Antirhea borbonica* J.F. Gmel. and placed in the sister group of *Timonius*, while *Tinadendron*

grouped with the *Guettardella–Antirhea megacarpa* clade. *Timonius* is a species-rich genus with a wide distribution in the Indian Ocean, Asia, and the Pacific, and a close relationship between this genus and the two Malagasy/Mascarene taxa would suggest that their present distribution stems from a shared Paleotropical ancestor following a dispersal event from the Neotropics.

Our analysis suggests that the pantropical distribution of the seashore plant *Guettarda speciosa* represents a separate dispersal event; this event has been attributed to its fruits, which have air-filled cavities that enhance their dispersal by flotation over water (Achille et al. 2006). Remarkably, no other members of Guettardeae are found in Africa, and the reconstructed Neotropical-Paleotropical dispersals were probably westward. As in Chiococceae, the estimated divergence time (the Miocene) indicates a long-distance, trans-Pacific dispersal.

Conclusions

Our findings do not support Neotropical Rubiaceae as being Gondwana-derived taxa that migrated from Africa to the New World, as suggested by Raven and Axelrod (1974), nor do they support the recent suggestion that Rubiaceae used the NALB to reach the Neotropics in the Late Paleocene to Early Eocene (Antonelli et al. 2009). Instead, our results suggest that the ancestor of Cinchonoideae and Ixoroideae was already present in South America during the Late Cretaceous, with multiple subsequent dispersals to other regions in the Neotropics. Following dispersal to Central America no later than in the Early Paleocene, Late Eocene to Miocene dispersals to the Caribbean island can be inferred in Rondeletieae, Chiococceae, Hamelieae, *Chione* + *Colleteria*, and, to some extent, Hillieae. In Guettardeae, back dispersal to South America is estimated for the same time period. Their early presence and long persistence in the Caribbean islands are congruent with hypotheses of exposed land areas in the region from the Late Eocene.

Our results further suggest that the ancestor of Naucleeae and Hymenodictyeae dispersed from the Neotropics to the Paleotropics during the Paleocene or Eocene. The remains of *Cephalanthus* fruits support a presence in Eurasian boreotropical forests, from where it is reconstructed that Naucleeae and Hymenodictyeae are to have spread to Africa, Madagascar, and Asia, possibly due to decreasing temperatures in Eurasia in the Late Eocene–Oligocene.

The Neotropical and Paleotropical distributions of Chiococceae and Guettardeae are best explained by long-distance dispersals from the Neotropics to the Paleotropics during the Miocene. The dispersals were probably westward across the Pacific, and both tribes have their major Paleotropical distribution on islands in the Pacific Ocean while being absent in Africa.

Even though some dispersal events seem to coincide with temporary land connections between continents, long-distance dispersals have played a major role in the distribution of Cinchonoideae. Dispersals between remote regions or continents have been successful within the Neotropics and from the Neotropics to and within the Paleotropics for taxa with capsular fruits and wind-dispersed seeds and for taxa with fleshy fruits.

Acknowledgments

Appendix A

Information on GenBank Accession Numbers for Taxa Included in This Study

GenBank accession numbers for markers are listed in following order: *atp*B-*rbc*L, *ndh*F, *rbc*L, *rps*16, and *trn*T-F. Missing data are indicated by a dash.

Acranthera siamensis (Kerr) Bremek., EU145311, EU145399, EU145450, EU145476, EU145523; *Acrosynanthus latifolius* Standl., GQ851966, GQ852160, GQ852301, AF242900, GQ852457; *Acrosynanthus minor* Urb., GQ851967, GQ852161, GQ852302, AF242901, GQ852458; *Acunaeanthus tinifolius* (Griseb.) Borhidi, GQ851969, GQ852163, -, -, GQ852451; *Adina pilulifera* (Lam.) Franch. ex Drake, GQ851971, GQ852165, AJ346964, GQ852366, AJ414548; *Adina rubella* Hance, DQ131698, -, AJ346965, -, AJ346910; *Adinauclea fagifolia* (Teijsm. & Binn. ex Havil.) Ridsdale, GQ851970, GQ852164, AJ346966, GQ852365, AJ346911; *Alberta magna* E.Mey., -, AJ236282, Y18708, EU145491, AJ620118; *Alstonia scholaris* (L.) R.Br., DQ359161, AJ011982, X91760, AJ431032, AJ430907; *Amphidasya ambigua* (Standl.) Standl., EU145337, -, Y11844, AF129271, EU145576; *Antirhea inconspicua* (Seem.) Christoph., GQ852024, GQ852212, GQ852331, GQ852398, GQ852508; *Antirhea madagascariensis* Chaw, GQ851973, GQ852167, GQ852304, GQ852367, GQ852460; *Antirhea megacarpa* Merr. & L.M.Perry, GQ851974, GQ852168, -, AM117284, -; *Arachnothryx buddleioides* (Benth.) Planch., GQ851975, GQ852169, -, AF242960, GQ852461; *Arachnothryx chimboracensis* (Standl.) Steyerm., GQ851976, GQ852170, GQ852305, GQ852368, GQ852462; *Arachnothryx hondurensis* (Donn.Sm.) Lorence, -, -, -, AF243013, AF152716; *Arachnothryx leucophylla* (Kunth) Planch., GQ851977, GQ852171, GQ852306, AF242910, GQ852453; *Arachnothryx spectabilis* (Steyerm.) Rova, Delprete & B.Bremer, GQ852009, GQ852195, GQ852320, AF242934, GQ852488; *Argostemma hookeri* King, AJ234032, EU145419, Z68788, EU145497, EU145545; *Asemnantha pubescens* Hook.f., GQ851978, GQ852172, -, GQ852369, AF152713; *Augusta rivalis* (Benth.) J.H.Kirkbr., -, AM949846, AM949842, -, FM207118; *Badusa corymbifera* (G.Forst.) A.Gray, GQ851979, GQ852173, GQ852307, GQ852370, GQ852463; *Balmea stormae* Martínez, GQ851980, -, GQ852308, GQ852371, GQ852464; *Bikkia artensis* (Montrouz.) Guillaumin, GQ851981, GQ852174, GQ852309,

GQ852372, GQ852465; *Bikkia macrophylla* (Brongn.) K.Schum., GQ851982, GQ852175, -, GQ852373, GQ852466; *Bikkia tetrandra* (L.f.) A.Rich., GQ851983, -, -, GQ852374, GQ852467; *Blepharidium guatemalense* Standl., -, GQ852176, GQ852310, AF242916, GQ852468; *Bobea gaudichaudii* (Cham. & Schltdl.) H.St.John & Herbst, GQ851984, -, AM117209, -, -; *Bouvardia ternifolia* (Cav.) Schltdl., EU542925, FJ695292, X83626, EU543022, DQ359165; *Breonadia salicina* (Vahl) Hepper & J.R.I.Wood, GQ851987, GQ852178, AJ346967, GQ852377, AJ346912; *Breonia chinensis* (Lam.) Capuron, Adansonia, GQ851985, GQ852291, AJ346968, GQ852375, AJ346913; *Burttdavya nyasica* Hoyle, GQ851988, GQ852179, AJ346973, GQ852378, AJ346918; *Calycophyllum candidissiumum* (Vahl) DC., DQ131708, AJ236285, X83627, AF004030, AF152646; *Canthium inerme* (L.f.) Kuntze, -, -, AM117212, -, AJ620125; *Catesbaea holacantha* C.Wright ex Griseb., GQ851989, GQ852180, GQ852311, AF242920, GQ852469; *Catesbaea parviflora* Sw., GQ851990, GQ852181, GQ852312, GQ852379, GQ852470; *Cephalanthus natalensis* Oliv., GQ851992, GQ852182, Y18711, GQ852380, AJ414549; *Cephalanthus occidentalis* L., DQ131710, AJ236288, X83629, AF004033, AJ346955; *Cephalanthus salicifolius* Humb. & Bonpl., GQ851993, GQ852183, AJ346975, GQ852381, AJ346920; *Ceratopyxis verbenacea* (Griseb.) Hook.f., GQ851994, GQ852184, GQ852313, AF242921, GQ852472; *Chiococca alba* (L.) Hitchc., DQ131711, AJ130835, L14394, AF004034, GQ852473; *Chione venosa* (Sw.) Urb., GQ851995, GQ852185, AM117215, AF242965, GQ852474; *Chomelia angustifolia* Benth., GQ851998, GQ852189, GQ852315, GQ852385, GQ852478; *Chomelia spinosa* Jacq., GQ851999, GQ852190, -, GQ852386, GQ852454; *Chomelia tenuiflora* Benth., GQ852000, GQ852191, GQ852316, -, GQ852479; *Ciliosemina pedunculata* (H.Karst.) Antonelli, GQ852001, GQ852292, AY538506, AY538444, GQ852480; *Cinchona calisaya* Wedd., GQ852003, GQ852293, AY538478, AF242927, GQ852482; *Cinchona officinalis* L., GQ852004, -, AY538480, GQ852387, GQ852483; *Cinchona pubescens* Vahl, AJ233990, AJ235843, X83630, AF004035, AJ346963; *Cinchonopsis amazonica* (Standl.) L.Andersson, GQ852002, -, AY538482, AY538428, GQ852481; *Coffea arabica* L., X70364, AJ236290, X83631, AF004038, DQ153845; *Colleteria seminervis* (Urb. & Ekman) David W. Taylor, GQ852005, GQ852192, GQ852317, GQ852388, GQ852484; *Colletoecema dewevrei* (De Wild) E.M.A.Petit, DQ131713, EU145409, EU145457, AF129272, EU145532; *Condaminea corymbosa* (Ruiz & Pav.) DC., -, AJ236291, Y18713, AF004039, AF102406; *Coptosapelta diffusa* (Champ.) Steenis, EU145315, EU145403, EU145452, EU145482, EU145527; *Coptosapelta flavescens* Korth., EU145317, EU145405, EU145454, EU145485, EU145528; *Coptosperma neurophyllum* (S.Moore) Degreef, -, -, Z68861, -, AJ847403; *Corynanthe mayumbensis* (R.D.Good) N.Hallé, DQ131715, GQ852296, AJ346976, GQ852434, AJ346921; *Corynanthe paniculata* Welw., -, -, AJ346978, -, AJ346923; *Cosmibuena grandiflora* (Ruiz & Pav.) Rusby, GQ852007, GQ852193, AY538483, AM117295, GQ852485; *Cosmibuena valerioi* (Standl.) C.M.Taylor, X81683, GQ852194, GQ852319, GQ852390, GQ852486; *Coussarea hydrangeifolia* (Benth.) Benth. & Hook.f. ex Müll.Arg., EU145326, EU145422, EU145460, EU145501, EU145549; *Coutaportla ghiesbreghtiana* (Baill.) Urb., -, -, -, AF242931, AF152693; *Coutarea hexandra* (Jacq.) K.Schum., GQ852008, AM117344, AM117221, AF242933, GQ852487; *Cremaspora triflora* (Thonn.) K.Schum., DQ131718, AM949850, Z68856, AF200990, AF201040; *Crossopteryx febrifuga* (Afzel. Ex G.Don) Benth., DQ131719, AM949851, AM117223, FM204717, FM207123; *Cubanola daphnoides* (Graham) Aiello, GQ852010, -, GQ852321, AF242935, GQ852489; *Deppea blumenaviensis* (K.Schum.) Lorence, GQ852011, GQ852196, AJ288622, AF004046, GQ852491; *Deppea erythrorhiza* Schltdl. & Cham., GQ852012, GQ852197, GQ852322, GQ852391, GQ852492; *Deppea grandiflora* Schltdl., GQ852013, GQ852198, X83633, AM117299, Q852493; *Deppea splendens* Breedlove & Lorence, GQ852014, GQ852199, GQ852323, GQ852392, GQ852494; *Emmenopterys henryi* Oliv., DQ131728, AJ236294, Y18715, AF242941, FM207125; *Eosanthe cubensis* Urb., DQ131729, -, -, GQ852393, GQ852495; *Erithalis fruticosa* L., DQ131730, AJ236295, X83635, AF242942, GQ852496; *Erithalis harrisii* Urb., -, GQ852200, GQ852324, GQ852394, GQ852497; *Exacum affine* Balf.f., -, AJ011983, L11684, -, AJ490204; *Exostema acuminatum* Urb., GQ852015, GQ852201, AY205359, GQ852395, GQ852498; *Exostema caribaeum* (Jacq.) Schult. in J.J.Roemer & J.A.Schultes, AJ233991, AJ236296, X83636, -, -; *Exostema lineatum* (Vahl) Schult., DQ131732, GQ852202, AY205353, AF242944, AJ346924; *Exostema longiflorum* (Lamb.) Schult. in J.J.Roemer & J.A.Schultes, GQ852016, GQ852203, AY205352, AF242945, GQ852499; *Exostema spinosum* (Le Vavass.) Krug & Urb., GQ852017, GQ852204, AY205350, AF242947, GQ852500; *Faramea multiflora* A.Rich., EU145328, EU145424, Z68796, AF004048, AF102422; *Ferdinandusa speciosa* PohL, DQ131735, EU145412, AM117226, AM117304, EU145534; *Gelsemium sempervirens* (L.) J.St.-Hil., AJ233985, AJ011984, L14397, AJ431033, AF102428; *Gonzalagunia affinis* Standl. ex Steyerm., -, -, Y11848, AM117310, AJ847405; *Gonzalagunia cornifolia* (Kunth.) Standl., -, GQ852205, -, AF242958, GQ852501; *Gonzalagunia dependens* Ruiz & Pav., GQ852018, GQ852206, GQ852325, GQ852396, GQ852502; *Gonzalagunia rosea* Standl., GQ852019, GQ852207, GQ852326, AF242950, GQ852503; *Guettarda boliviana* Standl., GQ852021, GQ852209, GQ852328, AF242962, GQ852505; *Guettarda crispiflora* Vahl, GQ852022, GQ852210, GQ852329, AF004052, GQ852506; *Guettarda ferruginea* Griseb., GQ852023, GQ852211, GQ852330, GQ852397, GQ852507; *Guettarda speciosa* L., GQ852025, GQ852213, AY538485, AF242964, GQ852509; *Guettarda tournefortiopsis* Standl., GQ852026, GQ852214, GQ852332, -, GQ852510; *Guettarda uruguensis* Cham. & Schltdl., DQ131739, AJ236297, X83638, EU145489, EU145533; *Gyrostipula comorensis* J.-F.Leroy, GQ852027, GQ852215, AJ346979, AM117312, AJ346925; *Gyrostipula foveolata* (Capuron) J.-F. Leroy, GQ852028, GQ852216, AJ346980, GQ852399, AJ346926; *Haldina cordifolia* (Roxb.) Ridsdale, GQ852029, GQ852217, X83639, GQ852400, AJ346956; *Hamelia cuprea* Griseb., -, -, X83641, AM117313, AM117361; *Hamelia papillosa* Urb., AJ233992, GQ852218, AY538487, AF004053, GQ852511; *Hamelia patens* Jacq., GQ852030, GQ852219, GQ852333, al046 x, al046 x; *Heinsia crinita* (Afzel.) G.Taylor, DQ131740, -, Y11849, -, AJ847376; *Hillia illustris* (Vell.) K.Schum., GQ852031, -, -, GQ852401, GQ852565; *Hillia parasitica* Jacq., -, GQ852220, AM117233, -, GQ852512; *Hillia triflora* (Oerst.) C.M.Taylor, AJ233993, AJ236298, X83642,

AM117315, GQ852513; *Hintonia latiflora* (Sessé & Moç. ex DC.) Bullock, GQ852032, GQ852221, GQ852334, GQ852402, GQ852514; *Hodgkinsonia ovatiflora* F.Muell., GQ852033, GQ852222, AM117234, -, GQ852515; *Hoffmannia ghiesbreghtii* (Lem.) Hemsl., GQ852034, GQ852223, GQ852335, GQ852403, GQ852516; *Hymenodictyon floribundum* (Hochst. & Steud.) B.L.Rob., DQ131742, EU145411, AJ347015, AF004058, GQ852517; *Hymenodictyon orixense* (Roxb.) Mabb., GQ852035, GQ852224, GQ852336, GQ852404, GQ852518; *Isertia coccinea* (Aubl.) J.F.Gmel., GQ852036, GQ852225, GQ852337, GQ852405, AF152689; *Isertia laevis* (Triana) Boom, GQ852037, GQ852226, Y11852, AM117319, GQ852519; *Isertia parviflora* Vahl, GQ852038, GQ852227, GQ852338, GQ852406, GQ852520; *Isertia pittieri* (Standl.) Standl., GQ852039, GQ852228, -, AM117320, AJ847404; *Isidorea pedicellaris* Urb. & Ekman, -, -, -, AF242968, AF152703; *Ixora coccinea* L., AM412400, AJ236299, X83646, AM117321, AJ620117; *Ixora finlaysoniana* Wall. ex G.Don, DQ131744, -, EU817423, EF205643, EU817466; *Janotia macrostipula* (Capuron) J.-F.Leroy, -, GQ852229, AJ346982, AM117322, AJ346928; *Joosia umbellifera* H.Karst., GQ852040, GQ852294, AY538492, AY538433, GQ852521; *Keriantera preclara* J.H.Kirkbr., -, -, AY538493, AF242970, AY538459; *Knoxia platycarpa* Arn., -, -, AJ288631, AM266826, AM266915; *Kraussia floribunda* Harv., DQ131746, -, Z68858, AM117325, AM117368; *Ladenbergia amazonensis* Ducke, -, -, AY538494, AY538434, AY538460; *Ladenbergia oblongifolia* (Humb. ex Mutis) L.Andersson, GQ852041, GQ852295, AY538497, AY538436, AY538462; *Ladenbergia pavonii* (Lamb.) Standl., GQ852042, -, Z68801, AY538437, GQ852522; *Lasianthus pedunculatus* E.A.Bruce, AJ234003, EU145427, Z68802, EU145504, EU145555; *Lasianthus strigosus* Wight, -, EU145428, AM117239, EU145505, EU145556; *Lerchea bracteata* Valeton, AJ233997, EU145433, AJ288610, EU145508, EU145561; *Lorencea guatemalensis* (Standl.) Borhidi, -, -, -, AF242930, AF152694; *Luculia grandifolia* Ghose, AJ233986, AM117346, X83648, DQ662205, DQ662149; *Luculia pinceana* Hook., DQ131749, EU145395, EU145447, EU145472, AM117371; *Ludekia borneensis* Ridsdale, GQ852043, GQ852230, AJ346983, GQ852407, AJ346962; *Machaonia acuminata* Humb. & Bonpl., GQ852044, GQ852231, GQ852339, GQ852408, GQ852523; *Machaonia erythrocarpa* (Standl.) Borhidi, GQ851972, GQ852166, -, -, GQ852452; *Machaonia portoricensis* Baill., -, -, -, AF242976, AF152733; *Machaonia williamsii* Standl., GQ852045, GQ852232, -, -, GQ852524; *Malanea forsteronioides* Müll.Arg., -, GQ852233, AM117245, GQ852409, -; *Mazaea phialanthoides* (Griseb.) Krug & Urb., GQ852047, GQ852235, GQ852340, AF242980, GQ852525; *Metadina trichotoma* (Zoll. & Moritzi) Bakh.f., GQ852049, -, AJ346984, GQ852411, AJ346930; *Mitragyna inermis* (Willd.) Kuntze, DQ131751, GQ852237, AJ346986, GQ852412, AJ346932; *Mitragyna rotundifolia* (Roxb.) Kuntze, GQ852050, GQ852238, AJ346987, GQ852413, AJ346933; *Mitragyna rubrostipulata* (K.Schum.) Havil., -, GQ852239, X83640, AY538429, AJ346957; *Mitragyna stipulosa* (DC.) Kuntze, -, GQ852240, AJ346981, -, AJ346927; *Morierina montana* Vieill., GQ852051, GQ852241, GQ852341, GQ852414, GQ852527; *Morinda citrifolia* L., AJ234014, AJ236300, AJ318448, AJ320078, AF152616; *Mussaenda erythrophylla* Schumach. & Thonn., DQ131754, AJ130836, X83652, EU145493, EU145535; *Mussaenda scratchleyi* Wernham, -, -, AJ318447, AJ320079, AJ847412; *Myrmeconauclea strigosa* (Korth.) Merr., GQ852052, GQ852242, AJ346989, GQ852415, AJ346934; *Nauclea diderrichii* (De Wild.) Merr., -, GQ852243, AJ346994, -, AJ346935; *Nauclea orientalis* (L.) L., EU145320, EU145410, X83653, AJ320080, AJ346958; *Neolamarckia cadamba* (Roxb.) Bosser, GQ852054, GQ852245, AJ346990, AF242985, AJ346938; *Neonauclea brassii* S.Moore, GQ852053, GQ852244, AJ346991, GQ852416, AJ346939; *Neonauclea clemensiae* Merr. & L.M.Perry, GQ852055, GQ852246, AJ318450, AJ320081, AJ346940; *Nernstia mexicana* (Zucc. & Mart. ex DC.) Urb., GQ852057, GQ852248, GQ852342, GQ852418, GQ852529; *Ochreinauclea maingayi* (Hook.f.) Ridsdale, GQ852058, GQ852249, AJ346997, GQ852419, AJ346943; *Ophiorrhiza mungos* L., FJ226541, AJ130838, X83656, AF004064, DQ662151; *Osa pulchra* (D.R.Simpson) Aiello, GQ852060, GQ852251, GQ852343, GQ852421, GQ852531; *Ottoschmidtia microphylla* (Griseb.) Urb., GQ852061, GQ852252, GQ852344, GQ852422, GQ852532; *Palicourea crocea* (Sw.) Schult., AM945247, AM945280, AM117253, AF147510, AM945359; *Paracorynanthe antankarana* Capuron ex J.-F.Leroy, GQ852062, GQ852253, AJ347017, GQ852423, GQ852533; *Pausinystalia johimbe* (K.Schum.) Pierre ex Beille, DQ131760, GQ852254, AJ346998, GQ852424, AJ346945; *Pauridiantha paucinervis* (Hiern) Bremek., AJ233998, AJ236302, Z68811, AM900600, EU145578; *Pausinystalia macroceras* (K.Schum.) Pierre ex Beille, GQ852063, GQ852255, AJ347000, GQ852425, AJ346944; *Pavetta lanceolata* Eckl., -, -, Z68865, AM117325, AM117377; *Pertusadina malaccensis* Ridsdale, GQ852064, GQ852257, AJ347003, GQ852427, AJ346949; *Phialanthus ellipticus* Urb., GQ852065, GQ852258, AM117257, GQ852428, AQ852534; *Phyllacanthus grisebachianus* Hook.f., GQ852067, GQ852260, GQ852345, GQ852430, GQ852536; *Phyllomelia coronata* Griseb., GQ852066, GQ852259, AM117258, GQ852429, GQ852535; *Pinarophyllon bullatum* Standl., GQ852068, GQ852261, GQ852346, GQ852431, GQ852456; *Pinckneya bracteata* (Bartram) Raf., -, AJ130839, X83661, AF242995, AM117381; *Plocaniophyllon flavum* Brandegee, GQ852069, GQ852262, GQ852347, GQ852432, GQ852537; *Portlandia platantha* Hook.f., -, -, -, AF242997, AF102469; *Posoqueria latifolia* (Rudge) Schult., -, AM949855, Z68850, FM204728, FM207135; *Pravinaria leucocarpa* Bremek., AJ234001, EU145441, AJ288617, AM900613, EU145580; *Pseudomiltemia filisepala* (Standl.) Borhidi, GQ852059, GQ852250, AM117251, GQ852420, GQ852530; *Psilanthus manni* Hook.f., DQ131772, -, Z68852, -, DQ153760; *Psychotria kirkii* Hiern, AM945246, AM945278, X83663, AM945327, AY538469; *Randia aculeata* L., by016, by016, by016, by016, by016; *Remijia chelomaphylla* G.A.Sullivan, GQ852071, GQ852297, AY538503, GQ852435, GQ852539; *Remijia macrocnemia* (Mart.) Wedd., DQ131775, GQ852298, AY538504, GQ852436, GQ852540; *Rhachicallis americana* (Jacq.) Hitchc., GQ852072, GQ852264, X83664, AF004073, GQ852541; *Rogiera amoena* Planch., GQ852073, GQ852265, GQ852349, AF243000, GQ852542; *Rogiera cordata* (Benth.) Planch., GQ852074, GQ852266, -, AF242999, GQ852543; *Roigella correifolia* (Griseb.) Borhidi & M.Fernández Zeq., GQ852075, GQ852267, GQ852350, GQ852437, GQ852544; *Rondeletia deamii* (Donn.Sm.) Standl., GQ852076, GQ852268, GQ852351, AJ786765, GQ852545; *Rondeletia hameliifolia*

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