TIME TREE OF RUBIACEAE: PHYLOGENY AND DATING THE FAMILY, SUBFAMILIES, AND TRIBES

Birgitta Bremer[1] and Torsten Eriksson

Bergius Foundation, Royal Swedish Academy of Sciences and Department of Botany, Stockholm
University, SE-106 91 Stockholm, Sweden

Rubiaceae are one of the largest families of plants, with ~13,000 species. In this study, we have estimated the phylogeny for 534 Rubiaceae taxa from 329 genera with up to five different chloroplast regions by Bayesian analysis. It resulted in a highly resolved tree with many strongly supported nodes. There is strong support for the three subfamilies (Cinchonoideae, Ixoroideae, Rubioideae) and most of the 44 included tribes. A scaled-down data set of 173 Rubiaceae taxa was used with a Bayesian approach to estimate divergence times for clades classified as tribes and subfamilies. Four fossils were used as minimum age priors, one inside each subfamily and one for Rubiaceae as a whole (*Faramea*-type pollen, *Scyphiphora* pollen, *Cephalanthus pusillus* fruits, and *Paleorubiaceophyllum eocenicum* leaves). The estimated lineage (stem) divergence time for Rubiaceae is 90.4 Ma. The estimated lineage divergence times for the subfamilies are 84.4 (86.6) Ma for Rubioideae, 73.1 Ma for Ixoroideae, and 73.1 Ma for Cinchonoideae. The estimated lineage divergence times for the tribes vary between 86.6 and 14.2 Ma. Classification, relationships, geographical distribution, and age estimates are presented and discussed for all tribes.

Keywords: Rubiaceae, dating, divergence times, Bayesian inference, phylogeny.

Online enhancements: appendixes.

Introduction

Rubiaceae represent one of the five most species-rich flowering plant families, with ~13,000 species (Goevarts et al. 2006) classified in ~620 genera, more than 40 tribes, and three subfamilies. They occur on all continents, even on the Antarctic Continent, with a few species of *Coprosma*, *Galium*, and *Sherardia* (Goevarts et al. 2006), but most taxa are in tropical or subtropical areas. One group of genera (tribe Rubieae) is widely distributed in temperate regions. The species occupy many types of habitat in several biogeographical regions. The diversity in the family is huge, with a span of lifeforms from small, weedy herbs to large rainforest trees, flower types adapted to a wide range of pollinators, different fruit types with many kinds of dispersal mechanisms, and accumulation of different chemical substances in the plants. Despite knowledge of such biological traits in the family, very few studies have dealt with explanations for this variability. Further, little is known of the correlation of these characters to each other or to external factors or when and where the various traits have evolved.

During the past 17 yr, as molecular data have accumulated, we have seen a tremendous increase in our understanding of the phylogeny of Rubiaceae. More than 60 different phylogenetic reconstructions at different taxonomic levels have been published (Bremer and Jansen 1991; Natali et al. 1995; Andreasen and Bremer 1996; Bremer and Thulin 1998; Andersson and Rova 1999; Bremer et al. 1999; Nepokroeff et al.

[1] Author for correspondence; e-mail: birgitta.bremer@bergianska.se.

1999; Bremer and Manen 2000; Persson 2000*a*, 2000*b*; Anderson et al. 2001; Malcomber 2002; Razafimandimbison and Bremer 2002; Rova et al. 2002; Church 2003; Delprete and Cortes-B 2004; Andersson and Antonelli 2005; Lantz and Bremer 2005; Motley et al. 2005; Achille et al. 2006; Backlund et al. 2007; Davis et al. 2007), and we are getting increasingly better pictures of the phylogeny of the family. An overview of earlier published phylogenies of the family can be seen in the supertree construction presented by Robbrecht and Manen (2006). A few of the phylogenies have also been used for evolutionary studies about biogeography (Anderson et al. 2001; Malcomber 2002; McDowell et al. 2003; Negrón-Ortiz and Watson 2003; Nepokroeff et al. 2003; Alejandro et al. 2005; Nie et al. 2005), ecological traits (Eriksson and Bremer 1991; Bremer and Eriksson 1992; McDowell and Bremer 1998; Novotny et al. 2002; Motley et al. 2005; Razafimandimbison et al. 2005), and morphological traits in the family (Jansen et al. 2002, 2003; Huysmans et al. 2003). With a phylogenetic approach to ecological and evolutionary studies, the origin of specific traits can be inferred, but to understand the evolutionary and ecological context in which these traits evolved, it is important to take into account when these events took place. We need estimated divergence times for the nodes in the tree. So far, little is known about the age of Rubiaceae and the lineages they include.

There are several more or less confidently identified fossils known from the Rubiaceae. Fossils have been described from South America and North America to Europe, Africa, Asia, Australia, and Oceania, potentially indicating an ancient worldwide distribution of the family. Most fossils are from the Miocene (23.0–5.3 Ma), but the oldest reported Rubiaceae fossil is

from the Paleocene (65–54.8 Ma), described by Baykovskaya (1984). The age of a few living genera has been estimated to be ~5 Myr on the basis of distribution patterns and fossils (Malcomber 2002; Nie et al. 2005).

As a result of growing knowledge of angiosperm phylogeny, based on molecular data and new analytical tools, there has been an increasing interest in the past 10–15 yr in dating the major clades of the angiosperms (Goremykin et al. 1997; Sanderson and Doyle 2001; Wikström et al. 2001; Leebens-Mack et al. 2005; Magallon and Sanderson 2005; Kay et al. 2006) and specific subgroups corresponding to different taxonomic levels (e.g., Asteraceae [Bremer and Gustafsson 1997], monocots [Bremer 2000], Lauraceae [Chanderbali et al. 2001], Liliales [Vinnersten and Bremer 2001], Crypteroniaceae [Conti et al. 2002], asterids [Bremer et al. 2004], Myrtaceae [Sytsma et al. 2004], basal eudicots [Anderson et al. 2005], Dipsacales [Bell and Donoghue 2005], Leguminosae [Lavin et al. 2005], Burseraceae [Week et al. 2005], Nymphaeales [Yoo et al. 2005], Moraceae [Zerega et al. 2005], and basal angiosperms [Moore et al. 2007]). In some of these studies Rubiaceae have been included. In the comprehensive effort to calibrate the angiosperm family tree, Wikström et al. (2001) analyzed three molecular markers and calibrated their phylogeny with one fossil from the Fagales lineage (rosids). These authors estimated the stem node age of Rubiaceae to be 61–64 Myr and the crown node age to be 55–56 Myr. In another study focusing on the asterid families, the group to which Rubiaceae belong (Olmstead et al. 1993), Bremer et al. (2004) investigated six molecular markers for more than 100 families, using six different fossils from within the asterids as calibration points. In that analysis Rubiaceae were represented by a single taxon (*Luculia*) together with four representatives of the rest of the order Gentianales. The crown age of the Gentianales was estimated to be 78 Myr, and because Rubiaceae are the sister group to the rest (Bremer 1996b), the crown age of the Gentianales corresponds to the stem age of the Rubiaceae. Because only one taxon (*Luculia*) was included in the study, the crown age of the family could not be estimated.

Initial attempts of dating clades used a strict molecular-clock assumption and a global rate of evolution (usually obtained from some other group of organisms). When tests for rate constancy (Felsenstein 1981) were more generally applied to data sets used for dating, a common result was that rate constancy was rejected. This spurred the development of dating methods that do not rely on a strict molecular clock, such as local-clock methods (Yoder and Yang 2000), nonparametric rate smoothing (Sanderson 1997), penalized likelihood (Sanderson 2002), and Bayesian dating methods (Thorne et al. 1998). Many of these methods (and the corresponding software packages) minimize rate changes between ancestors and descendants (rates are considered autocorrelated in time; Sanderson 1997), although there are exceptions (Drummond et al. 2006; Britton et al. 2007). The Bayesian relaxed-clock package "the Beast," of Drummond et al. (2006), has the desirable qualities of taking topological uncertainty into account as well as giving confidence intervals for the divergence time estimates.

The main goal for this study was to estimate the age of different branches within Rubiaceae, with a particular focus on clades that correspond to well-supported subfamilies and tribes. Secondary goals were to produce a robust phylogenetic tree in-

dicating relationships and support for those tribes and subfamilies and to present short comments about all tribes. Previous phylogenetic analyses of the Rubiaceae have been either limited in scope, covering only a portion of the clade, or based on restricted sampling in terms of taxa or DNA regions (Bremer and Jansen 1991; Bremer et al. 1995; Natali et al. 1995; Rova et al. 2002). This study is the first to cover all major subclades of Rubiaceae with extensive sampling and several DNA regions in a single analysis. Supertrees, such as the construction of Robbrecht and Manen (2006) of Rubiaceae, have very limited utility as compared with a full analysis using the real data. Supertrees suffer from several problems that we consider critical in relation to the goals of this study. Because supertrees are not directly based on real data, they have no branch lengths and cannot in themselves be used for estimating divergence times. Furthermore, supertrees incorporate (but do not show) any weaknesses of the separate trees they are built from, and there is no simple way to estimate support for their clades. A phylogenetic analysis using as much as possible of available data will always be preferable and more useful in evolutionary studies. Because sampling is focused on a good selection of taxa for evaluating the potential monophyly of subfamilies and tribes and documenting relationships among them, it may not be optimal for studying more detailed relationships among genera within tribes. Such an analysis would require denser sampling at lower taxonomic levels.

Material and Methods

Data Set

We analyzed a data set comprising 534 taxa from 329 Rubiaceae genera representing all three subfamilies and most tribes, and we included as many genera as possible available from our lab or from GenBank/EMBL. We used the full data set for this analysis. Our focus was on tribal and family relationships, and because high numbers of taxa as well as high numbers of characters are positively correlated with clade support (Bremer et al. 1999), we included as many genera as possible and also several species of each genus. All new sequences presented here represent the first sampling of that genus (*Cladoceras subcapitatum* [K. Schum. & K. Krause] Bremek., *Boholia nematostylis* Merr., *Omiltemia filisepala* [Standl.] C.V. Morton, *Paracephaelis* sp., *Robbrechtia grandifolia* De Block, *Schizostigma hirsutum* Arn.), a species sampled for the first time, or a new DNA marker of a problematic taxon. As outgroups we used two taxa of Loganiaceae (*Strychnos* and *Spigelia*) and one of Gelsemiaceae (*Mostuea*), in agreement with earlier phylogenetic studies (Olmstead et al. 1993; Bremer et al. 1995). Altogether, 959 sequences were included in the analysis: 605—of which 174 are new—from our lab, 107 sequences from Andersson and Rova (1999), 87 from Persson (2000a), 70 from Rova et al. (2002), and the remaining 90 sequences from many different studies (for references and GenBank/EMBL accession numbers, see app. A, in the online edition). The final data set included 9420 characters from five chloroplast regions: *rbc*L, 1402 characters (388 taxa); *trn*T-F, 2922 (225 taxa); *rps*16, 1868 (276 taxa; 424 ambiguously aligned characters were not included); *atp*B-*rbc*L, 925 (44 taxa), and *ndh*F, 2303 (50 taxa). All data were combined into one data set, despite the fact that some markers had

not been sequenced for many taxa. We relied on studies such as that of Wiens and Moen (2008), suggesting that even highly incompletely sequenced taxa can be safely included in Bayesian phylogenetic analyses. Each chloroplast region was tested to determine the model of evolution that best fit the data by using MrAIC (Nylander 2004) with PHYML (Guindon and Gascuel 2003) and the AICc criterion (Posada and Buckley 2004). We also used a reduced data set with fewer taxa but the same number of characters for divergence time (dating) analysis.

Downscaling

Because each separate run of the full data set used more than 20 d of computer time on our cluster, any tests and reruns were very time-consuming. For this reason we used a scaled-down data set for the divergence time analysis. In scaling down the full data set into the reduced data set, we followed a number of steps, outlined below. The decisions on what taxa to keep were based on results from phylogeny estimates of the full data set, using MrBayes. The three outgroups were kept in the reduced data set. In total, 362 species were removed, leaving 176 species in the reduced data set.

Step 1. We removed all but a single representative species from monophyletic genera (with posterior probability [PP] $\geq$ 0.95), selecting the species with most available sequence data. In this step, 77 species were removed.

Step 2. Because the main purpose for this study was the dating of clades corresponding to tribes and subfamilies and other major clades, we removed all but three to six species from well-supported clades (PP 0.95 or higher) corresponding to tribes. More species were kept in large tribes. The remaining species were selected in order to span the basal clade of which we were reducing the sampling, and we preferably selected those with more available sequence data over those with less data. In this step, 248 species were removed.

Comments on the treatment of certain tribal clades in step 2. A few small tribal clades (such as Sipaneeae) with only a few species sampled were not reduced. In the Guettardeae, two well-supported internal subclades were reduced to a single species each. The large Psychotrieae was reduced to six species. In the large Spermacoceae clade, two small clades in the first splits were reduced to a single species each, and two large clades were reduced to two species each. In the Coussareeae clade there was little support for internal clades, and it was not possible to determine which taxa to select for spanning the first split. For this reason and because one of the calibration points belonged here, we refrained from applying step 2 to Coussareeae. In the Hamelieae clade, only two species were kept.

Step 3. Five well-supported clades were identified among the clades commonly classified as Gardenieae. These clades were reduced separately in the same way as in step 2, while the rest of the species were kept. An additional 34 species were removed in step 3.

Step 4. An arbitrary lowest level of 800 BP was set, and those few (three) species still remaining with less than 800 BP of available sequence data were removed.

Phylogeny Estimation

We estimated the phylogeny with Bayesian inference of phylogeny (Yang and Rannala 1997), using MrBayes 3.1.1.

(Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). For the full data set, four MrBayes runs were conducted, each with four chains performing 16,000,000 generations, drawing a sample every 100 generations. The DNA regions were allowed to estimate model parameter values separately. From the tree sample, we removed 50% as chain burn-in, and because of problems with using the "sumt" command in MrBayes on 64-bit processors, we used PAUP* 4.0b10 (Swofford 2003) to compute 90% majority-rule consensus trees for the remaining sample. The runs were seeded with a user-specified tree, which was the single tree resulting from a parsimony analysis in PAUP* set to run 1000 addseq = random starting trees, branch swapping with the tree bisection reconnection algorithm, and saving a single best tree in each replicate. More recent versions of MrBayes were unable to load our large user-specified tree. To evaluate topological differences between the four runs, we used PAUP* to obtain a 95% majority-rule consensus tree for each run. Then, we computed a 50% majority-rule consensus tree based on those four trees, and this was used to pinpoint similarities and differences between the runs. For the reduced data set, MrBayes was set to run two parallel runs with 10,000,000 generations and four chains, sampling once every 100 generations.

Divergence Time Estimation

To check whether the data sets could be assumed to have evolved under rate constancy, we performed a likelihood ratio test, evaluating likelihood scores for three sampled trees (selected at random after burn-in) for the scaled-down data set. The test statistic was computed as two times the absolute difference between the likelihood scores for the tree with and without enforcing a molecular clock (Felsenstein 1981). We used PAUP* to optimize the likelihood scores for the trees. The test statistic was assumed to be distributed as χ^2, with the number of degrees of freedom corresponding to the number of sequences minus 2 (Page and Holmes 1998).

The divergence time analysis used the reduced data set and a relaxed-clock model under a Bayesian framework in the Beast 1.4.8 package (Drummond et al. 2006; Drummond and Rambaut 2007). The Beast input file was created using the Beauti 1.4.8 utility, bundled with the Beast. However, owing to a bug in this version of the program, the resulting XML file had to be manually edited before it could be successfully submitted to Beast (A. Drummond, personal communication). The Beast analysis used the same molecular model as the MrBayes analysis. Four age priors were attached to nodes of relevant taxa based on fossil ages. All four were treated as minimum age constraints by using uniform priors with the lower (younger) bound set to the minimum age of the fossil and the upper (older) bound set high enough (we used 1,000 Myr), effectively not restricting the upper bound. Three separate Beast runs were set to perform 10,000,000 generations, sampling once every 100 generations, and each run was checked for convergence with the Tracer v1.4 utility. The samples after burn-in for the three runs were then pooled into a joint tree file, and mean divergence ages were assigned to the nodes of a chronogram using the TreeAnnotator utility. Because of problems with TreeAnnotator default memory allocation for our big tree files, we started TreeAnnotator manually from

the (Linux) command line in order to assign extra memory: "java -Xmx2048m -Xms2048m -classpath/usr/local/src/BEAST.v1.4.8/lib/beast.jardr.app.tools.TreeAnnotator." The estimates and uncertainty levels for the nodes (table 1) were fetched directly from the TreeAnnotator chronogram tree file.

Following Bremer et al. (2004) and taking other previous estimates for the age of the Gentianales crown node into account (Wikström et al. 2001; Davies et al. 2004), we applied a normally distributed prior with a mean of 78 Myr for the root of our tree. The standard deviation of the prior distribution was set to be relatively large (10 Myr), which means that the 95% confidence interval of the mean was 61.6–94.5 Myr—covering previous estimates with a large margin.

Calibration Points and Rubiaceae Fossils

The ages of certain Rubiaceae genera have previously been estimated on the basis of Rubiaceae fossils (Malcomber 2002; Negrón-Ortiz and Watson 2003; Nie et al. 2005), and there are at least a dozen described fossils of Rubiaceae from Paleocene to younger strata of the Tertiary. Some of these fossils will be discussed, while others not used in our study are discussed in appendix B (in the online edition). In order to use the age of a fossil as a calibration point within a tree, it is necessary that the fossil has characteristics to link it to a specific clade in the tree. We used only the oldest fossils and those that could be unambiguously placed in the tree. Consequently, we selected four fossils, one from each subfamily, as internal minimum age calibration points, and one fossil for the Rubiaceae as a whole. Calibration points here were minimum age priors under the Bayesian framework. In addition, we used previous age estimates for the Gentianales crown node to calibrate the root of our trees.

A very well-preserved and well-described leaf fossil, *Paleorubiaceophyllum eocenicum* (Berry) Roth and Dilcher, was published from middle Eocene (55.8–33.9 Ma) by Roth and Dilcher (1979). This taxon has several typical Rubiaceae characters that are described and shown in the illustration, but, as also indicated by the authors, these traits occur in many parts of the family, and it is not possible to assign this fossil to any specific branch within the Rubiaceae clade. This middle Eocene fossil (Claiborn Formation of western Tennessee and Kentucky) is probably the oldest "certain" Rubiaceae leaf fossil. Previously, several leaf fossils have been assigned to Rubiaceae, but these are based on superficial similarities. Without careful morphological analysis, many leaf fossils have been erroneously determined. Before the study of Roth and Dilcher (1979), the same fossil had been placed in seven different families. Roth and Dilcher, however, investigated the specimens in detail with modern methods, and their classification of the fossil was based on many characters. The suite of characters they identified were, e.g., adnate stipules with glandular tips (=colleters, unique characters of Gentianales [our comment]), entire leaf margins, characteristic venation and cuticula, and paracytic stomata. However, because these characters occur in genera of all three subfamilies, the fossil cannot be more specifically placed than to Rubiaceae, and it cannot be used for internal calibration in our study. We used it as a minimum age prior for the family.

Most of the reported Rubiaceae fossils (see app. B) are dispersed pollen grains of a rather common tricolporate type, oc-

curring in many parts of the family; these were not used in our analysis. However, two pollen fossils have been included in our analysis. The oldest is pollen of *Faramea* type from the Upper Eocene, ~34–40 Myr; the other is of *Scyphiphora*, ~23 Myr. The *Faramea* pollen (two-porate) is reported from the Upper Eocene Gatuncillo Formation near Alcalde Diaz in Panama (Graham 1985). The description and images support the hypothesis that the grains are from *Faramea*. The *Faramea* pollen type is very characteristic and was already described by Erdtman (1966). His description is based on more than 20 different recent species of the genus, and the whole study includes 120 genera of Rubiaceae. Because of the orientation of the bacula at the apertures (two- to four-porate) and the size and the shape of the pollen, Erdtman (1966, p. 384) concluded that these grains were more or less unique among recent plants (and "at the same time exhibit a remarkable similarity to certain Cretaceous sporomorphs [*Tricolporites protrudens* and others]"). We consider the *Faramea* fossil reported by Graham (1985) from the Upper Eocene to be reliable, and we have used the mean age of the Upper Eocene, 37 Ma, as a minimum age prior in our dating analysis for the stem node of *Faramea*.

Scyphiphora was reported by Leopold (1969) from different layers of the Miocene sediments. Saenger (1998), in a discussion of fossil mangrove floras, gave two age estimates for the *Scyphiphora* fossils from the Miocene: ~16 Ma from Japan and ~23 Ma from the Marshall Islands collection. *Scyphiphora* is the only extant Rubiaceae genus that belongs to mangrove vegetation. It occurs in the Old World and in Australia and New Caledonia. *Scyphiphora* pollen is of the rather common tricolporate type in Rubiaceae but is characterized by distinct pores with a protruding papilla-like rim (Leopold 1969; Puff and Rohrhofer 1993). Because of this characteristic, in combination with the specific habitat, we include these fossils in our analysis with the approximate age of 23 Ma, using it as a minimum age prior for the *Scyphiphora* stem node.

The most convincing Rubiaceae fossil that could be linked to a specific internal branch in the tree is *Cephalanthus pusillus* Friis (1985). The fossil was described from the Odderup Formation in Denmark and is rather young, from the Middle Miocene. It was described on the basis of one well-preserved fruit and from almost 40 mericarps. The description and the illustration definitely point to the recent genus *Cephalanthus*. The fruit is a schizocarp composed of two one-seeded mericarps. The apex and the upper dorsal side of the seed are surrounded by a strophiole (a funicular outgrowth). The genus was previously described from the fossil record from the Upper Miocene from Konin in Poland by Raniecka-Bobowska (1959), as well as from Tertiary floras from Kireevski in western Siberia by Dorofeev (1960, 1963). Contrary to the view of Dorofeev, Friis (1985) indicated that Dorofeev's material was of Oligocene and Miocene age. Dorofeev's (1960) article about new data on the Tertiary flora from Kireevski listed several species stated to occur in the Oligocene, but *Cepahalanthus kireevskianus* was not mentioned. Later in the article, while discussing opinions by various authors about the age of the clay layer of Kireevski, he argued that the flora could not be older than Miocene because several characteristic new fossils of the Kireevski were missing from the other, older Oligocene floras. Our conclusion about the *Cephalanthus* fossils is

Table 1

Estimated Mean Divergence Times (Ma) for Nodes, Corresponding to Clades in Rubiaceae Classified as Tribes and Subfamilies, with Some Additional Major Clades and Calibration Points

Clade/taxon	Lineage age (stem)	95% HPD of lineage age	Alternative lineage age in fig. 8	Node age (crown)	95% HPD of crown node age	Notes
Rubiaceae	90.4	76.5–104.7		86.6	72.9–100.8	Prior age minimum, 45 Myr
Rubioideae	84.4	72.9–100.8	86.6	77.9	65.3–90.7	
Ophiorrhizeae	77.9	65.3–90.7		44.3	22.4–70.1	
Urophylleae	66.3	50.0–83.2	77.9	34.8	14.2–56.6	
Lasiantheae	66.3	50.0–83.2	77.9	34.8	20.3–53.5	
Coussareae	65.4	54.3–76.6		52.2	42.6–62.8	
Faramea	41.0	37.0–47.8				Prior age minimum 37 Myr; single accession
SPE alliance	63.0	52.0–75.2		54.8	45.4–65.1	
Danaideae	54.8	45.4–65.1		20.1	4.9–41.0	
Knoxieae	44.3	35.6–53.7		17.3	9.1–26.5	
Spermacoceae	44.3	35.6–53.7		28.5	21.7–36.0	
Anthospermeae	47.6	38.3–57.6		31.8	16.7–46.6	
Argostemmateae	44.8	35.8–54.2		21.0	9.0–38.1	
Paederieae	41.2	32.5–50.6		30.7	20.4–40.7	
Putorieae	34.4	25.5–44.0				Single accession
Rubieae	28.6	20.2–37.6		18.1	12.0–24.7	
Theligoneae	28.6	20.2–37.6				Single accession
PSY alliance	63.0	52.0–75.2		48.7	34.9–60.7	
Craterispermeae	34.8	34.9–60.7	48.7			Single accession
Gaertnereae	37.3	26.2–48.7	48.7	18.2	9.1–29.3	
Psychotrieae	48.7	34.9–60.7		35.6	25.5–46.9	
Morindeae	36.8	24.4–49.9	48.7	25.5	13.0–36.8	
Schradereae	30.0	34.9–60.7	48.7			Single accession
IXOR + CINC	80.3	65.9–94.6	84.4	73.1	58.4–88.7	
Ixoroideae	73.1	58.4–88.7		59.6	45.7–73.7	
Condamineeae	58.9	45.7–73.7	59.6	19.7	7.6–35.4	
Posoquerieae	28.7	10.5–47.1	46.5	17.9	5.8–33.4	
Sipaneae	46.5	30.3–63.5		30.0	16.7–47.4	
Sabiceeae	59.6	45.7–73.7		26.9	10.9–43.5	
Mussaendeae	54.9	42.5–68.0		24.0	9.0–41.8	
Scyphiphora	31.6	23.0–40.4				Prior age minimum, 23 Myr; single accession
Ixoreae	29.0	19.9–38.7		14.4	6.0–23.4	
Vanguerieae	29.0	19.9–38.7		15.5	7.3–24.6	
Retiniphylleae	44.7	34.0–56.0				Single accession
Alberteae	22.5	13.0–33.0	31.4			Single accession
Bertiereae	14.2	21.3–33.3	27.3			Single accession
Coffeeae	27.3	21.3–33.3		14.4	5.7–22.7	
Cremasporeae	16.7	8.2–25.0				Single accession
Octotropideae	16.7	8.2–25.0		7.1	2.9–12.3	
Pavetteae	23.9	21.3–33.3	27.3	11.8	6.5–17.3	
Cinchonoideae	73.1	58.4–88.7		38.7	28.1–52.5	
Chiococceae	34.4	23.3–47.8	38.7	27.6	15.4–40.5	
Cinchoneae	34.2	23.6–46.3	38.7	15.6	5.3–28.6	
Guettardeae	27.5	18.3–37.3		23.0	14.5–31.2	
Rondeletieae	27.5	18.3–37.3		22.4	12.1–32.0	
Hamelieae	18.7	11.5–27.4	38.7	13.5	7.4–20.8	
Hillieae	18.7	11.5–27.4	38.7	11.7	5.1–18.8	
Hymenodictyeae	19.7	14.9–25.3		3.6	.1–9.0	
Naucleeae	19.7	14.9–25.3		16.0	14.0–19.6	=*Cephalanthus* stem node
Cephalanthus	16.0	14.0–19.6				Prior age minimum, 14 Myr
Isertieae	34.7	24.0–47.6	38.7			Single accession
Luculieae	80.3	65.9–94.6	86.6			Single accession
Coptosapelteae	86.6	72.9–100.8		51.2	26.2–76.0	

Note.   See figure 8. The divergence time analysis in the Beast used the scaled-down data set of 176 taxa. For some taxa, the node in the dating tree had lower support than posterior probability = 0.95 or was collapsed when taxa were pruned for figure 8. In those cases, the estimated age of the alternative (better-supported) node shown in figure 8 is also given. One tribe, Gardenieae, was resolved as nonmonophyletic in the analysis and is not included here. Other tribes were represented only by a single accession in the analysis (Alberteae, Bertiereae, Craterispermeae, Cremasporeae, Isertieae, Luculieae, Putorieae, Retiniphylleae, Schradereae, and Theligoneae) and lack crown node age. Levels of uncertainty are indicated by 95% highest probability density (HPD) for the clades, except for a few clades not occurring in a majority of the trees (underlined). In those cases, the HPD for the alternative node is reported. Taxon abbreviations: CINC, Cinchonoideae; IXOR, Ixoroideae; PSY, Psychotrieae; SPE, Spermacoceae.

that the oldest and most specific age estimate is the Middle Miocene estimate for *C. pusillus* by Friis (1985). In our study, the prior age of the stem node of *Cephalanthus* is set to be 15 Myr (mean value of the Miocene period) or older.

Results

Method for Phylogeny Estimation

The general time reversible model (GTR; Tavaré 1986) with gamma distribution of rates (GTR+G; Yang 1993) was preferred for all regions. The PAUP* analysis resulted in a single tree that was used only as a starting topology ("usertree") for the analysis of the full data set in MrBayes. By drawing a sample every 100 generations in each of the MrBayes runs, we obtained a total tree sample of 160,000 trees per run. The runs quickly leveled off on an approximately even (not sloping) log-likelihood score level (after ~1 million generations). This level, however, was slightly different for each run and was not noticeably improved even after 16 million generations (−lnL ~106,300, 105,600, 106,050, and 106,050, respectively, for the four runs). The tree in figure 1 is a 90% majority-rule consensus tree based on the tree sample (80,000 trees) remaining after the Markov chain Monte Carlo (MCMC) burn-in had

been removed in the run that sampled at the highest likelihood score level.

Phylogeny

The phylogenetic analysis resulted in a highly resolved consensus tree, but many weakly supported nodes were collapsed (figs. 1–6). However, there is strong support for the clades that correspond to the three subfamilies (Cinchonoideae, Ixoroideae, Rubioideae) and most of more than 40 tribes. Figure 7 is a pruned version of figures 1–6 that illustrate the relationships among the subfamilies and the tribes. Only strongly supported nodes (with clade credibility [PP] of 0.95–1.0) are resolved in this tree. All other nodes are collapsed in figure 7 and will be mentioned as unresolved, unless results from figures 1–6 are discussed. All clades shown in figures 1–7 that were more inclusive than tribal clades were supported by PP 0.95 or higher in all four MrBayes runs. Except for the two tribal clades mentioned below, all other differences between the separate runs were found inside tribal clades or among the Gardenieae clades (23 differences). Of all tribes, five could not be tested for monophyly because they are monogeneric and represented by single taxa in the analysis (Alberteae, Cremasporeae, Retiniphylleae, Schradereae, and Theligoneae). Guettardeae
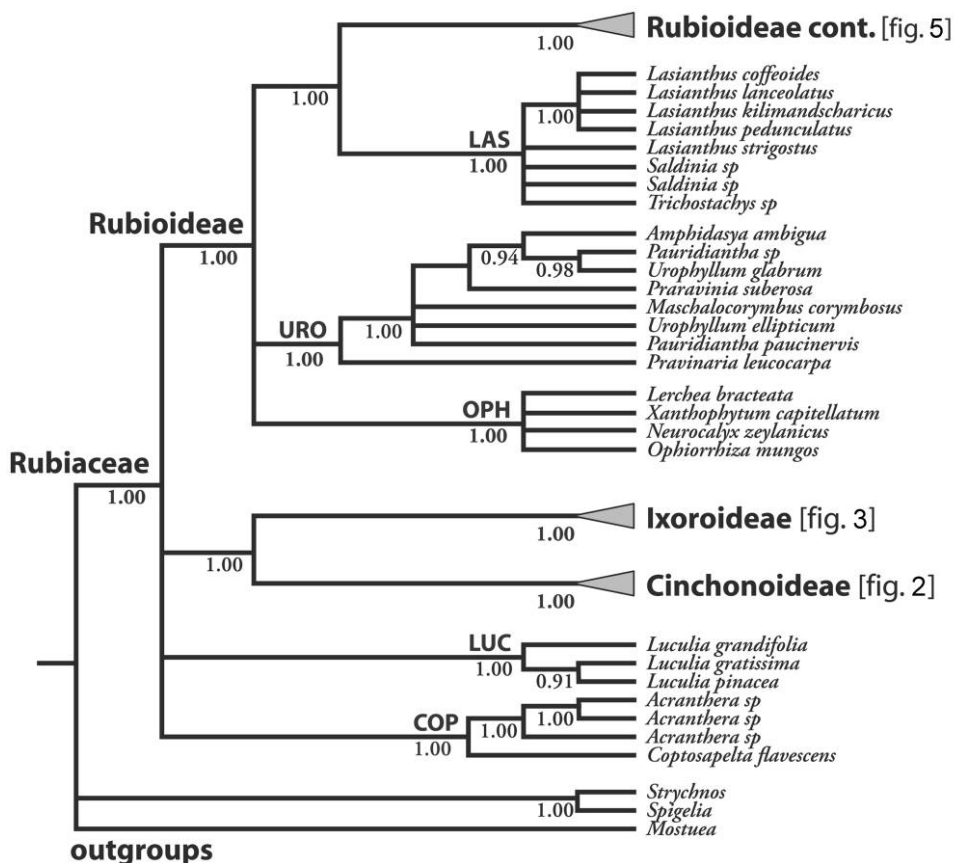
Fig. 1 The 90% majority-rule consensus tree from the Bayesian analysis. Root portion with outgroups, first major splits, and part of Rubioideae. Clade posterior probabilities are indicated below branches. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *LAS*, Lasiantheae; *URO*, Urohylleae; *OPH*, Ophiorrhizeae; *LUC*, Luculieae; *COP*, Coptosapelteae. Cinchonoideae are shown in fig. 2. Ixoroideae are shown in figs. 3, 4. The subclade of Rubioideae labeled "Rubioideae cont." is shown in figs. 5, 6.

Table 2

Rubiaceae Genera Included in the Study, with Current Tribal and Subfamily Classification

Genus	Tribe	Subfamily	Genus	Tribe	Subfamily
Acranthera Arn. ex Meisn.	COP	No subfamily	*Luculia* Sweet	LUC	No subfamily
Acrosynanthus Urb.	RON	CINC	*Ludekia* Ridsdale	NAU	CINC
Adina Salisb.	NAU	CINC	*Macbrideina* Standl.	CON	IXOR
Adinauclea Ridsdale	NAU	CINC	*Machaonia* Humb. & Bonpl.	GUE	CINC
Aidia Lour.	GAR[a]	IXOR	*Macrosphyra* Hook. f.	GAR[a]	IXOR
Alberta E. Mey.	ALB	IXOR	*Maguireothamnus* Steyerm.	SIP	IXOR
Aleisanthia Ridl.	No tribe	IXOR	*Malanea* Aubl.	GUE	CINC
Aleisanthiopsis Tange	No tribe	IXOR	*Manettia* Mutis ex L.	SPE	RUBI
Alibertia A. Rich. ex DC.	GAR[a]	IXOR	*Manostachya* Bremek.	SPE	RUBI
Allenanthus Standl.	GUE	CINC	*Mapouria* Aubl.	PSY	RUBI
Alseis Schott	CON	IXOR	*Margaritopsis* C. Wright	PSY	RUBI
Amaioua Aubl.	GAR[a]	IXOR	*Maschalocorymbus* Bremek.	URO	RUBI
Amaracarpus Blume	PSY	RUBI	*Massularia* (K. Schum.) Hoyle	GAR[a]	IXOR
Amphiasma Bremek.	SPE	RUBI	*Mazaea* Krug & Urb.	RON	CINC
Amphidasya Standl.	URO	RUBI	*Melanopsidium* Colla	GAR[a]	IXOR
Anthospermum L.	ANT	RUBI	*Metadina* Bakh. f.	NAU	CINC
Antirhea Comm. ex A. Juss.	GUE	CINC	*Meyna* Roxb. ex Link	VAN	IXOR
Aoranthe Somers	GAR[a]	IXOR	*Mitchella* L.	No tribe	RUBI
Appunia Hook. f.	MOR	RUBI	*Mitracarpus* Zucc. ex Schult. & Schult. f.	SPE	RUBI
Arachnothryx Planch.	GUE	CINC	*Mitragyna* Korth.	NAU	CINC
Arcytophyllum Willd. ex Schult. & Schult. f.	SPE	RUBI	*Mitrasacmopsis* Jovet	SPE	RUBI
Argostemma Wall.	ARG	RUBI	*Mitriostigma* Hochst.	GAR[a]	IXOR
Asperula L.	RUB	RUBI	*Molopanthera* Turcz.	POS	IXOR
Atractocarpus Schltr. & K. Krause	GAR[a]	IXOR	*Morelia* A. Rich. ex DC.	GAR[a]	IXOR
Atractogyne Pierre	GAR[a]	IXOR	*Morinda* L.	MOR	RUBI
Augusta Pohl	No tribe	IXOR	*Mussaenda* L.	MUS	IXOR
Badusa A. Gray	CHI	CINC	*Mussaendopsis* Baill.	CON	IXOR
Bathysa C. Presl	CON	IXOR	*Mycetia* Reinw.	ARG	RUBI
Batopedina Verdc.	KNO	RUBI	*Myonima* Comm. ex Juss.	IXO	IXOR
Benkara Adans.	GAR[a]	IXOR	*Myrmecodia* Jack	PSY	RUBI
Bertiera Aubl.	BER	IXOR	*Myrmeconauclea* Merr.	NAU	CINC
Bikkia Reinw.	CHI	CINC	*Nauclea* L.	NAU	CINC
Bobea Gaudich.	GUE	CINC	*Nenax* Gaertn.	ANT	RUBI
Boholia Merr.	No tribe	IXOR	*Neobertiera* Wernham	SIP	IXOR
Borojoa Cuatrec.	GAR[a]	IXOR	*Neoblakea* Standl.	GUE	CINC
Borreria G. Mey.	SPE	RUBI	*Nesohedyotis* (Hook. f.) Bremek.	SPE	RUBI
Bouvardia Salisb.	SPE	RUBI	*Neolamarckia* Bosser	NAU	CINC
Breonadia Ridsdale	NAU	CINC	*Neonauclea* Merr.	NAU	CINC
Breonia A. Rich. ex DC.	NAU	CINC	*Nertera* Banks & Sol. ex Gaertn.	ANT	RUBI
Burchellia R. Br.	GAR[a]	IXOR	*Neurocalyx* Hook.	OPH	RUBI
Burttdavya Hoyle	NAU	CINC	*Normandia* Hook. f.	ANT	RUBI
Bremeria Razafim. & Alejandro	MUS	IXOR	*Ochreinauclea* Ridsdale & Bakh. f.	NAU	CINC
Calochone Keay	GAR[a]	IXOR	*Oldenlandia* L.	SPE	RUBI
Calycophyllum DC.	CON	IXOR	*Oligocodon* Keay	GAR[a]	IXOR
Calycosiphonia Pierre ex Robbr.	GAR[a]	IXOR	*Omiltemia* Standl.	HAM	CINC
Canthium Lam.	VAN	IXOR	*Ophiorrhiza* L.	OPH	RUBI
Capirona Spruce	CON	IXOR	*Oreopolus* Schltdl.	COU	RUBI
Carpacoce Sond.	ANT	RUBI	*Otiophora* Zucc.	KNO	RUBI
Casasia A. Rich.	GAR[a]	IXOR	*Otomeria* Benth.	KNO	RUBI
Catesbaea L.	CHI	CINC	*Oxyanthus* DC.	GAR[a]	IXOR
Catunaregam Wolf	GAR[a]	IXOR	*Oxyceros* Lour.	GAR[a]	IXOR
Cephalanthus L.	NAU	CINC	*Paederia* L.	PAE	RUBI
Ceratopyxis Hook. f.	CHI	CINC	*Pagamea* Aubl.	GAE	RUBI
Ceriscoides (Hook. f.) Tirveng.	GAR[a]	IXOR	*Palicourea* Aubl.	PSY	RUBI
Chassalia Poir.	PSY	RUBI	*Paracephaelis* Baill.	PAV	IXOR
Chazaliella E.M.A. Petit & Verdc.	PSY	RUBI	*Parachimarrhis* Ducke	CON	IXOR
Chimarrhis Jacq.	CON	IXOR	*Paracorynanthe* Capuron	HYM	CINC
Chiococca P. Browne	CHI	CINC	*Parapentas* Bremek.	KNO	RUBI
Chione DC.	HAM/HIL-c	CINC	*Paratriaina* Bremek.	KNO	RUBI
Chomelia Jacq.	GUE	CINC	*Pauridiantha* Hook. f.	URO	RUBI

Table 2

(*Continued*)

Genus	Tribe	Subfamily	Genus	Tribe	Subfamily
Cinchona L.	CIN	CINC	*Pausinystalia* Pierre ex Beille	NAU	CINC
Cladoceras Bremek.	PAV	IXOR	*Pavetta* L.	PAV	IXOR
Coccocypselum P. Browne	COU	RUBI	*Pentagonia* Benth.	CON	IXOR
Coddia Verdc.	GAR[a]	IXOR	*Pentanisia* Harv.	KNO	RUBI
Coelospermum Blume	MOR	RUBI	*Pentanopsis* Rendle	SPE	RUBI
Coffea L.	COF	IXOR	*Pentas* Benth.	KNO	RUBI
Condaminea DC.	CON	IXOR	*Pentodon* Hochst.	SPE	RUBI
Conostomium (Stapf) Cufod.	SPE	RUBI	*Peponidium* (Baill.) Arènes	VAN	IXOR
Coprosma J.R. Forst. & G. Forst.	ANT	RUBI	*Pertusadina* Ridsdale	NAU	CINC
Coptosapelta Korth.	COP	No subfamily	*Phialanthus* Griseb.	CHI	CINC
Coptosperma Hook. f.	PAV	IXOR	*Phuopsis* (Griseb.) Hook. f.	RUB	RUBI
Corynanthe Welw.	NAU	CINC	*Phyllacanthus* Hook. f.	CHI	CINC
Cosmibuena Ruiz & Pav.	HIL	CINC	*Phyllis* L.	ANT	RUBI
Coussarea Aubl.	COU	RUBI	*Phyllomelia* Griseb.	RON	CINC
Coutarea Aubl.	CHI	CINC	*Phylohydrax* Puff	SPE	RUBI
Craterispermum Benth.	CRA	RUBI	*Picardaea* Urb.	CON	IXOR
Cremocarpon Boivin ex Baill.	PSY	RUBI	*Pinckneya* Michx.	CON	IXOR
Cremaspora Benth.	CRE	IXOR	*Plectroniella* Robyns	VAN	IXOR
Crossopteryx Fenzl	No tribe	IXOR	*Plocama* Aiton	PUT	RUBI
Crucianella L.	RUB	RUBI	*Pogonopus* Klotzsch	CON	IXOR
Cruciata Mill.	RUB	RUBI	*Pomax* DC.	ANT	RUBI
Cruckshanksia Hook. & Arn.	COU	RUBI	*Porterandia* Ridl.	GAR[a]	IXOR
Cubanola Aiello	CHI	CINC	*Portlandia* P. Browne	CHI	CINC
Damnacanthus C.F. Gaertn.	No tribe	RUBI	*Posoqueria* Aubl.	POS	IXOR
Danais Comm. ex Vent.	DAN	RUBI	*Pouchetia* DC.	OCT	IXOR
Deccania Tirveng.	GAR[a]	IXOR	*Praravinia* Korth.	URO	RUBI
Declieuxia Kunth	COU	RUBI	*Pravinaria* Bremek.	URO	RUBI
Dentella J.R. Forst. & G. Forst.	SPE	RUBI	*Preussiodora* Keay	GAR[a]	IXOR
Deppea Cham. & Schltdl.	HAM	CINC	*Prismatomeris* Thwaites	No tribe	RUBI
Dialypetalanthus Kuhlm.	CON	IXOR	*Pseudosabicea* N. Hallé	SAB	IXOR
Dibrachionostylus Bremek.	SPE	RUBI	*Psilanthus* Hook. f.	COF	IXOR
Dictyandra Welw. ex Hook. f.	PAV	IXOR	*Psychotria* L.	PSY	RUBI
Didymaea Hook. f.	RUB	RUBI	*Psydrax* Gaertn.	VAN	IXOR
Didymosalpinx Keay	GAR[a]	IXOR	*Psyllocarpus* Mart. & Zucc.	SPE	RUBI
Diodia L.	SPE	RUBI	*Pyrostria* Comm. ex Juss.	VAN	IXOR
Dioicodendron Steyerm.	CON	IXOR	*Ramosmania* Tirveng. & Verdc.	OCT	IXOR
Dirichletia Klotzsch	KNO	RUBI	*Randia* L.	GAR[a]	IXOR
Discospermum Dalzell,	COF	IXOR	*Readea* Gillespie	PSY	RUBI
Dolichodelphys K. Schum. & K. Krause	CON	IXOR	*Retiniphyllum* Humb. & Bonpl.	RET	IXOR
Dolicholobium A.Gray	CON	IXOR	*Rhachicallis* DC.	RON	CINC
Duperrea Pierre ex Pit.	GAR[a]	IXOR	*Richardia* L.	SPE	RUBI
Duroia L. f.	GAR[a]	IXOR	*Robbrechtia* De Block	PAV	IXOR
Durringtonia R.J.F. Hend. & Guymer	ANT	RUBI	*Rogiera* Planch.	RON	CINC
Emmenopterys Oliv.	CON	IXOR	*Roigella* Borhidi & M. Fernández Zeq.	RON	CINC
Erithalis P. Browne	CHI	CINC	*Rondeletia* L.	RON	CINC
Ernodea Sw.	SPE	RUBI	*Rosenbergiodendron* Fagerl.	GAR[a]	IXOR
Euclinia Salisb.	GAR[a]	IXOR	*Rothmannia* Thunb.	GAR[a]	IXOR
Exostema (Pers.) Bonpl.	CHI	CINC	*Rubia* L.	RUB	RUBI
Fadogia Schweinf.	VAN	IXOR	*Rudgea* Salisb.	PSY	RUBI
Faramea Aubl.	COU	RUBI	*Rustia* Klotzsch	CON	IXOR
Ferdinandusa Pohl	CON	IXOR	*Rutidea* DC.	PAV	IXOR
Feretia Delile	OCT	IXOR	*Sabicea* Aubl.	SAB	IXOR
Fernelia Comm. ex Lam.	OCT	IXOR	*Saldinia* A. Rich. ex DC.	LAS	RUBI
Gaertnera Lam.	GAE	RUBI	*Sarcocephalus* Afzel. ex R. Br.	NAU	CINC
Galium L.	RUB	RUBI	*Schismatoclada* Baker	DAN	RUBI
Galopina Thunb.	ANT	RUBI	*Schizocolea* Bremek.	No tribe	RUBI
Gardenia Ellis	GAR[a]	IXOR	*Schizomussaenda* H.L. Li	MUS	IXOR
Genipa L.	GAR[a]	IXOR	*Schmidtottia* Urb.	CHI	CINC
Geophila D. Don	PSY	RUBI	*Schradera* Vahl	SCH	RUBI
Gleasonia Standl.	POS-c	IXOR	*Schumanniophyton* Harms	GAR[a]	IXOR
Glossostipula Lorence	GAR[a]	IXOR	*Scolosanthus* Vahl	CHI	CINC

Table 2

(*Continued*)

Genus	Tribe	Subfamily	Genus	Tribe	Subfamily
Gonzalagunia Ruiz & Pav.	GUE	CINC	*Scyphiphora* C.F. Gaertn.	IXO/VAN-c	IXOR
Greenea Wight & Arn.	No tribe	IXOR	*Serissa* Comm. ex A. Juss.	PAE	RUBI
Guettarda L.	GUE	CINC	*Sherardia* L.	RUB	RUBI
Gynochthodes Blume	MOR	RUBI	*Sherbournia* G. Don	GAR[a]	IXOR
Gyrostipula J.-F. Leroy	NAU	CINC	*Siemensia* Urb.	CHI	CINC
Haldina Ridsdale	NAU	CINC	*Simira* Aubl.	CON	IXOR
Hamelia Jacq.	HAM	CINC	*Sinoadina* Ridsdale	NAU	CINC
Hedyotis L.	SPE	RUBI	*Sipanea* Aubl.	SIP	IXOR
Hedythyrsus Bremek.	SPE	RUBI	*Sipaneopsis* Steyerm.	SIP	IXOR
Heinisa DC.	MUS	IXOR	*Sommera* Schltdl.	CON	IXOR
Heinsenia K. Schum.	GAR[a]	IXOR	*Spermacoce* L.	SPE	RUBI
Hekistocarpa Hook. f.	SAB	IXOR	*Spermadictyon* Roxb.	PAE	RUBI
Hemidiodia	SPE	RUBI	*Sphinctanthus* Benth.	GAR[a]	IXOR
Heterophyllaea Hook. f.	COU	RUBI	*Squamellaria* Becc.	PSY	RUBI
Hillia Jacq.	HIL	CINC	*Stachyarrhena* Hook. f.	GAR[a]	IXOR
Hindsia Benth. ex Lindl.	COU	RUBI	*Steenisia* Bakh.f.	No tribe	IXOR
Hintonia Bullock	CHI	CINC	*Stenosepala* C. Perss.	GAR[a]	IXOR
Hippotis Ruiz & Pav.	CON	IXOR	*Streblosa* Korth.	PSY	RUBI
Hodgkinsonia F. Muell.	GUE	CINC	*Strumpfia* Jacq.	CHI	CINC
Hoffmannia Sw.	HAM	CINC	*Suberanthus* Borhidi & M. Fernández Zeq.	RON	CINC
Houstonia L.	SPE	RUBI	*Sukunia* A.C. Sm.	GAR[a]	IXOR
Hydnophytum Jack	PSY	RUBI	*Synaptantha* Hook. f.	SPE	RUBI
Hydrophylax L. f.	SPE	RUBI	*Tamilnadia* Tirveng. & Sastre	GAR[a]	IXOR
Hymenocoleus Robbr.	PSY	RUBI	*Tamridaea* Thulin & B. Bremer	SAB	IXOR
Hymenodictyon Wall.	HYM	CINC	*Tapiphyllum* Robyns	VAN	IXOR
Hyperacanthus E. Mey. ex Bridson	GAR[a]	IXOR	*Tarenna* Gaertn.	PAV	IXOR
Hypobathrum Blume	OCT	IXOR	*Tarennoidea* Tirveng. & Sastre	GAR[a]	IXOR
Ibetralia Bremek.	GAR[a]	IXOR	*Theligonum* L.	THE	RUBI
Isertia Schreb.	ISE	CINC	*Timonius* DC.	GUE	CINC
Isidorea A. Rich. ex DC.	CHI	CINC	*Tocoyena* Aubl.	GAR[a]	IXOR
Ixora L.	IXO	IXOR	*Triainolepis* Hook. f.	KNO	RUBI
Janotia J.-F. Leroy	NAU	CINC	*Tricalysia* A. Rich. ex DC.	COF	IXOR
Javorkaea Borhidi & Jarai-Koml.	GUE	CINC	*Trichostachys* Hook. f.	LAS	RUBI
Kailarsenia Tirveng.	GAR[a]	IXOR	*Uncaria* Schreb.	NAU	CINC
Keetia E. Phillips	VAN	IXOR	*Urophyllum* Wall.	URO	RUBI
Knoxia L.	KNO	RUBI	*Valantia* L.	RUB	RUBI
Kraussia Harv.	OCT	IXOR	*Vangueria* Juss.	VAN	IXOR
Kutchubaea Fisch. ex DC.	GAR[a]	IXOR	*Versteegia* Valeton	IXO	IXOR
Ladenbergia Klotzsch	CIN	CINC	*Virectaria* Bremek.	SAB	IXOR
Lasianthus Jack	LAS	RUBI	*Warszewiczia* Klotzsch	CON	IXOR
Leptactina Hook. f.	PAV	IXOR	*Wendlandia* DC.	No tribe	IXOR
Leptodermis Wall.	PAE	RUBI	*Wittmackanthus* Kuntze	CON	IXOR
Leptostigma Arn.	ANT	RUBI	*Xanthophytum* Reinw. ex Blume	OPH	RUBI
Lerchea L.	OPH	RUBI			

Note. Abbreviations: c, close to (taxon is sister group to or close to one or two tribes); ALB, Alberteae; ANT, Anthospermeae; ARG, Argostemmateae; BER, Bertiereae; CHI, Chiococceae; CIN, Cinchoneae; COF, Coffeeae; CON, Condamineeae; COP, Coptosapelteae; COU, Coussareeae; CRA, Craterispermeae; CRE, Cremasporeae; DAN, Danaideae; GAE, Gaertnereae; GAR, Gardenieae; GUE, Guettardeae; HAM, Hamelieae; HIL, Hillieae; HYM, Hymenodictyeae; ISE, Isertieae; IXO, Ixoreae; KNO, Knoxieae; LAS, Lasiantheae; LUC, Luculieae; MOR, Morindeae; MUS, Mussaendeae; NAU, Naucleeae; OCT, Octotropideae; OPH, Ophiorrhizeae; PAE, Paederieae; PAV, Pavetteae; POS, Posoquerieae; PSY, Psychotrieae; PUT, Putorieae; RET, Retiniphylleae; RON, Rondeletieae; RUB, Rubieae; SAB, Sabiceeae; SCH, Schradereae; SIP, Sipaneeae; SPE, Spermacoceae; THE, Theligoneae; URO, Urophylleae; VAN, Vanguerieae; CINC, Cinchonoideae; IXOR, Ixoroideae; RUBI, Rubioideae. No tribe, without tribal position. No subfamily, without subfamilial position (taxon has been sequenced but has not been placed within any described subfamily).

[a] Tribe is not monophyletic according to our analysis.

received somewhat lower support in two of the four runs (0.94 and 0.93), and Ixoreae also received lower support in two of the four runs (0.87 and 0.83). Gardenieae are not monophyletic and are currently under investigation (A. Mouly, A. Davis, C. Persson, C. Kainulainen, and B. Bremer, personal communication), so we refrain from discussing them in detail. The support for a monophyletic Gardenieae in our data is only 8%. All investigated genera are listed in table 2 with tribal affiliation.

The basalmost node of Rubiaceae (fig. 1) is not resolved; it is a polytomy of four branches with Coptosapelteae, Luculieae (Rydin et al. 2009), the subfamily Rubioideae, and a clade including subfamilies Cinchonoideae and Ixoroideae. These four clades, as well as the monophyly of Cinchonoideae and Ixoroideae, are strongly supported.

The subfamily Cinchonoideae includes nine well-supported clades corresponding to nine tribes. Most interrelationships among these are unresolved, but there is support for three pairs of sister taxa in the subfamily: Guettardeae + Rondeletieae, Hamelieae + Hillieae, and Hymenodictyeae + Naucleeae. These three pairs of sister clades, together with the three clades corresponding to Chiococceae, Cinchoneae, and Isertieae, are in a polytomy at the base of the subfamily. One genus (*Chione*) is without tribal position (fig. 2) but is supported as sister to the clade joining the Hamelieae and the Hillieae.

The subfamily Ixoroideae (figs. 3, 4, 7) includes two monogeneric tribes (Cremasporeae, Retiniphylleae), one represented by a single taxon (Alberteae), 12 well-supported monophyletic clades corresponding to tribes, several taxa that we refer to as the tribe Gardenieae (fig. 4; not monophyletic), and a few taxa without tribal position (*Boholia*, here sequenced for the first time; *Burchellia*, *Didymosalpinx*, *Gleasonia*, *Scyphiphora*, *Steenisia*). A polytomy at the base of the subfamily consists of Condamineeae, Posoquerieae + Sipaneeae + *Gleasonia*, Sabiceeae, and a clade including the rest of the subfamily. In the latter, the Mussaendeae are the first branch to split off, followed by *Steenisia*, Retiniphylleae, and a clade consisting of a trichotomy of *Boholia*, Ixoreae + Vanguerieae + *Scyphiphora*, and a clade including the remaining tribes of the subfamily. The latter includes Alberteae as sister to Bertiereae + Coffeeae, *Burchellia*, *Didymosalpinx*, and a clade of Cremasporeae + Octotropideae, Gardenieae, and Pavetteae.

The subfamily Rubioideae (figs. 1, 5–7) includes two monogeneric tribes (Schradereae, Theligoneae), 16 supported clades corresponding to tribes, and also a few taxa with unresolved phylogenetic relationships (*Mitchella* + *Damnacanthus*, *Schizocolea*, and *Prismatomeris*). After the completion of our study, these genera have been placed in newly described tribes Mitchelleae (Razafimandimbison et al. 2008) and Schizocoleeae (Rydin et al. 2009) or placed in a reestablished tribe Prismatomerideae. In our study *Schizocolea* is treated as an unplaced genus, and the other genera are discussed under Morindeae, in which they were earlier classified. At the base of the subfamily Rubioideae, there is an unresolved node of three branches: Ophiorrhizeae, Urophylleae, and a clade comprising Lasiantheae plus the rest of the subfamily. In the latter clade (fig. 5), Coussareeae are sister to two main branches, the Psychotrieae alliance (Craterispermeae, Gaertnereae + Schradereae, Morindeae, Psychotrieae, *Schizoclea*, *Mitchella* + *Damnacanthus*, *Prismatomeris*) and the Spermacoceae alliance. In the latter (figs. 5, 6), Danaideae are sister to the rest, which are grouped in two clades; one includes Knoxieae and Spermacoceae, and the other is a clade of tribes forming a grade, with Anthospermeae diverging first, followed by Argostemmateae, Paederieae, Putorieae, Rubieae, and Theligoneae.

Although the scaled-down data set was used mainly for estimating divergence times, we checked the consensus trees resulting from the Beast analysis and the MrBayes analysis of the scaled-down data set for any supported discrepancies. We found no well-supported (PP ≥ 0.95) topological conflicts between any of the analyses of the scaled-down data set and the full data set, although the Beast analysis in particular had less resolution in some areas of the tree. We did not find any well-supported differences between the MrBayes and the Beast analyses of the scaled-down data set (not shown).

Divergence Time Estimation

The likelihood ratio tests for the scaled-down data set rejected evolution under rate constancy with a wide margin ($P \ll 0.001$ for all three trees tested). The χ^2 statistic was 10 times higher than the critical level for $P = 0.001$.

The results from the three separate Beast dating runs were similar when checked in the Tracer utility, although their levels of burn-in differed. The burn-in was determined by checking the lnL level as traced by Tracer. We removed 50%, 10%, and 30% of the sampled trees as burn-in and pooled the remaining sampled trees into one joint tree file (210,000 trees). This joint tree file was subsequently input to Tree-Annotator, and the estimated divergence time results are presented in figure 8, with levels of uncertainty in table 1.

The dating analysis (fig. 8; table 1) resulted in an estimated lineage (stem) divergence time for Rubiaceae of 90.4 Ma. The estimated lineage divergence times for the subfamilies were 84.4 (86.6) Ma for Rubioideae, 73.1 Ma for Ixoroideae, and 73.1 Ma for Cinchonoideae. The estimated lineage divergence times for the tribes of Rubioideae vary between 77.9 and 28.6 Ma, between 59.6 and 14.2 Ma for the tribes of Ixoroideae, and between 34.7 and 19.7 Ma for the tribes of Cinchonoideae. The two tribes Coptosapelteae and Luculieae that were placed unresolved at the base of the tree and also unplaced to subfamily have estimated lineage divergence times of 86.6 and 80.3 Ma, respectively.

Discussion

Very big data sets are difficult and time-consuming to analyze. Although the Bayesian approach to phylogeny estimation scales well when number of taxa are increased, obtaining good results can still be problematical when dealing with data sets as large as our full data set. Initial trials using the default random starting trees in MrBayes indicated that it would be difficult to get convergence for our large data set. MrBayes has the possibility of using a "usertree" that the MCMC chain uses as a kind of topological prior instead of a random topology (which is the default) when the run starts. We received much more consistent results using this approach, and the runs reached stationarity much faster. Analyses of this size (537 taxa and 9420 characters) take a long time to run, even in MrBayes, and although our chains ran for 16 million generations, the difference between them (as measured by average standard deviation of split frequencies) did not go below 0.05. Also, even though each run seemed to reach "stationarity" after ~1 million generations and remained at this level for the rest of the run, the level was not exactly the same for the separate runs.

ISE

Isertia hypoleuca
Isertia laevis
1.00 1.00 *Isertia pittieri*

Adina pilulifera
1.00 *Pertusadina hainanensis*
1.00 *Adinauclea fagifolia*
Metadina trichotoma
1.00 *Haldina cordifolia*
0.96 *Pertusadina euryncha*
Sinoadina racemosa
Ludekia borneensis
Myrmeconauclea strigosa
1.00 *Ochreinauclea mangayi*
Neonauclea forsterii
Breonadia salicina
0.95 *Janotia macrostipula*
0.97 *Gyrostipula comoriensis*
1.00 *Breonia chinensis*

NAU

1.00

Burttdavya nyasica
1.00 *Sarcocephalus latifolius*
Nauclea orientalis
1.00 *Nauclea subdita*
1.00 *Neolamarckia cadamba*
Uncaria africana
Uncaria rhynchophylla
Corynanthe mayumbensis
1.00 *Corynanthe pachyceras*
1.00 *Pausinystalia johimbe*
Pausinystalia lane
Mitragyna rubrostipulata
1.00 *Mitragyna rotundifolia*
Mitragyna speciosa
Cephalanthus glabratus
1.00 *Cephalantus occidentalis*
Cephalanthus natalensis

HYM *Paracorynanthe antakanrana*
1.00 *Hymenodictyon floribundum*

1.00

Chione sylvicola
Cosmibuena grandiflora
HIL 1.00 *Cosmibuena valerii*
1.00 *Hillia parasitica*
1.00 *Hillia triflora*
1.00 *Deppea blumenaviensis*
HAM *Deppea grandiflora*
Hamelia sp
1.00 *Hamelia papillosa*
1.00 *Hamelia cuprea*
Hoffmannia ghiesbreghtii x refulgens
1.00 *Omiltemia filisepala*

Cinchonoideae

1.00

Acrosysanthus sp
Mazaea phialanthoides
Mazaea shaferi
1.00 *Phyllomelia coronata*
Rhachicallis americana
RON *Roigella correifolia*
1.00 0.98 *Rondeletia intermixta*
1.00 *Rondeletia portoricensis*
Rondeletia odorata
Suberanthus brachycarpum
Rogiera suffrutescens

1.00

Allenanthus hondurensis
Neoblakea venezuelensis
Antirhea lucida
Bobea elatior
Hodgkinsonia ovatiflora
Guettarda crispiflora
0.98 *Timonius sp*
Timonius timon
GUE *Antirhea megacarpa*
0.95 *Chomelia sp*
0.91 *Guettarda sp*
Guettarda uruguensis
Arachnothryx fosbergii
Gonzalagunia affinis
Javorkaea hondurensis
Malanea sp
Machaonia sp

CIN *Cinchona pubescens*
1.00 *Ladenbergia pavonii*

Catesbaea fuertesii
Catesbaea spinosa
0.97 *Phyllacanthus grisebachianus*
Portlandia plaiantha
0.96 *Cubanola domingensis*
0.93 *Isidorea cf brachyantha*
Coutarea hexandra
Ceratopyxis verbenacea
0.97 *Phialanthus ellipticus*
1.00 *Schmidtottia*
Chiococca alba
Scolosanthus lucidus
0.94 *Badusa corymbifera*
CHI 1.00 *Erithalis fruticosa*
Bikkia tetrandra
Siemensia pendula
Exostema ellipticum
Exostema salicifolium
Exostema nitens
0.96 *Exostema spinosum*
Exostema parviflorum
Strumpfia maritima

1.00

Phylogeny and Dating the Rubiaceae

The following part of the discussion is structured according to the resulting phylogeny of the family (figs. 1–7), starting with the family in general and followed by three main sections corresponding to the three subfamilies. Under each subfamily there are descriptions of each investigated tribe; these are discussed from the bottom to the top according to figures 7 and 8. These descriptions include information about phylogeny, geographic distribution, and the age and dating of the group (fig. 8; table 1).

This Bayesian analysis is so far the most taxon-rich phylogenetic analysis of Rubiaceae, including 534 taxa. A large super-tree of the family was also assembled by Robbrecht and Manen (2006). Our trees (figs. 1–7) have many similarities to their trees but also many different positions of tribes, e.g., that of Alberteae, Gaertnereae, Danaideae, Octotropideae, and Pavettteae. For some parts they also present more resolution between tribes, e.g., in the Cinchonoideae and in the Psychotrieae alliance. However, the tree and relationships Robbrecht and Manen (2006) compiled are without support values, while our analysis presents only strongly supported relationships (PP ≥ 0.95). Hence, we find no reason to compare our results in more detail with their supertree. Our phylogenetic tree (figs. 1–6) is highly resolved, with many strongly supported nodes but also many weakly supported ones. There is strong support for the three subfamilies and most of the tribes, as illustrated in figure 7 (with PP = 0.95–1.0). Most of our phylogenetic results are in agreement with earlier, more restricted studies of the family, but here we have more taxa and stronger support for most groups.

In the first phylogenetic analyses of Rubiaceae (Bremer and Jansen 1991; Ehrendorfer et al. 1994; Bremer et al. 1995; Rova et al. 2002), many new relationships were proposed that disagreed with earlier classifications (Bremekamp 1954, 1966; Verdcourt 1958; Bridson and Verdcourt 1988; Robbrecht 1988). Bremer et al.'s (1995) study included Rubiaceae and many outgroups from Gentianales as well as Oleaceae; Rubiaceae were found to be a sister group to the rest of Gentianales (congruent with a study of Asteridae by Olmstead et al. [1993]). Further, the family was classified into three subfamilies, Rubioideae, Cinchonoideae, and Ixoroideae (Bremer et al. 1995, 1999), as in the present investigation, and the genera *Luculia* and, in some cases, *Coptosapelta* were unresolved at the base of the family. There is now very strong support for three large subclades corresponding to the subfamilies Rubioideae, Ixoroideae, and Cinchonoideae (fig. 7). However, some authors prefer to accept just two subfamilies, Cinchonoideae (including former Ixoroideae) and Rubioideae (Robbrecht and Manen 2006). The basalmost node in the family is still unresolved, with four branches. We do not know exactly how the tribes Luculieae and Coptosapelteae are related to the other subfamilies (Rydin et al. 2009). The oldest-known fossil for which there is convincing evidence for a membership in Rubiaceae is *Paleorubiaceophyllum eocenicum* (Berry) Roth

and Dilcher (1979), from the middle Eocene (49–37 Ma). Rubiaceae have been proposed to be much older than this by molecular studies of angiosperms calibrated with fossils outside of Rubiaceae. In these studies the divergence time of the family (the stem age) has been estimated to be 61–64 Ma (Wikström et al. 2001) or 78 Ma (Bremer et al. 2004). In our study the estimated stem age of the family is 90.4 Myr (table 1), but the focus is not on dating the age of the family per se but on dating the different branches of the family. Here, we have used the age of 78 Myr as the mean of the prior estimate for the stem node of Rubiaceae (Bremer et al. 2004) because that study is based on several fossils and a large set of asterids, the angiosperm group to which Rubiaceae belong, and also because the fossils used (as calibration points) are from within the asterids. The prior age of the crown node of the family (=the age of the fossil *Paleorubiaceophyllum*) was set to at least 45 Myr. However, our estimated age of the crown node of Rubiaceae was much older, 86.6 Myr, and without the root prior, the age of the Rubiaceae crown node would have been estimated at ~200 Myr. It is difficult to estimate the age of a diversification in a lineage (the crown group) because some or all of the taxa spanning the crown node (the "basal" taxa in a clade) may not have been sampled or they are extinct. Our sampling is obviously not complete, and the crown node age estimates should therefore be considered tentative. In the discussion that follows, we focus on the age estimate for the lineages (stem ages). However, estimated crown ages are also reported in table 1.

Coptosapelteae and Luculieae

The relationships of Coptosapelteae and Luculieae to each other and to the other subfamilies are not understood; in this analysis Coptosapelteae (*Coptosapelta* and *Acranthera*) and Luculieae (*Luculia*) are placed unresolved at the base of the family (figs. 1, 7), outside the three subfamilies, similar to earlier studies (Bremer et al. 1999). In other studies these have been placed as sister taxa (Robbrecht and Manen 2006; Rydin et al. 2009) but with low support. There are no obvious morphological characters supporting these taxa as a monophyletic group; e.g., they have different fruits and corolla aestivations. However, they all have a Southeast Asian distribution. The estimated divergence time (stem age) of Coptosapelteae is 86.6 Ma, and the estimated divergence time of Luculieae is 80.3 Ma (fig. 8).

Subfamily Cinchonoideae

The Cinchonoideae are the smallest subfamily, with ~1500 species. Plants are mostly small trees or shrubs, with imbricate or valvate corolla aestivation (several exceptions occur, e.g., right-contorted in Hamelieae and Hillieae). The subfamily includes nine well-supported clades corresponding to nine tribes

Fig. 2 The 90% majority-rule consensus tree of the Cinchonoideae portion of the tree from the Bayesian analysis. Clade posterior probabilities are indicated below branches. This is a continuation of fig. 1. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *ISE*, Isertieae; *NAU*, Naucleeae; *HIL*, Hillieae; *HAM*, Hamelieae; *RON*, Rondeletieae; *GUE*, Guettardeae; *CIN*, Cinchoneae; *CHI*, Chiococceae.

Ixoroideae cont. [fig. 4]

1.00

1.00

1.00

Burchellia bubalina
Didymosalpinx norae
Coffea arabica
1.00 *Paracoffea melanocarpa*
Coffea buxifolia
1.00 *Psilanthus ebracteolatus*
COF *Psilanthus mannii*
1.00 1.00 *Tricalysia cryptocalyx*
1.00 BER *Bertiera breviflora*
1.00 *Bertiera guianensis*
ALB *Alberta magna*
Augusta rivalis
Augusta longifolia
1.00 *Augusta rivalis*
Crossopteryx febrifuga
Wendlandia thyrsoidea

0.99

1.00

1.00

1.00

VAN *Canthium coromandelicum*
Cantium inerme
Fadogia tetraquetra
Vangueria lasiantha
1.00 1.00 *Vangueria madagascariensis*
Tapiphyllum velutinum
1.00 *Meyna tetraphylla*
Plectroniella armata
Keetia gueinzii
Peponidium spnov
Pyrostria bibracteata
Pyrostria hystrix
Psydrax livida
Keetia zanzibarica

0.98

1.00

IXO *Aleisanthia rupestris*
Aleisanthiopsis distantiflora
Greenea corymbosa
Ixora biflora
Ixora coccinea
0.99 *Ixora hookeri*
0.94 *Ixora parviflora*
Myonima violacea
Versteegia grandifolia

Scyphiphora hydrophyllacea

Boholia nematostylis
RET *Retiniphyllum pilosum*
Steenisia pleurocarpa

1.00

MUS *Mussaenda uniflora*
1.00 *Mussaenda arcuata*
Mussaenda erythrophylla
0.97 1.00 *Mussaenda scratchleyi*
1.00 *Pseudomussaenda flava*
Bremeria erectiloba
1.00 *Schizomussaenda dehiscens*
Heinsia crinita

SAB *Pseudosabicea arborea*
Sabicea diversifolia
Sabicea villosa
Sabicea ceylanica
Pseudosabicea floribunda
1.00 *Pseudosabicea nobilis*
Sabicea calycina
Tamridaea capsulifera
Virectaria major
0.99 *Hekistocarpa minutiflora*

SIP *Maguireothamnus speciosus*
1.00 *Neobertiera gracilis*
Sipaneopsis rupicola
1.00 1.00 *Sipanea biflora*
1.00 POS *Molopanthera paniculata*
Posoqueria latifolia
1.00 1.00 *Gleasonia prancei*

Capirona decorticans
0.93 *Dolichodelphys chlorocrater*
Wittmackanthus stanleyanus
Alseis lugonis
Bathysa peruviana
Condaminea corymbosa
Simira tinctoria
Warszewiczia cordata
0.90 *Chimarrhis hookeri*
Calycophyllum candidissimum
Dioicodendron
Hintonia latiflora
Parachimarrhis breviloba
Picardaea haitiensis
Pogonopus speciosus
CON *Rustia splendens*
1.00 *Hippotis scarlatina*
Sommera sabiceoides
Dialypetalanthus fuscescens
Simira viridiflora
Hippotis sp
Pentagonia macrophylla
Sommera sabiceoides
Dolicholobium oxylobum
Emmenopterys henryi
Mussaendopsis beccariana
Macbrideina peruviana
Pinckneya pubens
Ferdinandusa speciosa

Ixoroideae
1.00

in our analysis (figs. 2, 7). Seven of these have their main distribution in the New World, from North America and the West Indies to Central and South America. Only Naucleeae and Hymenodictyeae are mainly paleotropic. In our analysis most interrelationships among the tribes are unresolved, but there is support for three pairs of sister taxa in the subfamily: Hamelieae + Hillieae, Guettardeae + Rondeletieae, and Hymenodictyeae + Naucleeae. These three sister clades, together with the three clades of Chiococceae, Cinchoneae, and Isertieae, are all unresolved in a polytomy at the base of the subfamily. So far, no study has explicitly been focused on the entire subfamily Cinchonoideae, but two studies on the whole family (Bremer et al. 1995; Rova et al. 2002) or specific groups (Bremer and Thulin 1998; Razafimandimbison and Bremer 2002; Andersson and Antonelli 2005; Motley et al. 2005) have contributed to phylogenetic knowledge of the subfamily. From these, there is support for the same nine tribes in our analysis. The oldest certain fossil from the subfamily Cinchonoideae is *Cephalanthus pusillus* Friis (1985) from the Middle Miocene (16.0–11.6 Ma). In our analysis the subfamily is estimated to be much older; the divergence time of the Cinchonoideae lineage is estimated to be 73.1 Ma, and the crown age is estimated to be 38.7 Myr. The crown age, when the subfamily radiates, is unexpectedly young compared with the crown age of its sister group, Ixoroideae, which is 59.6 Myr (fig. 8).

Chiococceae are a mainly tropical American tribe, with most taxa in the West Indies, but a few genera are Pacific, with *Morierina* in New Caledonia; *Badusa* in Palawan, New Guinea, and the West Pacific; and *Bikkia* from East Malesia to the West Pacific. The genera *Badusa*, *Bikkia*, *Catesbaea*, *Ceratopyxis*, *Chiococca*, *Coutarea*, *Cubanola*, *Erithalis*, *Exostema*, *Hintonia*, *Isidoria*, *Phialanthus*, *Phyllacanthus*, *Portlandia*, *Schmidtottia*, *Scolosanthus*, *Siemensia*, and also *Strumpfia* are included in our analysis (fig. 2, 7), and we found strong support for Chiococceae sensu lato (1.0), congruent with earlier studies (Bremer et al. 1995; Andersson and Rova 1999; Rova et al. 2002), and the two tribes, Chiococceae and Catesbaeeae, earlier proposed by Delprete (1996) are not supported. Motley et al. (2005), in their comprehensive study of the tribe (although they called it the Catesbaeeae-Chiococceae complex), left *Strumpfia* outside the complex because of the anther and ovule structure. However, our results imply that the stamens inserted at the base of the corolla, a character state that is rare in the family, are a synapomorphy for *Strumpfia* and Chiococceae, and hence we do not hesitate to include it in the tribe, even if *Strumpfia* has many unique morphological characters (Igersheim 1993). The estimated divergence time of Chiococceae is 34.4 Ma (fig. 8).

Cinchoneae are a tropical American tribe with most taxa in the Andes. However, *Cinchona* has been introduced into many tropical countries around the world. We included only two genera (fig. 2), *Cinchona* and *Ladenbergia*, which are sister taxa (1.0). Andersson and Antonelli (2005) included five more genera in their study (*Cinchonopsis*, *Joosia*, *Remijia*, *Stilpnophyllum*,

and a new genus, *Ciliosemina*). The estimated divergence time of the tribe is 34.2 Ma (fig. 8).

One species of the tribe Guettardeae is a pantropical seashore plant (*Guettarda speciosa*), but most other species occur in Southeast Asia, on islands in the Pacific Ocean, and in the Neotropics. Guettardeae and Rondeletieae are strongly supported as sister taxa. In our analysis, Guettardeae include 14 genera (*Allenanthus*, *Anthirhea*, *Arachnothryx*, *Bobea*, *Chomelia*, *Gonzalagunia*, *Guettarda*, *Hodgkinsonia*, *Javorkaea*, *Machaonia*, *Malanea*, *Neoblakea*, *Stenostomum*, *Timonius*), but the relationships of the genera are poorly resolved, except for a clade joining *Guettarda crispifolia* with two specimens of *Timonius* (fig. 2). The split between Guettardeae and Rondeletieae and the estimated divergence time of Guettardeae are at 27.5 Ma (fig. 8).

Rondeletieae occur in Central America, with a predominant Antillean distribution. In our analysis (figs. 2, 7), with limited sampling within genera, Rondeletieae and Guettardeae are strongly supported as sister groups, and Rondeletieae are strongly supported as a clade including eight genera (*Rondeletia*, *Acrosynanthus*, *Mazaea*, *Phyllomelia*, *Rachicallis*, *Rogiera*, *Roigella*, and *Suberanthus*), much in agreement with earlier studies (Rova et al. 2002, 2009). The estimated divergence time of Rondeletieae is 27.5 Ma (fig. 8).

The tribe Hamelieae has an American (mainly Central American) distribution. The tribe has never been the focus of molecular study, but a few species have been investigated as representatives of the tribe (Bremer et al. 1995; Andersson and Rova 1999). We included four of the five genera of the tribe (fig. 2): *Deppea*, *Hamelia*, *Hoffmannia*, and *Omiltemia* (*Pinarophyllon* was not included). Of these, *Hamelia* is monophyletic, with three species analyzed, and *Deppea*, with two species, is unresolved. The tribe is well supported (1.0), with the sister relationship to tribe Hillieae (1.0). The estimated divergence time of the Hamelieae is 18.7 Ma (fig. 8).

The tribe Hillieae is geographically distributed in the Neotropics. Hillieae include two genera, *Hillia* and *Cosmibuena*, and both have recently been revised (Taylor 1992, 1994). Our analysis, albeit with very few species investigated, supports a split between these genera. Our analysis (figs. 2, 7) also strongly supports the sister relationship between Hillieae and Hamelieae, which is also further supported by other data, e.g., the occurrence of raphides (the only two tribes of Cinchonoideae with this trait), bird pollination, and right-contorted corolla aestivation. The estimated divergence time of Hillieae is 18.7 Ma (fig. 8).

The genus *Chione* is sister to the two tribes Hamelieae and Hillieae (fig. 2). The molecular support for this is strong (1.0), but we know of no obvious morphological support for this relationship. *Chione* has imbricate corolla aestivation and drupaceous fruits, whereas Hamelieae and Hillieae generally have right-contorted corolla aestivation and baccate or capsular fruits; at least *Chione venosa* also seems to lack raphides (B. Bremer, personal observation). However, *Chione* shares a

Fig. 3 The 90% majority-rule consensus tree of the Ixoroideae portion of the tree from the Bayesian analysis. Clade posterior probabilities are indicated below branches. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *COF*, Coffeeae; *BER*, Bertiereae; *ALB*, Alberteae; *VAN*, Vanguerieae; *IXO*, Ixoreae; *RET*, Retiniphylleae; *MUS*, Mussaendeae; *SAB*, Sabiceeae; *SIP*, Sipaneeae; *POS*, Posoquerieae; *CON*, Condamineeae. One subclade, labeled "Ixoroideae cont.," is shown in fig. 4.
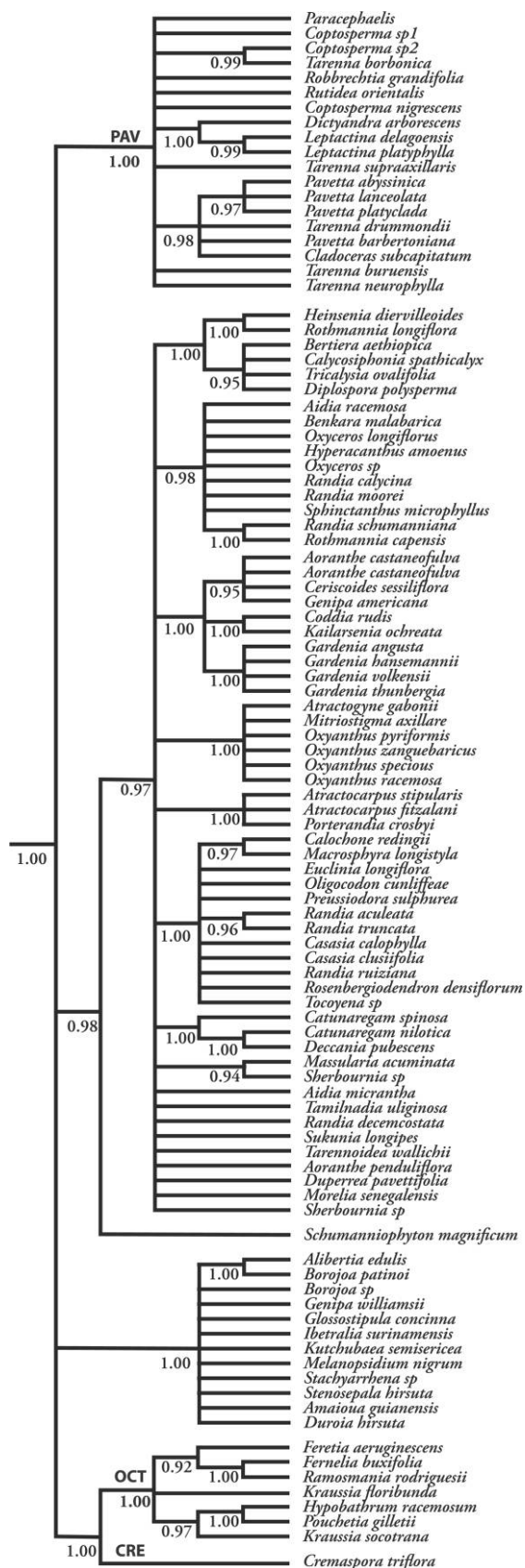
Neotropical distribution (i.e., Central America and the West Indies) with Hamelieae and Hillieae.

Hymenodictyeae include two genera, *Paracorynanthe* (two species) and *Hymenodictyon* (22 species), distributed in Madagascar and in Madagascar, mainland Africa, and tropical Asia, respectively (Razafimandimbison and Bremer 2001). Both genera are included in our study and form a strongly supported monophyletic group (fig. 2). The sister group relationship to Naucleeae is also strongly supported (fig. 7; as also shown by Razafimandimbison and Bremer [2002]). The split between Hymenodictyeae and Naucleeae and the estimated divergence time of Hymenodictyeae are at 19.7 Ma (fig. 8). Interestingly, this tribe diversified or radiated much later than its sister group Naucleeae. The estimated diversification age (crown age) of Hymenodictyeae is 3.6 Myr, compared with the diversification age (crown age) of Naucleeae, which is 16.0 Ma.

The main geographical distribution of the tribe Naucleeae is paleotropical, with most species (134; Razafimandimbison and Bremer 2002) in Southeast Asia, followed by Madagascar (24), mainland Africa (22), and America (5). Our result is in full agreement with that of Razafimandimbison and Bremer (2002). In this analysis 22 of 25 accepted genera are included (fig. 2). The tribe is strongly supported (1.0), and a number of the earlier redefined subtribes (Razafimandimbison and Bremer 2002) are also well supported. In our analysis the tribe includes the genera *Adina*, *Adinauclea*, *Breonadia*, *Breonia*, *Burttdavya*, *Cephalanthus*, *Gyrostipula*, *Haldina*, *Janotia*, *Ludekia*, *Metadina*, *Mitragyna*, *Myrmeconauclea*, *Nauclea*, *Neolamarckia*, *Neonauclea*, *Ochreinauclea*, *Pausinystalia*, *Pertusadina*, *Sarcocephalus*, *Sinoadina*, and *Uncaria*. The fossil from the subfamily Cinchonoideae, *Cephalanthus pusillus* Friis (1985), belongs to the genus *Cephalanthus* of this tribe. Hence, the minimum age of the tribe is Middle Miocene (16.0–11.6 Ma). In our dating analysis the estimated divergence time of the tribe is 19.7 Ma (fig. 8). The prior age of the crown node of Naucleeae (=stem node of *Cephalanthus*) was set to at least 14 Myr. It was estimated to be 16 Myr.

With the present bigeneric circumscription (restricted to *Isertia* [incl. *Yutajea*] and *Kerianthera*), the tribe Isertieae is restricted to tropical America (Bremer and Thulin 1998). In our analysis (fig. 2) we investigated three *Isertia* species, and the genus was monophyletic (1.0). The estimated divergence time of the tribe is 34.7 Ma (fig. 8).

Subfamily Ixoroideae

The Ixoroideae are trees or shrubs; corolla aestivation varies in the subfamily, but most tribes have left-contorted or valvate aestivation (e.g., Vanguerieae). Many tribes have secondary pollen presentation (e.g., Alberteae, Gardenieae, Ixoreae, Octotropideae, Pavetteae, Retinophylleae, Vanguerieae).

Fig. 4 The 90% majority-rule consensus tree of a subclade of Ixoroideae from the tree from Bayesian analysis. This is a continuation of Ixoroideae as shown in fig. 3. Clade posterior probabilities are indicated below branches. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *PAV*, Pavetteae; *OCT*, Octotropideae; *CRE*, Cremasporeae.

The subfamily has a pantropical and pansubtropical distribution. The subfamily was the subject of earlier molecular investigations (Andreasen and Bremer 1996, 2000; Andreasen et al. 1999). These studies found that the Vanguerieae are part of the subfamily; the Octotropideae, Pavetteae, and Coffeeae were found to be monophyletic, but the latter two have different circumscriptions compared with earlier classifications. *Ixora* (together with *Myonima* and *Versteegia*) is not part of Pavetteae. Further, Coffeeae include *Tricalysia* and probably also *Bertiera*. Subtribe Diplosporineae (*Cremaspora* and *Tricalycia*) and *Posoqueria* were excluded from the tribe Gardenieae. Robbrecht and Puff (1986) suggested that the informal "tetrad group" within Gardenieae is not monophyletic. Alberteae (*Alberta*) was shown to be part of the subfamily, and the mangrove genus *Scyphiphora* was shown to be close to Ixoreae. Several previous Cinchonoideae tribes have also been found to be closer to or within Ixoroideae: Condamineeae (Bremer et al. 1995), Mussaendeae (Bremer and Thulin 1998), Sabiceeae (Bremer and Thulin 1998), Retiniphylleae (Rova et al. 2002), and Sipaneeae (Rova et al. 2002). In this analysis (fig. 7), 11 Ixoroideae clades, corresponding to 11 of 15 recognized tribes, were monophyletic. Three tribes are represented by single taxa (Alberteae, Cremasporeae, Retiniphylleae), and most of the remaining taxa do not form a monophyletic group, but for convenience we will use the name Gardenieae for them here. The subfamily is unresolved at the base (fig. 7) with a polytomy of Condamineeae, Posoquerieae + Sipaneeae, Sabiceeae, and a clade including the rest of the subfamily. In the latter, Mussaendeae are the first branch to split off, followed by Retiniphylleae, which are sister to Ixoreae + Vanguerieae, and a clade including Alberteae and their sister group, including Bertiereae + Coffeeae, and an unresolved clade including Cremasporeae + Octotropideae, Gardenieae, and Pavetteae. The oldest certain fossil from the subfamily is *Scyphiphora* (Leopold 1969; Saenger 1998), from different layers of the Miocene sediments. The oldest is ~23 Myr and was found on the Marshall Islands. The Ixoroideae are estimated to be much older in our dating analysis; the divergence time is estimated to be 73.1 Ma, and the crown age is estimated to be 59.6 Myr (fig. 8).

Most genera of the tribe Condamineeae occur in South or Central America, but a few occur in South Asia (*Emmenopterys*, *Mussaendopsis*), Africa (*Simira* pp.), or Fiji (*Dolicholobium*). The circumscription of Condamineeae, as well as their rank, has differed markedly between classifications (Bremekamp 1954; Verdcourt 1958; Robbrecht 1988; Delprete 1999). In our analysis the tribe is well supported (1.0), but relationships among the 24 genera included here are poorly resolved (fig. 3). The estimated divergence time of Condamineeae is 58.9 Ma (fig. 8).

Posoquerieae were recently erected by Delprete (in Delprete et al. 2004) for the two genera *Posoqueria* and *Molopanthera*, which were earlier placed in Gardenieae and Cinchonoideae, respectively. The tribe is restricted to tropical America (with *Molopanthera* only in Brazil). In our analysis, these two genera are sister to *Gleasonia*, another tropical American taxon usually included in Henriquezieae, a tribe of uncertain status because the type genus has not yet been included in a phylogenetic study. The three genera together are sister taxa to tribe Sipaneeae (figs. 3, 7), and this relationship is well supported (1.0). The estimated divergence time of Posoquerieae is 28.7 Ma (fig. 8).

Sipaneeae are geographically restricted to the Guayana and Brazilian shields and Central America (Delprete and Cortes-B 2004). In our phylogenetic analysis only four genera were included (*Sipanea*, *Maguireothamnus*, *Neobertiera*, *Sipaneopsis*), but our tree (fig. 3) is congruent with those of earlier studies (Rova et al. 2002; Delprete and Cortes-B 2004). The estimated divergence time of Sipaneeae is 46.5 Ma (fig. 8).

Sabiceeae are pantropical in distribution. Most of the ~150 *Sabicea* species occur in mainland Africa or in the Neotropics, and the remaining genera are restricted to Africa; the monotypic genera *Hekistocarpa* and *Tamridaea* are endemic to Cameroon and Nigeria and to Socotra (Yemen), respectively, and *Virectaria*, with eight species, occurs in tropical West Africa (Khan et al. 2008). In our study the tribe was monophyletic (1.0), in agreement with Khan et al. (2008), but relationships among the four genera were not strongly supported, except for a clade joining the single accessions of *Hekistocarpa*, *Tamridaea*, and *Virectaria* (fig. 3). The estimated divergence time is 59.6 Ma (fig. 8).

Mussaendeae (Alejandro et al. 2005; Bremer and Thulin 1998) are distributed in tropical Africa and Asia. We investigated representatives from five of the six genera (*Bremeria*, *Heinsia*, *Mussaenda*, *Pseudomussaenda*, *Schizomussaenda*), and results (fig. 3) were congruent with those of Alejandro et al. (2005). The estimated divergence time is 54.9 Ma (fig. 8).
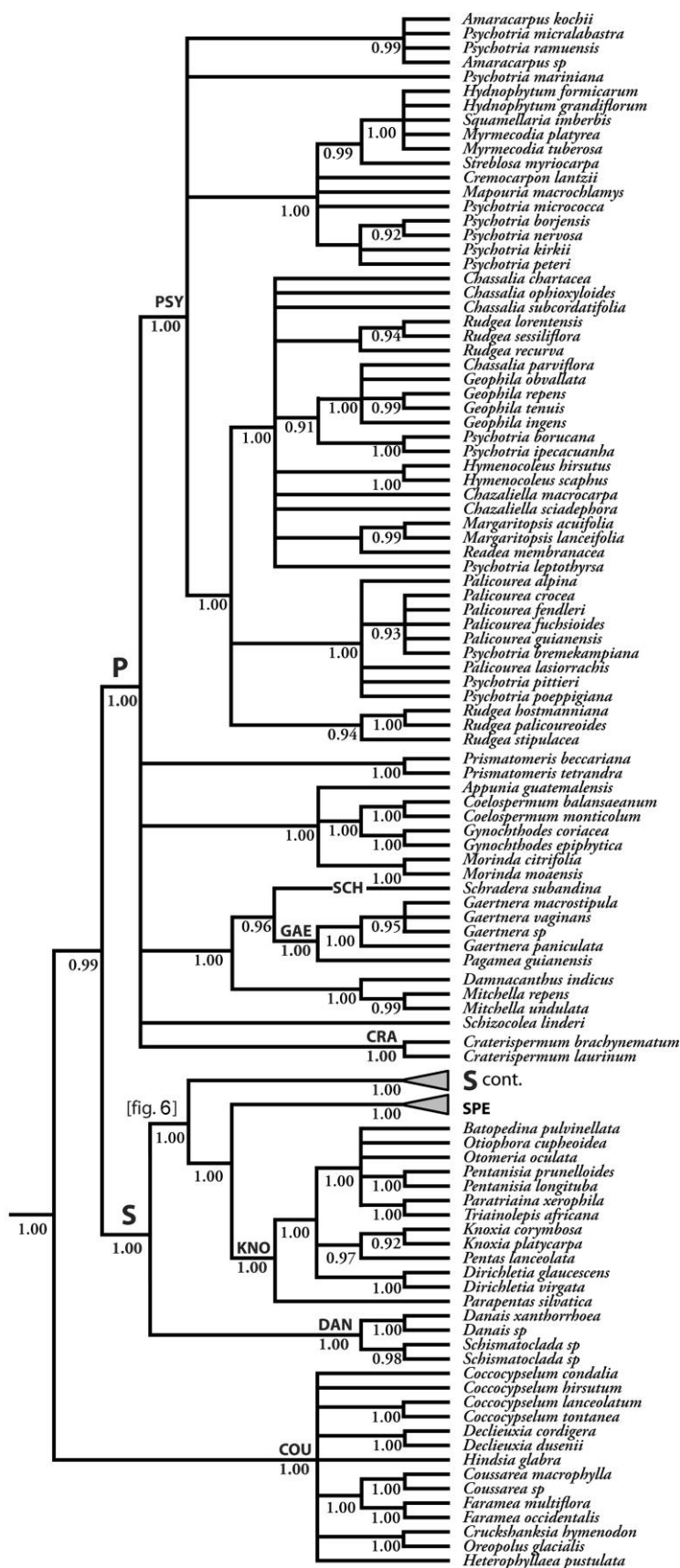
The tribe Retiniphylleae is monogeneric, with geographical distribution in tropical South America. Another genus, *Botryarrhena*, from Brazil and Venezuela, was believed to be close to *Retiniphyllum* when it was described (Ducke 1933). However, this genus has not been included in phylogenetic work and remains to be tested. The estimated divergence time of Retiniphylleae is 44.7 Ma (fig. 8). In our analysis (fig. 3), *Retinophyllum* falls in a grade of taxa; the other two belong to *Steenisia* and *Boholia*.

Ixoreae have a tropical distribution, with most taxa in Asia. Our analysis also supports inclusion of the Asian genera *Greenea*, *Aleisanthia*, and *Aleisanthiopsis* in the tribe, a result first shown by Rova et al. (2002). Of the other Ixoreae taxa, we sampled only *Ixora*, *Myonima*, and *Versteegia*, and our analysis (fig. 3) did not resolve relationships within the tribe, perhaps because of sparse sampling. The estimated divergence time of Ixoreae is 29.0 Ma (fig. 8).

Vanguerieae have a paleotropical distribution (Verdcourt and Bridson 1991). The Vanguerieae are strongly supported as monophyletic (1.0). The sister group to Vanguerieae is Ixoreae (fig. 7), and these together are sister to the genus *Scyphiphora*, unclassified to tribe. We investigated only 14 species (fig. 3), representing nine genera, and we could not clearly identify any subgroups (as earlier identified in Lantz et al. 2002; Lantz and Bremer 2004, 2005), probably because of the small sample. The estimated divergence time of the tribe is 29.0 Ma (fig. 8). For the dating analysis, we set a prior age of 23 Myr on the stem node of *Scyphiphora*. This node was estimated to be considerably older in the analysis, 31.6 Myr.

The following tribes of Ixoroideae, Alberteae, Bertiereae + Coffeeae, Cremasporeae + Octotropideae, Gardenieae, and Pavetteae form a strongly supported clade (fig. 7). A similar set of taxa, but including Ixoreae, was earlier referred to as "Ixoroideae s.str." (Andreasen 1997; Andreasen and Bremer 2000; Rova et al. 2002). However, because it has been shown that the

Amaracarpus kochii
Psychotria micralabastra
Psychotria ramuensis
Amaracarpus sp
Psychotria mariniana
Hydnophytum formicarum
Hydnophytum grandiflorum
Squamellaria imberbis
Myrmecodia platyrea
Myrmecodia tuberosa
Streblosa myriocarpa
Cremocarpon lantzii
Mapouria macrochlamys
Psychotria micrococca
Psychotria borjensis
Psychotria nervosa
Psychotria kirkii
Psychotria peteri
Chassalia chartacea
Chassalia ophioxyloides
Chassalia subcordatifolia
Rudgea lorentensis
Rudgea sessiliflora
Rudgea recurva
Chassalia parviflora
Geophila obvallata
Geophila repens
Geophila tenuis
Geophila ingens
Psychotria borucana
Psychotria ipecacuanha
Hymenocoleus hirsutus
Hymenocoleus scaphus
Chazaliella macrocarpa
Chazaliella sciadephora
Margaritopsis acuifolia
Margaritopsis lanceifolia
Readea membranacea
Psychotria leptothyrsa
Palicourea alpina
Palicourea crocea
Palicourea fendleri
Palicourea fuchsioides
Palicourea guianensis
Psychotria bremekampiana
Palicourea lasiorrachis
Psychotria pittieri
Psychotria poeppigiana
Rudgea hostmanniana
Rudgea palicoureoides
Rudgea stipulacea
Prismatomeris beccariana
Prismatomeris tetrandra
Appunia guatemalensis
Coelospermum balansaeanum
Coelospermum monticolum
Gynochthodes coriacea
Gynochthodes epiphytica
Morinda citrifolia
Morinda moaensis
Schradera subandina
Gaertnera macrostipula
Gaertnera vaginans
Gaertnera sp
Gaertnera paniculata
Pagamea guianensis
Damnacanthus indicus
Mitchella repens
Mitchella undulata
Schizocolea linderi
Craterispermum brachynematum
Craterispermum laurinum
S cont.
SPE
Batopedina pulvinellata
Otiophora cupheoidea
Otomeria oculata
Pentanisia prunelloides
Pentanisia longituba
Paratriaina xerophila
Triainolepis africana
Knoxia corymbosa
Knoxia platycarpa
Pentas lanceolata
Dirichletia glaucescens
Dirichletia virgata
Parapentas silvatica
Danais xanthorrhoea
Danais sp
Schismatoclada sp
Schismatoclada sp
Coccocypselum condalia
Coccocypselum hirsutum
Coccocypselum lanceolatum
Coccocypselum tontanea
Declieuxia cordigera
Declieuxia dusenii
Hindsia glabra
Coussarea macrophylla
Coussarea sp
Faramea multiflora
Faramea occidentalis
Cruckshanksia hymenodon
Oreopolus glacialis
Heterophyllaea pustulata

Ixoreae do not belong to this clade (Andreasen 1997; Andreasen and Bremer 2000), using the name Ixoroideae s.str. would be inappropriate unless Ixoreae, Vanguerieae, and their close relatives are included.

The geographical distribution of the tribe Alberteae (including *Alberta* and *Nematostylis*) is in South Africa, southeast Africa, and Madagascar. We included only a single taxon (*Alberta magna*) of the tribe (figs. 3, 7). However, it was found to be placed in an unresolved clade with taxa of uncertain taxonomic positions (*Augusta*, *Crossopteryx*, *Wendlandia*). The estimated divergence time of Alberteae is 22.5 Ma (fig. 8).

Bertiereae are a monogeneric tribe and mainly distributed in Africa but with one species (*Bertiera guianensis*; the types species) widespread in the New World tropics. Bertiereae and Coffeeae (fig. 7) are strongly supported as sister taxa (1.0). In our analysis one of the species, *Bertiera aetiopica* (a sequence from GenBank by Dessein et al. [2001]), is found in the non-monophyletic Gardenieae (fig. 4). Excluding that sequence, the tribe Bertiereae has an estimated divergence time of 14.2 Ma (fig. 8).

Coffeeae are geographically distributed in the Old World, mainly in Africa but also in Indomalaya and Australia. We sampled only four genera (fig. 3) of the tribe (*Coffea*, *Diplospora*, *Psilanthus*, *Tricalysia*), and these were found to constitute a monophyletic tribe Coffeeae (1.0), congruent with the result of Davis et al. (2007), who included 10 genera. However, one accession of *Tricalysia*, as well as one of *Diplospora*, falls in the Gardenieae, close to the *Bertiera* species mentioned above. The estimated divergence time of Coffeeae is 27.3 Ma (fig. 8).

Cremasporeae are distributed in tropical Africa, Comoro Islands, and Madagascar. It is a monogeneric tribe with few species. It is placed as sister to the Octotropideae (fig. 7), as in the study of Andreasen and Bremer (1996). The estimated divergence time of Cremasporeae is 16.7 Ma (fig. 8).

The tribe Octotropideae has an Old World distribution, with genera occurring in tropical Africa, Comoro Islands, Madagascar, Mascarenes, Rodriguez, and Indomalaya (*Octotropis* and *Hypobatrum*). We sampled six genera (*Feretia*, *Fernelia*, *Hypobathrum*, *Kraussia*, *Pouchetia*, *Ramosmania*), and they comprise a strongly supported Octotropideae (fig. 4), which is sister to the tribe Cremasporeae (fig. 7), congruent with earlier results (Andreasen and Bremer 1996, 2000; Davis et al. 2007). The estimated divergence time of Octotropideae is 16.7 Ma (fig. 8).

Gardenieae are not monophyletic, and thus it is not meaningful to discuss phylogeny or geographical distribution in detail. Gardenieae, in a modern sense, were first studied by Robbrecht and Puff (1986), using morphological data. However, several molecular studies have indicated that their circumscription is not monophyletic (Andreasen and Bremer 1996, 2000; Persson 2000*a*, 2000*b*). In our study, including 50 Gardenieae genera, we found very low support (0.8%) for
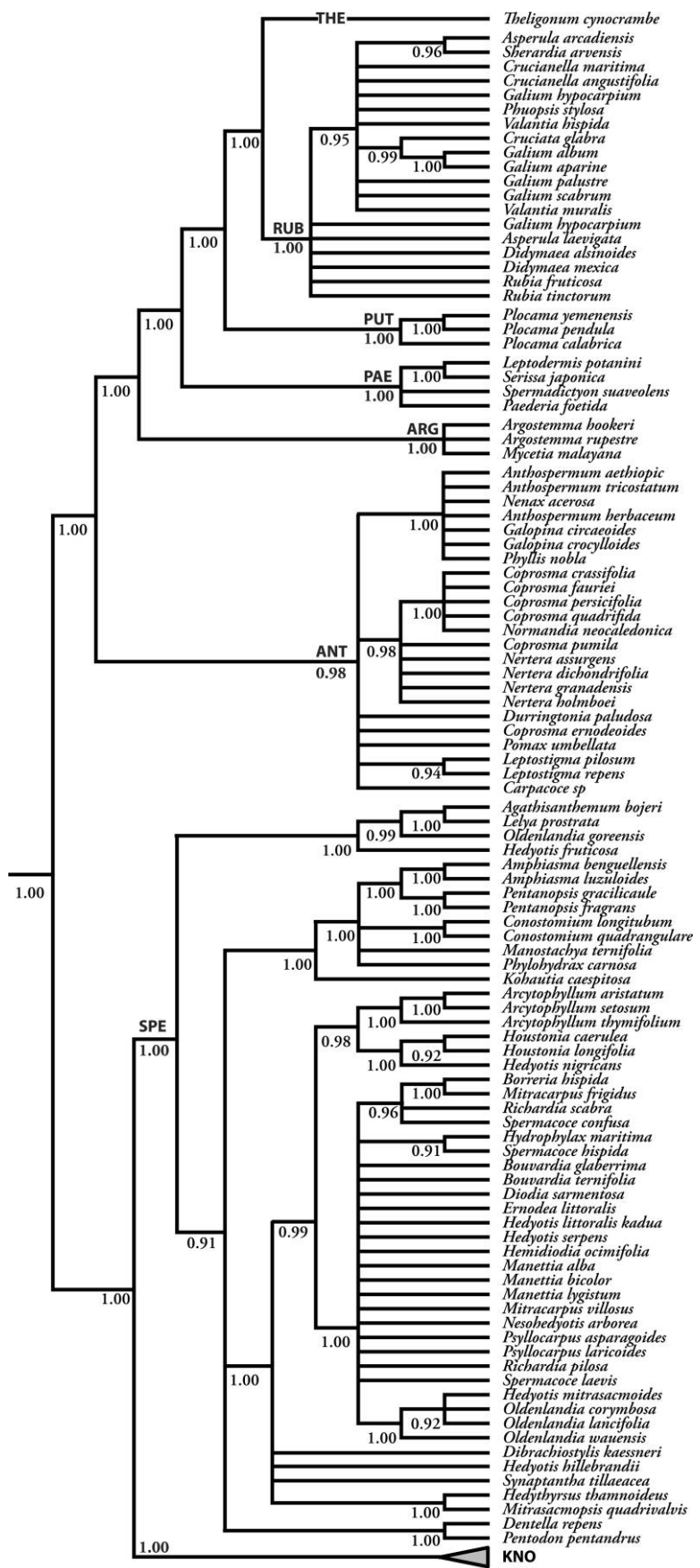
a monophyletic tribe. The "tribe" is mixed among Cremasporeae + Octotropideae and Pavetteae. However, there is support (1.0) for several Gardenieae groups (fig. 4), some of which have been previously identified from molecular data as the "*Alibertia* clade" (here including *Alibertia*, *Amaioua*, *Borojoa*, *Duroia*, *Genipa* p.p., *Glossostipula*, *Ibetralia*, *Kutchubaea*, *Melanopsidium*, *Stachyarrhena*, *Stenosepala*; Andreasen and Bremer 1996; Andreasen 1997; Persson 2000*a*, 2000*b*), the "*Randia* clade" (*Calochone*, *Casasia*, *Euclinia*, *Oligocodon*, *Macrosphyra*, *Preussiodora*, *Randia* p.p., *Rosenbergiodendron*), the "*Gardenia* clade" (*Aoranthe* p.p., *Ceriscoides*, *Coddia*, *Gardenia*, *Genipa*, *Kailarsenia*; Persson 2000*a*), and the "*Aidia* clade" (*Aidia*, *Benkara*, *Hyperacanthus*, *Oxyceros*, *Randia* p.p., *Sphinctanthus*). Because the Gardenieae are not monophyletic, divergence time is not estimated (there is no clade to date).

In Pavetteae, the large genera *Pavetta* and *Tarenna* both have wide Old World tropical distributions, while the other, smaller genera investigated here occur in mainland Africa or Madagascar. The current circumscription of Pavetteae, excluding Ixoreae, was proposed by Andreasen and Bremer (2000). No molecular analysis focusing on the tribe has yet been published. In our analysis the tribe is well supported (1.0), but relationships among taxa are not resolved or supported to the extent that there are indications of para/polyphyletic genera (fig. 4). We included the following genera: *Dictyandra*, *Pavetta*, *Coptosperma*, *Cladoceras* (here sequenced for the first time), *Leptactina*, *Robbrechtia* (here sequenced for the first time), *Paracephaelis* (here sequenced for the first time), *Rutidea*, and *Tarenna*. The estimated divergence time of Pavetteae is 23.9 Ma (fig. 8).

Subfamily Rubioideae

The Rubioideae are characterized as herbs or shrubs and with raphides (calcium oxalate crystals), valvate corolla aestivation, and indumentum of septate (articulated) hairs. The subfamily has a worldwide distribution; most tribes and species are tropical or subtropical, but a few tribes of the Spermacoceae alliance (Anthospermeae, Putorieae, Rubieae, and Theligoneae) also have many species in Mediterranean and temperate regions. A new phylogeny and comprehensive classification of Rubioideae were presented by Bremer and Manen (2000). They analyzed 151 genera and accepted 16 tribes. The tribes Ophiorrhizeae, Urophylleae, Lasiantheae, and Coussareeae form a grade to the rest of the subfamily, which consists of two informal groups: the Psychotrieae alliance (Craterispermeae, Gaertnereae, Morindeae [polyphyletic], Psychotrieae, Schradereae) and the Spermacoceae alliance (Anthospermeae, Argostemmateae, Danaideae, "Paederieae" [paraphyletic], Rubieae, Spemacoceae, Theligoneae). There is support for most tribes and also support for many relationships among these (fig. 7). Rubioideae are probably the most well-understood subfamily in terms of phylogeny, but still only a minority of genera and species

Fig. 5 The 90% majority-rule consensus tree of a part of Rubioideae from the tree from Bayesian analysis. This is a continuation of Rubioideae as shown in fig. 1. Clade posterior probabilities are indicated below branches. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *PSY*, Psychotrieae; *SCH*, Schradereae; *GAE*, Gaertnereae; *CRA*, Craterispermeae; *KNO*, Knoxieae; *DAN*, Danaideae; *COU*, Coussareeae. P, Psychotrieae alliance clade; S, Spermacoceae alliance clade. Two subclades of the latter continue in fig. 6, labeled "S cont." and "SPE." The root of fig. 6 corresponds to the clade marked "fig. 6."

THE — *Theligonum cynocrambe*

0.96

Asperula arcadiensis
Sherardia arvensis
Crucianella maritima
Crucianella angustifolia
Galium hypocarpium
Phuopsis stylosa
Valantia hispida
Cruciata glabra
Galium album
Galium aparine
Galium palustre
Galium scabrum
Valantia muralis
Galium hypocarpium
Asperula laevigata
Didymaea alsinoides
Didymaea mexica
Rubia fruticosa
Rubia tinctorum

RUB 1.00
0.95
0.99
1.00
1.00

1.00

PUT 1.00
Plocama yemenensis
Plocama pendula
Plocama calabrica
1.00

PAE 1.00
Leptodermis potanini
Serissa japonica
Spermadictyon suaveolens
Paederia foetida
1.00

ARG 1.00
Argostemma hookeri
Argostemma rupestre
Mycetia malayana

1.00

ANT 0.98

Anthospermum aethiopic
Anthospermum tricostatum
Nenax acerosa
Anthospermum herbaceum
Galopina circaeoides
Galopina crocylloides
Phyllis nobla
1.00
Coprosma crassifolia
Coprosma fauriei
Coprosma persicifolia
Coprosma quadrifida
Normandia neocaledonica
1.00
Coprosma pumila
Nertera assurgens
Nertera dichondrifolia
Nertera granadensis
Nertera holmboei
0.98
Durringtonia paludosa
Coprosma ernodeoides
Pomax umbellata
Leptostigma pilosum
Leptostigma repens
0.94
Carpacoce sp

1.00

SPE 1.00

0.91

Agathisanthemum bojeri
Lelya prostrata
Oldenlandia goreensis
Hedyotis fruticosa
1.00
0.99
1.00

Amphiasma benguellensis
Amphiasma luzuloides
Pentanopsis gracilicaule
Pentanopsis fragrans
Conostomium longitubum
Conostomium quadrangulare
Manostachya ternifolia
Phylohydrax carnosa
Kohautia caespitosa
1.00
1.00
1.00
1.00
1.00

Arcytophyllum aristatum
Arcytophyllum setosum
Arcytophyllum thymifolium
Houstonia caerulea
Houstonia longifolia
Hedyotis nigricans
0.98
1.00
0.92
1.00

Borreria hispida
Mitracarpus frigidus
Richardia scabra
Spermacoce confusa
Hydrophylax maritima
Spermacoce hispida
0.96
1.00
0.91

Bouvardia glaberrima
Bouvardia ternifolia
Diodia sarmentosa
Ernodea littoralis
Hedyotis littoralis kadua
Hedyotis serpens
Hemidiodia ocimifolia
Manettia alba
Manettia bicolor
Manettia lygistum
Mitracarpus villosus
Nesohedyotis arborea
Psyllocarpus asparagoides
Psyllocarpus laricoides
Richardia pilosa
Spermacoce laevis
1.00

Hedyotis mitrasacmoides
Oldenlandia corymbosa
Oldenlandia lancifolia
Oldenlandia wauensis
0.92
0.99

Dibrachiostylis kaessneri
Hedyotis hillebrandii
Synaptantha tillaeacea
Hedythyrsus thamnoideus
Mitrasacmopsis quadrivalvis
Dentella repens
Pentodon pentandrus
1.00
1.00

1.00

1.00

1.00

KNO

have been included in phylogenetic studies. It will be an important task in coming years to analyze the large and problematic genera of Rubioideae, among them 11 of the 20 largest genera of the family (*Psychotria*, *Galium*, *Ophiorrhiza*, *Palicourea*, *Spermacoce*, *Oldenlandia*, *Lasianthus*, *Faramea*, *Asperula*, *Argostemma*, and *Coussarea*). These genera together contain ~40% (~5000 species) of all species in the family. Hence, understanding their phylogenetic status and relationships will be important for evolutionary studies. Since Bremer and Manen's (2000) study was published, the tribe Putorieae (Backlund et al. 2007) has been split from the former para/polyphyletic "Paederieae." Further, the emended tribe Spermacoceae (fide Bremer and Manen 2000) has been restricted to the group of taxa excluding the Knoxieae and the so-called *Pentas* group (Kårehed and Bremer 2007). After the submission of this article, three new tribes have been described from the subfamily (Rydin et al. 2009), namely, Dunnieae and Colletocemateae (not represented in this study) and Schizocoleeae (represented by one accession in our study; fig. 5). The present analysis included 115 genera representing 18 tribes. The result (fig. 7) is very similar to that of Bremer and Manen (2000), with four early-diverging tribes, although relationships among those are partly unresolved (Ophiorrhizeae, Urophylleae, Lasiantheae, Coussareae), followed by two well-supported sister groups, the Spermacoceae alliance and the Psychotrieae alliance. In the Psychotrieae alliance, the relationships among the tribes are mostly unresolved (Craterispermeae, Gaertnereae, Morindeae, Psychotrieae, Schradereae), whereas in the Spermacoceae alliance (Danaideae, Knoxieae + Spermacoceae, Anthospermeae, Argostemmateae, Paederieae, Putorieae, and Rubieae + Theligoneae), the relationships are resolved with strong support (fig. 7). The oldest fossil from the subfamily that we consider to be reliable is *Faramea* (Graham 1985) from the Upper Eocene (we have used the mean age of the Upper Eocene, 37 Myr, as a minimum age constraint in our dating analysis). The estimated divergence time of the subfamily is, however, much older, 84.4 Ma (fig. 8).

Ophiorrhizeae occur in tropical Asia, from India and China to Southeast Asia and Fiji. Our analysis supports the earlier circumscription of the tribe (fig. 1) proposed by Bremer and Manen (2000), including *Ophiorrhiza*, *Neurocalyx*, *Lerchea*, and *Xanthophytum*. The present analysis shows increased support for the group, but there are still no sequences available for the allegedly closely related genera *Coptophyllum* and *Spiradiclis*. In our analysis the tribe is placed unresolved at the base of the subfamily (fig. 7). The estimated divergence time of the tribe is 77.9 Ma (fig. 8).

Urohylleae occur in the Old World tropics, from West Africa to Central Malesia and Japan, but also in the Neotropics. In our analysis six genera are included (*Amphidasya*, *Maschalocorymus*, *Praravinia*, *Pravinaria*, *Pauridiantha*, *Urophyllum*). The tribe is well supported (1.0), but relationships among genera are not, and there are indications that neither *Pauridiantha* nor *Urophyllum* is monophyletic (fig. 1). In a recent study of

Urophylleae, Smedmark et al. (2008) found strong support for also including the small genera (*Poecilocalyx*, *Stelechantha*, *Pentalonchia*, *Pleiocarpida*, *Raritebe*, and, with less confidence, *Temnopteris*) that sometimes have been included in the tribe. The estimated divergence time of Urophylleae is 66.3 Ma (fig. 8).

Most species of the tribe Lasiantheae occur in the Paleotropics, from Africa, Madagascar, and Indomalaya to Australia, with few species in Panama and the West Indies. In our analysis (fig. 1) the tribe is monophyletic, including *Lasianthus*, *Saldinia*, and *Trichostachys*, and it is sister to a clade including the rest of the Rubioideae (except for Ophiorrhizeae and Urophylleae). The dating analysis was less resolved than the full analysis in relation to the position of Lasiantheae. The estimated divergence time of Lasiantheae is 66.3 Ma (fig. 8).

The tribe Coussareeae (Bremer and Manen 2000) is distributed in Mexico and Central and South America, including temperate regions of South America; most species are South American. Our analysis shows that the tribe is well supported and sister to a clade joining the Spermacoceae alliance and the Psychotrieae alliance (fig. 7), but relationships among the genera (*Coussarea*, *Coccocypselum*, *Declieuxia*, *Cruckshanksia*, *Faramea*, *Hindsia*, *Heterophyllaea*, and *Oreopolus*) are only partly resolved (fig. 5). Only *Faramea* + *Coussarea* and *Oreopolus* + *Cruckshanksia* form clades. Andersson and Rova (1999) studied representatives of Coussareae and showed that the group, as delimited here, is monophyletic. However, these authors classified the genera in three tribes and left two (*Hindsia* and *Heterophyllaea*) without tribal assignment. The fossil discussed above as belonging to the subfamily Rubioideae, *Faramea* (Graham 1985), indicates a minimum age of the tribe from the Upper Eocene (mean age 37 Myr). In our dating analysis, the divergence time is much older, 65.4 Myr (fig. 8). The node to which the prior age of the fossil was attached was estimated in the analysis to be slightly older, 41 Myr.

Craterispermeae, Gaertnereae, Morindeae, Psychotrieae, and Schradereae belong to the Psychotrieae alliance (fide Bremer and Manen 2000; or Psychotriidinae Robbr. & Manen 2006). There is strong support for the alliance (1.0) but not for relationships among the tribes (figs. 5, 7), except for a clade joining Schradereae and Gartnereae. The estimated divergence time of the Psychotrieae alliance is 63.0 Ma (fig. 8).

In Craterispermeae, the single genus *Craterispermum* occurs in tropical Africa, Seychelles, and Madagascar. The genus was found to be polyphyletic by Robbrecht and Manen (2006). In our study we included two species that are sister species in our analysis (fig. 5), in agreement with Razafimandimbison et al. (2008). These together were placed unresolved in the Psychotrieae alliance clade. The estimated divergence time of Craterispermeae is 34.8 Ma (fig. 8).

The tribe Gaertnereae has a disjunct geographical distribution, with one genus in the Old World tropics (*Gaertnera*) and the other genus in the Neotropics (*Pagamea*). Further, the highest diversity of the tribe is in Madagascar, with 25 species.

Fig. 6 The 90% majority-rule consensus tree of a part of Rubioideae from the tree from Bayesian analysis. This is a continuation of Rubioideae as shown in figs. 1 and 5. Clade posterior probabilities are indicated below branches. Tribal abbreviations are shown in bold capital letters above branches of the corresponding clades. *THE*, Theligoneae; *RUB*, Rubieae; *PUT*, Putorieae; *PAE*, Paederieae; *ARG*, Argostemmateae; *ANT*, Anthospermeae; *SPE*, Spermacoceae. The condensed clade labeled "KNO" (Knoxieae) is shown in detail in fig. 5.
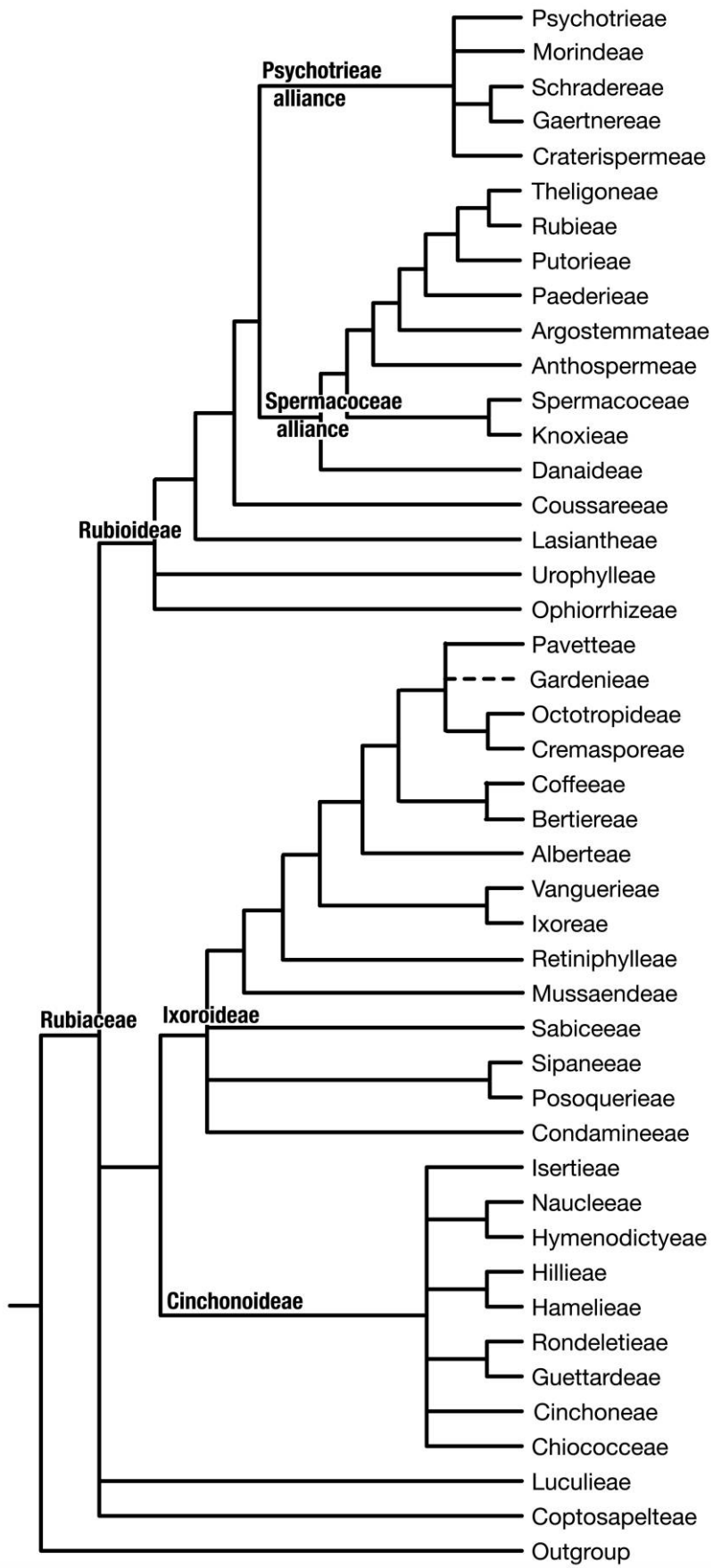
The result of this study places the tribe in a clade together with *Schradereae* and with the two genera *Mitchella + Damnacanthus* (figs. 5, 7). Malcomber (2002) hypothesized that the genus *Gaertnera* represents a case of rapid radiation beginning ~5.2 Myr ago. This radiation of the genus is much later than our estimated divergence time of the tribe, which is 37.3 Ma (fig. 8).

The Morindeae s.str. is mainly pantropical. Earlier circumscription (and at hand when we performed this study) of Morindeae also included Prismatomerinae (*Prismatomeris*) and the *Mitchella* group (*Mitchella, Damnacanthus*). Our analysis does not support any close relationships between these groups and Morindeae s.str. (fig. 5), in agreement with earlier indications (Bremer 1996*b*; Bremer and Manen 2000). In our study the Morindeae s.str. are monophyletic (1.0) and include *Morinda, Gynochtodes, Coelospemum*, and *Appunia* (fig. 5). After this study was performed, a new circumscription of Morindeae s.l. has been proposed (Razafimandimbison et al. 2008). *Mitchella* and *Damnacanthus* are treated as tribe Mitchelleae, and *Primatomeris* has been moved to an earlier-described tribe Primatomerideae. The estimated divergence time of Morindeae s.str. is 36.8 Ma (fig. 8); it is 30.0 Ma for the *Michella* group (Mitchelleae) and 36.9 Ma for *Prismatomeris* (Prismatomerideae).

The type genus of the tribe Psychotrieae, *Psychotria*, is one of the largest of all angiosperm genera, with almost 2000 species, and, together with the other genera of the tribe, it occurs in both the Old and the New World tropics. It has recently been suggested to split the Psychotrieae into two tribes (Robbrecht and Manen 2006), but here we maintain a conservative approach until more taxa of the Psychotrieae have been investigated, in particular because our analysis does not support the subdivision in two clades according to Robbrecht and Manen (2006). We included 16 genera of the Psychotrieae in our analysis (fig. 5), and data support the tribe (1.0) and also several subclades (fig. 5), e.g., *Psychotria* s.str. and allied genera (*Cremocarpon, Hydnophytum, Mapouria, Myrmecodia, Squamellaria, Streblosa*), and most of the other taxa in another strongly supported subclade including several *Psychotria* species and also many other genera (*Chassalia, Chazaliella, Geophila, Hymenocoleus, Margaritopsis, Palicourea, Readea, Rudgea*), mainly in agreement with the earlier studies (Nepokroeff et al. 1999; Andersson 2002). The estimated divergence time of the Psychotrieae is 48.7 Ma (fig. 8).

The tribe Schradereae includes three genera, with distribution in tropical America (*Schradera*) and Asia (*Leucocodon* in Sri Lanka and *Lecanathus* in West Malesia). So far, only *Schradera* has been included in any molecular analysis. In our analysis (fig. 5) *Schradera* is sister to *Gaertnera*, with the *Mitchella* group as sister to Schradereae and Gaertnereae. The dating analysis is resolved with much less support, and the estimated divergence time of Schradereae is 30.0 Ma (fig. 8).

The nine tribes Danaideae, Knoxieae, Spermacoceae, Anthospemeae, Argostemmateae, Paederieae, Putorieae, Rubieae, and Theligoneae belong to the informal group referred to as the Spermacoceae alliance by Bremer and Manen (2000; or Rubiidinae [Robbrecht and Manen 2006]). There is strong support for the alliance (1.0), as well as for most relationships among the tribes (fig. 7). In this alliance most species are herbaceous or subshrubs; fruits are variable but very commonly dry, capsular fruits with many seeds. A few groups have fleshy fruits (e.g., Paederieae, Putorieae, and Rubieae), and in some tribes one-seeded carpels are common (e.g., Anthospermeae, Paederieae, Putorieae, Rubieae, Theligoneae, Knoxieae, Spermacoceae p.p.). The estimated divergence time of the Spermacoceae alliance is 63.0 Ma (fig. 8).

In Danaideae, the genus *Danais* occurs in tropical East Africa and on islands in the western Indian Ocean, particularly on Madagascar, where the two other genera (*Schismatoclada, Payera*) also occur. The tribe is sister to the rest of the Spermacoceae alliance in our analysis (fig. 5), in agreement with Bremer (1996*b*) and Bremer and Manen (2000). Here we have sampled two of the genera, *Danais* and *Schismatoclada*; no analysis has yet included any representative of *Payera*. The estimated divergence time of the tribe is 54.8 Ma (fig. 8).

In Knoxieae, all genera occur in mainland Africa or in Madagascar; only *Knoxia* is also found in Indomalaya. Knoxieae have recently been investigated with molecular data (Kårehed and Bremer 2007), which resulted in a completely new circumscription (see also under Spermacoceae) of the tribe. It now includes 15 genera, several of which were recently described. We have included nine of these in our study (*Batopedina, Dirichletia, Knoxia, Otiophora, Otomeria, Parapentas, Pentanisia, Pentas, Triainolepis*). Our result is congruent with that of Kårehed and Bremer (2007), indicating several supported clades within the tribe (fig. 5). The estimated divergence time of Knoxieae is 44.3 Ma (fig. 8).

The tribe Spermacoceae is pantropical, with few taxa in temperate regions. Spermacoceae have been treated as a small tribe (Verdcourt 1958), up to a very large tribe, including Hedyotideae, Knoxieae, Manettieae, and Triainolepideae (Bremer 1996*b*; Bremer and Manen 2000). Following the results from recent analyses (Dessein 2003; Kårehed and Bremer 2007), Spermacoceae are now treated as a tribe of ~1000 species and ~60 genera (including Manettieae and most genera of Hedyotideae but not Knoxieae). We have included ~40 genera in our analysis (fig. 6). The tribe is strongly supported (1.0), and so are several internal clades. The estimated divergence time of the tribe is 44.3 Ma (fig. 8).

Anthospermeae are represented in temperate South America, on South Atlantic islands, and in Africa and Australia. Eleven genera (*Anthospermum, Carpacoce, Coprosma, Durringtonia, Galopina, Leptostigma, Nenax, Nertera, Normandia, Phyllis, Pomax*) are included in this analysis, and they form a well-supported group (0.98) that is sister to a clade of five tribes (Argostemmateae, Paederieae, Putorieae, Rubieae, Theligoneae). Anderson et al. (2001) analyzed Anthospermeae and found that Anthospermeae formed a weakly supported clade; *Carpacoce* was excluded from the tribe and placed sister to Knoxieae, a placement that is contradicted by our results, which instead

Fig. 7 Overview of Rubiaceae phylogeny based on the Bayesian analysis of the full data set. All clades shown have clade posterior probabilities of 0.95–1.0. Except for the nonmonophyletic Gardenieae (indicated with dashed line), tribal names correspond to well-supported clades of 0.95 or higher.
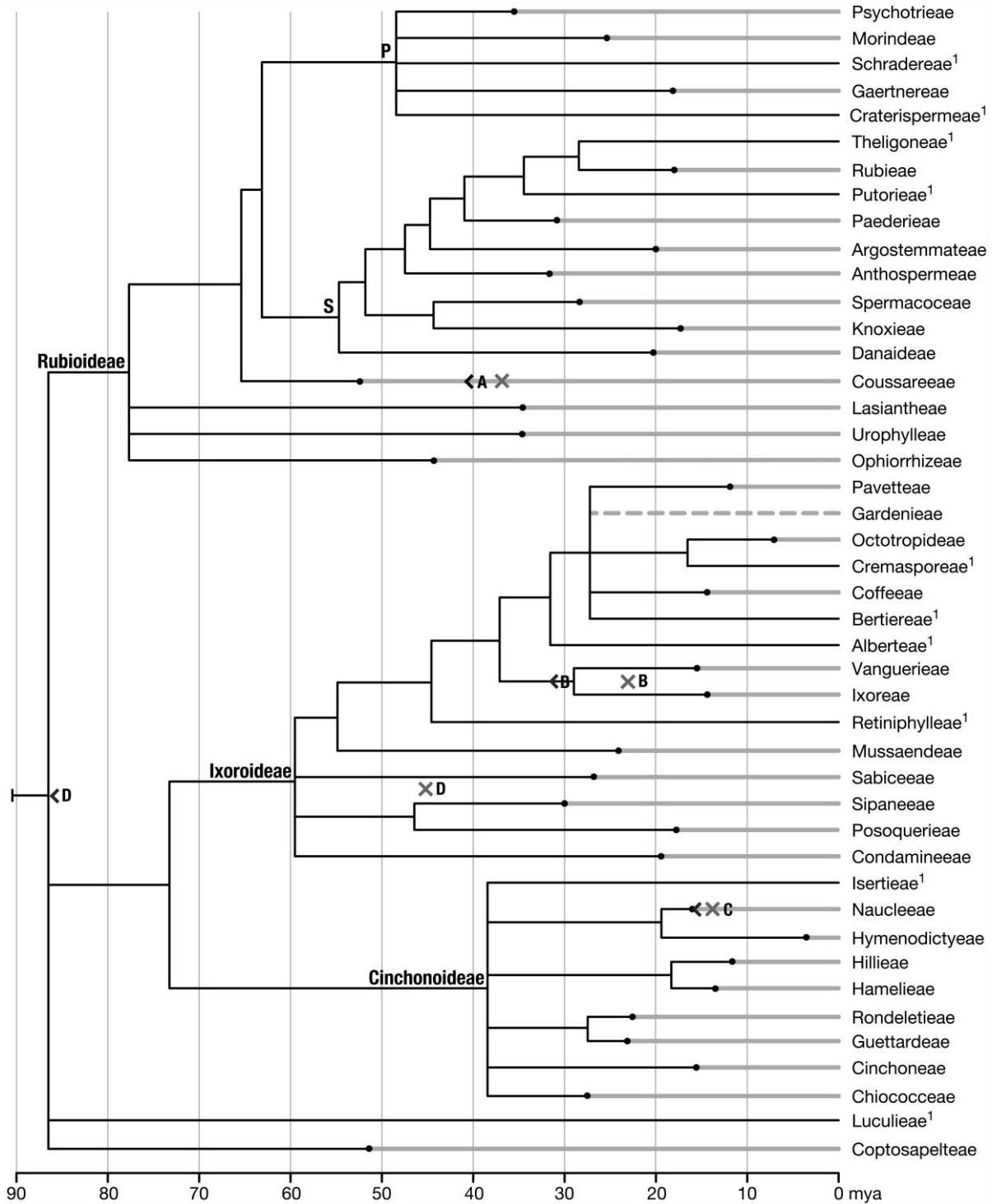
Fig. 8 Chronogram showing mean divergence times for nodes on the basis of the scaled-down data set of 176 taxa analyzed with the Beast. Note that in order to simplify this tree, several clades present in the original chronogram were condensed here—tribal clades in particular. The mean prior age of the root was 78 Myr and was estimated to 90.4 Myr. The prior ages for four nodes are marked with capital letters *A–D* and a cross. The corresponding estimated ages for those nodes are marked with arrowheads. The prior ages for those nodes were set to *A*, *Faramea* stem node, 37 Myr; *B*, *Scyphiphora* stem node, 23 Myr; *C*, *Cephalanthus* stem node, 14 Myr; *D*, Rubiaceae crown node, 45 Myr. The nodes were estimated to be 41.0, 31.6, 16.0, and 86.6 Myr (mean age), respectively. The *Faramea* node is collapsed here into Coussareae, but it was included in the analysis. Crown node divergence times are indicated by circles where crown nodes were condensed (for details, see table 1). Crown node divergence times were not available for tribes represented by a single taxon only in the scaled-down data set. The taxon labels of those tribes are marked with a superscript 1. *P*, Psychotrieae alliance clade; *S*, Spermacoceae alliance. The nonmonophyletic Gardenieae is shown here with a dashed line, indicating the oldest of the Gardenieae subclades.

support a position within Anthospermeae (fig. 6). In our study several nodes in the tribe are unresolved or resolved with low support values, but a few nodes have strong support (1.0). One of these includes *Anthospermum*, *Nenax*, *Galopina*, and *Phyllis*, a group in agreement with Puff's (1982) subtribe Anthosperminae, also in agreement with Anderson et al. (2001; but in our study also including *Carpacoce*). The estimated divergence time of the tribe is 47.6 Ma (fig. 8).

In Argostemmateae, one species of the large genus *Argostemma* (~200 species) occurs in West Africa, the other in tropical and subtropical Asia; *Mycetia* occurs in Indomalaya. Our analysis places Argostemmateae as sister to a clade including Paederieae, Putorieae, Rubieae, and Theligoneae (in Bremer 1996b the tribe is unresolved as part of a lower node in the tree; figs. 6, 7). The estimated divergence time of the tribe is 44.8 Ma (fig. 8).

Paederieae are pantropical but with most taxa in the Old World. In our analysis we included all four genera (*Paederia*, *Leptodermis*, *Serissa*, *Spermadictyon*), and the tribe and some interrelationships of the genera (fig. 6) were supported and congruent with the results of Backlund et al. (2007). The estimated divergence time of the tribe is 41.2 Ma (fig. 8).

The tribe Putorieae (Backlund et al. 2007) has a geographic distribution in southern and northern Africa, Macaronesia, and south-central and southwestern Asia. The tribe is now treated as monogeneric, with the single genus *Plocama* (including the former genera *Aitchisonia*, *Choulettia*, *Crocyllis*, *Gaillonia*, *Jaubertia*, *Pseudogaillonia*, *Pterogaillonia*). In our analysis the three included species (fig. 6) are well supported as monophyletic (1.0). The estimated divergence time of Putorieae is 34.4 Ma (fig. 8).

Rubieae are cosmopolitan, with many representatives in areas with a Mediterranean climate and in temperate areas. In our analysis we included 19 taxa representing 11 genera (fig. 6). The tribe is monophyletic (1.0), as found in other studies (Manen et al. 1994; Natali et al. 1995), but most internal clades have low support. Further, not a single genus represented by more than one taxon was monophyletic (*Rubia*, *Didymaea*, *Asperula*, *Galium*, *Crucianella*). The estimated divergence time of the tribe is 28.6 Ma (fig. 8).

Theligoneae are monogeneric, with species in Macaronesia, the Mediterranean, and also China and Japan. In this study (fig. 7), Theligoneae are the sister group to Rubieae (1.0), a phylogenetic position found in many earlier studies (Bremer and Jansen 1991; Bremer et al. 1995). Backlund et al. (2007) found *Theligonum* to be sister to Rubieae + *Kelloggia*, in agreement with a study by Nie et al. (2005; the latter genus not included in our study). We have investigated only one species, and the monophyly could not be tested. The estimated divergence time of the tribe is 28.6 Ma (fig. 8).

Conclusions

This study aims to estimate divergence times for internal lineages of Rubiaceae, corresponding mainly to subfamilies and tribes. A secondary goal is to produce a phylogenetic tree indicating relationships and support for tribes and subfamilies, with short comments on all tribes. We analyzed 534 Rubiaceae taxa from 329 genera with up to five different chloroplast regions by Bayesian analysis to get a robust phylogenetic tree. A scaled-down data set of 173 Rubiaceae taxa was used with a Bayesian relaxed-clock approach (the Beast of Drummond et al. 2006) to estimate divergence times for clades classified as tribes and subfamilies. Four different Rubiaceae fossils (*Faramea*-type pollen, *Scyphiphora* pollen, *Cephalanthus pusillus* fruits, and *Paleorubiaceophyllum eocenicum* leaves) were used as minimum age priors, one for each subfamily and one for Rubiaceae as a whole.

The phylogenetic analysis resulted in a highly resolved consensus tree but with many weakly supported nodes. However, there is strong support for the clades that correspond to the three subfamilies (Cinchonoideae, Ixoroideae, Rubioideae) and most of the 44 included tribes (fig. 7). Five of these could not be tested for monophyly because they are monogeneric or represented by single taxa in the analysis (Alberteae, Cremasporeae, Retiniphylleae, Schradereae, Theligoneae). One tribe, Gardenieae, is not monophyletic. Further, we have still not resolved the intriguing question about the basalmost relationships of the Rubiaceae. Our phylogenetic tree shows a polytomy of four branches at the base of the family with Rubioideae, Cinchonoideae + Ixoroideae, Coptosapelteae, and Luculieae.

The dating analysis (fig. 8; table 1) resulted in an estimated lineage (stem) divergence time for Rubiaceae of 90.4 Ma. The estimated lineage divergence times for subfamilies were 84.4 (86.6) Ma for Rubioideae, 73.1 Ma for Ixoroideae, and 73.1 Ma for Cinchonoideae. The estimated lineage divergence times for the tribes of Rubioideae vary between 77.9 and 28.6 Ma, between 59.6 and 14.2 Ma for Ixoroideae, and between 34.7 and 19.7 Ma for Cinchonoideae.

Rubiaceae occur on all continents, but of the 44 tribes included in our analysis, 18 are restricted to paleotropical or paleosubtropical areas, 10 are restricted to Neotropical or neosubtropical areas, and the remaining 16 tribes occur in both the Old and the New World. Among the tribes with these different distribution patterns there are both very young and very old tribes; among paleotropical or paleosubtropical areas, they vary in age between 86.6 and 18.7 Myr; among the Neotropical or neosubtropical areas, they vary between 65.4 and 18.7 Myr; and those tribes with more worldwide distribution vary between 66.3 and 14.2 Myr. However, both Coptosapelteae and Luculieae, which are placed unresolved at the base of the Rubiaceae tree, are from Southeast Asia, and because they represent the oldest-dated tribes in this analysis, with stem ages 86.6 and 80.3 (86.6) Myr, respectively, one might suggest that Southeast Asia is the cradle of the family.

Acknowledgments

Literature Cited

Achille F, TJ Motley, PP Lowry, J Jeremie 2006 Polyphyly in *Guettarda* L. (Rubiaceae, Guettardeae) based on nrDNA ITS sequence data. Ann Mo Bot Gard 93:103–121.

Alejandro GD, SG Razafimandimbison, S Liede-Schumann 2005 Polyphyly of *Mussaenda* inferred from ITS and *trn*T-F data and its implication for generic limits in Mussaendeae (Rubiaceae). Am J Bot 92:544–557.

Anderson CL, K Bremer, EM Friis 2005 Dating phylogenetically basal eudicots using *rbc*L sequences and multiple fossil reference points. Am J Bot 92:1737–1748.

Anderson CL, JHE Rova, L Andersson 2001 Molecular phylogeny of the tribe Anthospermeae (Rubiaceae): systematic and biogeographic implications. Aust Syst Bot 14:231–244.

Andersson L 2001 *Margaritopsis* (Rubiaceae, Psychotrieae) is a pantropical genus. Syst Geogr Plants 71:73–85.

——— 2002 Relationships and generic circumscription in the *Psychotria* complex (Rubiaceae, Psychotrieae). Syst Geogr Plants 72:167–202.

Andersson L, A Antonelli 2005 Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina*. Taxon 54:17–28.

Andersson L, JHE Rova 1999 The *rps*16 intron and the phylogeny of the Rubioideae (Rubiaceae). Plant Syst Evol 214:161–186.

Andersson L, JHE Rova, FA Guarin 2002 Relationships, circumscription, and biogeography of *Arcytophyllum* (Rubiaceae) based on evidence from cpDNA. Brittonia 54:40–49.

Andreasen K 1997 Phylogeny of Ixoroideae. PhD diss. Uppsala University.

Andreasen K, BG Baldwin, B Bremer 1999 Phylogenetic utility of the nuclear rDNA ITS region in subfamily Ixoroideae (Rubiaceae): comparisons with cpDNA *rbc*L sequence data. Plant Syst Evol 217:119–135.

Andreasen K, B Bremer 1996 Phylogeny of the subfamily Ixoroideae (Rubiaceae). Opera Bot Belg 7:119–138.

——— 2000 Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. Am J Bot 87:1731–1748.

Backlund M, B Bremer, M Thulin 2007 Paraphyly of Paederieae, recognition of Putorieae and expansion of *Plocama* (Rubiaceae-Rubioideae). Taxon 56:315–328.

Backlund M, B Oxelman, B Bremer 2000 Phylogenetic relationships within the Gentianales based on *ndh*F and *rbc*L sequences, with particular reference to the Loganiaceae. Am J Bot 87:1029–1043.

Baykovskaya TN 1984 Paleotsenovaya flora Roamankulsaya. Nauka, Leningrad.

Bell CD, MJ Donoghue 2005 Dating the dipsacales: comparing models, genes, and evolutionary implications. Am J Bot 92:284–296.

Bremekamp CEB 1954 Les sous-familles et les tribus des Rubiacées. Huitième Congrès International de Botanique, Rapports et Communications, Paris Sect 2–6, p 113–114.

——— 1966 Remarks on the position, the delimitation, and the subdivision of Rubiaceae. Acta Bot Neerl 15:1–33.

Bremer B 1996a Combined and separate analyses of morphological and molecular data in the plant family Rubiaceae. Cladistics 12:21–40.

——— 1996b Phylogenetic studies within Rubiaceae and relationship to other families based on molecular data. Opera Bot Belg 7:33–50.

Bremer B, K Andreasen, D Olsson 1995 Subfamilial and tribal relationships in the Rubiaceae based on *rbc*L sequence data. Ann Mo Bot Gard 82:383–397.

Bremer B, K Bremer, N Heidari, P Erixon, RG Olmstead, AA Anderberg, M Källersjö, E Barkhordarian 2002 Phylogenetics of asterids based on three coding and three noncoding chloroplast DNA markers and the utility of noncoding DNA at higher taxonomic levels. Mol Phylogenet Evol 24:273–300.

Bremer B, O Eriksson 1992 Evolution of fruit characteristics and dispersal modes in the tropical family Rubiaceae. Biol J Linn Soc 47:79–95.

Bremer B, RK Jansen 1991 Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within Rubiaceae. Am J Bot 78:198–213.

Bremer B, RK Jansen, B Oxelman, M Backlund, H Lantz, KJ Kim 1999 More characters or more taxa for a robust phylogeny in a case study from the coffee family (Rubiaceae). Syst Biol 48:413–435.

Bremer B, JF Manen 2000 Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). Plant Syst Evol 225:43–72.

Bremer B, M Thulin 1998 Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceeae (Rubiaceae): phylogenetic relationships based on *rbc*L data. Plant Syst Evol 211:71–92.

Bremer K 2000 Early Cretaceous lineages of monocot flowering plants. Proc Natl Acad Sci USA 97:4707–4711.

Bremer K, EM Friis, B Bremer 2004 Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification. Syst Biol 53:496–505.

Bremer K, MHG Gustafsson 1997 East Gondwana ancestry of the sunflower alliance of families. Proc Natl Acad Sci USA 94:9188–9190.

Bridson DM, B Verdcourt 1988 Rubiaceae. Pt 2. Pages 415–747 *in* RM Polhill, ed. Flora of tropical East Africa. Balkema, Rotterdam.

Britton T, CL Andersson, D Jacquet, S Lundqvist, K Bremer 2007 Estimating divergence times in large phylogenetic trees. Syst Biol 56:741–752.

Chanderbali AS, H van der Werff, SS Renner 2001 Phylogeny and historical biogeography of Lauraceae: evidence from the chloroplast and nuclear genomes. Ann Mo Bot Gard 88:104–134.

Church SA 2003 Molecular phylogenetics of *Houstonia* (Rubiaceae): descending aneuploidy and breeding system evolution in the radiation of the lineage across North America. Mol Phylogenet Evol 27:223–238.

Conti E, T Eriksson, J Schonenberger, KJ Sytsma, DA Baum 2002 Early tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. Evolution 56:1931–1942.

Cros J, MC Combes, P Trouslot, F Anthony, S Hamon, A Charrier, P Lashermes 1998 Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. Mol Phylogenet Evol 9:109–117.

Davies TJ, TG Barraclough, MW Chase, PS Soltis, DE Soltis, V Savolainen 2004 Darwin's abominable mystery: insights from a supertree of the angiosperms. Proc Natl Acad Sci USA 101:1904–1909.

Davis AP, M Chester, O Maurin, MF Fay 2007 Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae): the circumscription and phylogeny of Coffeeae based on plastid sequence data and morphology. Am J Bot 94:313–329.

Delprete PG 1996 Evaluation of the tribes Chiococceae, Condamineeae and Catesbaeeae (Rubiaceae) based on morphological characters. Opera Bot Belg 7:165–192.

——— 1999 Rondeletieae (Rubiaceae). Pt 1. Pages 1–226 *in* Flora neotropica monograph 77. New York Botanical Garden, Bronx.

Delprete PG, R Cortes-B 2004 A phylogenetic study of the tribe Sipaneeae (Rubiaceae, Ixoroideae), using *trn*L-F and ITS sequence data. Taxon 53:347–356.

Delprete PG, LB Smith, RM Klein 2004 Rubiaceae. Pages 1–344 *in* A Reis, ed. Flora ilustrada Catarinense. Herbário Barbosa Rodrigues, Santa Catarina.

Dessein S 2003 Systematic studies in the Spermacoceae (Rubiaceae). PhD diss. Katholieke University Leuven.

Dessein S, L Andersson, E Robbrecht, E Smets 2001 *Hekistocarpa* (Rubiaceae): a member of an emended tribe Virectarieae. Plant Syst Evol 229:59–78.

Dorofeev PI 1960 Novye dannye o treticnych florach Kireevskogo jara nar obi. Dokl Akad Nauk SSR 133:211–213.

——— 1963 Treticnye flory Zapadnoj Sibiri. Izvestiya Akademii Nauk SSR, Moscow.

Drummond AJ, SYW Ho, MJ Phillips, A Rambaut 2006 Relaxed phylogenetic and dating with confidence. PLoS Biol 4:e88.

Drummond AJ, A Rambaut 2007 BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214.

Ducke A 1933 Plantes nouvelles ou peu connues de la région Amazonienne. Arch Jard Bot Rio de Janeiro 6:1–108.

Ehrendorfer F, JF Manen, A Natali 1994 cpDNA intergene sequences corroborate restriction site data for reconstruction Rubiaceae phylogeny. Plant Syst Evol 190:245–248.

Erdtman G 1966 Pollen morphology and plant taxonomy. Hafner, New York. Corrected reprint of 1952 ed.

Eriksson O, B Bremer 1991 Fruit characteristics, life forms, and species richness in the plant family Rubiaceae. Am Nat 138:751–761.

Fay MF, B Bremer, GT Prance, M van der Bank, D Bridson, MW Chase 2000 Plastid *rbc*L sequence data show *Dialypetalanthus* to be a member of Rubiaceae. Kew Bull 55:853–864.

Felsenstein J 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376.

Friis EM 1985 Angiosperm fruits and seeds from the Middle Miocene of Jutland (Denmark). K Dan Vidensk Selsk Biol Skr 24:1–165.

Goevarts R, M Ruhsam, L Andersson, E Robbrecht, D Bridson, A Davis, I Schanzer, B Sonké 2006 World checklist of Rubiaceae. Royal Botanic Gardens, Kew. http://www.kew.org/wcsp/rubiaceae/

Goremykin VV, S Hansmann, WF Martin 1997 Evolutionary analysis of 58 proteins encoded in six completely sequenced chloroplast genomes: revised molecular estimates of two seed plant divergence times. Plant Syst Evol 206:337–351.

Graham A 1985 Studies in Neotropical paleobotany. IV. The Eocene communities of Panama. Ann Mo Bot Gard 72:504–534.

——— 1987 Fossil pollen of *Sabicea* (Rubiaceae) from the lower Miocene Culebra Formation of Panama. Ann Mo Bot Gard 74:868–870.

Guindon S, O Gascuel 2003 A simple, fast, and accurate algorithm to estimate phylogenies by maximum likelihood. Syst Biol 52:696–704.

Huelsenbeck JP, F Ronquist 2001 MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Huysmans S, S Dessein, E Smets, E Robbrecht 2003 Pollen morphology of NW European representatives confirms monophyly of Rubieae (Rubiaceae). Rev Palaeobot Palynol 127:219–240.

Huysmans S, E Robbrecht, E Smets 1994 Are the genera *Hallea* and *Mitragyna* (Rubiaceae-Coptosapelteae) pollen morphologically distinct? Blumea 39:321–340.

Igersheim A 1993 The character states of the Caribbean monotypic endemic *Strumpfia* (Rubiaceae). Nord J Bot 13:545–559.

Jansen S, E Robbrecht, H Beeckman, E Smets 2002 A survey of the systematic wood anatomy of the Rubiaceae. IAWA J 23:1–67.

Jansen S, T Watanabe, E Smets, E Robbrecht 2003 A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. Ann Bot 91:657–663.

Kårehed J, B Bremer 2007 The systematics of Knoxieae (Rubiaceae): molecular data and their taxonomic consequences. Taxon 56:1051–1076.

Kay KM, JB Whittall, SA Hodges 2006 A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. BMC Evol Biol 6:36.

Khan SA, SG Razafimandimbison, B Bremer, S Liede-Schumann 2008 Sabiceeae and Virectarieae (Rubiaceae, Ixoroideae): one or two tribes? new tribal and generic circumscriptions of Sabiceeae and biogeography of *Sabicea* s.l. Taxon 57:1–17.

Lantz H, K Andreasen, B Bremer 2002 Nuclear rDNA ITS sequence data used to construct the first phylogeny of Vanguerieae (Rubiaceae). Plant Syst Evol 230:173–187.

Lantz H, B Bremer 2004 Phylogeny inferred from morphology and DNA data: characterizing well-supported groups in Vanguerieae (Rubiaceae). Bot J Linn Soc 146:257–283.

——— 2005 Phylogeny of the complex Vanguerieae (Rubiaceae) genera *Fadogia*, *Rytigynia*, and *Vangueria* with close relatives and a new circumscription of *Vangueria*. Plant Syst Evol 253:159–183.

Lavin M, PS Herendeen, MF Wojciechowski 2005 Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. Syst Biol 54:575–594.

Leebens-Mack J, LA Raubeson, LY Cui, JV Kuehl, MH Fourcade, TW Chumley, JL Boore, RK Jansen, CW dePamphilis 2005 Identifying the basal angiosperm node in chloroplast genome phylogenies: sampling one's way out of the Felsenstein zone. Mol Biol Evol 22:1948–1963.

Leopold E 1969 Miocene pollen and spore flora of Eniwetok Atoll, Marshall Island. US Geol Surv Prof Pap 260-II:1133–1185.

Magallon SA, MJ Sanderson 2005 Angiosperm divergence times: the effect of genes, codon positions, and time constraints. Evolution 59:1653–1670.

Malcomber ST 2002 Phylogeny of *Gaertnera* Lam. (Rubiaceae) based on multiple DNA markers: evidence of a rapid radiation in a widespread, morphologically diverse genus. Evolution 56:42–57.

Manen JF, A Natali 1995 Comparison of the evolution of ribulose-1,5-biphosphate carboxylase (*rbc*L) and *atp*B-*rbc*L noncoding spacer sequences in a recent plant group, the tribe Rubieae (Rubiaceae). J Mol Evol 41:920–927.

Manen JF, A Natali, F Ehrendorfer 1994 Pylogeny of Rubiaceae-Rubieae inferred from the sequence of cpDNA intergene region. Plant Syst Evol 190:195–211.

McDowell T, B Bremer 1998 Phylogeny, diversity, and distribution in *Exostema* (Rubiaceae): implications of morphological and molecular analyses. Plant Syst Evol 212:215–246.

McDowell T, M Volovsek, P Manos 2003 Biogeography of *Exostema* (Rubiaceae) in the Caribbean region in light of molecular phylogenetic analyses. Syst Bot 28:431–441.

Moore MJ, CD Bell, PS Soltis, DE Soltis 2007 Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proc Natl Acad Sci USA 104:19363–19368.

Motley TJ, KJ Wurdack, PG Delprete 2005 Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae): flower and fruit evolution and biogeographic implications. Am J Bot 92:316–329.

Natali A, JF Manen, F Ehrendorfer 1995 Phylogeny of the Rubiaceae-Rubioideae, in particular the tribe Rubieae: evidence from a noncoding chloroplast DNA sequence. Ann Mo Bot Gard 82:428–439.

Negrón-Ortiz V, LE Watson 2003 Hypotheses for the colonization of the Caribbean basin by two genera of the Rubiaceae: *Erithalis* and *Ernodea*. Syst Bot 28:442–451.

Nepokroeff M, KJ Sytsma, B Bremer 1999 Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbc*L sequence data. Syst Bot 24:5–27.

Nepokroeff M, KJ Sytsma, WL Wagner, EA Zimmer 2003 Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. Syst Biol 52:820–838.

Nie ZL, J Wen, H Sun, B Bartholomew 2005 Monophyly of *Kelloggia* Torrey ex Benth. (Rubiaceae) and evolution of its intercontinental disjunction between western North America and eastern Asia. Am J Bot 92:642–652.

Novotny V, Y Basset, SE Miller, GD Weiblen, B Bremer, L Cizek, P Drozd 2002 Low host specificity of herbivorous insects in a tropical forest. Nature 416:841–844.

Nylander JAA 2004 MrAIC.pl. Evolutionary Biology Centre, Uppsala University. Program distributed by author.

Olmstead RG, B Bremer, KM Scott, JD Palmer 1993 A parsimony analysis of the Asteridae sensu lato based on *rbc*L sequences. Ann Mo Bot Gard 80:700–722.

Page RDM, EC Holmes 1998 Molecular evolution: a phylogenetic approach. Blackwell, Oxford. 346 pp.

Persson C 2000a Phylogeny of Gardenieae (Rubiaceae) based on chloroplast DNA sequences from the *rps*16 intron and *trn*L(UAA)-F(GAA) intergenic spacer. Nord J Bot 20:257–269.

——— 2000b Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. Am J Bot 87:1018–1028.

Piesschaert F, L Andersson, S Jansen, S Dessein, E Robbrecht, E Smets 2000 Searching for the taxonomic position of the African genus *Colletoecema* (Rubiaceae): morphology and anatomy compared to an *rps*16-intron analysis of the Rubioideae. Can J Bot 78:288–304.

Posada D, TR Buckley 2004 Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53:793–808.

Puff C 1982 The delimitation of the tribe Anthospermeae and its affinities to the Paederieae (Rubiaceae). Bot J Linn Soc 84:355–377.

Puff C, U Rohrhofer 1993 The character states and taxonomic position of the monotypic mangrove genus *Scyphiphora* (Rubiaceae). Opera Bot Belg 6:143–172.

Raniecka-Bobowska J 1959 Trzeciorzedowa flora nasienna z Konina. Biul Inst Geol 130:159–252.

Razafimandibison SG, B Bremer 2001 Tribal delimitation of Naucleeae (Cinchonoideae, Rubiaceae): inference from molecular and morphological data. Syst Geogr Plants 71:515–538.

——— 2002 Phylogeny and classification of Naucleeae s.l. (Rubiaceae) inferred from molecular (ITS, *rbc*L, and *trn*T-F) and morphological data. Am J Bot 89:1027–1041.

Razafimandibison SG, J Moog, H Lantz, U Maschwitz, B Bremer 2005 Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* s.s. (Rubiaceae). Mol Phylogenet Evol 34:334–354.

Razafimandibison SG, C Rydin, B Bremer 2008 Evolution and trends in the Psychotrieae alliance (Rubiaceae): a rarely reported evolutionary change of many-seeded carpels from one-seeded carpels. Mol Phylogenet Evol 48:207–223.

Robbrecht E 1988 Tropical woody Rubiaceae. Opera Bot Belg 1:1–271.

Robbrecht E, CF Manen 2006 The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Syst Geogr Plants 76:85–146.

Robbrecht E, C Puff 1986 A survey of the Gardenieae and related tribes (Rubiaceae). Bot Jarb Syst 108:63–137.

Ronquist F, JP Huelsenbeck 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Roth LJ, DL Dilcher 1979 Investigations of angiosperms from the Eocene of North America: stipulate leaves of the Rubiaceae including a probable polyploid population. Am J Bot 66:1194–1207.

Rova JHE, PG Delprete, L Andersson, VA Albert 2002 A *trn*L-F cpDNA sequence study of the Condamineeae-Rondeletieae-

Sipaneeae complex with implications on the phylogeny of the Rubiaceae. Am J Bot 89:145–159.

Rova JHE, PG Delprete, B Bremer 2009 The *Rondeletia* complex (Rubiaceae): an attempt to use ITS, *rps*16, and *trn*L-F sequence data to delimitate Guettardeae, Rondeletieae and sections within *Rondeletia*. Ann Mo Bot Gard 96:182–193.

Rydin C, K Kainulainen, SG Razafimandibison, JEE Smedmark, B Bremer 2009 Deep divergences in the coffee family and the systematic position of *Acranthera*. Plant Syst Evol 278:101–123.

Saenger P 1998 Mangrove vegetation: an evolutionary perspective. Mar Freshwater Res 49:277–286.

Salard-Cheboldaeff M 1978 Sur la palynoflore Maestrichtienne et tertiaire de bassin sédimentaire littoral du Cameroun. Pollen Spores 20:215–260.

Sanderson MJ 1997 A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol Biol Evol 14:1218–1231.

——— 2002 Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19:101–109.

Sanderson MJ, JA Doyle 2001 Sources of error and confidence intervals in estimating the age of angiosperms from *rbc*L and 18S rDNA data. Am J Bot 88:1499–1516.

Smedmark JEE, C Rydin, SG Razafimandibison, SA Khan, S Liede-Schumann, B Bremer 2008 Phylogeny of Urophylleae (Rubiaceae) based on *rps*16 intron data. Taxon 57:24–32.

Struwe L, M Thiv, JW Kadereit, S-R Pepper, TJ Motley, PJ White, JHE Rova, K Potgieter, VA Albert 1998 *Saccifolium* (Saccifoliaceae), an endemic of Sierra de la Neblina on the Brazilian-Venezuelan border, is related to a temperate-alpine lineage of Gentianaceae. Harv Pap Bot 3:199–214.

Swofford DL 2003 PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland, MA.

Sytsma KJ, A Litt, ML Zjhra, JC Pires, M Nepokroeff, E Conti, J Walker, PG Wilson 2004 Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the Southern Hemisphere. Int J Plant Sci 165(suppl):S85–S105.

Tavaré S 1986 Some probabilistic and statistical problems in the analysis of DNA sequences. Lect Math Life Sci 17:57–86.

Taylor CM 1992 Revision of *Cosmibuena* (Rubiaceae). Ann Mo Bot Gard 79:886–900.

——— 1994 Revision of *Hillia* (Rubiaceae). Ann Mo Bot Gard 81:571–607.

Thorne JL, H Kishino, IS Painter 1998 Estimating the rate of evolution of the rate of molecular evolution. Mol Biol Evol 15:1647–1657.

Thulin M, B Bremer 2004 Studies in the tribe Spermacoceae (Rubiaceae-Rubioideae): the circumscription of *Amphiasma* and *Pentanopsis* and the affinities of *Phylohydrax*. Plant Syst Evol 247:233–249.

Verdcourt B 1958 Remarks on the classification of the Rubiaceae. Bull Bot Etat 28:209–290.

Verdcourt B, DM Bridson 1991 Rubiaceae. Pt 3. Pages 749–933 in RM Polhill, ed. Flora of tropical East Africa. Balkema, Rotterdam.

Vinnersten A, K Bremer 2001 Age and biogeography of major clades in Liliales. Am J Bot 88:1695–1703.

Weeks A, DC Daly, BB Simpson 2005 The phylogenetic history and biogeography of the frankincense and myrrh family (Burseraceae) based on nuclear and chloroplast sequence data. Mol Phylogenet Evol 35:85–101.

Wiens JJ, DS Moen 2008 Missing data and the accuracy of Bayesian phylogenetics. J Syst Evol 46:307–314.

Wikström N, V Savolainen, MW Chase 2001 Evolution of the

angiosperms: calibrating the family tree. Proc R Soc B 268:2211–2220.

Xiang Q-Y, DE Soltis, PS Soltis, SR Manchester, DJ Crawford 2000 Timing the eastern Asian–eastern North American floristic disjunction: molecular clocks corroborate paleontological evidence. Mol Phylogenet Evol 15:462–472.

Yang Z 1993 Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. Mol Biol Evol 10:1396–1401.

Yang Z, B Rannala 1997 Bayesian phylogenetic inference using DNA sequences: a Markov chain Monte Carlo method. Mol Biol Evol 14:717–724.

Yoder AD, Z Yang 2000 Estimation of primate speciation dates using local molecular clocks. Mol Biol Evol 17:1081–1090.

Yoo MJ, CD Bell, PS Soltis, DE Soltis 2005 Divergence times and historical biogeography of Nymphaeales. Syst Biol 30:693–704.

Zerega NJC, WL Clement, SL Datwyler, GD Weiblen 2005 Biogeography and divergence times in the mulberry family (Moraceae). Mol Phylogenet Evol 37:402–416.