

4 A MAJOR RADIATION OF RAINFOREST TREES (*GUATTERIA*; ANNONACEAE) FOLLOWING DISPERSAL FROM CENTRAL INTO SOUTH AMERICA

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ABSTRACT

Several recent studies have suggested that a substantial portion of today's plant diversity in the Neotropics has resulted from the dispersal of taxa into that region rather than vicariance, but more data are needed to substantiate this claim. *Guatteria* (Annonaceae) is, with 265 species, the third largest genus of Neotropical trees after *Inga* (Fabaceae) and *Ocotea* (Lauraceae), and its widespread distribution and frequent occurrence makes the genus an excellent model taxon to study diversification patterns. This study reconstructed the phylogeny of *Guatteria* and inferred three major biogeographical events in the history of this genus: 1) A trans-oceanic Miocene migration from Central into South America before the closing of the Isthmus of Panama; 2) a major diversification of the lineage within South America; and 3) several migrations of South American lineages back into Central America via the closed Panamanian land bridge. Therefore, *Guatteria* is not an Amazonian centred-genus sensu Gentry but a major Miocene radiation that followed its dispersal into South America. This study provides further evidence that migration into the Neotropics was an important factor in the historical assembly of its biodiversity. Furthermore, it is shown that phylogenetic patterns are comparable to those found in *Ocotea* and *Inga* and that a closer comparison of these genera is desirable.

Keywords: radiation, dispersal, Neotropics, rainforest trees, *Guatteria*, Annonaceae

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INTRODUCTION

The Neotropics hold 30% of the world's plant diversity (Smith & al., 2004) but the origin of this diversity is still debated. Although this immense biodiversity could have arisen in situ, recent studies have suggested that immigration of taxa into South America in the Oligocene and Miocene may have contributed substantially to plant diversity on this continent (Chanderbali & al., 2001; Renner & Meyer, 2001; Davis & al., 2002; Pennington & Dick, 2004; Richardson & al., 2004; Nathan, 2006; Pirie & al., 2006). Molecular phylogenetic studies on the history of species-rich plant genera can contribute to our knowledge of speciation processes, key innovations, and biogeographic patterns. Such genera (e.g. the legume genus *Inga*, c. 300 species or *Eschweilera* (Lecythidaceae), c. 100 species), form a substantial component of the woody element in neotropical forests (in terms of biomass and species numbers) and contribute in an important way to the extraordinary biomass and species diversity in South America (Oliveira & Mori, 1999; Richardson & al., 2001a).

The genus *Guatteria* (Annonaceae) is, with approximately 265 species, one of the largest genera of Neotropical trees along with *Inga* and *Ocotea* (Lauraceae, c. 300 species; (Madriñán, 2004). Species of *Guatteria* are frequent constituents of Neotropical (lowland) forests (Morawetz & Waha, 1985) and the genus is widely distributed throughout Mesoamerica (c. 30 species), the Caribbean (2 species) and South America (c. 230 species). Its widespread distribution and frequent occurrence make the genus an excellent model taxon to study diversification.

Although *Guatteria* is the largest genus in Annonaceae, it can be seen as conservative with regard to its morphological evolution, especially when considering the wide range of morphological differentiation in other large genera of Annonaceae (e.g. *Annona*, *Uvaria*). Several plant features, such as floral and fruit type, pollen morphology, and chromosome number, are invariable. However, the genus does show a wide ecological distribution and can for instance be found in Amazonian *terra firme* forests, in *campina* types and inundated forests, in Atlantic rain forests, gallery forests, and semideciduous forests (Morawetz & Waha, 1985). It has therefore been suggested that the main cause of speciation could have been adaptation to a number of different ecological niches in the humid tropics by changes in vegetative characters such as growth form, height, leaf shape and size (Morawetz & Waha, 1985).

Morawetz and Waha (1985) generated the first biogeographic speciation hypothesis for the evolution of *Guatteria*. They suggested that the speciation centre of *Guatteria* could be found in the Amazon basin since the highest species diversity in the genus is there. This distribution pattern can be seen for other taxa as well and the term "Amazonian-centred taxon" was proposed by Gentry to account for this pattern (in contrast to extra-Amazonian, or Andean-centred, taxa; Gentry, 1982). The high species diversity in the Amazon basin is not surprising. The basin has differences in relief, soils, precipitation and seasonality, all leading to a fragmented landscape (Colinvaux, 1996). Furthermore, the dissections of the great river system provide barriers to dispersal (Colinvaux, 1996) and together these factors provide opportunities for allopatric speciation.

The term "Amazonian-centred taxon" could imply that a clade originated and produced

the bulk of its species in the Amazonian basin and subsequently dispersed throughout the rest of South and Central America. The idea that a taxon has originated at its point of highest diversity was first suggested by Vavilov (1935) and has been criticised since (reviewed in: Posadas & al., 2006). “Amazonian-centred taxon” could also mean that the Amazon basin functioned as a hotspot for speciation due to the fact that it is a dynamic and fragmented area but that the geographic origin of the genus might be found somewhere else. In the latter case, migration into the Amazon basin led to new opportunities for the evolving taxon, and possibly to a radiation of species.

The aims of this paper are to assess the evolutionary relationships between the major clades of *Guatteria*, to date their origin, test whether *Guatteria* indeed is an Amazonian-centred genus *sensu* Gentry (i.e. it both originated and diversified within the Amazon basin), and finally to gain insight into the causes of speciation.

MATERIALS AND METHODS

Taxon sampling strategy

Species of *Guatteria* were sampled throughout the geographic range of the genus. Furthermore, all major clades in the family as found by Richardson & al. (2004) are included in the analyses, as well as a representative of the sister family of the Annonaceae (Eupomatiaceae; *Eupomatia bennettii*). *Magnolia kobus* was selected as outgroup.

A preliminary analysis of 106 *Guatteria* accessions and based on four plastid regions (*matK*, *rbcl*, *trnL-trnF* and *psbA-trnH*) yielded many most parsimonious trees, and support for individual clades was generally low. Representatives of the larger putative clades from this preliminary analysis were included in matrix A (43 *Guatteria* accessions; voucher information in appendix 1) and sequenced for an additional chloroplast region (*trnT-L*). Matrix A thus contains a limited number of *Guatteria* species but sequence data from five plastid markers. The strict consensus tree resulting from phylogenetic analysis of matrix A was then used as a backbone constraint on the before mentioned larger matrix (matrix B; voucher information in appendix 1), with 106 species but only four plastid regions.

DNA extraction and sequencing

Total genomic DNA from silica-dried leaves and herbarium specimens was extracted from 121 specimens using a modified CTAB method (Doyle & Doyle, 1987). The *matK* gene was amplified and sequenced using primers 390F and 1326R (Cuénoud & al., 2002) and MintF and MintR (Pirie & al., 2005b). Where the 390F primer failed to amplify, the forward primer 390F-2 was used instead (5'-CGYCATCTGGAAATCTTGGTTC-3'). For *rbcl*, the primers 1F/724R (Olmstead & al., 1992), 636F/1460R (Fay & al., 1997; Fay & al., 1998) and 217F, 922F, 536R and 1104R (Pirie & al., 2005b) were used. The *trnT-trnF* region was amplified and sequenced using primers a, b, c, d, e and f (Taberlet & al., 1991). If problems occurred with the amplification of the *trnT-trnL* spacer the a2 primer designed by Cronn & al. (2002) was used as well. Finally, the *psbA-trnH* intergenic spacer was amplified and sequenced with primers *psbA* and *trnH* from Hamilton (1999).

A standard PCR protocol (35 cycles; 30 sec.: 94°C; 30 sec.: 53°C; 1 min.: 72°C; with an initial 5 min.: 94°C and final 10 min.: 72°C) was used and 0.4% BSA was added to the mixes. PCR products were purified using the QIAquick PCR purification kit (Qiagen) and cycle-

sequenced with DYE-ET terminators (Amersham) and run on an ABI 3730XL automated DNA sequencer.

Phylogenetic analyses

Sequences were edited and assembled in SeqMan 4.0 (DNASTar Inc., Madison, WI), alignment was done by eye and simple indel coding (Simmons & Ochoterena, 2000) was applied. Most-parsimonious trees were generated from 10,000 replicates of random taxon addition and swapped using tree bisection-reconnection (TBR), equal weights and a maximum of five trees held at each step, using PAUP* 4.0b10 (Swofford, 2003). Bootstrap analysis (Felsenstein, 1985) of 10,000 replicates with “full” heuristic searches of 10 random addition sequences was performed, equal weights and TBR swapping, saving a maximum of 5 trees at each replicate. Bootstrap support of 50–74% is considered to represent weak support, 75–89% moderate support and 90–100% strong support. The strict consensus from the analysis of matrix A was used as a backbone constraint on matrix B. The search for most parsimonious trees was repeated as described above, as well as the bootstrap analysis.

The data were also analysed with Bayesian inference using MrBayes version 3.1.1 (Huelsenbeck & Ronquist, 2001); analyses were run for 5,000,000 generations with six simultaneous Monte Carlo Markov Chains, and one tree sampled per 100 generations. Parameters for the general model of DNA substitution (GTR with gamma-distributed rate variation across sites) for the separate partitions were estimated by MrBayes. All partitions were allowed to have their unique model and the overall evolutionary rate was allowed to be (potentially) different across partitions. Only parsimony informative characters were coded as indels and MrBayes was informed of this coding bias in order to calculate the probability of the data correctly (using the “lset coding=informative” option).

Age estimation

Maximum likelihood as implemented in PAUP* was used to calculate branch lengths from both matrices with the DNA substitution models that were indicated as optimal for the combined data partitions of matrix A (TIM + I + G) and matrix B (TVM + I + G) by Modeltest v. 3.06 (Posada & Crandall, 1998). Both matrices were used to estimate ages of clades so that these ages could be compared between data sets. Rate heterogeneity among lineages was evaluated using the likelihood ratio test (Felsenstein, 1988). For both matrices a molecular clock was rejected because the log likelihoods of the unconstrained and constrained analyses were significantly different (matrix A: 19,363.05 versus 21,813.6, $P < 0.001$; matrix B: 16,135.65 versus 16,331.93, $P < 0.001$). Therefore, non parametric rate smoothing (NPRS; Sanderson, 1997) and penalized likelihood (PL; Sanderson, 2002) were used to estimate divergence times, using the software package r8s (Sanderson, 2004).

Three calibration points were used in this study: (1) the age estimate by Wikström & al. (2001) for the stem of Annonaceae (figure 1, marked “Wi”; 82 my), (2) fossil seeds from the Maastrichtian in Nigeria, well-characterised with their lamelliform ruminations (Chesters, 1955); figure 1, marked “Fo”; 68 my), and (3) the endemic occurrence of *Guatteria caribaea* on the Lesser Antilles (Puerto Rico, Guadeloupe, Dominica, Martinique, Saint Lucia and Nevis; (Fries, 1939). Although originating from the late Eocene, these islands may not have been permanently uplifted. In their present form the majority of them are younger than the

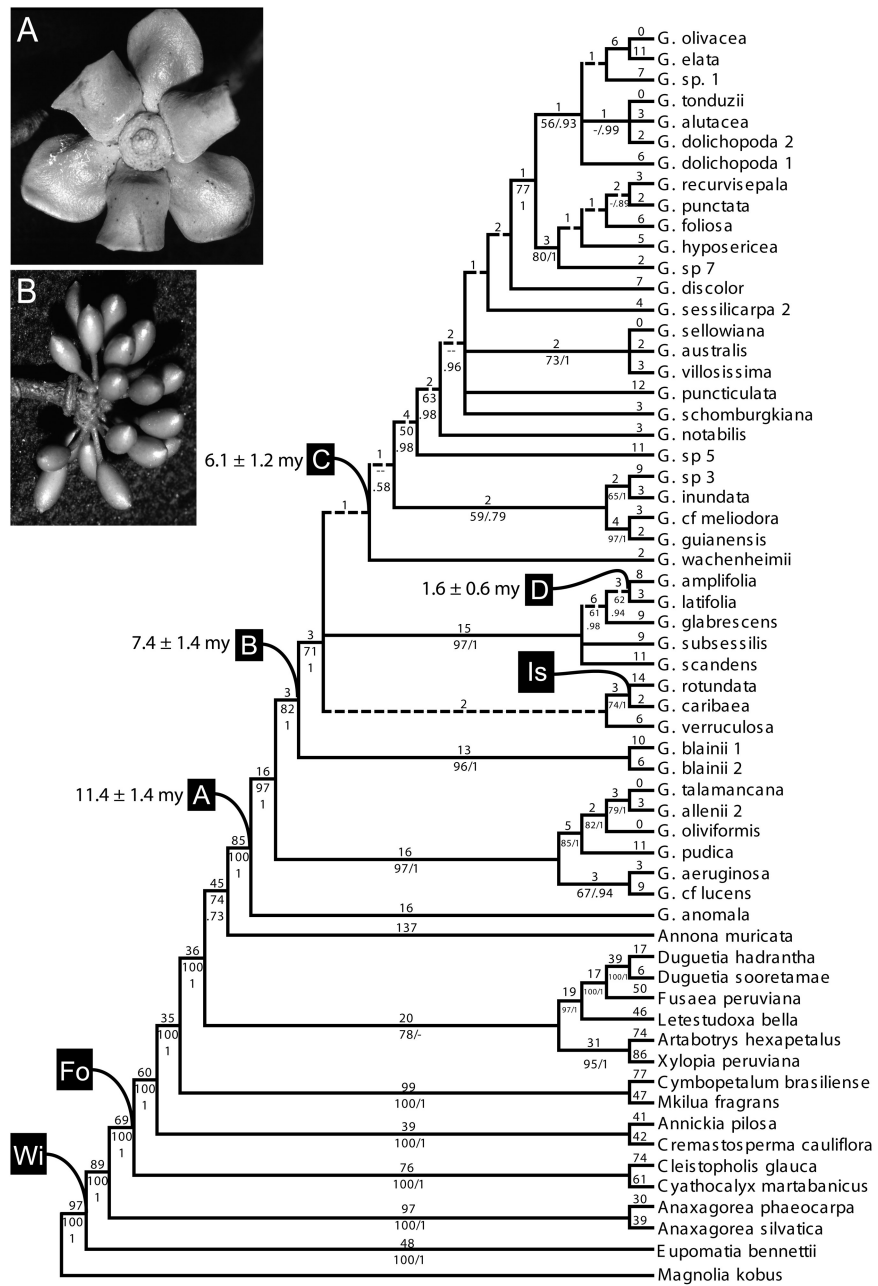


Figure 1. One of 41,968 most parsimonious trees from an analysis based on *matK*, *trnTL*, *trnLF*, *rbcL* and *psbA-trnH* (matrix A). Branch lengths are shown above the branches, bootstrap percentages and Bayesian posterior probabilities below. Branches absent in the strict consensus of all most parsimonious trees are indicated by dashed lines. Calibration points are marked with Wi (Wikström, 82 my), Fo (Maastrichtian seed, 68 my) and Is (maximum age of the Lesser Antilles, 6 my; see text). Dated nodes based upon PL are labelled A-D (node A: 11.4 ± 1.4 my, node B: 7.4 ± 1.4 my, node C: 6.1 ± 1.2 my, node D: 1.6 ± 0.6 my). Photograph A. open flower bud of *Guatteria oliviformis*; B. Fruits of *Guatteria allenii*.

Pliocene (Iturralde-Vinent & MacPhee, 1999). Although objections exist against the use of the age of strata on which endemic taxa occur for calibrating phylogenetic trees (see Heads (2005) for a critique), it is the only additional calibration point possible within *Guatteria* since there are no known fossils. Therefore, the split between *G. caribaea* and *G. rotundata* was constrained to a maximum age of 6 million years (figure 1, marked "Is"). For a discussion on the placement and accuracy of calibration point (1) and (2) see Richardson & al. (2004). Mean values and confidence intervals for all dates were calculated by reapplying NPRS and PL to 100 bootstrapped matrices (Sanderson, 2004).

Biogeography

Species distributions were scored using floristic regions as described by Takhtajan (Takhtajan, 1986). Within the Neotropical Kingdom he specifies the Caribbean, Guyana Highlands, Amazonian, Brazilian and Andean regions. The occurrences of the different species within these regions were mapped onto the cladogram in figure 2 and optimised over the tree with MacClade version 4.08 (Maddison & Maddison, 2000) using Fitch optimisation (Fitch, 1971).

RESULTS

Phylogeny and geography of speciation

For the 60 accessions in matrix A, the *matK*, *rbcl*, *trnT-trnF* and *psbA-trnH* plastid DNA regions were sequenced, with the exception of *Eupomatia bennettii* for which no *matK*, *psbA-trnH* and *trnTL* sequences were obtained, *Magnolia kobus*, *Guatteria cf. lucens* and *G. blainii* 1 of which the *trnTL* region failed to amplify, and *Cyathocalyx martabanicus* and *Annona muricata* for which only partial *trnTL* could be sequenced. Matrix B contained no missing data, except for the above mentioned outgroup sequences.

Phylogenetic analysis of matrix A resulted in 41,968 most parsimonious trees of 2192 steps, with a consistency index (CI) of 0.78 and retention index (RI) of 0.84. One of the most parsimonious trees is shown in figure 1. The relationships within the outgroup taxa are fully congruent with Richardson & al. (2004) and are in general strongly supported. *Guatteria* proves to be monophyletic, receiving bootstrap support (BS) of 100% and a posterior probability (PP) of 1.0 and is subtended by a relatively long branch of 85 substitutions. Early-diverging relationships within the genus generally have strong support but towards the tips of the tree support values decline.

The strict consensus of the trees from matrix A (figure 1) was used as a backbone constraint on matrix B and subsequent phylogenetic analysis of this matrix resulted in 39,305 most parsimonious trees of 1826 steps (CI 0.72; RI 0.83). One of the most parsimonious trees is shown in figure 2, including optimised geographical areas. Due to the backbone constraint used, relationships within the outgroups receive high bootstrap support (not shown) as

Figure 2. (next page) One of 39,305 most parsimonious trees from an analysis based on *matK*, *trnLF*, *rbcl* and *psbA-trnH* (matrix B). Branch lengths are shown above the branches, bootstrap percentages and Bayesian posterior probabilities below. Age estimates for nodes are based upon PL analysis of matrix A and are labelled A-D. Optimised geographical areas are indicated at the nodes as CA (Central American) or SA (South American). Geographic distribution of the species is also shown. Solid square boxes indicate species within the "South American Clade" that are of Central American distribution. The arrow indicates the possible onset of the radiation. Outgroups were pruned, except for *Annona muricata*. Relationships and support for pruned outgroups are equal to figure 1

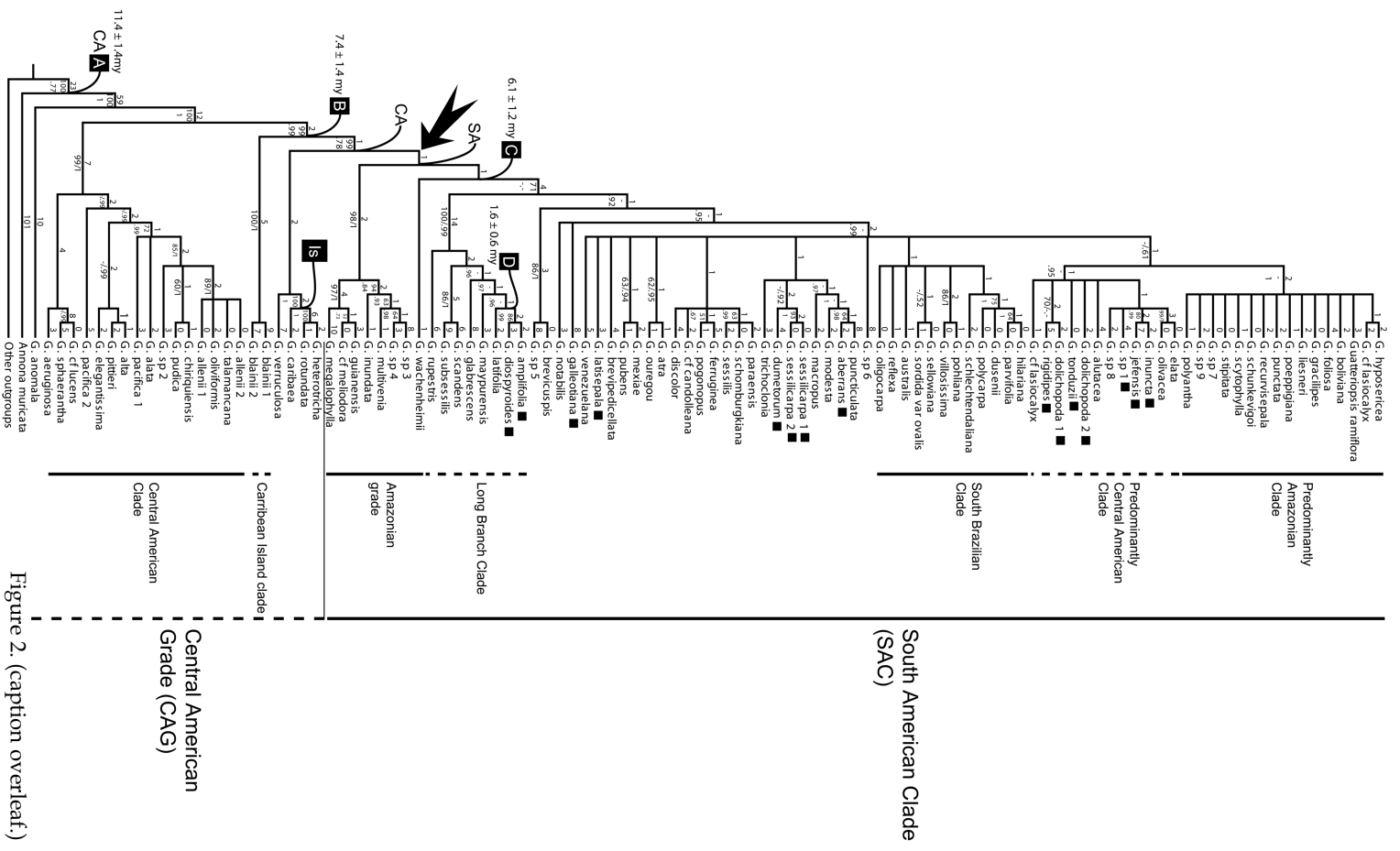


Figure 2. (caption overleaf.)

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well as the early diverging relationships within *Guatteria*. However, the unconstrained and independent Bayesian analysis corroborates the result found by the bootstrap analysis. *Guatteria anomala* from Mexico and Guatemala is sister to the rest of the genus and is part of a grade (Central American grade; CAG) consisting of species from Central America, the Caribbean Islands (*G. blainii* and *G. caribaea*) and some species from the west side of the Andes in Colombia. Therefore, the crown node of *Guatteria* resolves as Central American (figure 2, node A). From within this grade a clade is derived in which bootstrap support is in general weak or absent. This clade primarily contains South American species and hence this clade will be referred to as the South American Clade (SAC). The crown node of this clade resolves as South American (figure 2, indicated by SA). In contrast to the bootstrap analysis, the Bayesian yields a better supported backbone topology in the SAC. Generally, both analyses show lack of support for relationships towards the tips of the tree in the SAC. One clade is exceptional with regard to the length of the branch leading to it. The Long Branch Clade containing *G. scandens*, *G. subsessilis*, *G. glabrescens*, *G. latifolia*, and *G. amplifolia* (BS 100% ; PP 0.99) is subtended by a relatively long branch of 14 substitutions, the longest branch within the topology. Lastly, the node connecting the *G. verruculosa* - *G. heterotricha* clade with its sister is reconstructed as Central American (figure 2, indicated by CA).

Timing of diversification

Age estimates on the basis of matrix A were always younger than those based on matrix B. Also, estimates calculated with PL were always younger than those calculated with NPRS (table 1). Regarding variation in substitution rate PL is considered to provide the best estimates for ages because NPRS tends to over-smooth short branches (Sanderson, 1997; 2004) and many short branches are present in the tree in figure 2. Because age estimates must be seen as minimum ages only the youngest age estimates (based on PL and conducted on matrix A) will be reported in the text. The node connecting the *G. caribaea* - *G. rotundata* clade with its sister, *G. verruculosa*, does not have high bootstrap and/or Bayesian support values. It might be argued that this uncertainty in relationships might influence the dating of the tree because the occurrence of *G. caribaea* on the Caribbean islands is used as a calibration point. However, removing this calibration point from the analysis did not result in different age estimates (data not shown).

The crown node of *Guatteria* is dated at least 11.4 ± 1.4 my (figure 1, node A). The node connecting the *G. blainii* clade and the rest of the genus has an estimated age of at least 7.4 ± 1.4 my (figure 1, node B). The age of the node linking *G. wachenheimii* with its sister group was estimated at least 6.1 ± 1.2 my (figure 1, node C). Finally, the split between the Central American species *G. amplifolia* and its South American sister species *G. latifolia* was

Table 1. Mean age estimates with standard deviations (million years) from bootstrap resampled analyses for selected nodes of *Guatteria* based upon PL and NPRS analyses of matrix A and B. Node B did not occur in the trees from the bootstrap resampling analyses of matrix B. Age constraints for calibration are marked in figure 1 and 2. Nodes are those labelled in figure 1 and 2.

Node	Matrix A,	Matrix A, PL	Matrix B,	Matrix B, PL
A	21.5 ± 1.7	11.4 ± 1.4	29.9 ± 2.1	25.7 ± 5.2
B	16.0 ± 1.8	7.4 ± 1.4	-	-
C	13.1 ± 1.8	6.1 ± 1.2	21.0 ± 2.7	19.5 ± 5.5
D	3.3 ± 1.1	1.6 ± 0.6	5.0 ± 1.7	5.3 ± 2.5

dated at least 1.6 ± 0.6 my (figure 1, node D).

DISCUSSION

Lack of resolution indicates a radiation

Although it was possible to reconstruct the phylogenetic relationships between several clades within *Guatteria*, the majority of the relationships remain unclear. There is a lack of informative characters leading to many short branches and hence low resolution. This pattern is comparable to that found by Richardson & al. (2001) in *Inga*, noteworthy because both genera are comparable in their size and distribution. *Inga* is considered a rapidly diversifying, species rich genus because most estimates place diversification in *Inga* within the last 10 my (with many species arising as recently as 2 million years ago). The crown group of *Guatteria* was estimated to be at least 11.4 ± 1.4 my of age. The minimum age of the SAC, which contains most of the species diversity, is estimated at between 6.1 ± 1.2 and 7.4 ± 1.4 my (figure 1 and 2, node B and C). Thus, for *Guatteria* as well as *Inga* most speciation occurred relatively recent and *Guatteria* might therefore also be called a rapidly diversifying, species-rich genus. The fact that internal branch lengths in the SAC are significantly shorter than in the CAG (t-test, $P < 0.05$; matrix A and B) supports the hypothesis that the SAC represents a rapid diversification.

Geography of speciation

Amazonian-centred genera sensu Gentry are autochthonous South American and their main diversity is found in the Amazon (Gentry, 1982). Because *Guatteria* has its highest species diversity there, it could be argued that *Guatteria* is such a genus. Furthermore, most of the Amazonian-centred taxa are canopy trees and lianas and *Guatteria* for 99% comprises trees. Next to this, according to Gentry the Amazonian-centred taxa are poorly represented in Central America with only c. 15% of their species there. *Guatteria* contains only c. 30 Central-American species (c. 12%).

In order to test whether *Guatteria* is Amazonian-centred it is necessary to know where the genus originated and how it spread throughout the Neotropics. Optimisation of geographical distributions onto the tree in figure 2 shows that the crown node of the SAC is optimised as South American, while the more basal nodes are all optimised as Central American. These data suggest that the most recent common ancestor of all extant *Guatteria* species arose in the early Miocene of what now is called Central America (figure 2). The hypothesis that early diversification took place in proto-Central America is further supported by the fact that the basal lineages comprise solely of species from Central America, the Caribbean Islands or species from the west side of the Andes in Colombia. That the SAC is derived from within this clade implies that *Guatteria* dispersed into South America in the late Miocene (figure 2). Because of the timing of this migration *Guatteria* moved into South America before the complete closure of the Isthmus of Panama (c. 3-3.5 my ago) and subsequently diversified rapidly.

The more ancient evolutionary history of the CAG is reflected in the morphology of the species. The species in the CAG are mostly endemic to Central America, a high level of endemism that is expected from the isolation of a landmass for a considerable amount of time (Burnham & Graham, 1999). Morphologically they are easier to distinguish than the species in the large SAC clade. In contrast, the SAC contains many widespread species and

species complexes. Several of these species have entered Central America again (figure 2) and are part of morphologically difficult complexes (e.g. *G. dolichopoda*, *G. tonduzii* and *G. rigidipes* or *G. amplifolia* and *G. diospyroides*). Many intermediates are present between the putative Central American and South American species. The closer alliance of the remigrants to the South American, rather than to the other Central American, *Guatteria* species is corroborated by a seven base pair insertion in the *trnLF* region (the only supporting character on that particular node in figure 2), a synapomorphy for *G. anomala* and all the species in the CAC and the Caribbean clade.

Simpson (1975) generated a list of 14 genera that must have been immigrants to South America from the north (although she was unable to estimate the timing of arrival). She mentions *Bomarea*, *Ranunculus*, *Lupinus*, *Gentiana* and *Senecio* among others. In contrast to *Guatteria* only few of these genera show radiation following their arrival in South America. Central American distributional centres have also been suggested for genera as *Ocotea*, *Beilschmiedia*, *Cedrela*, *Luehea*, *Oreopanax*, and *Swartzia* on the basis of fossil data (Leopold & MacGinitie, 1972). Especially *Ocotea* (Lauraceae) is interesting in this respect. With c. 300 species (Madriñán, 2004) it is, next to *Guatteria* and *Inga*, one of the largest Neotropical genera that predominantly comprise trees. The fossil record is inadequate to show when the modern distribution was reached but phytogeographic and ecological evidence suggests that it was before the closure of the Panamanian land bridge (Gentry, 1982). This conclusion is supported by phylogenetic analyses that estimate an early Miocene arrival of the *Ocotea* complex in South America (Chanderbali & al., 2001; Renner, 2004). Furthermore, similar to *Guatteria* a Central America-centred species group in *Ocotea* is sister to a South America-centred species rich group and the split between these lineages is estimated at 20 my.

The predicted diversity for a group of plants isolated in South America until the establishment of the Panamanian land bridge would be a high number of species in South America, with only a few, perhaps widespread, taxa extending into Central America. This pattern has indeed been found for many genera (Burnham & Graham, 1999) but cannot be acknowledged for *Guatteria*. Most of the Central American *Guatteria* species are endemic. Only one or two species occur outside Central America (mostly in undercollected regions in Colombia), and just one species (*G. recurvisepala*) is probably widespread with its distribution throughout Central America, Venezuela and the Guianas.

Based on all of the above it can be concluded that *Guatteria* is not an autochthonous South American genus but has dispersed into that region at some time during the Miocene.

Migrations in the evolution of Guatteria

Three events are of particular importance in the history of the South American continent in the last 15-20 my (Gentry, 1982; Burnham & Graham, 1999): 1) the Andean orogony, 2) the closing of the Isthmus of Panama and 3) the Pleistocene climatic fluctuations.

The major period of uplift in northern South America occurred in the Miocene epoch (Burnham & Graham, 1999) and resulted in the formation of the Andes. By the late Miocene epoch the Cordillera Oriental in Colombia is estimated to have reached an altitude of around 1000m (Wijninga, 1996) but most of the uplift took place only in the last 5 my

(Gentry, 1982). The fact that the crown of the SAC is at least 6.1-7.4 my old might mean that migration of *Guatteria* into South America took place before the Andes proved an effective barrier. According to Chanderbali & al. (2001), the timing of the separation between the Central America-centred species group and the South America-centred species rich group in *Ocotea* coincides with increased uplift of the northern Andes in the early Miocene. Therefore they state that it is conceivable that the Andean orogeny divided the ancestral range of *Ocotea*. However, here it is believed that migration across the submerged Panamanian land bridge might have been a more important event in splitting the Central and South American lineages, at least for *Guatteria*, than the relatively low altitude of the Andes in the early Miocene. Nevertheless, the Andes might nowadays be an effective barrier against (re)migration.

Next to Andean orogeny the closing of the Isthmus of Panama was one of the most important events for the Latin American biota because it enabled easy migration between North and South America. The definitive closure of the isthmus has been estimated around 3-3.5 my ago (Coates & Obando, 1996) and is known to have caused the so-called "Great American Interchange" in animals. The land bridge appears to be far less significant in angiosperms and numbers of South American species derived from northern immigrants are estimated at about 10% (Burnham & Graham, 1999). Indeed, in the case of *Guatteria* the submerged land bridge did not seem an obstacle against migration. On the other hand, the estimate of at least 6.1 ± 1.2 my for the migration of *Guatteria* into South America is about the same age as a hypothesised short-lived terrestrial corridor around c. 4-7 my ago (Bermingham & Martin, 1998). In addition, Iturralde-Vinent and MacPhee (1999) suggested a land bridge that could have existed between c. 8.5 and 14 my and Pennington & Dick (2004) put forward even other possibilities for land bridges. Therefore, migration might not have taken place across a large water barrier but via stepping stones.

While the first arrival of *Guatteria* in South America took place prior to the definitive Pliocene closure of the Isthmus, the remigration into Central America could have happened after closure, as for example can be seen from the split between the Central American *G. amplifolia* - *G. diospyroides* lineage and its South American sister that is estimated at a minimum of 1.6 ± 0.6 my (figure 1 and 2, node D).

The third important event in the history of South America are the Pleistocene climatic fluctuations, associated with glacial advances and retreats at higher altitudes. Data of Pennington & al. (2004) show that Pleistocene diversification plays an important role in Central American semi-deciduous tropical forests. In *Inga* some evidence exist for Pleistocene speciation in Neotropical rain forests (Richardson & al., 2001a). *Guatteria* occurs nearly exclusively in wet evergreen forests and in this study the many recent speciation events seem to suggest that at least some part of the extant diversity in *Guatteria* is due to Pliocene or even Pleistocene speciation. However, the impact of the climatic fluctuations is difficult to assess on the basis of the current data set.

Ecological speciation and vicariance

The link between species proliferation and adaptive diversification in *Guatteria* seems very weak, especially in the SAC. This observation seems to corroborate the suggestion of (Morawetz & Waha, 1985) that the main cause of speciation in *Guatteria* could have been

adaptation to a number of different ecological niches by changes in vegetative characters. Phylogenetic analyses by Fine & al. (2005) showed that multiple putative sister taxa with parapatric distributions differ in their edaphic associations, suggesting that edaphic heterogeneity was an important driver of speciation in the tribe Protieae (Burseraceae) in the Amazon basin. A comparable mechanism might also be responsible for speciation in *Guatteria*. Morawetz and Waha (1985) might have been right by pinpointing the Amazon as speciation centre because the basin might have played a pivotal role in the rapid diversification of *Guatteria*. However, the ecological and phylogenetic data needed to test this hypothesis thoroughly is presently lacking.

An alternative explanation for species-richness in *Guatteria* is that its populations might have been subdivided on a regular basis due to abiotic factors. In such a case no one character or set of characters would be causally involved in promoting speciation (Cracraft, 1985). Although support is weak, a few geographically delimited clades can be seen in figure 2, suggesting at least some influence of geography on the origin of lineages. For instance with regard to the predominantly Amazonian or Central American clades, the fragmented landscape of the Amazon basin (Colinvaux, 1996) or the complex geological history of Central America (Iturralde-Vinent, 1999) would have provided ample opportunities for sequences of vicariant events leading to allopatric speciation, though with only negligible adaptive divergence between the vicariant species (Skelton, 1993).

CONCLUSIONS

It seems that the evolutionary history of *Guatteria* can be regarded as a three step process: 1) a trans-oceanic Miocene migration from Central into South America before the closing of the Isthmus of Panama, unhampered by the low altitude of the Andes; 2) a subsequent major diversification of tree species in South America; and 3) several small remigrations of South American lineages into Central America across the Andes and over the closed Panamanian land bridge. We can therefore conclude that *Guatteria* is not an Amazonian centred-genus *sensu* Gentry but a recent major radiation after dispersal into South America. As brought to attention by Pennington & Dick (2004) the contribution of immigrant taxa to the South American rainforest flora needs a re-evaluation in order to properly understand the historical assembly of its biodiversity. *Guatteria* proves the importance of their claim, whilst providing another case in which migration into South America might have contributed substantially to South American speciation. Furthermore, a closer comparison of the evolutionary history of *Ocotea*, *Inga* and *Guatteria* is highly desirable because all three large Neotropical tree genera might show comparable phylogenetic patterns and timings.

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