

# A rapid diversification 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 the 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 diversification 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.

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## 1. Introduction

The Neotropics hold 30% of the world's plant diversity (Smith et 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 et al., 2001; Renner and Meyer, 2001; Davis et al., 2002; Pennington and Dick,

2004; Richardson et al., 2004; Nathan, 2006; Pirie et 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 and Mori, 1999; Richardson et al., 2001).

The genus *Guatteria* (Annonaceae) is, with approximately 265 species, one of the largest genera of Neotropical

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trees along with *Inga* and *Ocotea* (Lauraceae, c. 300 species; Madriñán, 2004). Species of *Guatteria* are frequent constituents of Neotropical (lowland) forests (Morawetz and Waha, 1985) and the genus is widely distributed throughout Mesoamerica (c. 30 species), the Caribbean (3 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 semi-deciduous forests (Morawetz and 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 and 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 et 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.

## 2. Materials and methods

### 2.1. 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 et 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 Table 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 aforementioned larger matrix (matrix B; voucher information in Table 1), with 106 species but only four plastid regions.

### 2.2. 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 and Doyle, 1987). The *matK* gene was amplified and sequenced using primers 390F and 1326R (Cuénoud et al., 2002) and MintF and MintR (Pirie et al., 2005). Where the 390F primer failed to amplify, the forward primer 390F-2 was used instead (5'-CGYYCATCTGGAAATCTTGGTTC-3'). For *rbcL*, the primers 1F/724R (Olmstead et al., 1992), 636F/1460R (Fay et al., 1997; Fay et al., 1998) and 217F, 922F, 536R and 1104R (Pirie et al., 2005) were used. The *trnT-trnF* region was amplified and sequenced using primers a, b, c, d, e and f (Taberlet et al., 1991). If problems occurred with the amplification of the *trnT-trnL* spacer the a2 primer designed by Cronn et 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 s: 94 °C; 30 s: 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.

Table 1  
Taxa, voucher information and GenBank/EBI accession numbers of DNA sequences

Taxon	Geography	Voucher	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>psbA-trnH</i>	<i>trnTL</i>	Matrix A
<i>Magnolia kobus</i> DC.	Cultivated in Utrecht University Botanic Garden, originating from Japan	Chatrou, L.W. et al. 278 (U)	AY743438	AY743457	AY743476	AY841425	—	Yes
<i>Eupomatia bennettii</i> F.Muell.	Cultivated in Utrecht University Botanic Garden (1983GR00365)	Chatrou, L.W. et al. 278 (U)	DQ861790	DQ861842	—	—	—	Yes
<i>Anaxagorea phaeocarpa</i> Mart.	Ecuador	Maas, P.J.M. et al. 8592 (U)	AY238952	AY238944	AY238960	AY841426	DQ861643	Yes
<i>Anaxagorea silvatica</i> R.E.Fr.	Brazil	Maas, P.J.M. et al. 8836 (U)	AY743439	AY743458	AY743477	AY841427	DQ861644	Yes
<i>Annickia pilosa</i> (Exell) Setten & Maas	Gabon	Sosef, M.S.M. 1803 (WAG)	AY743450	AY743469	AY743488	AY841444	AY841572	Yes
<i>Annona muricata</i> L.	Cultivated in Utrecht University Botanic Garden (83GR00169), of Neotropical origin	Chatrou, L.W. 468 (U)	AY743440	AY743459	AY743478	AY841428	DQ861648	Yes
<i>Artabotrys hexapetalus</i> (L.f.) Bhandari	Cultivated in Utrecht University Botanic Garden (94GR01614), originating from India	Chatrou, L.W. 470 (U)	AY238953	AY238946	AY238962	AY841429	DQ861649	Yes
<i>Cleistopholis glauca</i> Engl. & Diels	Gabon	Wieringa, J.J. et al. 3278 (WAG)	AY841603	AY841681	AY841395	AY841432	DQ861645	Yes
<i>Crematosperma cauliflorum</i> R.E.Fr.	Peru	Chatrou, L.W. et al. 224 (U)	AY743519	AY743565	AY743542	AY841448	AY841574	Yes
<i>Cyathocalyx martabanicus</i> Hook.f. & Thoms.	Kabun Raya Bogor, cultivated, origin unknown	Mols, J.B. 11 (L)	AY841605	AY841683	DQ125054	DQ125120	—	Yes
<i>Cymbopetalum brasiliense</i> (Vell.) Benth. ex Baill.	Cultivated in Utrecht University Botanic Garden (84GR00275), originating from Brazil	Chatrou, L.W. 471 (U)	AY841608	AY841686	DQ125055	DQ125121	DQ861646	Yes
<i>Duguetia hadrantha</i> (Diels) R.E.Fr.	Peru	Chatrou, L.W. et al. 181 (U)	AY738161	AY740573	AY740541	DQ125123	DQ861650	Yes
<i>Duguetia sooretamae</i> Maas	Brazil	Maas, P.J.M. et al. 8827 (U)	AY738177	AY740589	AY740557	DQ861746	DQ861651	Yes
<i>Fusaea peruwiana</i> R.E.Fr.	Peru	Chatrou, L.W. et al. 179 (U)	AY743445	AY743464	AY743483	AY841436	DQ861652	Yes
<i>Letestudoxa bella</i> Pellegr.	Gabon	Wieringa, J.J. & T. Nzabi 2797 (WAG)	AY841629	AY841707	DQ125059	DQ125128	DQ861653	Yes
<i>Mkilua fragrans</i> Verdc.	Cultivated in Utrecht University Botanic Garden (E91GR01649), of tropical East African origin	Chatrou, L.W. 474 (U)	AY841634	AY841712	DQ125060	DQ861696	DQ861647	Yes
<i>Xylopia peruwiana</i> R.E.Fr.	Cultivated in Utrecht University Botanic Garden (84GR00271), originating from Peru	Chatrou, L.W. 483 (U)	AY238958	AY238951	AY238967	DQ125134	DQ861654	Yes
<i>Guatteria aberrans</i> Erkens & Maas	Panama	Maas, P.J.M. et al. 9570 (U)	DQ124987	DQ125045	DQ125111	DQ125227		
<i>Guatteria aeruginosa</i> Standl.	Costa Rica	Chatrou, L.W. et al. 66 (U)	AY740958	AY741007	AY740909	DQ125136	DQ861661	Yes
<i>Guatteria alata</i> Maas & Setten	Panama	Mori, S.A. 2894 (U)	AY740959	AY741008	AY740910	DQ125137		
<i>Guatteria allenii</i> R.E.Fr. #1	Panama	Mori, S.A. 2952 (U)	AY740960	AY741009	AY740911	DQ125138		
<i>Guatteria allenii</i> R.E.Fr. #2	Panama	Maas, P.J.M. et al. 9538 (U)	DQ861791	DQ861843	DQ861697	DQ861747	DQ861695	Yes
<i>Guatteria alta</i> R.E.Fr.	Colombia	Gentry, A.H. and M. Monsalve 48250 (U)	DQ124941	DQ124999	DQ125065	DQ125139		
<i>Guatteria alutacea</i> Diels	Bolivia	Chatrou, L.W. et al. 339 (U)	AY740961	AY741010	AY740912	DQ125140	DQ861682	Yes
<i>Guatteria amplifolia</i> Triana & Planch.	Costa Rica	Chatrou, L.W. et al. 111 (U)	DQ124942	DQ125000	DQ125066	DQ125141	DQ861664	Yes
<i>Guatteria anomala</i> R.E.Fr.	Mexico	Ishiki, M. et al. 2233 (U)	AY740962	AY741011	AY740913	AY841437	DQ861657	Yes
<i>Guatteria atra</i> Sandwith	Guyana	Gopaul, D. & Maas, P.J.M. 2791 (U)	AY740963	AY741012	AY740914	DQ125142		
<i>Guatteria australis</i> A.St.-Hil.	Brazil	Lobão, A.Q. & Fiaschi, P. 499 (U)	AY740964	AY741013	AY740915	AY841438	DQ861675	Yes
<i>Guatteria blainii</i> (Griseb.) Urb. #1	Dominican Republic	Maas, P.J.M. 6443 (U)	AY740965	AY741014	AY740916	DQ125143	—	Yes
<i>Guatteria blainii</i> (Griseb.) Urb. #2	Cuba	Rainer, H. 1620 (WU)	DQ861793	DQ861845	DQ861699	—	DQ861688	Yes

(continued on next page)

Table 1 (continued)

Taxon	Geography	Voucher	<i>rbcL</i>	<i>trnLF</i>	<i>matK</i>	<i>psbA-trnH</i>	<i>trnTL</i>	Matrix A
<i>Guatteria boliviana</i> H.J.P. Winkl.	Bolivia	Solomon, J.C. 10789 (U)	DQ124943	DQ125001	DQ125067	DQ125144		
<i>Guatteria brevicuspis</i> R.E.Fr.	Brazil	Prance, G.T. 16328 (U)	AY740966	AY741015	AY740917	DQ125145		
<i>Guatteria brevipedicellata</i> R.E.Fr.	Colombia	Lawrence, G.E. 771 (S)	DQ124944	DQ125002	DQ125068	DQ125146		
<i>Guatteria caribaea</i> Urb.	Dominican Republic	Tuxill, J. 89 (U)	AY740967	AY741016	AY740918	DQ125149	DQ861689	Yes
<i>Guatteria</i> cf <i>candolleana</i> Schldtl.	Brazil	Harley, R.M. et al. 17360 (U)	DQ124946	DQ125004	DQ125070	DQ125148		
<i>Guatteria</i> cf <i>hilariana</i> Schldtl.	Brazil	Gottsberger, G. & Gottsberger I. (U)	DQ124952	DQ125010	DQ125076	DQ125165		
<i>Guatteria</i> cf <i>lasiocalyx</i> R.E.Fr. #1	Brazil	Maas, P.J.M. et al. 9000 (U)	DQ124969	DQ125027	DQ125093	DQ125202		
<i>Guatteria</i> cf <i>lasiocalyx</i> R.E.Fr. #2	Bolivia	Krukoff, B.A. 11086 (U)	DQ124945	DQ125003	DQ125069	DQ125147		
<i>Guatteria</i> cf <i>meliodora</i> R.E.Fr.	Brazil	Maas, P.J.M. et al. 9231 (U)	DQ124955	DQ125013	DQ125079	DQ125177	DQ861677	Yes
<i>Guatteria chiriquiensis</i> R.E.Fr.	Costa Rica	Chatrou, L.W. et al. 43 (U)	AY740968	AY741017	AY740919	DQ125150		
<i>Guatteria diospyroides</i> Baill.	Costa Rica	Chatrou, L.W. et al. 84 (U)	AY740969	AY741018	AY740920	DQ125152		
<i>Guatteria discolor</i> R.E.Fr.	Brazil	Maas, P.J.M. et al. 9030 (U)	AY740970	AY741019	AY740921	DQ125153	DQ861676	Yes
<i>Guatteria dolichopoda</i> Donn.Sm. #1	Costa Rica	Maas, P.J.M. et al. 9484 (U)	DQ861800	DQ861852	DQ861704	DQ861751	DQ861693	Yes
<i>Guatteria dolichopoda</i> Donn.Sm. #2	Panama	Maas, P.J.M. et al. 9508 (U)	DQ861801	DQ861853	DQ861705	DQ861752	DQ861694	Yes
<i>Guatteria dumetorum</i> R.E.Fr.	Panama	FLORPAN 2497 (U)	AY740971	AY741020	AY740922	DQ125154		
<i>Guatteria dusenii</i> R.E.Fr.	Brazil	Dusén, P. 13752 (S)	DQ124948	DQ125006	DQ125072	DQ125155		
<i>Guatteria elata</i> R.E.Fr.	Peru	Chatrou, L.W. et al. 252 (U)	AY740972	AY741021	AY740923	DQ125156	DQ861671	Yes
<i>Guatteria elegantissima</i> R.E.Fr.	Colombia	Gentry, A.H. 56948 (U)	AY740973	AY741022	AY740924	DQ125157		
<i>Guatteria ferruginea</i> St. Hil.	Brazil	Lobão, A.Q. 643 (U)	DQ124949	DQ125007	DQ125073	DQ125158		
<i>Guatteria foliosa</i> Benth.	Bolivia	Chatrou, L.W. et al. 325 (U)	AY740974	AY741023	AY740925	DQ125159	DQ861681	Yes
<i>Guatteria galeottiana</i> Baill.	Mexico	Beaman, J.H. 6121 (U)	DQ124950	DQ125008	DQ125074	DQ125160		
<i>Guatteria glabrescens</i> R.E.Fr.	Brazil	Maas, P.J.M. et al. 8816 (U)	AY740975	AY741024	AY740926	DQ125161	DQ861656	Yes
<i>Guatteria gracilipes</i> R.E.Fr.	Brazil	Krukoff, B.A. 1156 (S)	DQ124951	DQ125009	DQ125075	DQ125162		
<i>Guatteria guianensis</i> (Aubl.) R.E.Fr.	Brazil	Webber, A.C. et al. 1884 (U)	AY740976	AY741025	AY740927	DQ125163	DQ861678	Yes
<i>Guatteria heterotricha</i> R.E.Fr.	Colombia	Monsalve B., M. 1262 (U)	AY740977	AY741026	AY740928	DQ125164		
<i>Guatteria hyposericea</i> Diels	Bolivia	Chatrou, L.W. et al. 375 (U)	AY740978	AY741027	AY740929	DQ125166	DQ861683	Yes
<i>Guatteria inuncta</i> R.E.Fr.	Costa Rica	Liesner, R.L. 14631 (U)	AY740979	AY741028	AY740930	DQ125167		
<i>Guatteria inundata</i> Mart.	Peru	Chatrou, L.W. et al. 191 (U)	AY740980	AY741029	AY740931	DQ125168	DQ861668	Yes
<i>Guatteria jefensis</i> Barringer	Panama	Valdespino, I.A. 685 (U)	AY740981	AY741030	AY740932	DQ125169		
<i>Guatteria latifolia</i> (Mart.) R.E.Fr.	Brazil	Lobão, A.Q. 544 (U)	AY740982	AY741031	AY740933	DQ125170	DQ861673	Yes
<i>Guatteria latsepala</i> R.E.Fr.	Colombia	Sánchez, D. et al. 404 (U)	DQ124953	DQ125011	DQ125077	DQ125171		
<i>Guatteria liesneri</i> D.M.Johnson & N.A. Murray	Brazil	Cid F., C.A. 8403 (U)	AY740983	AY741032	AY740934	DQ125172		
<i>Guatteria macropus</i> Mart.	Brazil	Pirani, J.R. 2725 (U)	AY740984	AY741033	AY740935	DQ125174		
<i>Guatteria maypurensis</i> Kunth	Guyana	Jansen-Jacobs, M.J. et al. 5416 (U)	AY740985	AY741034	AY740936	DQ125175		
<i>Guatteria megalophylla</i> Diels	Peru	Chatrou, L.W. et al. 216 (U)	AY740986	AY741035	AY740937	DQ125176		
<i>Guatteria mexiae</i> R.E.Fr.	Brazil	Mori, S.A. et al. 9722 (U)	DQ124956	DQ125014	DQ125080	DQ125178		
<i>Guatteria modesta</i> Diels	Colombia	Dulmen, A. van et al. 183 (U)	DQ124957	DQ125015	DQ125081	DQ125179		
<i>Guatteria multivenia</i> Diels	Ecuador	Maas, P.J.M. 8611 (U)	AY740987	AY741036	AY740938	DQ125180		
<i>Guatteria notabilis</i> Mello-Silva & Pirani	Brazil	Lobão, A.Q. 623 (U)	DQ124958	DQ125016	DQ125082	DQ125181	DQ861680	Yes
<i>Guatteria oligocarpa</i> Mart.	Brazil	Maas, P.J.M. 7006 (U)	AY740988	AY741037	AY740939	DQ125182		
<i>Guatteria olivacea</i> R.E.Fr.	Peru	Chatrou, L.W. et al. 209 (U)	AY740989	AY741038	AY740940	DQ125183	DQ861669	Yes
<i>Guatteria oliviformis</i> Donn.Sm.	Costa Rica	Chatrou, L.W. et al. 80 (U)	AY740990	AY741039	AY740941	DQ125184	DQ861662	Yes
<i>Guatteria ouegou</i> (Aubl.) Dunal	French Guiana	Scharf, U. 85 (U)	AY740991	AY741040	AY740942	DQ125185		
<i>Guatteria pacifica</i> R.E.Fr. #1	Colombia	Gentry, A.H. and D. Faber-Langendoen 62881 (U)	DQ124959	DQ125017	DQ125083	DQ125186		
<i>Guatteria pacifica</i> R.E.Fr. #2	Colombia	Cuatrecasas, J. 17150 (S)	DQ124960	DQ125018	DQ125084	DQ125187		
<i>Guatteria paraensis</i> R.E.Fr.	Brazil	Froes, R. 1753 (U)	DQ124961	DQ125019	DQ125085	DQ125188		
<i>Guatteria parvifolia</i> R.E.Fr.	Brazil	Gottsberger, G.K. 573007 (U)	AY740992	AY741041	AY740943	DQ125189		

<i>Guatteria pittieri</i> R.E.Fr.	Colombia	Werff, H.H. van der 9767 (U)	AY740993	AY741042	AY740944	DQ125190		
<i>Guatteria poeppigiana</i> Mart.	Brazil	Prance, G.T. and T.D. Pennington 1775 (U)	DQ124962	DQ125020	DQ125086	DQ125191		
<i>Guatteria pogonopus</i> Mart.	Brazil	Kollmann, L. et al. 202 (U)	DQ124963	DQ125021	DQ125087	DQ125192		
<i>Guatteria pohliana</i> Schldt.	Brazil	Anderson, W.R. et al. 35703 (U)	DQ124964	DQ125022	DQ125088	DQ125193		
<i>Guatteria polyantha</i> R.E.Fr.	Brazil	Costa, R.C. Monteiro da 284 (S)	DQ124965	DQ125023	DQ125089	DQ125194		
<i>Guatteria polycarpa</i> R.E.Fr.	Brazil	Dusén, P. 7414 (S)	DQ124966	DQ125024	DQ125090	DQ125195		
<i>Guatteria pubens</i> (Mart.) R.E.Fr.	Brazil	Spada, J. 198 (U)	DQ124967	DQ125025	DQ125091	DQ125196		
<i>Guatteria pudica</i> N.Zamora & Maas	Costa Rica	Chatrou, L.W. et al. 107 (U)	AY740994	AY741043	AY740945	DQ125197	DQ861663	Yes
<i>Guatteria punctata</i> (Aubl.) R.A.Howard	French Guiana	Molino, J.F. 1593 (U)	AY740995	AY741044	AY740946	DQ125198	DQ861672	Yes
<i>Guatteria puncticulata</i> R.E.Fr.	Peru	Chatrou, L.W. et al. 172 (U)	AY740996	AY741045	AY740947	DQ125199	DQ861666	Yes
<i>Guatteria recurvisepala</i> R.E.Fr.	Costa Rica	Chatrou, L.W. et al. 61 (U)	AY740997	AY741046	AY740948	DQ125200	DQ861659	Yes
<i>Guatteria reflexa</i> R.E.Fr.	Brazil	Glaziou, L.A. 5725 (S)	DQ124968	DQ125026	DQ125092	DQ125201		
<i>Guatteria rigidipes</i> R.E.Fr.	Costa Rica	Skutch, A.F. 2553 (S)	DQ124970	DQ125028	DQ125094	DQ125203		
<i>Guatteria rostrata</i> Erkens & Maas	Costa Rica	Chatrou, L.W. et al. 118 (U)	DQ124980	DQ125038	DQ125104	DQ125220		
<i>Guatteria rotundata</i> Maas & Setten	Panama	Mori, S.A. 5531 (U)	AY740998	AY741047	AY740949	DQ125204	DQ861687	Yes
<i>Guatteria rupestris</i> Mello-Silva & Pirani	Brazil	CFCR 4116 (U)	AY740999	AY741048	AY740950	DQ125205		
<i>Guatteria scandens</i> Ducke	Guyana	Jansen-Jacobs, M.J. et al. 5494 (U)	DQ124971	DQ125029	DQ125095	DQ125207	DQ861658	Yes
<i>Guatteria schlechtendaliana</i> Mart.	Brazil	Kollmann, L. et al. 871 (U)	DQ124972	DQ125030	DQ125096	DQ125208		
<i>Guatteria schomburgkiana</i> Mart.	Guyana	Scharf, U. 60 (U)	AY741001	AY741050	AY740952	DQ125209	DQ861686	Yes
<i>Guatteria schunkevigoi</i> D.R.Simpson	Peru	Schunke V., J. 3551 (S)	DQ124973	DQ125031	DQ125097	DQ125210		
<i>Guatteria scytophylla</i> Diels	Brazil	Maas, P.J.M. et al. 6956 (U)	AY741002	AY741051	AY740953	DQ125211		
<i>Guatteria sellowiana</i> Schldt.	Brazil	Lobão, A.Q. 557 (U)	AY741003	AY741052	AY740954	DQ125212	DQ861674	Yes
<i>Guatteria sessilicarpa</i> Maas & Setten #1	Panama	McPherson, G. 12599 (U)	AY741004	AY741053	AY740955	DQ125213		
<i>Guatteria sessilicarpa</i> Maas & Setten #2	Panama	Maas, P.J.M. et al. 9554 (U)	DQ124974	DQ125032	DQ125098	DQ125214	DQ861692	Yes
<i>Guatteria sessilis</i> R.E.Fr.	Venezuela	Liesner, R. 8546 (U)	DQ124975	DQ125033	DQ125099	DQ125215		
<i>Guatteria sordida</i> var. <i>ovalis</i> R.E.Fr.	Brazil	Riedel, L. 1689 (S)	DQ124976	DQ125034	DQ125100	DQ125216		
<i>Guatteria</i> sp. #1	Costa Rica	Chatrou, L.W. et al. 65 (U)	DQ124947	DQ125005	DQ125071	DQ125151	DQ861660	Yes
<i>Guatteria</i> sp. #2	Costa Rica	Gómez, L.D. et al. 23305 (U)	DQ124954	DQ125012	DQ125078	DQ125173	—	Yes
<i>Guatteria</i> sp. #3	Peru	Chatrou, L.W. et al. 174 (U)	DQ124981	DQ125039	DQ125105	DQ125221	DQ861667	Yes
<i>Guatteria</i> sp. #4	Peru	Chatrou, L.W. et al. 196 (U)	DQ124982	DQ125040	DQ125106	DQ125222		
<i>Guatteria</i> sp. #5	Peru	Chatrou, L.W. et al. 245 (U)	DQ124983	DQ125041	DQ125107	DQ125223	DQ861670	Yes
<i>Guatteria</i> sp. #6	Brazil	Lobão, A.Q. et al. 565 (U)	AY741000	AY741049	AY740951	DQ125206		
<i>Guatteria</i> sp. #7	Guyana	Prévost M.-F. 4247 (U)	DQ124984	DQ125042	DQ125108	DQ125224	DQ861685	Yes
<i>Guatteria</i> sp. #8	Peru	Pirie, M.D. et al. 50 (U)	DQ124985	DQ125043	DQ125109	DQ125225		
<i>Guatteria</i> sp. #9	Peru	Pirie, M.D. et al. 143 (U)	DQ124986	DQ125044	DQ125110	DQ125226		
<i>Guatteria sphaerantha</i> R.E.Fr.	Colombia	Faber-Langendoen, D. and J.A. Hurtado 1556 (U)	DQ124977	DQ125035	DQ125101	DQ125217		
<i>Guatteria stipitata</i> R.E.Fr.	Peru	Jaramillo, N. and D. Chamik 820 (U)	DQ124978	DQ125036	DQ125102	DQ125218		
<i>Guatteria subsessilis</i> Mart.	Bolivia	Maas, P.J.M. et al. 8684 (U)	DQ124979	DQ125037	DQ125103	DQ125219	DQ861655	Yes
<i>Guatteria talamancana</i> Zamora & Maas	Panama	Maas, P.J.M. et al. 9516 (U)	DQ861825	DQ861878	DQ861729	DQ861773	DQ861691	Yes
<i>Guatteria tonduzii</i> Diels	Costa Rica	Chatrou, L.W. et al. 121 (U)	AY741005	AY741054	AY740956	DQ125228	DQ861665	Yes
<i>Guatteria trichoclonia</i> Diels	Peru	Schunke V., J. 14061 (U)	DQ124988	DQ125046	DQ125112	DQ125229		
<i>Guatteria venezuelana</i> R.E.Fr.	Venezuela	Wingfield, R. and H.H. van der Werff 6688 (U)	DQ124989	DQ125047	DQ125113	DQ125230		
<i>Guatteria verruculosa</i> R.E.Fr.	Colombia	Fosberg, F.R. 19126 (S)	DQ124990	DQ125048	DQ125114	DQ125231	DQ861690	Yes
<i>Guatteria villosissima</i> A.St.-Hil.	Brazil	Lobão, A.Q. 630 (U)	AY741006	AY741055	AY740957	DQ125232	DQ861679	Yes
<i>Guatteria wachenheimii</i> Benoist	Guyana	Scharf, U. 43 (U)	DQ124991	DQ125049	DQ125115	DQ125233	DQ861684	Yes
<i>Guatterlopsis ramiflora</i> D.R.Simpson	Peru	Schunke V, J. 8073 (U)	DQ124940	DQ124998	DQ125064	DQ125135		

### 2.3. Phylogenetic analyses

Sequences were edited and assembled in SeqMan 4.0 (DNASar Inc., Madison, WI), alignment was done by eye and simple indel coding (Simmons and 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, 2000). 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 five 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 and 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).

### 2.4. 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 and 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 et al. (2001) for the stem of

Annonaceae (Fig. 1, marked “Wi”; 82 my), (2) fossil seeds from the Maastrichtian in Nigeria, well-characterised with their lamelliform ruminations (Chesters, 1955; Fig. 1, marked “Fo”; 68 my), and (3) the occurrence of *Guatteria caribaea* on the Lesser Antilles (Guadeloupe, Dominica, Martinique, Saint Lucia and Nevis) and Puerto Rico (Fries, 1939). Although originating from the late Eocene, the islands of the Lesser Antilles may not have been permanently uplifted. In their present form the majority of them are younger than the Pliocene (Iturralde-Vinent and 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 six million years (Fig. 1, marked “Is”). For a discussion on the placement and accuracy of calibration point (1) and (2) see Richardson et al. (2004). Mean values and confidence intervals for all dates were calculated by reapplying NPRS and PL to 100 bootstrapped matrices (Sanderson, 2004).

### 2.5. 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 Fig. 2 and optimised over the tree with MacClade version 4.08 (Maddison and Maddison, 2000) using Fitch optimisation (Fitch, 1971).

## 3. Results

### 3.1. 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* sp. 2 and *G. blainii* 1 of which the *trnTL* region failed to amplify, and *Cyathocalyx martinianicus* 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 Fig. 1. The relationships within the outgroup taxa are fully congruent with Richardson et 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

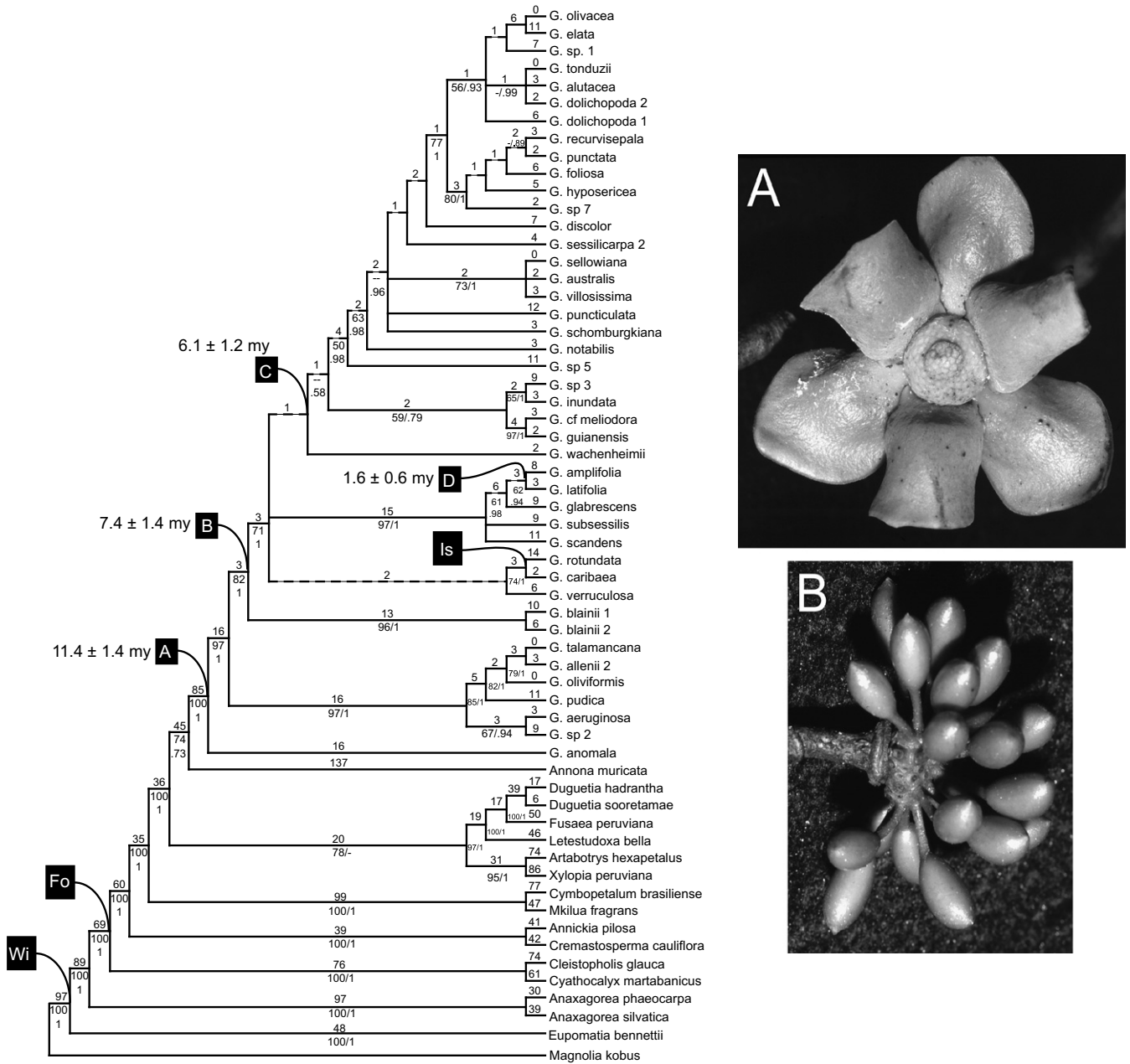


Fig. 1. One of 41,968 most-parsimonious trees from an analysis based on *matK*, *trnTL*, *trnLF*, *rbcL* and *psbA-trnH* (matrix A). Branch lengths are shown above the branches, bootstrap percentages and Bayesian posterior probabilities below. Branches absent in the strict consensus of all most-parsimonious 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:  $11.4 \pm 1.4$  my, node B:  $7.4 \pm 1.4$  my, node C:  $6.1 \pm 1.2$  my, node D:  $1.6 \pm 0.6$  my). Photograph A: open flower bud of *Guatteria oliviformis*; B: fruits of *Guatteria allenii*.

genus generally have strong support but towards the tips of the tree support values decline.

The strict consensus of the trees from matrix A (Fig. 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 Fig. 2, including optimised geographical areas. Due to the backbone constraint used, relationships within the outgroups receive high bootstrap support (not shown) as 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 (Fig. 2, node A). From within this

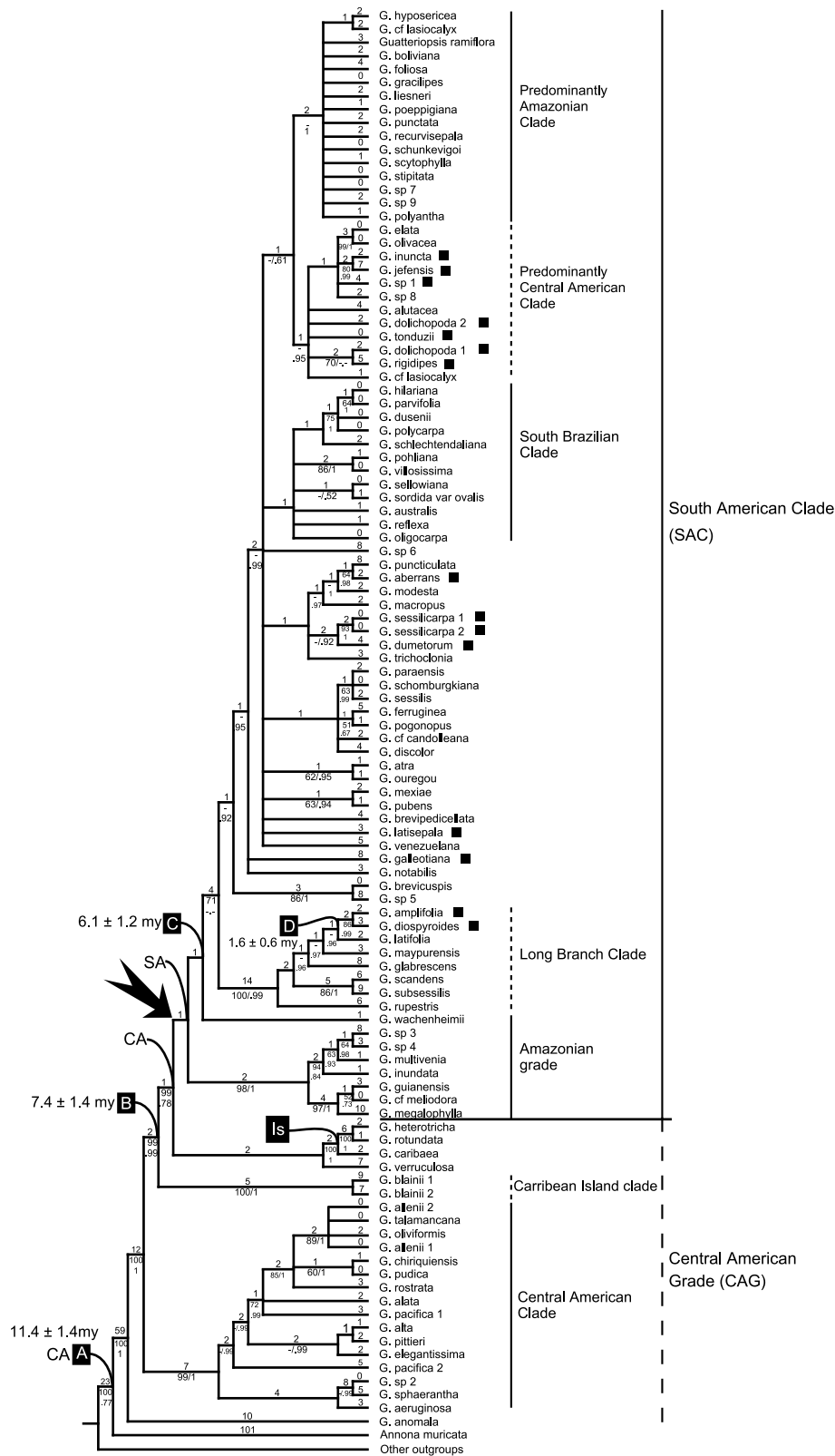


Fig. 2. One of 39,305 most-parsimonious trees from an analysis based on *matK*, *trnLF*, *rbcL* and *psbA-trnH* (matrix B). Branch lengths are shown above the branches, bootstrap percentages and Bayesian posterior probabilities below. Age estimates for nodes are based upon PL analysis of matrix A and 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 rapid diversification. Outgroups were pruned, except for *Annona muricata*. Relationships and support for pruned outgroups are equal to Fig. 1.



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 (Fig. 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 (Fig. 2, indicated by CA).

### 3.2. 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 2). 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 Fig. 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 fact that age estimates based on all three calibration points combined are consistent with age estimates based on only calibration points (1) and (2) is furthermore important because the use island ages for calibrating phylogenetic trees has been criticised. This result demonstrates that the

use of tricky calibration point (3) did not influence the age estimates.

The crown node of *Guatteria* is dated at least  $11.4 \pm 1.4$  my (Fig. 1, node A). The node connecting the *G. blainii* clade and the rest of the genus has an estimated age of at least  $7.4 \pm 1.4$  my (Fig. 1, node B). The age of the node linking *G. wachenheimii* with its sister group was estimated at least  $6.1 \pm 1.2$  my (Fig. 1, node C). Finally, the split between the Central American species *G. amplifolia* and its South American sister species *G. latifolia* was dated at least  $1.6 \pm 0.6$  my (Fig. 1, node D).

## 4. Discussion

### 4.1. Lack of resolution indicates a rapid diversification

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 et 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 two million years ago). The crown group of *Guatteria* was estimated to be at least  $11.4 \pm 1.4$  my of age. The minimum age of the SAC, which contains most of the species diversity, is estimated at between  $6.1 \pm 1.2$  and  $7.4 \pm 1.4$  my (Figs. 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.

### 4.2. 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 Fig. 2 shows that the crown node of the SAC is optimised as South American, while the more basal nodes are all optimised

Table 2

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

Node B did not occur in the trees from the bootstrap resampling analyses of matrix B. Age constraints for calibration are marked in Figs. 1 and 2. Nodes are those labelled in Figs. 1 and 2.

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 (Fig. 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 (Fig. 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 and 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 widespread species and species complexes have entered Central America again (Fig. 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 *trnL*F region (the only supporting character on that particular node in Fig. 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 rapid diversification 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 and MacGinitie, 1972). Especially *Ocotea* (Lauraceae) is interesting in this respect. With c. 300 species (Madrinán, 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 et 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 and 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.

#### 4.3. 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 and Graham, 1999): (1) the Andean orogeny, (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 and 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 1000 m (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 et 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 thought 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 and 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 and 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  $6.1 \pm 1.2$  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 and 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 and 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  $1.6 \pm 0.6$  my (Figs. 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 et 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 et al., 2001). *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.

#### 4.4. 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 and 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 et 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 (often clades are only supported by PP), a few geographically delimited clades can be seen in Fig. 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).

The data presented here might be interpreted as support for the Pleistocene refugia model of Prance (1987). However, on the basis of contradicting palaeo-ecological evidence this model has been contested (for an overview see Colinvaux and Oliveira, 2001). Also, several alternative explanations exist that try to explain the species diversity of for instance the Amazon, such as the ‘canopy density hypothesis’ (Cowling et al., 2001) and, proposed more recently, the ‘precessional-forced seasonality hypothesis’ (Maslin et al., 2005). Because of the lack of support within the SAC, it is on the basis of the current dataset impossible to test which of these hypotheses is most likely. However, in the future increased supported resolution in the SAC might enable the use of *Guatteria* as a model taxon for testing these competing hypotheses.

#### 4.5. 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 diversification after dispersal into South America.

As brought to attention by Pennington and 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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