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Biogeography of *Cedrela* (Meliaceae, Sapindales) in Central and South America[1]

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Dated phylogenies have helped clarify the complex history of many plant families that today are restricted to the world's tropical forests, but that have Eocene, Oligocene, and Miocene fossils from the northern hemisphere. One such family is the Meliaceae. Here we infer the history of the neotropical Meliaceae genus *Cedrela* (17 species), the sister clade of which today is restricted to tropical Asia. Sequences from the nuclear ribosomal spacer region and five plastid loci obtained for all ingroup species and relevant outgroups were used to infer species relationships and for molecular-clock dating under two Bayesian relaxed clock models. The clock models differed in their handling of rate autocorrelation and sets of fossil constraints. Results suggest that (1) crown group diversification in *Cedrela* started in the Oligocene/Early Miocene and intensified in the Late Miocene and Early Pliocene, and (2) Central American *Cedrela* species do not form a clade, implying reentry into Central America after the closure of the Panamanian Isthmus. At present, *Cedrela* is distributed in both dry and humid habitats, but morphological features suggest an origin in dry forest under seasonal climates, fitting with Miocene and Pliocene *Cedrela* fossils from deciduous forests.

Key words: *Cedrela*; internal transcribed spacer (ITS); Meliaceae; molecular clocks; *psbB* exon; *psbN* exon; *psbT* exon; *rpoC1*; Sapindales; *trnS-trnG* intergenic spacer.

The closure of the Panamanian Isthmus approximately three million years ago (Coates and Obando, 1996), joining together South America and Central America, led to the Great American Interchange, a concept developed mainly for animals (Webb, 2006). For plants, however, many paleobotanical and molecular biogeographic studies have demonstrated an exchange between the biota of North and South America long before the closing of the Isthmus, suggestive of a greater power of dispersal of plants compared to animals (Burnham and Johnson, 2004; Pennington et al., 2006). Among the most interesting systems in which to study the biotic exchange across the Panamanian Isthmus are clades endemic in Central and South America, hence likely to have originated there. Even with DNA sequencing from herbarium specimens, few such clades have been densely sampled (with most or all their species) because many are undercollected, with poorly documented species geographic ranges. An exception is the Meliaceae genus *Cedrela*, with 17 species in Central and South America (Pennington and Muellner, in press). Some of these species, in particular *C. odorata*, have long been valued for their fine timber, and in the course of preparing a monograph of this economically important genus, we were able to document the habitats and geographic ranges of all species. Most *Cedrela* species have restricted distributions in tropical deciduous forest; *C. fissilis*, however, is a widespread lowland species occurring in both rain forests and drier areas.

Cedrela is a member of the Cedreleae, which also comprise the Old World *Toona*, with four or five species in India, Indo-China, Malesia, and Australasia (Edmonds, 1993; Mabberley et al., 1995; our Fig. 1). The tribe Cedreleae and both genera are well characterized morphologically (Muellner et al., 2009), and molecular phylogenetic data have supported the monophyly of Cedreleae (Muellner et al., 2003, 2006).

Here we use plastid and nuclear data for most of the species of Cedreleae, together with information on species ranges from our monographic work (Pennington and Muellner, in press) and additional fossils not included in earlier dating efforts to resolve two questions: (1) How old is the crown group of *Cedrela*? and (2) Did *Cedrela* enter Mesoamerica and South America once or multiple times and from which source area(s)? To better understand the ecological evolution of *Cedrela*, we also optimized habitat preferences of the species, which mostly occur in particular forest types (Pennington and Muellner, in press), on the phylogeny. A final focus was the history of the economically important species *C. odorata*, a seemingly widespread and correspondingly variable species until recently thought to range from the Caribbean to Brazil and for which RFLP data from

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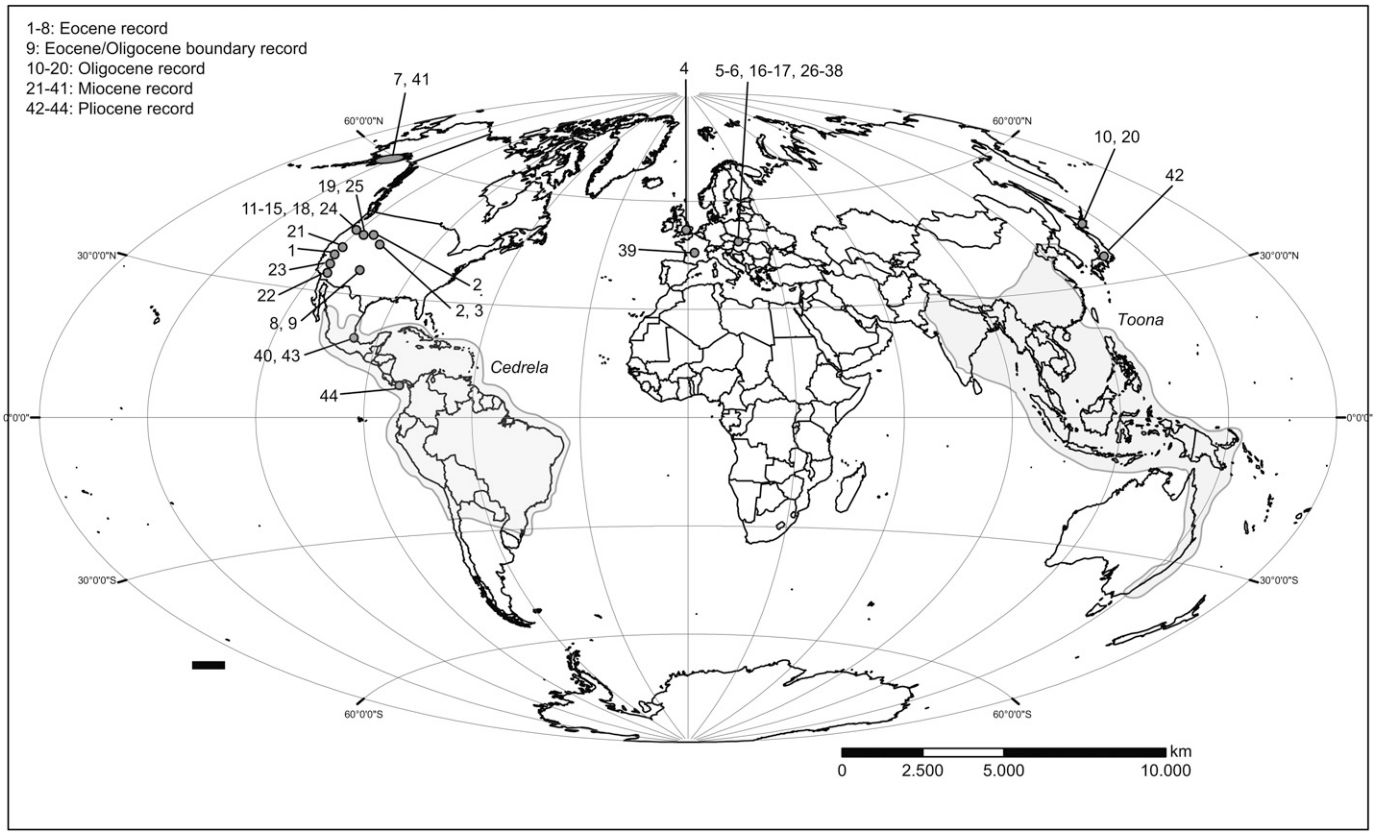

Fig. 1. Map of the global distribution of Cedreleae (*Cedrela*, *Toona*) fossil findings (Eocene to Quaternary). Areas encircled in light gray: modern distribution of *Cedrela* (New World) and *Toona* (Old World).

Mesoamerica have demonstrated unusually high population divergence (Cavers et al., 2003).

MATERIALS AND METHODS

Taxon sampling—Plant material was obtained from K and FHO or collected and preserved in silica gel during collecting expeditions in Peru (Table 1). Determination of plant material involved an examination of morphology and comparison with herbarium material borrowed from major world herbaria (A, AAU, BM, E, F, GH, K, MO, NY, S, UC, US).

We included up to 18 accessions that represent over 90% of the currently known species of *Cedrela*. The names applied to these accessions follow the species delimitations of Pennington and Muellner (in press; Table 1). Depending on the number of successfully sequenced accessions for the different DNA data sets, *Toona* was represented in the matrices by up to four accessions, representing two of its four to five species. As outgroups for the combined ITS/plastid marker analyses, we used *Khaya* and *Swietenia*, both from the tribe Swietenieae in subfamily Cedreloideae. As outgroups for analyses of ITS only, we used *Azadirachta indica* and *Melia azedarach,* both belonging to Melieae in subfamily Melioideae. These choices of ingroup and outgroup taxa were based on the findings of Muellner et al. (2003, 2006, 2008). The ITS matrices included a total of 32 or 35 accessions (30 or 33 ingroup and two outgroup samples), the combined ITS/*trnS-G*/*psbB-T-N* matrix and ITS/*trnS-G*/*psbB-T-N*/*rpoC1* matrix 23 or 24 accessions (21 or 22 ingroup and two outgroup samples). The number of included samples was dependent on the number of successfully sequenced accessions for the different DNA data sets.

Isolation of DNA, amplification, and sequencing—The protocol used for DNA extractions is that of Muellner et al. (2006); that for PCR amplification of ITS1, 5.8S, ITS2 plus the flanking 18S and 26S regions, the *trnS-trnG* intergenic spacer and the *psbB*, *psbT*, and *psbN* genes follows Muellner et al. (2009). For *rpoC1*, PCR amplifications were carried out using the primers rpoC1-1, rpoC1-2, rpoC1-3, and rpoC1-4 and the PCR reaction mix suggested by the CBOL Plant Working Group (http://www.kew.org/barcoding), applying the following PCR programs: the program described in Muellner et al., 2003, or alternatively, initial denaturation for 1 min at 94°C, followed by 40 cycles of denaturation for 30 s at 94°C, annealing for 40 s at 53°C and extension for 40 s min at 72°C; followed by final extension for 5 min at 72°C (http://www.kew.org/barcoding). PCR products were cleaned using a NucleoSpin Extract II kit (Macherey-Nagel, Dueren, Germany), or an E.Z.N.A. Gel Extraction kit (Peqlab, Erlangen, Germany). Sequencing reactions were run on an ABI 3730 capillary sequencer (Applied Biosystems, Warrington, Cheshire, UK) or a CEQ 8800 Genetic Analysis System (Beckman Coulter, Krefeld, Germany), following the manufacturers' protocols. Sequence editing and alignment followed Muellner et al. (2009). New sequences generated for this study have been deposited in GenBank (accessions GU295814–GU295836 and GU338247; http://www.ncbi.nlm.nih.gov/), and alignments for this study are in TreeBASE (http://treebase.org, submission SN4814; Sanderson et al., 1994).

Phylogenetic analysis—Individual and combined phylogenetic analyses were performed using parsimony (MP), likelihood (ML), and Bayesian optimization. We relied on visual inspection of the individual bootstrap consensus trees to determine combinability of the nuclear and plastid data sets. Given the absence of topological conflict (defined as nodes with <85% bootstrap support), we combined the chloroplast and nuclear data partitions. Throughout this paper, 75–84% bootstrap support is considered moderate and 85–100% strong support. Parsimony analyses were carried out in the program PAUP*4.0b10 (Swofford, 2002). Heuristic searches used 1000 random taxon additions, tree-bisection-reconnection (TBR) branch swapping, and MulTrees on (keeping multiple, shortest trees). Robustness of clades was assessed by bootstrap analysis with 1000 replicates, each with simple sequence addition, TBR swapping, and holding only 10 trees per replicate to reduce time spent in swapping on large numbers of suboptimal trees.

Maximum likelihood analyses were performed with the program GARLI version 0.96 under the GTR + G + I model (Zwickl, 2006; http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html) and Bayesian analyses with the program MrBayes version 3.1.2 under the same model (Ronquist and Huelsenbeck,

TABLE 1. Species included in this study, with their author names, voucher information, geographic origin, and GenBank accession numbers.

Taxon	Voucher information	Origin	GenBank accessions			
			ITS	*trnS-G*	*psbB-T-N*	*rpoC1*
Cedreloideae						
Tribe Cedreleae						
Cedrela angustifolia C.DC.	Wood et al., 19222 (K)	Bolivia	FJ462478	FJ462508	FJ462540	GU295825
Cedrela balansae C.DC.	Zapater & Castillo 2406 (K)	Argentina	FJ462473	FJ462505	FJ462537	GU295823
Cedrela dugesii S. Watson	German et al., 450 (K)	Mexico	FJ462483	FJ462513	FJ462545	GU295828
Cedrela fissilis Vell.	Muellner 2064 (K), Pennington 17752 (K)	Peru	FJ462475	FJ462507	FJ462539	GU295824
Cedrela montana Moritz ex Turczaninov	Pennington et al., 17623 (K)	Peru	FJ462480	FJ462510	FJ462542	GU295826
Cedrela oaxacensis C.DC. & Rose	Mendoza et al., 291 (K)	Mexico	FJ462482	FJ462512	—	GU295827
Cedrela oaxacensis C.DC. & Rose	Cedillo et al., 880 (FHO)	Mexico	—	—	FJ462544	—
Cedrela odorata L. s.str.	Styles 166 (K)	Belize	FJ462467	FJ462499	FJ462531	GU295818
Cedrela odorata L. s.str.	Villacorta & Berendsohn 271 (K)	El Salvador	FJ462468	FJ462500	FJ462532	GU295819
Cedrela odorata L. s.str.	Pratt 0010 (K)	West Indies, Antigua	GU338247	—	—	—
Cedrela saltensis M.A. Zapater & del Castillo	Zapater 2348 (K)	Argentina	FJ462462	FJ462494	FJ462526	GU295815
Cedrela salvadorensis Standley	Formoso 2 (K)	Costa Rica	FJ462484	FJ462514	FJ462546	GU295829
Cedrela tonduzii C.DC.	Styles 82 (K)	Costa Rica	FJ462485	FJ462515	FJ462547	GU295830
Cedrela weberbaueri Harms	Daza 4012 (K)	Peru	FJ462472	FJ462504	FJ462536	GU295822
Cedrela sp. nov. 1	Muellner 2056 (K), Pennington 17727 (K)	Peru	FJ462461	FJ462493	FJ462525	GU295814
Cedrela sp. nov. 2	Pennington et al., 17765 (K)	Peru	FJ462466	FJ462498	FJ462530	GU295817
Cedrela sp. nov. 3	Pennington et al., 17583 (K)	Peru	FJ462470	FJ462502	FJ462534	GU295820
Cedrela sp. nov. 4	Monro & Alexander 3081 (K)	El Salvador	FJ462486	FJ462516	FJ462548	GU295831
Cedrela sp. unnamed 5	Agra et al., 5014 (K)	Brazil	FJ462471	FJ462503	FJ462535	GU295821
Cedrela sp. unnamed 6	Neill et al., 6230 (K)	Ecuador	FJ462464	FJ462496	FJ462528	GU295816
Toona ciliata M. Roem. 1	PIF25085, AQ606666 (K)	Australia	FJ462488	FJ462517	FJ462549	GU295832
Toona ciliata M. Roem. 2	Hennessey 6634 (K)	South Africa, cult.	FJ462489	—	FJ462550	—
Toona sinensis M. Roem. 1	Wan & Chow 79175 (K)	China, cult.	FJ462490	FJ462518	FJ462551	GU295833
Toona sinensis M. Roem. 2	Troupin 16314 (K)	Rwanda, cult.	FJ462491	FJ462519	FJ462552	GU295834
Cedreloideae						
Tribe Swietenieae						
Capuronianthus mahafalensis J.-F. Leroy	Fosberg 52439 (MO)	Madagascar	FJ518868	—	—	—
Chukrasia tabularis A. Juss.	Hu & Yung 603 (MO)	Hong Kong	FJ518894	—	—	—
Entandrophragma angolense C. DC.	Sainge 1685 (MO)	Cameroon	FJ518895	—	—	—
Entandrophragma utile (Dawe and Sprague) Sprague	Sainge 1684 (MO)	Cameroon	FJ518896	—	—	—
Khaya anthotheca C.DC.	Roy. Bot. Gardens Kew, living coll. 1967-35601, Chase 2859 (K)	source plant: Amherst College, Massachusetts	DQ861608	FJ462520	FJ462553	—
Lovoa trichilioides Harms	Sainge 1678 (MO)	Cameroon	FJ518899	—	—	—
Neobeguea mahafalensis J.-F. Leroy	Labat & Du Puy 2032 (MO)	Madagascar	FJ518901	—	—	—
Pseudocedrela kotschyi (Schweinf.) Harms	Kenfack & Amponsah 2088 (MO)	Ghana	FJ518903	—	—	—
Schmardaea microphylla Karst. ex C.Muell.	Kenfack & Quizpe 2162 (MO)	Ecuador	FJ518904	—	—	—
Swietenia macrophylla King	Chase 250 (NCU)	USA	DQ861609	FJ462521	FJ462554	—
Swietenia macrophylla King	Samuel 10-01-01 (WU)	Sri Lanka, cult.	—	—	—	GU295835
Cedreloideae						
Tribe Xylocarpeae						
Carapa guianensis Aubl.	Forget 577 (MO)	Guiana	FJ518873	—	—	—
Xylocarpus moluccensis (Lam.) M. Roem.	Mwangoka et al. 3338 (MO)	Tanzania	FJ518907	—	—	—
Melioideae						
Tribe Melieae						
Azadirachta indica A. Juss.	Samuel 5 (WU)	Sri Lanka	AY695594	FJ462522	FJ462555	—
Melia azadirachta L.	Roy. Bot. Gardens Kew, living coll. 1953-37801 (K)	donation from KYGH	AY695595	FJ462523	FJ462556	—

2003; http://mrbayes.csit.fsu.edu/). Bayesian analyses used two sets of four simultaneous chains (three cold and one heated; the default in MrBayes) and two to four million cycles, sampling one tree every 1000th generation. The program AWTY (Wilgenbusch et al., 2004; http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php) was used to check if stationarity had been achieved, and trees that preceded stabilization of the likelihood value were excluded as burn-in. The remaining trees were used to calculate posterior probabilities via the construction of a majority rule consensus tree in PAUP*.

Habitat (forest type) preference optimization—For ancestral habitat optimization, we binned habitats into wet forests and dry forests, with the latter including cool montane forest habitats. Information about the habitat preferences of the species came from fieldwork and herbarium labels (Pennington and Muellner, in press). Optimization relied on parsimony and was carried out in the program MacClade version 4.0 (Maddison and Maddison, 2000).

Divergence dating—We used two Bayesian relaxed-clock approaches, the uncorrelated-rates model implemented in the program BEAST version 1.4.8 (Drummond et al., 2006; Drummond and Rambaut, 2007) and the autocorrelated-rates model implemented in the program multidivtime (Thorne and Kishino, 2002; http://statgen.ncsu.edu/thorne/). These approaches were applied to data partitions with somewhat different taxon sampling, which permitted the use of different sets of fossil constraints. The dating runs in BEAST used a concatenated data set comprising nrITS, *psbB*, *psbN*, *psbT* exon, *trnS-trnG* spacer, and *rpoC1* (run 2) sequences for 23–24 taxa (an alignment of 2476 to 2980 nucleotides). The best-fit substitution model for these data were identified with the program jModelTest version 0.1.1 (Posada, 2008) on a fixed tree estimated with the BIONJ algorithm and the JC model. Of the 88 models implemented in jModelTest, the general time-reversible model with rate variation and an invariant site parameter (GTR + G + I) was selected under the Bayesian information criterion. Analyses in BEAST used a speciation model that followed a Yule tree prior, with rate variation across branches uncorrelated and lognormally distributed. Markov chain Monte Carlo (MCMC) chains were run for between 2 and 10 million generations (burn-in 10%), with parameters sampled every 500th or 1000th generation. Results from individual runs were combined, and effective sample sizes for all relevant estimated parameters and node ages were well above 100.

BEAST accommodates calibration uncertainty by applying a prior probability distribution (defined in terms of means and standard deviations) on the age of nodes to which calibration fossils are assigned. From the range of prior probability distributions available, we chose normal distributions, which place less prior probability on a relatively narrow time frame than do exponential or lognormal distributions. The age of the most recent common ancestor (MRCA) of *Cedrela* and *Toona* was set to 48.6 Myr, with a SD of 1 and a 95% CI of 46.64 to 50.56 Myr, based on fruit and seed fossils from the London Clay (Chandler, 1964; Reid and Chandler, 1933) that have been ascribed to *Toona* but that are intermediate between *Toona* and *Cedrela* (insertion of the seed on the columella; T. D. Pennington, personal observation). The normal mean age of the MRCA of *Cedrela* was set to 33.62 Myr with a SD of 1 and a 95% CI of 31.66–35.58, based on leaflets and seeds of *Cedrela merillii* from the Oligocene Bridge Creek Flora of the John Day Formation, Oregon, USA (Meyer and Manchester, 1997). The age of the MRCA of *Swietenia* and *Khaya* was set to 22.5 Myr with a SD of 1 and a 95% CI of 20.54–24.46, based on a Late Oligocene-Early Miocene flower from Simojovel de Allende, Chiapas, Mexico, attributed to *Swietenia* and dated to 22.5–26.0 Ma (Castañeda-Posadas and Cevallos-Ferriz, 2007). We also ran an analysis that assumed a globally constant molecular clock (and the same three node age priors) to assess whether the relaxed clock analyses were returning anomalous results due to over-parameterization. Lastly, we ran an analysis without the DNA data to check that the effective priors were similar to the original priors and to assess the informativeness of the data by comparing these effective priors with posteriors obtained when data are added.

The dating runs in multidivtime used 32 ITS sequences, 30 representing the ingroup species and two representing outgroups. A likelihood-ratio test rejected the null hypothesis of rate constancy for ITS; we therefore allowed rates to vary between ancestors and descendants. The input topology for the time estimation was the ITS ML tree obtained with PAUP*. Parameter values in multidivtime were estimated with PAML's baseml version 3.14 (Yang, 1997; http://abacus.gene.ucl.ac.uk/software/paml.html). The program estbranches (Thorne et al., 1998) was then used to calculate branch lengths and their variance, given the sequence data (32 ITS sequences of a length of 941 nt), the model parameter values from PAML, and the specified rooted topology. Branch lengths from estbranches became the priors for MCMC searches in multidivtime (Thorne and Kishino, 2002) that sought to find the most likely model of rate change (with rate change assumed to be log-normally distributed), given the topology, time constraints on nodes (below), and a Brownian motion parameter (ν) that controls the magnitude of autocorrelation per million years along the descending branches of the tree. Prior gamma distributions on parameters of the relaxed clock model were as follows: The mean and SD of the prior distribution for the root age were set to 75 Myr based on fossils (below); the mean and SD of the prior distribution for the ingroup root rate were set to 0.0014 substitutions/site/Myr by dividing the median of the distances between the ingroup root and the tips by 75 Myr; the prior and SD for ν were set to 0.013. Markov chains in multidivtime were run for 1 million generations, sampling every 100th generation for a total of 10 000 trees, with a burn-in of 10 000 generations before the first sampling of the Markov chain. To check for convergence, we ran analyses of different chain lengths. We also tested the effect of the root rate by running another analysis with a rate of 0.0012.

Multidivtime dating relied on four to six simultaneous node constraints (numbered 1–6 below) of which constraints 1–2 and 5–6 were applied in run 1 and constraints 1–6 in run 2. (1) The root node of our data set (i.e., the MRCA of Melioideae and Cedreloideae) was constrained maximally to 90 Myr, based on previous estimates (Muellner et al., 2006, 2007, 2008), and fruit and seed fossils from Senegal "similar to living Meliaceae" that date back to the Campanian/Maastrichtian boundary (Monteillet and Lappartient, 1981). Because it is not possible to say whether any older fossils may be found in the future, this date may be regarded as a minimum estimate. (2) The stem of Cedreleae was constrained to minimally 48.6 Myr (with the rationale as in the BEAST analyses). (3) The clade comprising *Lovoa* and *Capuronianthus* was constrained to minimally 40.4 Myr (the upper bound of the Middle Eocene), based on fossil wood of *Lovoaxylon princeps* from northern Africa reported as similar to living *Lovoa trichilioides* (Boureau et al., 1983). (4) The crown group of *Entandrophragma* was likewise constrained to minimally 40.4 Myr, based on fossil wood of *Entandrophragmoxylon normandii* from northern Africa reported as similar to living *Entandrophragma angolense* (Boureau et al., 1983). (5) The crown group of *Cedrela* was constrained to minimally 33.62 Myr (as in the BEAST analyses). (6) Finally, the crown group of *Swietenia* and *Khaya* was constrained to minimally 22.5 Myr (as in the BEAST analyses).

For absolute ages, we relied on the geologic time scale of Gradstein et al. (2004).

RESULTS

Divergence time estimation—Table 2 provides a summary of the divergence times obtained with the two clock approaches. Estimates obtained from the autocorrelated-rates approach are generally older than those obtained from the uncorrelated-rates approach, which may be due to the different clock models or the slightly different gene and species sampling. Figure 2 shows a chronogram for the 23-taxon–3-gene data set, with 95% highest posterior density intervals around age estimates for the nodes of interest. The two parameters that provide information on the clock-likeness of the data, namely, the uncorrelated rates standard deviation and the coefficient of variation, were relatively close to 0.0 (both were 0.7), and the data therefore exhibit limited rate heterogeneity among lineages. The chronogram shows that there are two South American *Cedrela* subclades of highly different crown group ages, namely 15.7 vs. 6.9 Myr (Fig. 2).

Cedreleae fossil map—Figure 1 shows the global distribution of Cedreleae fossils; the gray lines with shaded areas delimit the modern distribution of *Cedrela* and *Toona*. All fossils currently ascribed to *Cedrela* or *Toona* come from the northern hemisphere (North America including Alaska and Mexico, Europe, Japan); the southernmost fossils are from Panama. Records viewed as doubtful with regard to their taxonomic assignment to Cedreleae were not considered (T. D. Pennington, personal observation). Fossil localities depicted on the map (Fig. 1) come from the following studies: Alaska and Western

TABLE 2. Age estimates (million years ago) for key events in the history of Cedreleae based on (i) an uncorrelated-rates relaxed clock (BEAST) and based on (ii) a correlated-rates relaxed clock (Multidivtime); n.a. = not applicable. Numbered columns under the clock models (i) and (ii) refer to different taxon sets as follows: Run 1 under model (i) is based on 21 ingroup and 2 outgroup sequences (combined data set), Run 2 on 21 ingroup and 2 outgroup sequences (combined data set incl. plastid rpoC1; *excluding *C. weberbaueri* and *C.* sp. nov. 2), Run 3 on 22 ingroup and 2 outgroup sequences (combined data set), Run 4 on 33 ingroup and 2 outgroup sequences (ITS data set only). Runs 1 and 2 under model (ii) are based on 30 ingroup and 2 outgroup sequences (ITS data set only). See Materials and Methods for all fossil constraints. Node numbers refer to the nodes in Fig. 2.

	(i) BEAST				(ii) Multidivtime	
Node	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2
1. Crown Cedreleae	48.4	48.4	48.4	48.5	54.3	55.3
2. Crown *Cedrela*	33.8	33.8	33.7	33.6	40.5	41.2
3. Crown *Cedrela* major clade	22.6	22.1	22.0	27.8	33.6	34.3
4. South American clade 1	15.7	14.7*	16.4	16.6	25.6	26.1
5. Central American clade 2	12.1	13.5	11.4	10.3	25.4	26.1
6. South American clade 2	6.9	6.6	6.9	3.3	10.4	10.7
7. Central American clade 1	3.2	3.1	3.0	2.8	n.a.	n.a.

USA—Wolfe (1977), Leopold (1984), MacGinitie (1941, 1974), Manchester (2001), Meyer and Manchester (1997), Becker (1961, 1962), Axelrod (1939, 1985, 1991), Chaney (1944), Chaney and Axelrod (1959); Central America—Graham (1976, 1991, 1999); Europe—Reid and Chandler (1933), Hably (2006), Kvacek and Hably (1991), Vörös (1955), Roiron (1991); Japan—Tanai (1970), Tanai and Suzuki (1963), Ozaki (1991).

Habitat (forest type) preference optimization—Most *Cedrela* species have restricted distributions in tropical, deciduous forest. Only *C. fissilis* is widespread (Colombia to Brazil) and occurs in diverse forest types from semideciduous forest to gallery forest and cerrado vegetation. In the western part of its range, it is a rare species of lowland, evergreen, seasonal forest (Peru) or nonseasonal, evergreen forest (Ecuador, Colombia). In southern Brazil (Paraná), it is a component of *Araucaria* forest

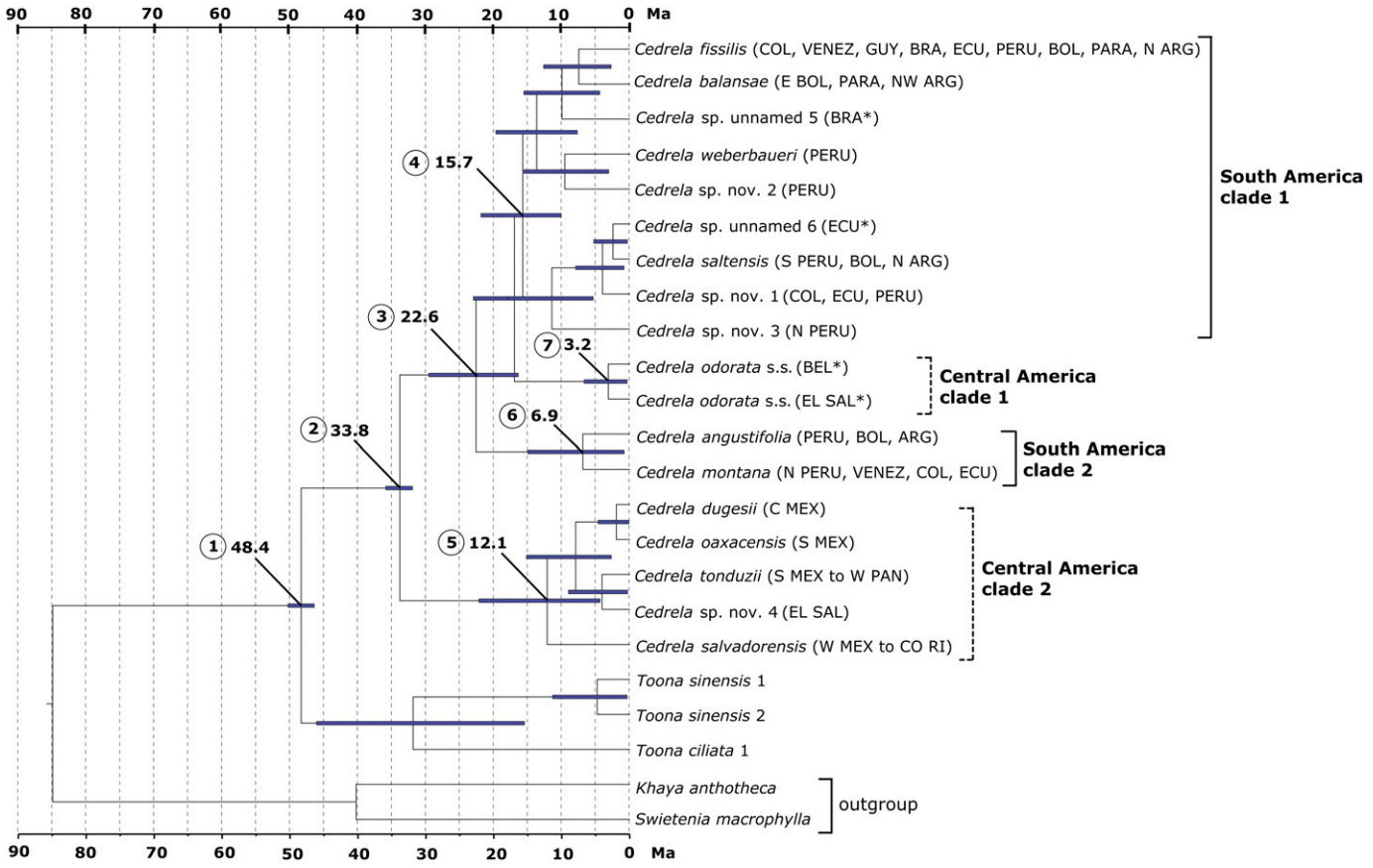

Fig. 2. Maximum clade credibility chronogram from combined nuclear and plastid DNA data modeled under a relaxed clock with uncorrelated rates. Node heights represent mean ages, and bars are the 95% confidence intervals around the estimates. Abbreviations for distribution areas (in brackets): ARG = Argentina, BEL = Belize, BOL = Bolivia, BRA = Brazil, CO RI = Costa Rica, COL = Colombia, ECU = Ecuador, EL SAL = El Salvador, GUY = Guyana, MEX = Mexico, PARA = Paraguay, PERU = Peru, PAN = Panama, VENEZ = Venezuela; *N* = North, E = East, S = South, C = Central, W = West, NW = Northwestern; *specimen locality.

(Pennington and Muellner, in press). *Cedrela odorata* in its traditional wide circumscription (Styles, 1981) had a similarly large range; however, the molecular data (this study) reject that wide species concept, indicating instead that at least three species are hiding under this name. The type of *C. odorata* is from Jamaica, and we therefore generated an ITS sequence for a specimen from the West Indies. It falls in a clade with the sequences from El Salvador and Belize (100% posterior probability; tree not shown), indicating that the Mesoamerican accessions represent *C. odorata* in the strict sense, while the Brazilian and Ecuadorian accessions represent species for which applicable names can probably be found among the numerous synonyms of *C. odorata* (see Discussion). In Fig. 2, we therefore labeled the Central American entity as *C. odorata* s.s. and the other two as *Cedrela* sp. unnamed 5 and *Cedrela* sp. unnamed 6.

Table 3 shows the results of optimizing the occurrence of species in either wet or dry forests on the topologies of the (a) ML tree, (b) Bayesian tree, (c) MP strict consensus tree, and (d) MP majority rule consensus tree. The accessions of *Cedrela odorata* from Belize and El Salvador were coded separately from the Ecuadorian and Brazilian ones because these are clearly three distinct species. On the ML and Bayesian trees, the habitat preferences of the MRCA of *Cedrela* remained ambiguous (equivocal habitat optimization). The optimization on the MP strict consensus tree suggested an ancestral preference for a dry forest habitat; that on the MP majority rule consensus suggested wet forest. The ambiguous results did not change when the three forms of *C.* "*odorata*" were coded slightly differently (see Table 3).

DISCUSSION

***Separation of* Cedrela *and* Toona**—The MRCA of *Cedrela* and *Toona* lived between 46.6 and 50.6 Ma, judging from fruit and seed fossils from the London Clay (Materials and Methods), and both genera thus already existed when Europe still supported tropical vegetation. Fossil fruits intermediate between extant *Cedrela* and *Toona*, such as the London Clay *Toona sulcata* (Reid and Chandler, 1933; T. D. Pennington, personal observation), support a scenario in which the ancestor of Cedreleae moved from Africa to Europe or, alternatively, originated in Europe, and then separated into two lineages, one moving westward into North America (*Cedrela*), the other into Asia (*Toona*). A detailed investigation of more fossils from North America and East Asia is needed to test this hypothesis.

TABLE 3. Results of the habitat (forest type) preference optimization for the most recent common ancestor of *Cedrela* based on four topologies: (a) the maximum likelihood tree; (b) the Bayesian tree; (c) the maximum parsimony (MP) strict consensus tree; and (d) the MP majority rule consensus tree. The difference between approaches 1 and 2 is the coding of the Mesoamerican accessions of *Cedrela odorata* and the Ecuadorian and Brazilian *C.* "*odorata*" as predominantly occurring in "wet" (approach 1) vs. "wet as well as dry" (approach 2) forest.

Optimization on tree	Approach 1	Approach 2
(a) ML tree	equivocal	wet
(b) Bayesian tree	equivocal	wet
(c) MP strict consensus tree	dry	dry
(d) MP majority rule consensus tree	wet	wet

Diversification in Cedrela and the invasion of Central and South America—Diversification in *Cedrela* apparently began in the Oligocene/Early Miocene and intensified in the Late Miocene and Early Pliocene (Table 2, Fig. 2). Assuming spread from North America, the *Cedrela* lineage entered South America between 33.8 and 22.6 Myr ago, diversified there, and the *C. odorata* s.s. lineage (labeled Central America clade 1 in Fig. 2) then reentered Central America at about 3 Ma. *Cedrela odorata* s.s. occurs in Central America and on the Caribbean islands, including Jamaica, an island that has been above water since at least the Early Miocene (Iturralde-Vinent et al., 2006). The Isthmus of Panama started forming 12.8–9.5 Ma, and by ~6 Ma the Darien region and a large part of the Isthmus already existed (Coates et al., 2004), although shoaling was probably not completed until 3.0–3.5 Ma (Coates and Obando, 1996). Central American *Cedrela odorata* has exceptionally high levels of population differentiation, as found in a study of RFLP haplotypes from 580 individuals from 29 populations throughout Mesoamerica (Cavers et al., 2003). Miocene or even earlier dispersal across the Panamanian Isthmus as inferred here for *Cedrela* has also been inferred for other plant clades, with varying dispersal abilities, including Melastomeae (Renner and Meyer, 2001), the Polygonaceae *Ruprechtia*, and the legume genera *Nissolia* (Pennington et al., 2004) and *Platymiscium* (Saslis-Lagoudakis et al., 2008).

Of the species sampled here, most have relatively restricted distributions (Fig. 2, lists the species' full range). For South American *Cedrela*, the Amazonian forest of Peru constitutes a center of species diversity, although they are certainly not a center of old *Cedrela* diversity (Fig. 2). Although the ancestral habitat preference of *Cedrela* could not be inferred (probably in part because of the crude binary coding of habitats), morphological adaptations, such as the deciduous habit, shoot apices protected by a cluster of bud scales, and capsular fruits with dry, winged and wind-dispersed seeds of extended viability, all point to a long evolutionary history of *Cedrela* in dry forest habitats.

Conclusions—Diversification in the neotropical genus *Cedrela* started in the Oligocene/Early Miocene and intensified in the Late Miocene and Early Pliocene. Central America contains two lineages, which suggests repeated northern and southern migration in the genus, although extinction is difficult to factor into this scenario. Morphological adaptations imply an origin of *Cedrela* in dry forest under seasonal climates, and micro- as well as macrofossil data from North America and Mesoamerica (Axelrod, 1991; Graham, 1991, 1999) indeed show that *Cedrela* was present in Miocene and Pliocene deciduous forests. By the Pliocene, the clade had adapted to moist and premontane wet forests (Graham, 1991). The fact that an important tropical timber, such as *C. odorata*, includes at least three biological species illustrates the importance of adding molecular to morphological data, which may be especially important for large tree species, for which the traits often cannot be inferred from standard herbarium sheets (Hopkins et al., 1999).

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