Spatio-temporal Evolution of *Cedrela* (Meliaceae)

Climatic Niche Dynamics, Phylogeography and Taxonomy

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Declaration

I confirm that this thesis was entirely written by myself. The contributions by coauthors and other colleagues are specified (in percentage) in a table at the beginning of each of the three chapters, respectively. The use of any materials from other sources is also indicated and fully acknowledged throughout the thesis.

Ich bestätige, dass ich diese Doktorarbeit eigenhändig verfasst und geschrieben habe. Der jeweilige Beitrag von KoautorInnen und weiteren KollegInnen ist (in Prozent) in einer Tabelle vor jedem Kapitel dieser Arbeit dargelegt. Die Nutzung von anderweitigen Materialien ist an den entsprechenden Stellen mit Quellenangaben gekennzeichnet.

Anna Valerie Köcke, Frankfurt am Main, Oktober 2014

The content of the three chapters is identical to the manuscripts which were published or submitted to the following journals, as indicated below with the following exceptions: A footnote * was added to Chapter I in the Material and Methods section (on page 40). The numbers of the figure and table captions were adjusted throughout the thesis to a reasonable matching order and therefore deviate from the numbers in the original manuscripts. The fonts and subtitles were adjusted to a uniform style.

Chapter I was published in the American Journal of Botany, St. Louis, MO, U.S., 2013, volume 100 (9), as: Koecke, A.V., Muellner-Riehl, A.N., Pennington, T.D., Schorr, G., Schnitzler, J. "Niche evolution through time and across continents: The story of tropical *Cedrela* (Meliaceae)" DOI: 10.3732/ajb.1300059.

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Abstract

Understanding major causes of biodiversity and range dynamics requires research on evolutionary processes under consideration of environmental changes. In my thesis, I investigated the spatio-temporal evolution of the Neotropical tree genus *Cedrela* from the Meliaceae family by studying its genetic diversity, taxonomy, colonization history, climatic niche changes and dynamics of species distributions. My results show that climatic and geological changes are major drivers of biological diversification in *Cedrela*.

The first chapter of my thesis was dedicated to understanding the climatic niche evolution of *Cedrela* by testing the capacity of species to switch their climatic niche. Distribution models of extant species of *Cedrela* based on climate layers were combined with a dated molecular multilocus phylogeny in order to reconstruct evolutionary dynamics of climatic niches. Relative disparity of climatic tolerances was measured to test for niche evolution within subclades or divergence between subclades and conservatism among closely related groups. The climatic niche reconstructions were compared to climatic conditions from fossil occurrences of *Cedrela* and corresponding paleosol studies. The fossil history of *Cedrela* provided support for an origin in high northern latitudes of the Early Eocene and a major biome shift from paratropical conditions into warm-temperate seasonal climates during the Early Oligocene of western North America. In the Miocene, *Cedrela* extended from North America (John Day, USA) to southern Central America (Gatún, Panama). South America was colonized at latest in the Pliocene (as indicated by fossil wood from the Iquitos region, Peru). Diversification in the early evolutionary history was mainly driven by changes in precipitation, which was comfirmed by both, the modeled niche reconstructions and fossil evidences. According to the models, temperature had an increasing impact on ecological diversification of the genus from the Miocene onwards, but based on fossil findings species might have already occurred under different temperature and seasonality by then. Direct comparison of closely related extant species revealed that speciation events may be related to divergence of climatic tolerances. This study highlights the complexity of climatic niche dynamics, and shows how conservatism and evolution act on different temporal scales and climatic parameters.

Within the second chapter of my thesis, I have analyzed genetic variation within and among species of *Cedrela*, in order to test if genetic variation corroborates the geographical structure of species distributions, and if gene flow occurs at intra- and/or interspecific levels. The continent-wide phylogeographical analysis of *Cedrela* unveils distinct levels of genetic variability and structure in different groups of the genus. Species-specific and geographically structured genetic patterns are common among some Andean species and wide-spread lowland species complexes of Central and South America (*C.* aff. *odorata* and *C. fissilis* sensu lato) suggesting vicariant diversification. These patterns are contrary to the geographically unstructured polymorphic patterns of two closely related Andean species, which could be a result of chloroplast capture through several events of hybridization, or persistence of ancestral polymorphisms through speciation. Furthermore, plastidial haplotype homogeneity in South America suggested gene flow at interspecific levels and recent speciation predating sequence differentiation.

The latest monographic treatment of *Cedrela* is relatively new. In western South America and northwestern Central America, species distributions and taxonomic circumscriptions are comparatively well-understood. However, specimens from southern Central America were rather scarce in major herbarium collections and less well studied. Major confusion prevailed upon taxonomy of the broad species complex *C. odorata*, which has been shown to consist of paraphyletic entities in the past. The third chapter of my thesis was therefore dedicated to a thorough examination of *Cedrela* specimens with special focus on morphological diversity of taxa from southern Central America and adjacent regions. The study material comprised approximately 500 herbarium specimens, some of them being my own collections from central and western Panama (in 2009). As a result, a new species was described, *Cedrela ngobe* Köcke, T.D. Penn. & Muellner-Riehl, which occurs in Panama and Costa Rica.

Zusammenfassung

Um Biodiversitäts- und Verbreitungsdynamiken verstehen zu können, ist die Erforschung evolutionsbiologischer Vorgänge in Zusammenhang mit Veränderungen der Umwelt notwendig. In meiner Doktorarbeit habe ich die räumlich-zeitliche Evolution von *Cedrela*, einer prominenten Baumgattung der Neotropis aus der Familie Meliaceae untersucht. Dabei habe ich mich der Analyse genetischer Diversität, der phylogenetischen und klassischen Taxonomie, der Kolonisierungsgeschichte, sowie den Dynamiken klimatischer Nischen und Artverbreitungsmustern gewidmet. Die Ergebnisse meiner Arbeit zeigen, dass klimatische und geologische Veränderungen Einfluss auf die biologische Vielfalt von *Cedrela* hatten. Im ersten Kapitel meiner Arbeit habe ich mich mit der Evolution von Klima-Nischen auseinandergesetzt. Für die Rekonstruktion der Nischendynamiken wurden Verbreitungsmodelle von rezenten Arten erstellt, die auf mehreren Klimaoberflächen basierten. Aufgrund dieser Verbreitungsmodelle war es möglich, für die unterschiedlichen Arten Toleranzkurven (predicted niche occupancy profiles) gegenüber klimatischen Parametern zu bestimmen. Aufgrund dieser Toleranzkurven wurden in Kombination mit einer datierten molekularen Phylogenie (bestehend aus verschiedenen nukleären und plastidären Markern) über einen statistischen Prozess die anzestralen klimatischen Nischen rekonstruiert. Über die Messung relativer Disparität der einzelnen Klimaparameter konnte auf Divergenz zwischen Subkladen und Konservatismus zwischen nahe verwandten Arten oder auf Divergenz zwischen nahe verwandten Arten getestet werden. Darüber hinaus wurden die Rekonstruktionen anzestraler Nischen verglichen mit den klimatischen Bedingungen, denen einst fossile Arten von *Cedrela* ausgesetzt waren, welche anhand paläobotanischer Studien (von Florenkomplexen) und Paleosolstudien evaluiert wurden. Fossilien belegen einen Ursprung *Cedrelas* in hohen nördlichen Breitengraden des frühen Eozäns. Beim Übergang vom Eozän in das Oligozän deuten Fossilien auf einen Biomwechsel hin, von paratropischen Bedingungen hin zu warm-temperiertem saisonalem Klima. Im Miozän war *Cedrela* bereits von Nordamerika (John Day, USA) bis in den Süden Zentralamerikas verbreitet (Gatún, Panama). Diversifizierungen der frühen Evolutionsgeschichte *Cedrelas* wurden vermutlich über Veränderungen der Niederschlagsmengen gesteuert. Den

modellierten Nischenrekonstruktionen zufolge hatten ab dem Miozän Temperaturwechsel einen zunehmenden Einfluss auf ökologische Diversifizierung. Fossilfunde hingegen deuten darauf hin, dass die klimatischen Nischen bereits im Miozän unterschiedlichen Temperaturen und unterschiedlicher Saisonalität unterworfen waren. Schwestergruppenvergleiche ergaben, dass jüngere Artbildungsprozesse unter anderem entlang divergierender klimatischer Gradienten entstanden sein könnten. Diese Studie zeigt wie komplex Nischendynamiken sind, inbesondere unter Berücksichtigung mehrerer klimatischer Paramater, denn sowohl die Entstehung evolutiver Divergenzen als auch Nischenkonservatismus verfolgen innerhalb der jeweiligen Parameter eigene Dynamiken.

Das zweite Kapitel meiner Arbeit befasste sich mit der Analyse der genetischen Vielfalt *Cedrelas* auf innerartlicher und zwischenartlicher Ebene über das gesamte neotropische Verbreitungsgebiet. Die genetischen Muster wurden phylogeographisch ausgewertet. Hierbei zeigte sich, dass genetische Diversität in unterschiedlichen Gruppen der Gattung unterschiedlich ausgeprägt ist. Dies widerum deutete auf unterschiedliche Evolutionsgeschichten und Artbildungsprozesse innerhalb der Gattung *Cedrela* hin. Das artspezifische und geografisch strukturierte genetische Muster einzelner andiner Arten, sowie die ebenfalls geografisch strukturierten genetischen Muster von weit verbreiteten Artkomplexen in Zentral- und Südamerika (*C.* aff. *odorata* und *C. fissilis* sensu lato) weisen auf vikariante Artbildungsprozesse hin und sind entgegengesetzt zu den geografisch unstrukturierten polymorphen Strukturen zweier andiner Arten. Diese könnten entweder aus Hybridisierungen hervorgegangen sein oder von Vorfahren mit plastidär polymorpher genetischer Ausrüstung abstammen. Demgegenüber steht die plastidäre Haplotypenhomogenität einiger südamerikanischer Arten, was sowohl auf möglichen interspezifischen Genfluss, als auch auf jüngere Artbildungsprozesse hindeutete.

In Südamerika, aber vor allem in Peru, sowie im Nordwesten Zentralamerikas sind Verbreitungsmuster sowie die Taxonomie zahlreicher Arten von *Cedrela* relativ gut untersucht. Aus dem Süden Zentralamerikas hingegen, vor allem aus Panama, gab es kaum Kenntnis über die Vielfalt und Verbreitung *Cedrelas*, und die Anzahl herbarisierter Belege schien sehr gering. Verwirrung resultierte zudem aus der unklaren taxonomischen Einordnung von *C. odorata*, einer aus

molekularphylogenetischer Sicht aus mehreren paraphyletischen Einheiten bestehenden Art. Aus diesem Grund widmete ich mich einer gründlichen Untersuchung von ca. 500 Herbarbelegen aus dem Süden Zentralamerikas und angrenzender Gebiete. Das Untersuchungsmaterial umfasste zum größten Teil Herbaranleihen, aber auch einige eigene Aufsammlungen aus Panama. Ein Ergebnis ist die im dritten Kapitel dieser Arbeit beschriebene neue Art, *Cedrela ngobe* Köcke, T.D. Penn. & Muellner-Riehl, welche in Panama und Costa Rica verbreitet ist.

General Introduction

1. 1. What is Biodiversity?

Knowledge about biological diversity (biodiversity) has always been essential to human beings, e.g. the ability to distinguish between poisonous and nutritious, or even medicinal plants and pass this knowledge to next generations. From the European point of view, our interest in all kinds of living forms considerably increased during the colonial period, when the exploration of the different continents expanded. Until then, the use of e.g. plants for food, medicines or decoration was more important, than the study of diversity itself. This specifically changed from the 18th century onwards: Famous naturalists of this epoch, such as Carl Linnaeus, who formally introduced the binary system of nomenclature, or Maria Sibylla Merian, who painted and studied numerous plants and insects in its different stages of metamorphosis, were pioneers of classical taxonomy. Great explorers of that time, e.g. Daniel Solander, Joseph Banks and the Forster family, who accompanied the Cook expeditions, and not to forget the expeditions of Alexander von Humboldt, Alfred Russel Wallace or Charles Darwin, brought thousands of new species to us. The question of how this astonishing diversity might have arisen became illuminated from a new side, when Lamarck stated within his *Philosophie Zoologique* (1809) that all species, including man, descended from other species, highlighting, for the first time, the probability of changes through time (Darwin, 1852). Darwin elaborated the idea of how these changes to species might occur and presented his theory of evolution by natural selection (Darwin, 1852, sixth edition from 1872 in which he used the term evolution). It was then Gregor Mendel, who for the first time analyzed the mechanisms of heredity in the 19th century (Mendel, 1866). His studies were only rediscovered in the early 20th century by Morgan et al. (1922) as genetics became a new research field in science (Moore, 1983). By the 1960s, it became clear that the underlying cause of diversity among and within species was genetic variation emerging through mutation and recombination (e.g. Crick et al., 1961; Crick, 1963; Crick et al., 1976). Mutation and recombination therefore are pre-conditions of evolutionary changes. These, in conjunction with extinction, are the key to changing patterns of biological diversity, ultimately ranging

from the variation of genes to the variation of entire ecosystems (Heywood, 1995). Therefore, the term biodiversity might broadly be defined as the sum total of all biotic variation (Purvis and Hector, 2000). Biotic variation is not evenly distributed and mainly concentrates in the tropics (Mittelbach et al., 2007). Regions harboring highest levels of species richness and endemism, but at the same time face exceptional degress of threat, were declared as biodiversity hotspots (Fig. 1, Myers, 1988, 1990; Mittermeier et al., 1998; Myers et al., 2000). Our notion of biodiversity has fundamentally changed, since we realized its massive loss within short time (Myers, 1988; Davis et al., 1995; Mittermeier et al., 1998; Myers et al., 2000; Mittermeier et al., 2011) while, at the same time many ecosystems (e.g. deep sea environments, coral reefs or rain forests) remain largely unexplored and an estimated number of 5+/-3 Million eucaryotic species on Earth outranges the number of ca. 1.5 Millions validly described species (Costello, May, and Stork, 2013). The main threats to biodiversity through human impact are habitat loss, fragmentation and climate change (Thuiller, 2007; Davis et al., 2011). Deforestation for timber exploitation, cattle pastures and agricultural land use is on-going. For instance, very recently, satellite pictures revealed that the state of Brazil has lost 5843 km^2 of its former forests, and deforestation increased in 2013 (15.11.13 on wwf.panda.org). After the publication of the World Conservation Strategy (IUCN 1980), “conservation, preservation and sustainable use of biodiversity” would thus ideally become a guiding principle worldwide.

We can, however, only protect what we are aware of, meaning that biodiversity needs to be captured from the smallest scale of genetic variation to the diversity of species and their niches, which again form part of interconnected ecosystems. Finally, sustainable use of biodiversity is only implementable if we aim at understanding the consequences of abiotic changes (e.g. climatic changes or geo-tectonics) and biotic interactions (e.g. gene flow, or the emergence of competitors) as these might trigger evolutionary changes or lead to extinction, thereby altering current patterns of biodiversity. Much on this focus, the mutual benefit of greater integration between different research fields dealing with biodiversity research, e.g. phylogenetics, taxonomy, historical biogeography, ecology, paleobiology and geology, has been discussed within the past few years (Wiens and Donoghue, 2004; Wiens and Graham,

2005; Mittelbach et al., 2007; Burnham, 2008; Warren, Glor, and Turelli, 2008; Crisp, Trewick, and Cook, 2011; Losos, 2011; Hoorn et al., 2013).

1. 2. Biodiversity hotspots in the Neotropics

Thirty-five regions were declared as biodiversity hotspots worldwide, in order to assess regions of conservation at least cost. The selection of a hotspot was based on two criteria, 1st holding at least 1500 endemic plant species and 2nd having lost 70%, or more, of its original area (Figure 1, Myers et al., 2000; Mittermeier et al., 2011). The neotropical region of Central and South America harbours eight of the hotspots defined by Mittermeier et al. (Mittermeier et al., 1998; Myers et al., 2000; Mittermeier et al., 2011): The Central American Madrean Pine-Oak woodlands, Mesoamerica, the Caribbean Islands, the tropical Andes, Tumbes/Choco/Magdalena, Brazil's Cerrado, Brazil's Atlantic Forest and the Chilean winter rainfall/Valdivian forests. These hotspots harbour altogether an estimated amount of 152000 species of vascular plants, which is more than the double amount of plant species in tropical Africa or Australasia (Gentry in Davis et al., 1995; Mittermeier et al., 2011). Besides their high species diversity, hotspots harbour unique climatic and edaphic properties, e.g. the vegetation of the Brazilian Atlantic coast adjoins the huge biome of the Cerrado Savannah, which underlies frequent fires during the dry seasons and hence, mainly consists of fire-adapted plants, such as shrubs and grasses. This in turn contributes to the geographical isolation of the neighbouring moist Atlantic side, which approximately comprises an amount of 53% of endemic tree species (Peixoto and Silva in Davis et al., 1995).

Another factor to landscape heterogeneity is mountain-building, inducing severe climatic changes through topography and the emergence of ecological gradients and physical habitats (Hoorn et al., 2010; Hoorn et al., 2013). In Central America, altitudinal differences raise from 0 to over 4000 m on a comparatively small geographic scale of which highlands comprise 77% (Toledo in Davis et al., 1995). The interior uplands consist of cooler damp or cloud forests with transitional climatic zones towards the seasides (Toledo in Davis et al., 1995). Both sea sides underlie distinct climates, which contributes to the heterogeneity of Central America. The

lowlands of the Caribbean are hot and humid, receiving moisture throughout the year, whereas the pacific coast has rainy and dry seasons.

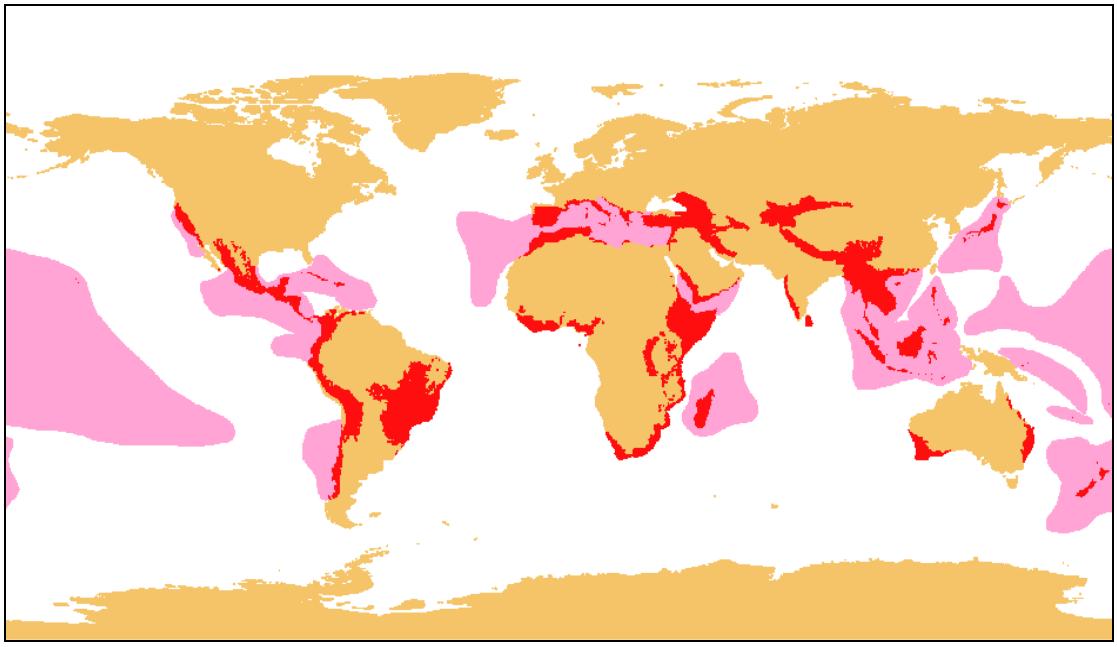

Figure 1. The Earth's Biodiversity hotspots marked in red. (Data for map creation were downloaded from the Conservation Biology Institute, 2013, on http://databasin.org/datasets/23fb5da1586141109fa6f8d45de0a260).

At the southernmost tip of Central America, the Isthmus of Panama cuts through a continental divide of only 100 m elevation, causing biological discontinuities between Central and South American taxa (Gentry in Davis et al., 1995). Due to the former separation of Central and South America, the Isthmus played a major role in the American biotic interchange (Cody et al., 2010). Plant clades that are distributed on both continents were able to migrate between them at least for the past 50 My, whereas the majority of animals crossed the Isthmus only until after 10 My ago (Cody et al., 2010). The tropical Andes and its adjacent regions are among the most species rich hotspots with highest levels of endemism (Mittermeier et al., 2011). The Andean orogeny began in the early Miocene at ca. 20 My ago, which gave rise to a variety of discontinous habitats. Steep physical gradients were formed and edaphic mosaics arose through the upheaval of rocks and transport of sediments from the mountains to

the Amazon basin (Hoorn et al., 2010). The Amazon region and the Central American lowlands mainly consist of tropical rain forests. Seasonally dry tropical forests (SDTF's sensu Pennington and Ratter, 2010) occur in disjunct patches throughout the neotropics, where rainfall is less than 1600 mm/year. The interandean valleys, which mostly consist of seasonally dry forests are considered to be particularly rich in endemics due to their geographical isolation (Pennington et al., 2004) and comprise several lineages dating back to the Miocene (Särkinen et al., 2012). These old species lineages co-occur with lineages from rapid and recent radiations in Andean foothills, which were reported e.g. for the genus *Inga* (Richardson et al., 2001). Rapid and recent radiations within the past 4 My are, otherwise, more characteristic for high elevation Andean grasslands (Särkinen et al., 2012). Migration of Northern American lineages into the Andes considerably contributed to species richness, too, e.g. boreotropical lineages, such as Malphigiaceae, Fabaceae and Annonaceae entered the Neotropics via the mountain chains of Central America and the newly formed Andes (Antonelli et al., 2009). An analysis of the tree community of an Amazon forest in Ecuador revealed that it consisted of 20% of immigrated taxa (Pennington and Dick, 2004).

These few examples may highlight that biotic variation underlies both, rapid changes (such as immigration of taxa) and large scale dynamics (such as topographic changes), which naturally act together as selective pressures over species compositions. Correspondingly, Särkinen et al. (2012) found that diverse species compositions (such as the “hottest hotspots”) are tightly coupled to the heterogeneous evolutionary histories of lineages they are composed of. If we try to better understand the evolutionary histories of organisms by reconstructing well-sampled phylogenies and integrating paleontological, geological and ecological data, we might be able to explain how, when and why species distributions have changed, perhaps predict their future dynamics and aim at understanding how these dynamics have influenced genetic differentiation and diversification. The widespread ranges of some Neotropical tree genera in SDTF’s and wet rain forests raise fundamental questions about their origin, history and contribution to Neotropical plant diversity.

1. 3. Introduction to the Neotropical genus *Cedrela* (Meliceae)

The Meliaceae family is pantropically distributed and comprises several hardwood timber tree genera, forming part of the major constituents of (sub-) tropical forests (Pennington and Styles, 1975). The economically important genus *Cedrela* is distributed in Central and South America (mainly throughout the Neotropical hotspot regions), in wet and (semi-) deciduous (sub-) tropical rain forests. It is closely related to the Indo-Malayan genus *Toona*, forming the monophyletic tribe of Cedreleae together with *Cedrela*, the latter which belongs to the subfamily Swietenioideae (Muellner et al., 2003; Muellner, Pennington, and Chase, 2009). *Cedrela* and *Toona* are morphologically very similar, but can be distinguished by the position of the seed wing, the structure of the androgynophore and the division of the first true leaves on the seedling (Pennington and Styles, 1975). The typical leaves are pinnate and the leaflets mostly have asymmetric bases (Figure 2.A). Shape, size, and the number of leaflet pairs, as well as sizes of capsules and flowers constitute important morphological characteristics for the determination of the 17 species of *Cedrela*, which were described in Pennington and Muellner (2010). The inflorescence of *Cedrela* (Figure 2.B) is a monoecious flower-rich thyrse (much-branched panicle) with cymose branches consisting of unisexual flowers that comprise rudimental organs of the opposite sex (Pennington and Styles, 1975). The flowers are tiny (0.5-1.2 cm long) and rather inconspicous in shape and colour. The androgynophore serves as a nectary attracting short-tongued insects, such as Lepidoptera, Hymenoptera and Diptera (Pennington and Muellner, 2010). Non-specifity of pollinators promotes cross-pollination, particularly in conjunction with protogyny, which protects species from self-pollination (Pennington and Muellner, 2010). The characteristic fruits of *Cedrela* are woody, septicidal, five-valved (star-like) capsules (Figure 2.D). The winged seeds (Figure 2.C) are attached towards the apex of the central columella (unlike *Toona*, whose seeds are attached towards the base of the columella) and disperse with the wind (Pennington and Muellner, 2010). All species of *Cedrela* are deciduous and the shoot apices are protected by bud scales when the trees are leafless (Pennington and Muellner 2010). This, together with the morphological properties of the fruit, led to

the hypothesis that *Cedrela* originated in a strongly seasonal dry forest habitat (Pennington and Muellner, 2010).

Common habitats of *Cedrela* are seasonally dry forests (Figure 3.A), but several species also, or exclusively, occur in wet rain forest (such as *C. tonduzii*, which is distributed throughout the mountain ranges of Central America in wet forests, Figure 3.B). Seasonally dry forests can be broadly divided into either strongly seasonally dry forests, where rainfall is less than 1600 mm per year with five to six months of drought, or semi-deciduous forests with less sharp seasons and more rain (Pennington et al., 2004). When compared to the more continuous geographical distribution of evergreen forests, the different types of seasonally dry forests are geographically much more disjunct (but see Pennington et al., 2004). This characteristic has probably persisted for the past several million years and probably did not change (as previously assumed) during drier glacial periods through habitat contraction. Within the wet rain forest regions, associated genera growing close with *Cedrela* seem to be diverse, but are yet not being well documented. Along the mountain ranges of Central America, *Cedrela* is often associated with *Pinus*, *Quercus* and *Liquidambar*, or to the South with *Podocarpus* (Pennington and Muellner, 2010). In South America, associations in montane habitats occur with *Weinmannia* and *Oreopanax*, or in northern Peru with *Ceroxylon* (Pennington and Muellner 2010).

Figure 2. Morphological characteristics of *Cedrela*

A. Leaf of *Cedrela* sp. (left), **B.** Flowers (middle), **C.** seeds (right), photographs taken by A. Muellner-Riehl

D. Mature open capsule of *C. ngobe* Köcke, Penn. & Muellner-Riehl (left), photograph taken by M. Piepenbring in Chiriqui, Panama; **E.** Dry capsules (right) hanging on a leafless tree of *C.* aff. *odorata* (group II) close to Almirante in Panama, photograph taken by V. Köcke

Figure 3. Typical habitats of *Cedrela*

A. Tropical deciduous forest in the Comarca Ngöbe-Buglé in Panama

B. Wet montane forest, Vulcan Barú, Panama, both photographs taken by V. Köcke

1. 4. Niche Evolution of *Cedrela* (Chapter I)

The Meliaceae family most likely is of western Gondwanan origin (Muellner et al. 2006). Fossil records of *Cedrela*, or *Toona*, or "intermediate forms" show that Cedreleae were once wide-spread throughout the northern hemisphere from the early Eocene to the Miocene, indicating an African or European origin of the group with subsequent splits into the East (Old World *Toona*) and West (New World *Cedrela*), respectively (Muellner et al., 2006; Muellner et al., 2010). The oldest fossil records of *Cedrela* date back to the early Eocene of North America (ca. 50 My ago, MacGinitie, 1941), thus, from a time when subtropical forests were present at northern latitudes and climates were rather equally warm and humid (Zachos et al., 2001; Zachos, Dickens, and Zeebe, 2008; Hren et al., 2010). However, based on morphological traits of *Cedrela* (deciduous habit and wind-dispersed seeds), an evolutionary origin within a strongly seasonal dry forest habitat was proposed (Pennington and Muellner, 2010). From the early Eocene to the Miocene (ca. 50-7 my ago), distributional patterns of *Cedrela* shifted towards the south, leading to its current distribution in Central- and South America, whereas northern lineages went extinct (see ancient patterns of distribution, Figure 4). It stands to reason that the severe climatic changes since the Eocene had an influence on shifts of species distributions. For instance, a pronounced temperature drop at the Eocene-Oligocene-boundary, which was supposedly correlated with the expansion of the Antarctic ice shields led to an increasing seasonality with colder winters and less precipitation throughout North America and thereby initiating a decline of tropical elements (Axelrod, 1985; Graham, 1993; Wolfe, 1994; Zachos et al., 2001; Sheldon and Retallack, 2004; Zanazzi et al., 2007; Eldrett et al., 2009). Temperatures rised again from the late Oligocene onwards (23-25 my ago); the global warming peaked in the late middle Miocene climatic optimum (17 to 15 my ago), although not reaching Eocene temperatures anymore, followed again by gradual cooling and reestablishment of a major ice-sheet on Antarctica (Zachos et al., 2001; Zachos, Dickens, and Zeebe, 2008).

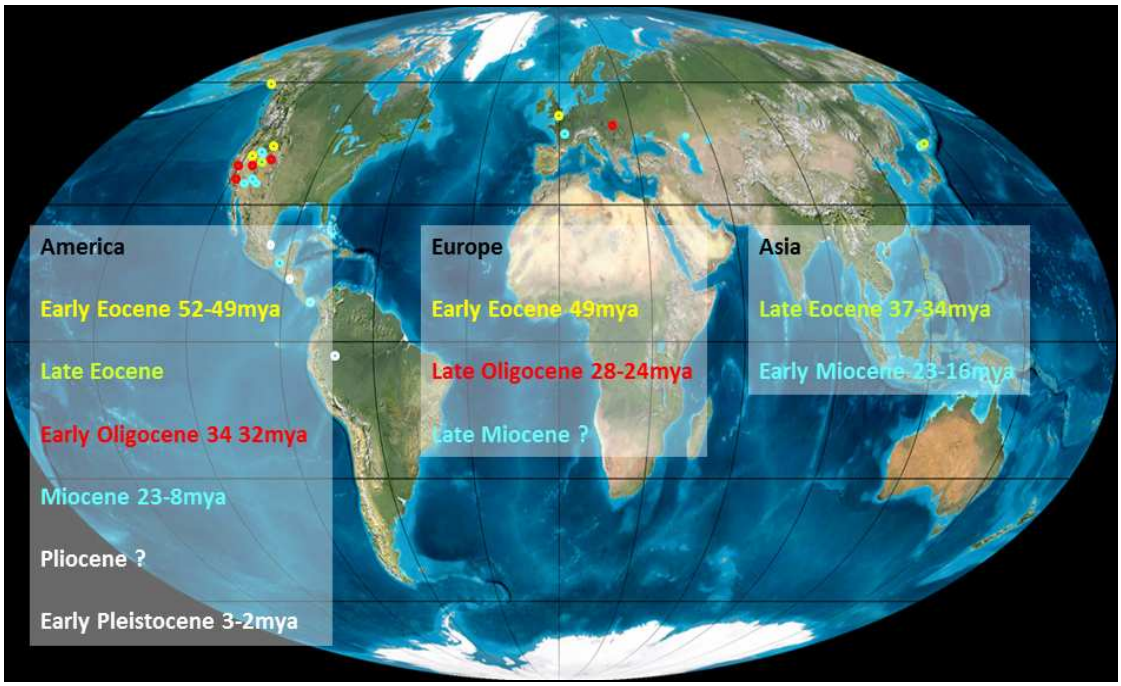

Figure 4. Fossil records of Cedreleae (*Cedrela* or *Toona*), modified from http://cpgeosystems.com/presentmoll.jpg.

As suggested by Wiens and Donoghue (2004), even crude information about past climates together with information about climatic tolerances of a group of taxa can give insights into understanding past events of dispersal (opportunities to niche expansions) and extinction. Climatic tolerances of species are limited by minimum and maximum values (e.g. temperature tolerance), thus having a specific width. The width of one specific tolerance would represent the fundamental niche of a species to this specific trait. In the sense of Hutchinson (1957) the fundamental niche is a multidimensional environmental space, where species are theoretically able to live in, and is thus governed by numerous species traits. The fundamental niche of a species can be different from the realized niche; e. g. expulsion from part of the fundamental niche might be caused by competition with other species. The acquisition of a new trait (e.g. frost tolerance) leads to a shift of the fundamental niche. This process has been called ‘niche evolution’, acting opposedly to ‘niche conservatism’ (Kawecki, 1995; Holt, 1996a; Holt, 1996b). Niche conservatism is the tendency of species to retain ancestral traits through time, whereas niche evolution describes evolutionary diversification (Wiens and Graham, 2005; Losos, 2008; Warren, Glor, and Turelli, 2008). Although both, niche conservatism and niche evolution highly depend on the

scale at which they are analyzed (Wiens and Graham, 2005; Losos, 2008), generally niche conservatism seems to be much more common (Kozak and Wiens, 2006; Losos, 2008), e.g. on large scales it has constrained dispersal of plants between major biomes (Figure 5, from Crisp et al., 2009) and has also been associated with events of mass extinctions (Wiens and Graham, 2005).

Techniques to infer historical patterns of diversification made huge progress within the past years. Pagel (1999) first wrote an overview about methods of combining molecular phylogenetics (based on gene-sequence information) with statistical approaches (based on Maximum Parsimony or Maximum Likelihood). By then, these particularly referred to: 1st estimation of the timing of diversification, 2nd reconstruction of ancestral character states, and 3rd assessment of the tempo of evolution. More recent advances have opened other approaches to reconstructing temporal dynamics of evolution (e. g. analyses of trait divergence) by combining dated phylogenies, nowadays using Bayesian tree inference as state-of-the-art approach (Huelsenbeck and Ronquist 2001, Drummont and Rambaut 2007, Ronquist et al. 2012) and post-tree analyses (Graham et al., 2004; Graham and Fine, 2008; Harmon et al., 2008; Harmon et al., 2010, and references cited herein). Specifically, the combination of species distribution models based on climate layers with time-calibrated trees allows for the reconstruction of climatic niches (Hijmans et al., 2005; Elith et al., 2006; Warren, Glor, and Turelli, 2008; Evans et al., 2009). For my study, the approach of Evans et al. (2009) represented an ideal method to investigate evolutionary dynamics of processes of niche evolution within *Cedrela*. The measurement of relative disparity furthermore allowed for testing if disparification of trait divergence (= ecomorphological disparity) is higher or lower than expected under the Brownian motion model of evolution (as explained in Harmon et al., 2003).

To my knowledge, very little attention has been paid to fossil findings, when studying phylogenetic dynamics of trait evolution of plant organisms. However, information from palaeontological studies can provide additional information upon ancestral traits and distributions. Concerning fossil plant remains, reliability of the records highly depends on the accuracy of taxon determination and the dating method of the geological setting. If fossil findings are judged to be reliable, fossils can additionally provide highly valuable insights into the evolution of species by unveiling, for

example, ancestral traits of morphological characters of leaves, fruits, flowers, stomatal architecture and even climatic tolerances. Climatic conditions under which fossil taxa occurred can be either estimated from the general composition of a fossil plant assemblage (e.g. Wolfe, 1978; Wolfe, 1985, 1994; Graham, 1999b, 2010; Teodoridis et al., 2011), corresponding paleosol studies (e. g. Sheldon and Retallack, 2004; Mulch et al., 2010), or marine temperature changes (Mosbrugger, Utescher, and Dilcher, 2005). Estimates of ancestral climatic tolerances might thus be inferred in two different ways: 1st from niche reconstructions based on extant information of species distributions and phylogenetic relationships; and 2nd by thoroughly evaluating data from geological and palaeobotanical studies that comprise information of ancestral distributions and extinct taxa. The results from these (independent) analyses can be compared to each other allowing conclusions upon their conformity. As far as I know, using this array of methods, specifically to elucidate the climatic niche evolution of a genus, is to date unique.

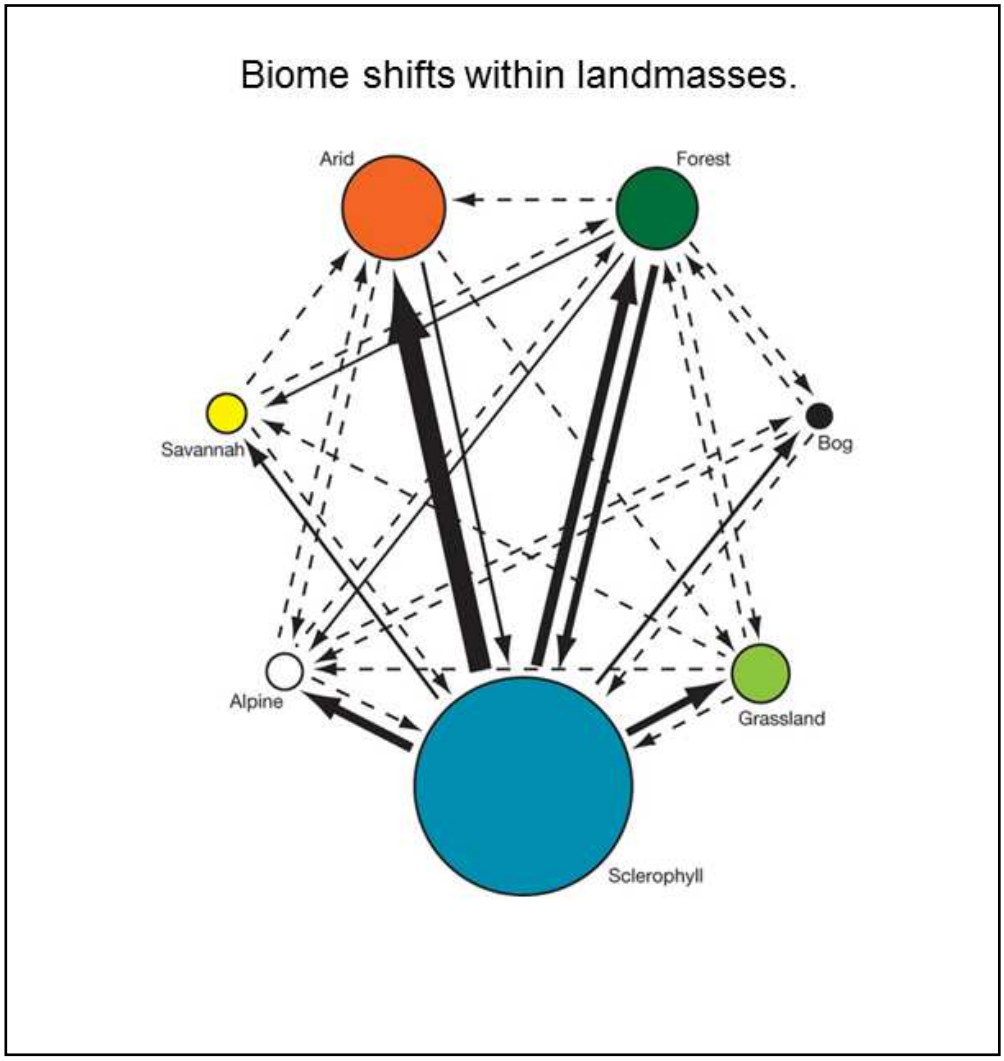

Figure 5. Biome shifts of plant species are rare

"Shifts occurred with only 356 of 10,800 speciation events within landmasses. Number of species sampled within each biome is proportional to the area of each circle: sclerophyll, 7,250; arid, 1,683; wet forest, 1,005; temperate grassland, 504; savannah, 242; montane, 186; bog, 84. Arrow thickness is proportional to the number of transitions in each direction, ranging from 6 to 95 events; dashed lines indicate 1–5 events and lack of an arrow indicates that there was no event. "

from Crisp *et al. Nature* **458**, Fig. 1 (2009)

1. 5. Phylogeography

The geographic distribution of plant species is tightly coupled to historical aspects of dispersal abilities and establishment possibilities, extinction, biotic and abiotic forces (e.g. climatic changes). Depending upon the maternal inheritance of genes (DNA sequences from the plastids) or biparental inheritance (DNA sequences from nuclei), genetic structures might reflect different historical processes. Reconstructions of molecular phylogenetic trees and network analyses are both widely used to unveil genetic relationships among and within groups of organisms. Although molecular phylogenetic trees serve in manifold ways to unravel evolutionary histories of organisms, they might also be insufficient, depending on the purpose of the scientific analysis. If, for instance, genetic variability is low, phylogenetic signal becomes increasingly misleading as e.g. compensatory base changes may lead to homoplasy, thereby obscuring phylogenetic distance (Álvarez and Wendel, 2003). Furthermore, within phylogenetic trees speciation is reflected by bifurcated branches, although descendent genes might coexist with persistent ancestors. Hence, several descendants might be derived from one single ancestor, which contradicts the bifurcated nature of phylogenetic trees (Posada and Crandall, 2001; Igic, Bohs, and Kohn, 2006). Also, processes of reticulate evolution do not follow bifurcations and thus cannot be immediately detected on a tree (Posada and Crandall, 2001). Therefore, network analyses are sometimes more appropriate, particularly when genetic variability of the selected markers is low, when studying reticulate relationships and/ or analyzing patterns of phylogeography.

Under the assumption of neutral molecular evolution, there is a direct relationship between the frequency of gene sequences (haplotypes) and time, meaning that higher frequencies of certain haplotypes indicate their longer presence within a group of taxa (Posada and Crandall, 2001). Exceptions might account for drift and bottleneck effects, or strong selective pressures. But if species-specific or geographically structured genetic diversity are found, restrictions to gene-flow and direction of colonization events can be inferred. On the other hand, the lack of geographic structure within a set of haplotypes might unravel different consequences of either past or on-going gene-flow.

Species distributions of *Cedrela* throughout the Neotropics with adaptations to different climates in lowlands and on mountains, the Peruvian centre of species diversity, that is certainly different to ancient centres of species diversity further to the North, and unclear species delimitations within widespread lowland taxa bear several questions: Can we detect geographical structures of genetic diversity and were there (potentially long-lasting) geographical dispersal barriers? How variable is genetic diversity within maternally and biparentally inherited haplotypes? Do the haplotypes from plastid and nuclear markers show congruent patterns? Will haplotype networks corroborate what we already know about the evolutionary history of the genus or unveil surprising results, and are there potential conclusions that might contribute to explain Neotropical species diversity?

1. 6. The role of Classical Taxonomy

Classical taxonomy refers to the description and recognition of discrete traits from morphology, anatomy and ecology, which classify different taxa (e.g. species, genera, families) and serve to order them hierarchically. Based on hierarchy, species represent the lowest unit of taxonomic variation (although species can also be further subdivided into subspecies and varieties). This describes what the taxonomic species concept is. There is, however, no rule to the selection of traits for the taxonomic description of species and not even scientific agreement upon species concepts in general. For instance, the evolutionary species concept suggests that morphological distinctiveness is not necessary (e.g. due to the existence of cryptic species), but that reproductive isolation from one population to another (interruption of gene flow) gives rise to speciation and maintains the identity of a lineages through time (Wiley, 1978; Mayr, 1996). In a phylogenetic sense, species therefore represent a group of individuals that evolve together (Simpson, 1951; Wiley, 1978). However, the phylogenetic perspective does not consider reticulate relationships (Posada and Crandall, 2001). Thus, the concept of ‘integrative taxonomy’, which unifies species diversity from multiple and complementary perspectives (e.g. from ecology, genetics and taxonomy) seems to best fit modern species circumscriptions (Dayrat, 2005; Weigand, Götze, and Jochum, 2012). Nevertheless, in the majority of sexual plant taxa, discrete entities that

correspond to reproductively independent lineages do exist at species levels, endorsing the usefulness of taxonomy (Rieseberg, Wood, and Baack, 2006). When capturing patterns of diversity, e.g. sampling individuals for research studies, recognition of species in the field is still the first, most essential step. Classical taxonomy therefore still is fundamentally important, considering that an estimated amount of 5+/-3 Million eukaryotic species probably outranges the number of ca. 1.5 Million validly described species (Costello, May, and Stork, 2013). In particular, large taxonomic groups lack taxonomic revisions, probably due to the time-consuming effort of taxonomic revision and the relatively little output of publishable information (Zizka et al., 2013).

Within the comparatively small genus *Cedrela*, species distributions are relatively well-understood and taxonomic circumscriptions up to date (Pennington et al. 2010). Naturally, some regions are better studied than others, or simply more accessible, which might explain why records of species occurrences can never be 100% accurate. In *Cedrela*, very little herbarium material is available from southern Central America, northwestern and central Mexico and the West Indies (personal observation and communication with T. Pennington, 2009). Considering the origin of *Cedrela* and the major role of the Panamanian Isthmus to its historical biogeography, I assumed southern Central America and northern South America would be a special area of interest for studying species diversity.

1. 7. Thesis aims

The aim of my doctoral thesis is to understand the evolutionary biology and diversity of the genus *Cedrela*, an economically important hardwood timber tree of the Meliaceae family. It is distributed throughout the neotropical hotspot regions, across the Central American Madrean Pine-Oak woodlands, Mesoamerica, the Caribbean Islands, Tumbes/Choco/Magdalena, Brazil's Atlantic Forest, and the tropical Andes in subtropical to tropical (semi-) deciduous or evergreen rain forests. These diverse habitats are found in lowlands as well as mountains, where species of *Cedrela* occur under different climatic conditions. The first chapter of my thesis is dedicated to understanding the climatic niche evolution of *Cedrela* by testing the capacity of

species to switch their climatic niche. The niche reconstructions were compared to the paleoclimatic conditions of fossil sites as described in publications of fossil records of *Cedrela*. The second chapter of my thesis was dedicated to analyzing genetic variation within and among species of *Cedrela*, in order to test if genetic variation corroborates the geographical structure of species distributions, and if gene flow is observable at intra- and/or interspecific levels. The third chapter of my thesis was dedicated to classical taxonomical studies of taxa from Central and northern South America resulting in the description of a new species, *Cedrela ngobe* Köcke, T.D. Penn. & Muellner-Riehl from Panama and Costa Rica.

Chapter I

Niche evolution through time and across continents: The story of Neotropical *Cedrela* (Meliaceae)

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Key words. *ancestral niche; Cedrela; climatic niche modeling*; *Eocene*; *fossil*; *Meliaceae*; *Miocene*; *Oligocene*; *relative disparity*

2. 0. Declaration of contribution/ Erklärung über die jeweiligen Autorenanteile

Erklärung über Anteile an Kapitel 1 (**Chapter I**) der Promotionsarbeit.
Promovierende, VK, **Anteile in Prozent und in Klammern ( ) Prozentanteile der KoautorInnen und weiterer KollegInnen**

(1) Entwicklung und Planung
Promovierende, VK: 10% (90% AM-R, TP und JS)

(2) Durchführung der einzelnen Untersuchungen/ Experimente
Promovierende, VK: 100% der Phylogenetischen Analysen und Divergence Dating (nach Anleitung durch JS); 100% der folgenden Analysen: Climatic Niche Models, Nischenrekonstruktionen basierend auf den Nischenmodellen und der datierten Phylogenie und Disparification; 100% der Nischenrekonstruktionen basierend auf Literaturrecherche über Fossilien und Paläoklimatische Bedingungen; 50% der Literaturrecherche (50% AM-R und JS).

(3) Generierung der Daten und Erstellung der Abbildungen
Promovierende, VK: 25% der Sequenzgenerierung (75% Sequenzen von AM-R, GS und Steven Cavers aus Edinburgh), im Detail: 43% des ITS Datensatzes von VK (47% von AM-R, GS und Stephen Cavers), 90% des *psb*A-*trn*H Datensatzes (10% Gertrud Schorr, AM-R generierte 100% des Datensatzes von *trnS-G* und *psbB-T-N*); 50% des Sequence Editing/Alignment (50% AM-R); 5% Verbreitungsdaten (95% TP und Anna Becker); 80% der Abbildungen (20% Optimierung JS); 100% der Tabellen.

(4) Analyse/Interpretation der Daten
Promovierende, VK: 75% Analyse der phylogenetischen Resultate (25% JS), 100% Analyse der Datierung, 55% Analyse und Interpretation der Nischenrekonstruktionen und Disparification (45% JS in gemeinsamen Diskussionen über die Interpretation der vorliegenden Ergebnisse), 100% Interpretation der Nischenrekonstruktionen basierend auf der Literaturrecherche über Fossilien und Paläoklima.

(5) Übergeordnete Einleitung/Ergebnisse/Diskussion
Promovierende, VK: 100% Entwurf von Einleitung, Material und Methoden, Ergebnisse und Diskussion, mit 100% Überprüfung und Optimierung (mind. 50%) durch JS und AM-R.

#Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben
*Mehrfacheintragungen möglich

2. 1. Abstract

Premise of the Study

Climatic and geological changes have been considered as major drivers of biological diversification. However, it has been generally assumed that lineages retain common environmental affinities, suggesting a limited capacity to switch their climatic niche. We tested this assumption with a study of the evolution of climatic niches in the Neotropical tree genus *Cedrela* (Meliaceae).

Methods

We combined distribution models of extant *Cedrela* with a dated molecular phylogeny based on one nuclear (ITS) and three plastid markers (*psbA-trnH*, *trnS-G* and *psbB-T-N*) to reconstruct the evolutionary dynamics of climatic niches. We calculated relative disparity of climatic tolerances over time to test for niche evolution within subclades or divergence between subclades and conservatism among closely related groups. Published fossil records and studies on paleosols were evaluated for the distribution and climatic conditions of extinct *Cedrela*.

Key results

The fossil record of *Cedrela* suggested a major biome shift from paratropical conditions into warm-temperate seasonal climates in the Early Oligocene of western North America. In the Miocene, *Cedrela* extended from North America (John Day, USA) to southern Central America (Gatún, Panama). Diversification in the early evolutionary history was mainly driven by changes in precipitation. Temperature had an increasing impact on ecological diversification of the genus from the Miocene onwards. Sister species comparisons revealed that recent speciation events may be related to divergence of climatic tolerances.

Conclusions

Our study highlights the complexity of climatic niche dynamics, and shows how conservatism and evolution act on different temporal scales and climatic parameters in *Cedrela*.

2. 2. Introduction

Current patterns of biodiversity are the result of long-term dynamics of speciation, dispersal, adaptation, and ultimately extinction, driven by geological and climatic changes. On a time scale of millions of years, large-scale (e.g. biome) shifts in plant species have been proposed to be rare events, indicating a limited capacity of species to switch their ecological niche (Crisp et al., 2009; Wiens et al., 2010). It has also been proposed that the evolutionary success of many plants might have resulted not from adaptation to new biomes but from expansion of biomes as climate changed (Crisp et al., 2009). This means that species tend to track particular climates in times of environmental change and implies retention of ancestral ecological characteristics through speciation events which is known as phylogenetic niche conservatism (Ackerly, 2003; Wiens and Graham, 2005). Thus, niche conservatism is a pattern of high similarity of niche-related traits over macroevolutionary time, but has also been considered a process that affects evolutionary dynamics (but see Crisp and Cook, 2012). For example, the failure of species to adapt to changing environmental conditions or to invade new habitats could be also seen as an effect of niche conservatism (Wiens and Donoghue, 2004; Wiens et al., 2010). However, only niche evolution enables species to establish in new habitats and climatic regimes or to persist within a changing environment. Accordingly, the interplay between processes leading to niche conservatism or evolution has an impact on large scale patterns of species distributions and their historical biogeography (Wiens and Donoghue, 2004).

Several niche concepts have been used in the context of ecology and evolution, but the niche definition of Hutchinson (1957) has found wide acceptance as it quantifies the fundamental and the realized niche of species (Ackerly, 2003; Wiens and Graham, 2005; Pearman et al., 2008; Holt, 2009; Wiens et al., 2010). The distinction is important because the fundamental niche is a volume that comprises a spectrum of abiotic and biotic factors where a species can persist whereas the realized niche is the result of dispersal ability and biotic interactions that effectively limit species distributions (Pearson and Dawson, 2003; Soberón and Peterson, 2005). In the face of global climate change and biodiversity loss, the question of how species distributions

change through time and how species evolve in different habitats becomes increasingly important.

The Neotropical tree genus *Cedrela* (Meliaceae) comprises 17 currently described species that occur in a wide range of habitats from northern Mexico to Paraguay, including tropical rainforests, tropical deciduous forests with pronounced dry seasons, and warm-temperate forests (Pennington and Muellner, 2010). While most species are restricted to mountain regions, several taxa can be found in lowland habitats. All species are deciduous with characteristic dry fruits that release wind-dispersed seeds, which led to the suggestion that *Cedrela* originated in seasonal dry forests (Pennington and Muellner, 2010).

Based on fossil occurrences, Muellner et al. (2010) showed that Cedreleae (*Cedrela* and *Toona*) were wide-spread in the northern hemisphere during the Eocene with occurrences in Europe (Reid and Chandler, 1933), North America (MacGinitie, 1974; Hickey and Hodges, 1975), and Japan (Tanai, 1970). Since then, global temperatures have decreased with intermittent warmer episodes (Zachos et al., 2001) and the lack of fossils in North America (north of Mexico) from the Late Miocene onwards suggests that *Cedrela* had gone extinct there by that time (Muellner et al., 2010). In southern Central America *Cedrela* was present by that time as evident from fossil pollen found in the Gatún Formation (Graham, 1991b, a, 2010). First evidence for *Cedrela* in South America is given by a Pliocene fossil from the Amazonas Formation of the Iquitos region in Peru (Pons and Franceschi, 2007; Graham, 2010). Correspondingly, one would expect to find a signature of the southward migration of species, as shown by the fossil record, reflected by the topology of a dated phylogenetic tree. Specifically, one would expect extant South American species be nested among Central American species.

The range dynamics documented in the fossil record may have been driven by the pronounced cooling events at the Eocene-Oligocene transition (c. 34 million years ago, mya) and during the Miocene (c. 10 mya) leading to local extinction and/or niche tracking towards favorable climates in Central and South America. Under phylogenetic niche conservatism, species are assumed to retain ancestral climatic tolerances, and we would expect extant species to share similar climatic niches.

Furthermore, climatic conditions at present-day ranges should correspond to those at known fossil occurrences with major shifts being absent or rare. Alternatively, climate might have also triggered niche evolution in *Cedrela*. In mountain areas and especially along the Andes, steep climatic gradients could provide potential opportunities for speciation resulting in different ecophysiological adaptations among closely related species and separation of climatic niches.

To understand the evolution of the climatic niche of this economically important tree genus through time and across continents, we reconstruct the phylogenetic relationships of extant *Cedrela* species, compile information on species distributions (for both extant and extinct species), and paleoclimates. The mutual benefit of greater integration between these fields has been discussed within the past few years (Wiens and Donoghue, 2004; Wiens and Graham, 2005; Mittelbach et al., 2007; Burnham, 2008; Warren, Glor, and Turelli, 2008; Losos, 2011). Specifically, we will address the following questions: To what extent did climatic niches of *Cedrela* change through time, and which environmental factors might be related these changes? Can the historical biogeography of this group as documented in the fossil record be explained by stasis and/or evolution of climatic niches? And finally, is climate a potential driver of speciation? We use species distribution modeling to approximate species realized climatic tolerances and combine these with a dated molecular phylogeny to derive evolutionary dynamics of the climatic niche. Published information on fossils in combination with studies on paleosols contributes to our understanding of niche evolution with additional information on ecological and geographical diversity of the genus in the past.

2. 3. Materials and Methods

Taxon sampling

Plant material of Cedreleae (*Toona* and *Cedrela*) for molecular work was obtained from herbarium specimens and our own field collections. Additional sequence data of Cedreleae were available from Muellner et al. (2009; 2010), GenBank and Cavers et al. (pers. comm.; 2013). All sequences used in this study are listed in the Appendix (Table A). In addition to the species used in previous analyses (Muellner, Pennington, and Chase, 2009; Muellner et al., 2010; Pennington and Muellner, 2010), we also included *C. cubensis* Bisse from Cuba and a new, undescribed species from Panama and Costa Rica (Koecke et al. in prep.) for our phylogenetic analyses. We included all *Toona* species (Bahadur, 1988; Hua and Edmonds, 2008), except for *Toona fargesii* for which we were not able to obtain any material.

Isolation of DNA, amplification and sequencing

Total genomic DNA was isolated from dried plant material using the DNeasy Plant Mini Kit (Quiagen, Hilden, Germany) following the manufacturer's protocol instructions. The nuclear ITS region was amplified using the primer pair AB101/AB102 (Douzery et al., 1999). The plastid region *psbA-trnH* was amplified using the primer pair psbA3′f/ trnHf (Sang, Crawford, and Stuessy, 1997). A 25 µl PCR reaction mix for ITS amplification contained 0.5 - 2 µl DNA-isolate (20 - 30 ng/µl), 20.5 - 22 µl ReddyMix PCR Master Mix (2.5 mmol $MgCl_2$; Advanced Bioenzymes, Surrey, UK), 0.5 µl of each primer, 1 µl of dimethyl sulfoxide (DMSO), and 0.5 µl of bovine serum albumin (BSA 0.4%) as described in Muellner et al. (2003; 2005). A 25 µl reaction mix for *psbA-trnH* amplification contained 0.5 - 2 µl DNA-isolate (20 - 30 ng/µl), 21.5 - 23 µl ReddyMix PCR Master Mix (2.5 mmol $MgCl_2$; Advanced Bioenzymes, Surrey, UK), 0.25 µl of each primer, 1 µl of DMSO, and 0.5 µl of BSA. Cycling conditions for both markers were carried out following the protocol of Muellner et al. (2003). PCR products were purified using the Quiaquick gel extraction kit (Quiagen) or directly purified using the Nucleospin Extract kit

(Machery-Nagel, Düren, Germany). Purified PCR products were directly sequenced with the same primers used for PCR amplification and the BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Germany) and analyzed on an ABI 3730 DNA analyzer.

Sequence editing and alignment

Forward and reverse sequences were edited manually and assembled in Geneious 5.1.7 (Biomatters Ltd, Auckland, New Zealand). An alignment was built using the Muscle algorithm with default settings as implemented in Geneious and edited manually. New sequences have been deposited in GenBank under the accession numbers KC155953 - KC155982 (Appendix, Table A).

Phylogenetic analyses and molecular clock dating

For each species, several accessions (between 2 and 6) collected throughout the known geographic range were sequenced for a preliminary assessment (except for *C. dugesii*, a narrow endemic from Central Mexico for which only one accession was available). Phylogenetic trees were built including all accessions (independently for ITS and cpDNA). Particular attention was paid to the phylogenetic relationships of *Cedrela odorata* and *C. fissilis*, two complex and much debated species (Muellner, Pennington, and Chase, 2009; Garcia et al., 2011; Cavers et al., 2013). In addition to our own collections, we incorporated all currently available ITS sequences for these taxa, using *Capuronianthus mahafalensis*, *Neobeguea mahafalensis*, *Lovoa trichilioides* and *Swietenia macrophylla* as outgroups. Accessions from any single species that clustered together with moderate to strong support (or that were not recovered as paraphyletic with high support) were reduced to a single sequence for the final analyses on the niche evolution of *Cedrela*. The final ITS dataset consisted of 23 accessions with 703 characters, 95 of which were variable and 44 were parsimony-informative. The combined chloroplast matrix (*psbA-trnH*, *trnS-G* and *psbB-T-N*) consisted of 22 accessions with 2030 characters of which 69 were variable and 41 were parsimony-informative. After preliminary analyses showed no strongly supported incongruence

between the nuclear and plastid data, all four alignments were combined into a concatenated matrix (2733 characters, with 164 variable and 85 parsimony-informative sites, respectively).

Bayesian phylogenetic analyses were run on partitioned and combined datasets using MrBayes 3.2 (Ronquist et al., 2012) and BEAST version 1.7 (Drummond et al., 2012a) for relaxed molecular clock dating of Cedreleae (*Cedrela* and *Toona*, without any outgroup selection). The best-fit model of nucleotide substitution was selected according to the Akaike Information Criterion (AIC) using jModeltest 0.1.1 (Posada, 2008). The general time-reversible model with rate variation and an invariant site parameter (GTR + G + I) was selected for both the ITS and the combined dataset. Molecular clock dating was performed with the relaxed uncorrelated clock model. The stem age of the most recent common ancestor (MRCA) of Cedreleae was set to 50 mya based on the minimum age of the oldest known fossils (MacGinitie, 1941; Hickey and Hodges, 1975) with a lognormal distribution and a standard deviation of 1.1. The tree prior was set to follow a birth-death process. Markov Monte Carlo (MCMC) chains were run for 50 million generations with parameters sampled every 5000 generations. Effective sample size (ESS) values of the BEAST analyses were checked in Tracer (Drummond and Rambaut, 2007) and found to be all >350. After removing the first 10% of the samples as burn-in, all remaining trees were combined to build the maximum clade credibility (MCC) tree using TreeAnnotator (Drummond and Rambaut, 2007). The alignments and MCC tree are available from TreeBase (accession number S13846).

Climatic niche modeling

We performed maximum entropy modelling of geographic distributions of extant species of *Cedrela* using the program Maxent (v.3.3.3k; Phillips and Dudík, 2008). Our distribution data comprised more than 2500 georeferenced localities of species of *Cedrela*. Environmental data comprised 19 climatic variables that were compiled by Hijmans et al. (2005). Therefore, we refer to our niche modeling as “climatic niche modeling” following Evans et al. (2009). We performed initial analyses on all 19 variables and then chose seven climatic variables (Table 1) according to their

importance in the Maxent models and with a low coefficient of correlation ($r < 0.7$). All Maxent analyses were performed using 10 replicates. Having only a very few localities for a species may be a problem for species distribution modeling as the predictive potential decreases. Although a comparison of different distribution modeling approaches revealed that Maxent can compensate for incomplete, small species occurrence data sets due to its regularization procedure, at least 5 - 10 distribution points are required for accuracy (Hernandez et al., 2006). Therefore, four species of *Cedrela* were excluded from the Maxent analysis: *Cedrela discolor* is currently only known from the type collection (Durango, Mexico), *C.* sp. nov.* from Panama and Costa Rica was represented by only four localities, *C. molinensis* and *C. weberbaueri* only had seven and four georeferenced collection localities, respectively.

**Cedrela* sp. nov. was represented by only four localities when this study was published in AJB in 2013. Now, many more occurrences of the newly described species, *C. ngobe* Koecke, Penn. & Muellner-Riehl are known (but see Chapter III). An additional niche model was generated for this species and was attached to all other climatic niche models that are shown in the Appendix (Figure I a-q).

Bioclimatic variables	
BIO2	Mean diurnal range of temperature (mean of monthly maximum/minimum temperature)
BIO4	Annual temperature seasonality (standard deviation *100)
BIO6	Minimum temperature of the coldest month
BIO14	Precipitation of the driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter

Table 1. Bioclimatic variables used for the climatic niche models of *Cedrela* in Central and South America.

Niche dimensions of extant species and history of niche occupancy

We inferred the history of niche occupancy by applying the methodological approach of Evans et al. (2009). The results from the Maxent models were then used to derive predicted niche occupancy profiles (PNOs) for each climate variable. The PNOs summarize the climatic suitability of species with respect to climate variables. Ancestral environmental tolerances were estimated with a non-parametric approach using the maximum clade credibility tree produced in BEAST. For each climatic variable, suitability scores were randomly selected 100 times and estimated for all interior nodes using a ML-reconstruction under the assumption of Brownian motion evolution. Given that a value of suitability is randomly chosen, this method captures intraspecific variability and takes into account that species' tolerances to environmental factors do not always follow a normal distribution (Evans et al., 2009). The analyses were performed using the phyloclim package v.0.8.1 (Heibl, 2011) in R (R Core Team, 2012).The results were compared to the paleoecology of fossil records of Cedreleae from the Eocene until the Pliocene (see below).

Finally, we specifically compared ecological divergence between sister species, thus excluding deeper nodes in the phylogeny. These comparisons aim at avoiding the potential uncertainty associated with ancestral trait reconstructions. Furthermore, following the concept of phylogenetic niche conservatism, observed niche differences between sister species may reflect ecological divergences that led to speciation. All comparisons were restricted to well-supported sister species pairs (pp > 0.9).

Disparification

The disparity between sister-lineages is calculated as the average of the squared pairwise differences of weighted mean values of traits (Harmon et al., 2003). To measure relative disparity over time for a (sub)clade the value of n-1 (with n being the number of extant taxa) has to be divided by the squared pairwise differences (Harmon et al., 2003; Evans et al., 2009). Values close to 1 imply that subclades contain a substantial proportion of the total variation and thus are likely to overlap extensively, whereas values near 0 imply that subclades contain relatively little of the variation

present (Harmon et al., 2003). We measured relative disparity using the GEIGER package (Harmon et al., 2008) in R. The morphological disparity index (MDI) shows the difference between the expected disparity under the Brownian motion model of evolution and the values obtained by the procedure described above.

Fossil ecology

Information on vegetation types and benchmark data of paleoclimates (e.g. presence/absence of seasonality, summer/winter rain, mean annual temperatures) were derived from Axelrod (1985; 1991, 1995; 2000), Bestland et al. (2008), Chandler (1964), Daley (1972), Erdei et al. (2007), Graham (1976, 1991a, c, 1993, 1999b, a, 2010), Graham and Dilcher (1998), Hably (2006), Hickey and Hodges (1975), Legrand (2003), Lötschert and Mädler (1975), MacGinitie (MacGinitie, 1974), Manchester and McIntosh (2007), Meyer and Manchester (1997), Poole (2000), Pons and Franceschi (2007), Reid and Chandler (1933), Roiron (1991), Tanai (1970), Tanai and Suzuki (1963), Wing et al. (1991), Wing and Greenwood (1993) and Wolfe (Wolfe, 1977, 1978, 1985, 1994). These data were complemented by information from geological studies of paleosols, which also provide data on precipitation/drought and temperatures (Retallack, Bestland, and Fremd, 2000; Retallack, 2004; Retallack and Kirby, 2007; Bestland et al., 2008; Hren et al., 2010). We used these data to determine climatic conditions of ancient *Cedrela* and compare these to the ancestral niche reconstructions.

2. 4. Results

Phylogenetic analyses and divergence dating

Phylogenetic analyses recovered two major subclades within *Cedrela*. Clade I comprised species from Central America, clade II comprised species from Central and South America. Our preliminary analyses recovered *C. fissilis* as monophyletic, but

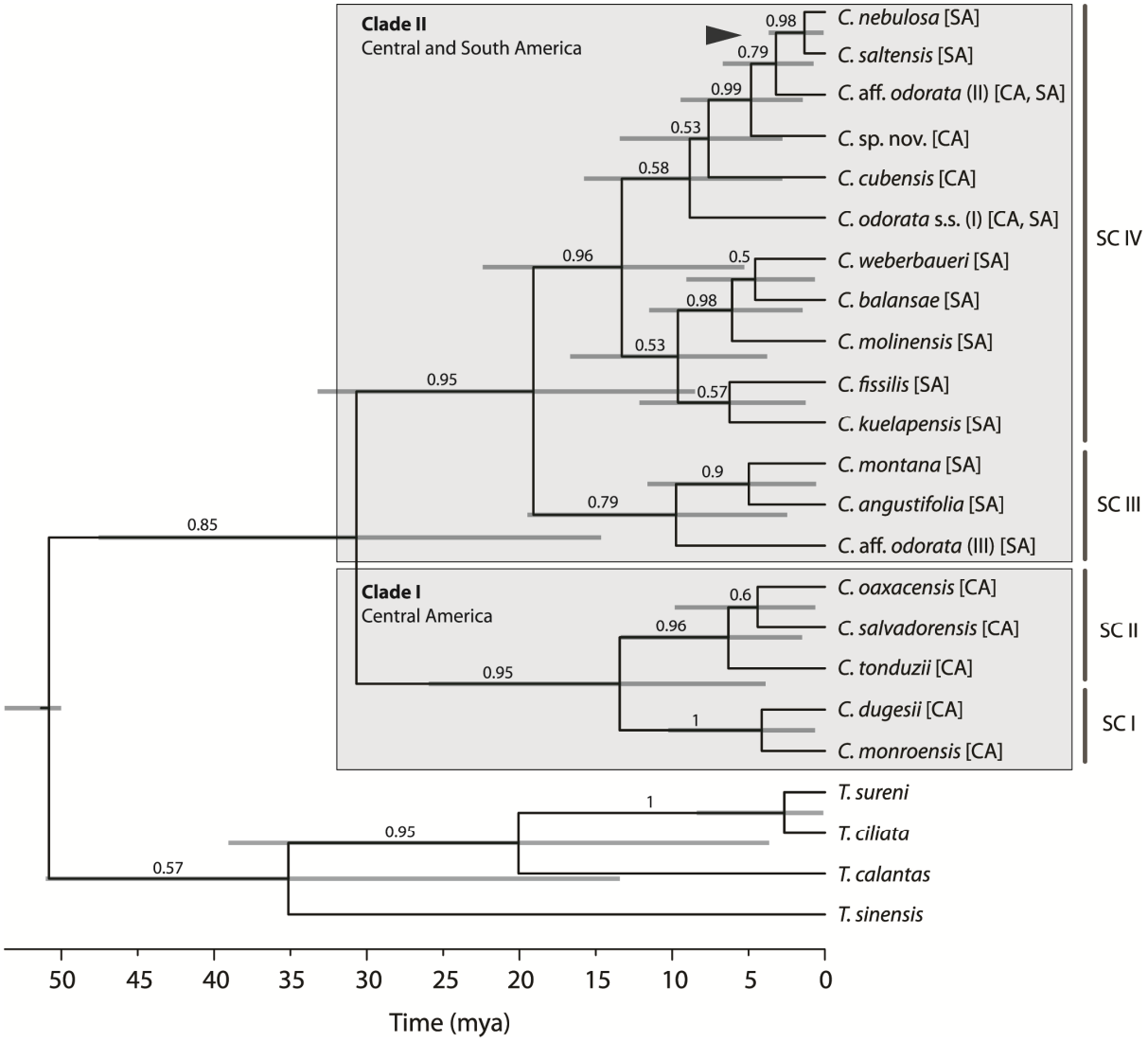
showed that *C. odorata* comprises at least three evolutionary lineages: *Cedrela odorata* groups I sensu stricto, II, and III (Figure 6). These genetic entities could be related to distinct geographic ranges in Central and South America (based on 236 collection localities of *C. odorata* sensu lato). Group I is mainly Central American, distributed from Mexico, Cuba and the West Indies across Central America to Nicaragua, but can also be found in Ecuador and Peru. The type of *Cedrela odorata* is from Jamaica. Group I therefore represents *C. odorata* in the strict sense (compare Muellner et al. 2010). Group II is similarly widespread from Costa Rica to French Guiana and along the Andes to Bolivia and Paraguay. We confirm that a third group (III) of the *Cedrela odorata* complex is endemic to Ecuador (Cavers et al., 2013). The three groups of *C. odorata* (I, II, III) represent distinct genetic entities, but can currently not be distinguished by morphological characters. All groups co-occur in Ecuador, but show slight habitat differentiation with groups I and II occurring in montane forests while group III appears to be restricted to western Ecuadorian lowland forests. One additional group that was also identified as *C. odorata* by Cavers et al. (2013) is morphologically and genetically identical to *C. cubensis* Bisse. Based on our phylogenetic analyses, we define the following operational taxonomic units (OTUs) of *Cedrela*, some of them being putatively new species that have not yet been formally described: 1. *C. dugesii*, 2. *C. monroensis*, 3. *C. tonduzii*, 4. *C. salvadorensis*, 5. *C. oaxacensis*, 6. *C.* aff. *odorata* (group III), 7. *C. angustifolia*, 8. *C. montana*, 9. *C. kuelapensis*, 10. *C. fissilis*, 11. *C. molinensis*, 12. *C. balansae*, 13. *C. weberbaueri*, 14. *C. odorata* (sensu stricto, group I), 15. *C. cubensis*, 16. *C.* spec. nov., 17. *C.* aff. *odorata* (group II), 18. *C. saltensis*, 19. *C. nebulosa*, 20. *C. longipetiolulata* and 21. *C. discolor*. Our sampling represents the currently most comprehensive sequence dataset that includes most of the taxonomic diversity within *Cedrela*.

The mean age estimate for the crown group of *Cedrela* is 31.7 mya (95% HPD: c. 46 - 13 mya; Figure 6). Divergence of subclades I and II was dated to 13.4 mya (95% HPD: 24.5 - 2.5 mya), divergence of subclades III and IV to 19.1 mya (95% HPD: 32 - 7 mya). Species of subclades I and II are of Late Miocene or Early Pliocene age. Divergence events within subclade III occurred in the Middle to Late Miocene. Diversification within subclade IV falls into the Miocene until the Early Pliocene. The youngest divergence event is that of *C. nebulosa*/*C. saltensis* at about 1.3 mya.

Climatic niche modeling and niche occupancy

The average test AUC (Area Under the receiver operating Curve) values of the models were above 0.9, except for the distribution model of *Cedrela fissilis* which obtained an AUC value of 0.85. The wide range of habitats inhabited by *Cedrela* (from very dry to everwet) was reflected in the variation of species' climatic tolerances. Temperature seasonality (the difference between the maximum temperature of the warmest month and the minimum temperature of the coldest month) was shown to be variable throughout *Cedrela*, ranging from very low (3.6 °C) to high (34.1 °C; Table B in Appendix). In general, temperature seasonality for species in clade II (Central and South America) was found to be more variable than in clade I, with *Cedrela saltensis*, *C. balansae* and *C. angustifolia* (all clade II) showing the highest variability. All species of *Cedrela* occur in frost-free areas, indicated by minimum winter temperatures at around 4 - 7 °C (e.g. *C. saltensis*, *C. weberbaueri*, and *C. kuelapensis*; Table B in Appendix). Most species experience precipitation seasonality with ample summer rain and less precipitation during the colder months, highlighted by the amount of precipitation during the coldest and the warmest quarter, respectively.

Figure 6. Maximum clade credibility tree based on nuclear and plastid DNA data under a relaxed molecular clock model with uncorrelated rates.



Node heights represent mean ages, and horizontal bars at the nodes indicate the 95% highest posterior density (HPD) intervals around the mean age estimates. Branch labels represent Bayesian posterior probabilities. The black arrow marks the re-entry into South America.

Numbers I to III in brackets indicate the different groups of *C . odorata* sensu lato I to III. Letters in square brackets indicate the distribution of each species (CA = Central America, SA = South America). Abbreviations: SC I to IV = subclades I to IV.

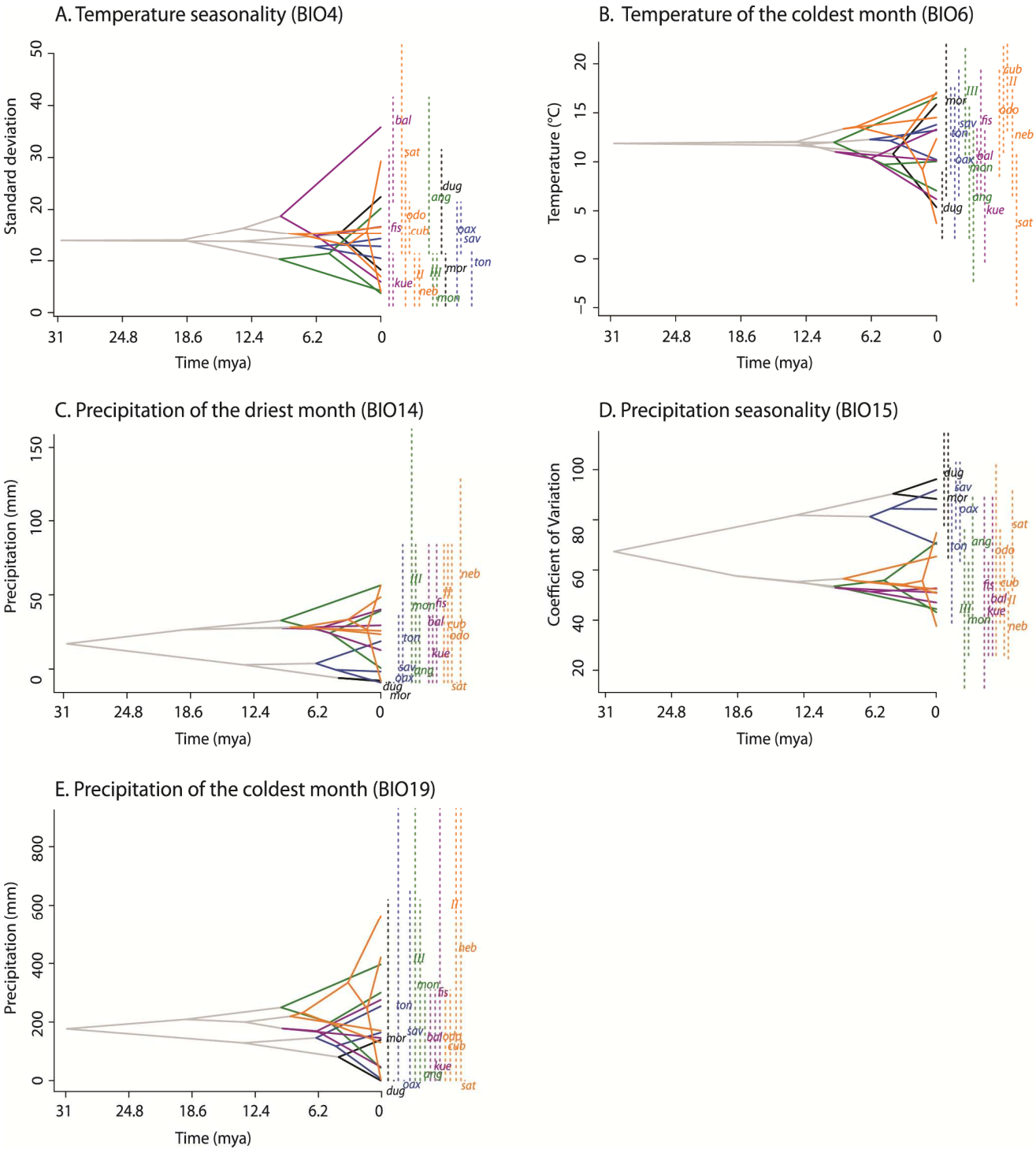
Ancestral niche reconstructions

Divergent evolution of annual temperature seasonality was apparent within all clades (Figure 7.A). Reconstructions of precipitation of the driest month and precipitation seasonality reveal an early divergence where clade I evolved towards generally drier conditions and higher seasonality whereas clade II evolved towards generally wetter conditions with less seasonality of rainfall (Figures 7.C-D). The sister species pairs *Cedrela saltensis*/*C. nebulosa*, *C. montana*/*C. angustifolia* and *C. dugesii*/*C. monroensis* all show high divergence in temperature seasonality with near complete separation of climatic tolerances (non-overlapping 80% density intervals; Figure 7.A). An increased disparification and adaptation to colder temperatures apparently occurred only within the last few million years (Figure 7.B). Again, the sister species pairs *C. dugesii*/*C. monroensis* and *C. saltensis*/*C. nebulosa* exhibit high divergence. For precipitation seasonality, only *Cedrela saltensis* and *C. nebulosa* show strong ecological divergence (non-overlapping 80% density intervals), while the other sister species pairs have congruent tolerances (Figure 7.D).

Accumulation of disparity

The morphological disparity index (MDI) was well above or close to 0 for all climatic variables (Figures 8.A-E). The positive MDI for temperature seasonality, precipitation of the driest month, and precipitation of the coldest quarter indicates that here overall disparity is distributed within rather than among subclades (Figure 8.A). Furthermore, for all variables except precipitation seasonality, mean subclade disparity was found to be extremely high at the most recent nodes, due to exceptional ecological divergence within all subclades that is likely to be associated with lineage divergence within the last 6 - 7 My.

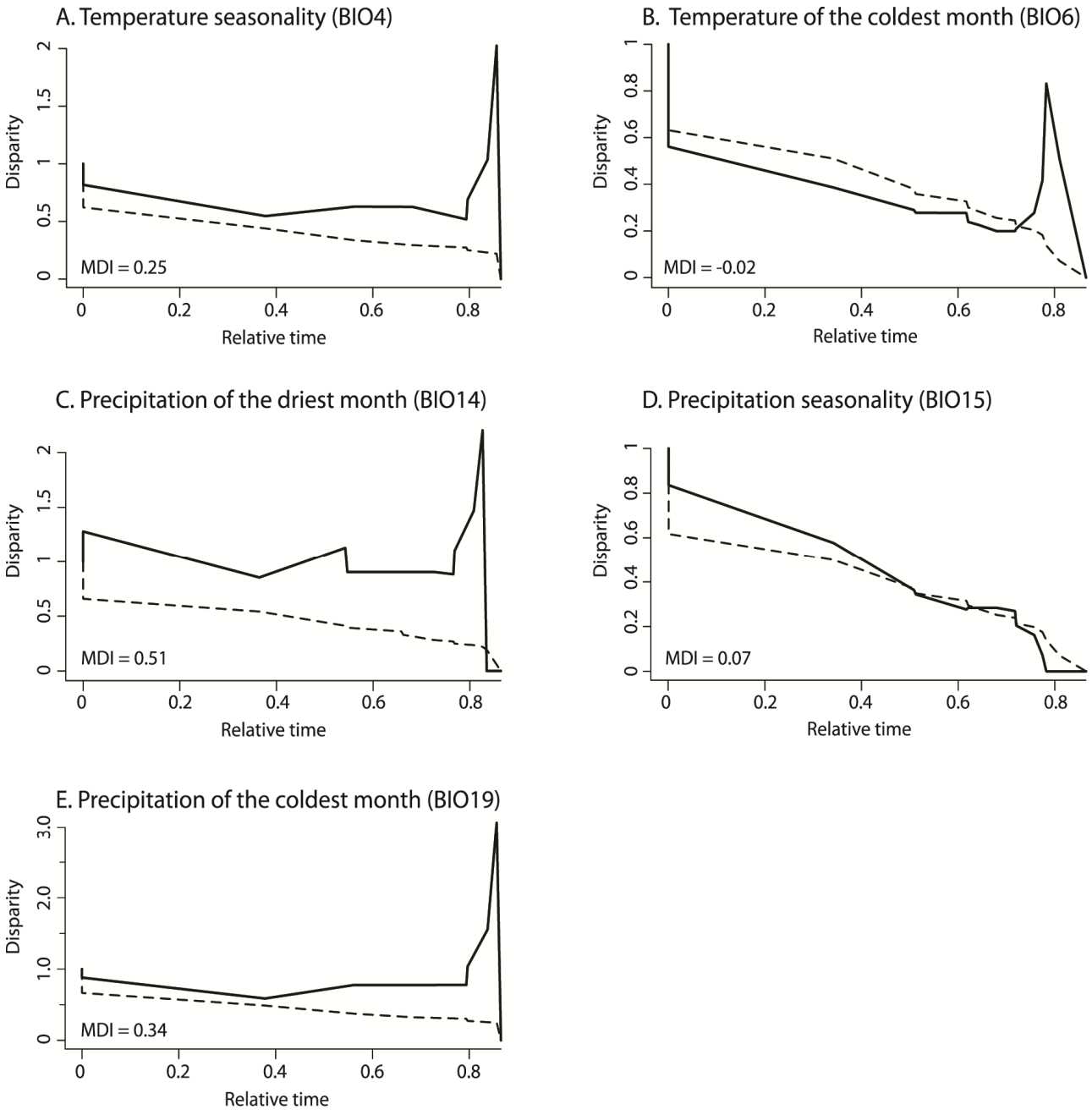
Figure 7. Ancestral niche reconstructions of *Cedrela*



The graphical representations of the ancestral niche reconstructions show the mean values and 80% central density intervals of the climatic tolerances of extant species (dashed lines) and the ML estimates for all internal nodes. →

→ continued legend **Figure 7.** The x-axis represents absolute time in millions of years, the y-axis represents the climatic tolerances: **A.** Temperature seasonality (BIO4), **B.** Minimum temperature of the coldest month in (BIO6), **C.** Precipitation of the driest month in (BIO14), **D.** Precipitation seasonality in (BIO15), **E.** Precipitation of the coldest quarter (BIO19). Subclade I = black, subclade II = blue (both clade I), subclade III = green, subclade IV = purple and orange (both clade II; purple = South America, orange = Central and South America). Abbreviations of species names of *Cedrela* are: *mor = monroensis* , *dug = dugesii* , *ton = tonduzii* , *sav = salvadorensis* , *oax = oaxacensis* , *ang = angustifolia* , *mon = montana* , III = aff. *odorata* (III), *kue = kuelapensis* , *fis = fi ssilis* , *bal = balansae* , *cub = cubensis* , *odo = odorata s.s.* (I), *sat = saltensis* , *neb = nebulosa,* II = aff. *odorata* (II).

Figure 8. Relative disparity through time plots for climatic tolerances in *Cedrela*.



The plots show the distribution of the relative disparity through time (black line), as well as the expected disparity under a Brownian motion model of evolution (dotted line). Morphological disparity indices (MDI) above 0 indicate niche evolution whereas MDI's below 0 indicate niche conservatism. **A.** Temperature seasonality (BIO4), **B.** minimum temperature of the coldest month in (BIO6), **C.** precipitation of the driest month in (BIO14), **D.** precipitation seasonality in (BIO15), **E.** precipitation of the coldest quarter (BIO19).

Fossil records

The oldest fossils of Cedreleae, which were found in northern Europe and northern America (Reid and Chandler, 1933; MacGinitie, 1941; Hickey and Hodges, 1975), date back to the Early Eocene. All fossil records assigned to *Cedrela* or *Toona* are listed in the Appendix (TABLES C-E). Fossil and paleoclimatic data suggest that species of extant Cedreleae descended from lineages that were living in tropical to subtropical conditions of higher northern latitudes. Climatic conditions of those high latitudes cannot be compared to any extant biome. It was a warm and humid environment without strong seasonality in terms of temperature, but long days in summer and lower intensity and duration of light in winter. Early Eocene fossils from the Chalk Bluffs Formation (52 - 49 mya, Appendix TABLE C: 2) were assigned to a wet montane subtropical forest type. Fossils from the Late Eocene until the Early Oligocene (Appendix TABLE C: 4 - 9) occurred under different climatic conditions marking a change towards colder and more seasonal habitats ("warm-temperate" or "temperate mixed mesophytic" forest). From the Early Miocene onwards habitats became increasingly diverse: habitats of western North American were mild and seasonal with either ample rain during the warm summer months and dry winters with lower temperatures, but no frost (Appendix TABLE C: 12, 15), or with dry summers and rain in winter (Appendix TABLE C: 13). The absence of fossils suggests that *Cedrela* went extinct in western North America by the Late Miocene, but its distributional range expanded towards southern Central America (Graham, 1991a, c, 2010). *Cedrela* appears in the fossil record of the Pliocene in South America (Pons and Franceschi, 2007). The fossil wood of *Cedrela* found in the Iquitos region in Peru has growth rings indicating seasonality, but we lack any further climatic details for Pliocene climatic conditions from this specific site. European lineages went extinct sometime after the Late Miocene (Appendix, TABLE D). Evidence for an early occurrence of Cedreleae in Asia (Appendix, TABLE E) is provided by two Japanese fossils, one from the Kushiro Coal Field (Late Eocene) and the other one from the Yoshioka Formation (Early Miocene). Altogether, the fossil findings reveal a formerly wide distribution of Cedreleae throughout the northern hemisphere from the Eocene until the Miocene.

2. 5. Discussion

Phylogeny of Cedrela

Our initial hypothesis that South American species should be nested within Central American species was not supported. The tree revealed two main clades of *Cedrela*, one from Central America (clade I) and another one comprising species from Central and South America (clade II). In accordance with the northern American origin and the gradual move into Central America in the Early Miocene (as described above), the extant South American species are most likely descendants from Central American taxa. Lineages of clade II may have colonized South America for the first time during the Early to Middle Miocene, but extant South American species are paraphyletic with both subclade III (*Cedrela montana, C. angustifolia and C.* aff. *odorata* (III)) as well as *C. nebulosa* and *C. saltensis* being endemic to the Andes, indicating that South America has been colonized at least two times independently (Figure 6).

Ancestral niche reconstructions and comparisons with the fossil record

Niche reconstructions reveal that the basal lineages of *Cedrela* diverged into different rainfall regimes (Figures 7.C and D), indicating a major niche shift at an early evolutionary stage. The shift occurred shortly after the Eocene-Oligocene boundary (C. 31 mya), a period of increasing seasonality, colder winters and decreasing precipitation (Wolfe, 1994; Sheldon and Retallack, 2004; Eldrett et al., 2009). This is corroborated by Late Eocene to Early Oligocene fossil sites (Appendix TABLE C: 1 - 9), which suggest a biome shift at the Eocene-Oligocene boundary from wet forest to seasonally dry forest. However, the reconstructed initial conservatism of temperature tolerances of the extant species is not reflected in the fossil record. Fossils from the Early Oligocene John Day Formation show that species experienced lower temperatures (warm-temperate habitats, Appendix TABLE C: 6 - 9) compared to the plants at Eocene fossils sites (subtropical habitats, Appendix S1(A): 1 - 5), followed by again increasing mean annual temperatures during the Miocene. The continued decrease of temperatures with increasing probabilities of frost in North America, but

also in Europe and Japan (Wolfe, 1994; Zanazzi et al., 2007; Doria et al., 2011), may have led to stepwise extinction in these regions, suggesting niche conservatism (i.e. the failure to adapt to lower temperatures) as a potential cause. Besides northern hemisphere extinctions, the fossil record indicates niche tracking into more southern areas. *Cedrela* species were present in La Quinta (southeastern Mexico, Appendix TABLE C: 10) and Gatún (Panamanian isthmus, Appendix TABLE C: 16) by the Miocene. These Central American fossils occurred in subtropical habitats of wet and seasonal conditions, respectively.

Our niche reconstructions reveal that climatic tolerances of species in clade II seem to be less conserved than in clade I. The increase in climatic disparity within clade II follows the major Andean uplift and the Miocene cooling 10 - 7 mya (Hoorn et al., 2010). Fossil evidence shows that *Cedrela* was present in South America in the Pliocene (fossil from the Iquitos region in Peru, Pons and Franceschi, 2007), but may have already colonized South America during the Middle Miocene as indicated by our molecular dating analysis. The initial Andean uplift (23 - 10 mya; Hoorn et al., 2010) provided habitats comparable to those north of the Panamanian isthmus. Furthermore, the Panamanian isthmus may have already closed much earlier (Early Miocene; Farris et al., 2011; Montes et al., 2012) than previously suggested (Late Pliocene to early Pleistocene; Bartoli et al., 2005), providing opportunities for the wind-dispersed *Cedrela* to disperse into South America.

Sister species comparisons

The youngest sister species pair, *Cedrela nebulosa* and *C. saltensis*, showed high climatic disparity based on our niche models. *Cedrela saltensis* occurs in a wider altitudinal range (from 800 m a.s.l. in Argentina to 2600 m a.s.l. in the Peruvian Andes) than *C. nebulosa* (1100 - 2400 m a.s.l.). In addition, *C. nebulosa* receives moisture and constant temperatures throughout the year whereas the habitat of *C. saltensis* is strongly seasonal. These results suggest that divergence along precipitation and temperature gradients may have driven speciation in this case. Similarly, sister lineages of dendrobatic frogs in the Andes were found to be separated along temperature, elevational or seasonality axes (Graham et al., 2004). This speciation

scenario matches Janzen's (1967) theory of a greater climatic zonation on tropical mountain slopes leading to higher species richness in tropical regions indicating that climate is a more effective barrier in the tropics. This idea was elaborated by Kozak and Wiens (2007) who studied the geographic isolation and speciation in two clades of North American salamanders. They found, however, that allopatric sister taxa generally inhabit very similar climatic niches and found no evidence for parapatric speciation along climatic gradients indicating that climatic niche conservatism may play a major role in speciation.

The Central American sister species *Cedrela dugesii* (N Mexico) and *C. monroensis* (El Salvador) share similar climatic tolerances and differ mostly in temperature preferences. *Cedrela dugesii* occurs in central Mexican pine-oak communities over volcanic rock at 1800 – 2200 m a.s.l. extending into tropical deciduous forest in lower elevations (Pennington and Muellner, 2010). *Cedrela monroensis* is only known from El Salvador in tropical semi-deciduous forest between 800 m and 1600 m a.s.l. Apart from temperature, ecological differences among these species exist for phenology, plant community and soil preferences. The flowering season of *C. dugesii* starts in spring (at the beginning of the rainy season) whereas *C. monroensis* only flowers in autumn (at the end of the wet season), but little is known in general about pollinators and pollination mechanisms in *Cedrela*.

Our study shows how large-scale environmental dynamics such as changes in (paleo-) topography and climate may have affected the evolution of *Cedrela*. Furthermore, the complexity of climatic niche evolutionary dynamics highlights how niche conservatism and evolution act on different temporal scales and climatic parameters. Conservatism may explain extinction of species in northern latitudes, range shifts into Central and South America, and the more restricted distribution of extant species from clade I in Central America. At the same time, fossil data from western North America suggest a major biome shift from paratropical warm and humid conditions into seasonal temperate climates as reflected in the ancestral niche reconstructions. Finally, sister species comparisons reveal divergence of climatic tolerances which might be related to recent speciation events.

2. 6. Acknowledgements

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Chapter II

A continent-wide phylogeographical analysis of *Cedrela* (Meliaceae)

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Key words. *Cedrela*; genetic diversity; gene flow; haplotype; Neotropics; network analyses; niche evolution; phenology; statistical parsimony; vicariance

3. 0. Declaration of contribution/ Erklärung über die jeweiligen Autorenanteile

Erklärung über Anteile an Kapitel 2 (**Chapter II**) der Promotionsarbeit.
Promovierende, VK, **Anteile in Prozent und in Klammern ( ) Prozentanteile der KoautorInnen und weiterer KollegInnen**

(1) Entwicklung und Planung
Promovierende, VK: 40% (60% JS und AM-R)

(2) Durchführung der einzelnen Untersuchungen/ Experimente
Promovierende, VK: 100 % der phylogenetischen und Netzwerk-Analysen

(3) Generierung der Daten und Erstellung der Abbildungen
Promovierende, VK: 100% des Datensatzes plastidäre Marker rpS16 und rpL16; 62% des Datensatzes plastidärer Marker *psb*A-*trn*H (38% Sequenzen von Gertrud Schorr und Patrick Teege); 29% des Datensatzes nukleärer Marker ITS (71% durch GS, PT, Steven Cavers und Genbankaccessions von *C. fissilis*/Garcia et al. 2013); die klonierten Sequenzen der ITS-Region für einen Teil der phylogenetischen Analysen wurden durch Gertrud Schorr generiert und im Gesamtkontext für die phylogenetischen Analysen durch VK ausgewertet; 63% des Sequence Editing/Alignment (37% AM-R); 100% der Abbildungen; 100% der Tabellen; 100% Literaturrecherche.

(4) Analyse/ Interpretation der Daten
Promovierende, VK: 90% Analyse und Interpretation der phylogenetischen Resultate und Netzwerke (10% Fernando Fernandez Mendoza).

(5) Übergeordnete Einleitung/Ergebnisse/Diskussion
Promovierende, VK: 100% Entwurf von Einleitung, Material und Methoden, Ergebnisse und Diskussion, mit 100% Überprüfung und Optimierung (mind. 50%) durch JS, AM-R und TP.

#Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben
*Mehrfacheintragungen möglich

3. 1. Abstract

Aim

We analysed the continent-wide genetic variation of the well-known timber genus *Cedrela* P. Browne to test if the structure of genetic variation corroborates the geographical distribution of species, if gene flow is observable at intra- and/or interspecific levels and better understand the evolutionary history of the genus.

Location

Central and South America

Methods

We applied network analysis using statistical parsimony of unrooted phylogenetic trees of haplotypes from 68 sequences of ITS and 74 sequences of *psbA-trnH*, repectively. In addition, infrageneric relationships of the tribe Swietenioideae (based on plastid rpS16 and rpL16) were analysed.

Results

Distinct levels of genetic variability suggested heterogeneous evolutionary histories of *Cedrela* species. Both haplotype networks (ITS and *psbA-trnH*) revealed a clear geographical structure with lineages distributed mainly in South (clade I) or Central America (clade II). Two major plastid haplotypes are frequent in South America (H1 and H2) and were shared by several species, but also species-specific structures and polymorphisms were found.

Main Conclusions

Migration from Central to South America might have initially led to a bottleneck-effect and reduction of genetic diversity, followed by radiations into Andean regions, adjacent lowland areas and re-entry into Central America. Despite being a small genus, *Cedrela* harbours lineages with very different evolutionary histories: Ancient polymorphisms persisted through speciation within *C. montana* Turcz. and *C. angustifolia* Moc & Sessé ex DC., and perhaps polyploidy contributed to these

polymorphisms, too. Contrarily, species specific genetic structures suggest that vicariance played a role in diversification of species from Andean valleys and within the "*C. fissilis*-complex". Differentiation of Central American taxa in clade II seems to be correlated with adaptation to different climates and distinct phenologies.

3. 2. Introduction

The Neotropical flora, extending from central Mexico to southern Brazil including the Caribbean islands, harbours an estimated 37% of all plant species (Myers et al., 2000; Antonelli and Sanmartín, 2011; Mittermeier et al., 2011). Various biotic and abiotic factors have driven the historical assembly and evolution of neotropical diversity (Antonelli and Sanmartín, 2011; Fine et al., 2013), with geotectonic events playing a key role in increasing habitat heterogeneity and thus species diversity (Hoorn et al., 2010; Antonelli and Sanmartín, 2011; Hoorn et al., 2013). The Andean uplift in particular has affected climate, hydrology, sedimentology and topology throughout its range and the Amazon basin (Hoorn et al., 2010). Gentry (1982) noted that the distribution of species throughout the Neotropics could mostly be described as either 'Andean-centred' or 'Amazonian-centred', a pattern which was later explained by the effect of the Palaeo-Orinoco and Lake Pebas as dispersal barriers between these two diversity centres (Antonelli, 2008). The Andean orogeny generated additional ecological barriers between Central and South American lowland taxa (Antonelli et al., 2009), although long distance dispersals between the two continents have also occurred (Dick et al., 2007). The uplift of the Andes first peaked in the early Miocene (ca. 23 My ago) and intensified in the middle Miocene and early Pliocene (ca. 12 My ago and ca. 4.5 My ago, Hoorn et al., 2010). Gentry (1982) hypothesized that 'Andean-centred' taxa were largely derived from Laurasian genera which had entered South America from the north and diversified in the course of the Andean orogeny. Phylogenetic analyses and divergence dating of 'Andean-centred' groups, such as *Guatteria* (Annonaceae), and members of the Arecaceae, Rubiaceae and Fabaceae indicated colonization of South America from Central America during the Miocene and subsequent diversification in the Andean regions (e.g. Hughes and Eastwood,

2006; Erkens et al., 2007; Cuenca, Asmussen-Lange, and Borchsenius, 2008; Antonelli et al., 2009; Koecke et al., 2013). Genetic diversity of 'Amazon centred' taxa was found to be generally lower and less geographically structured than of 'Andean centred' groups (Cavers, Navarro, and Lowe, 2003a, b; Dick, Abdul-Salim, and Bermingham, 2003; Novick et al., 2003; Dick et al., 2007; Dick and Heuertz, 2008; Fine et al., 2013). Although gene flow was found to be extensive in lowland Neotropical forests, genetic differentiation was still higher than in temperate forests, which was explained by the association of lower population densities, density dependent animal pollination and mixed mating systems (Dick et al., 2008). Given the lack of topographic variability in the Amazon, Fine et al. (2013) proposed that soil properties and spatial arrangements of habitats could also be major factors of genetic differentiation. The degree of gene flow in 'Andean centred' trees is less understood, but the higher degree of endemism in the Andes suggests that topographic barriers might exert a much stronger constraint on gene flow. In fact, Särkinen et al. (2012) found that Andean seasonally dry tropical forests (SDTF's) probably represent some of the most isolated and evolutionary persistent plant communities.

Cedrela (Meliaceae) is an 'Andean-centred' genus of North American origin, with highest species diversity in northwestern South America (Muellner et al., 2010; Pennington and Muellner, 2010). The latest systematic treatment of *Cedrela* comprised 17 species (Pennington and Muellner, 2010), but based on molecular phylogenetic results (Koecke et al., 2013), 21 operational taxonomic units (OTU's) of *Cedrela* were provisionally defined including one hitherto unrecognized species (*C. cubensis* Bisse) and one new species from Panama and Costa Rica (Koecke et al., in prep.). Because of the economic value of its wood and conservational issues, phylogeographical- and population genetic studies of the widespread species *C. odorata* L. and *C. fissilis* Vell. have been carried out, revealing surprisingly high intraspecific genetic variability with ecological differentiation (Gillies et al., 1997; Cavers, Navarro, and Lowe, 2003a, b; Garcia et al., 2011). However, genetic variability was later attributed to the paraphyly of *C. odorata* (Muellner, Pennington, and Chase, 2009; Cavers et al., 2013). Within *C. fissilis*, intraindividual polymorphism and incomplete concerted evolution of the internal transcribed spacer region (ITS) was detected, which could have arisen through interspecific hybridization, triggered by cross-pollination and long distance gene-flow through the wind-dispersed seeds (Garcia et al., 2011). Incomplete reproductive

barriers, sometimes between morphologically well-defined species, are a common phenomenon in plants, and might well lead to introgression or speciation (Rieseberg, Wood, and Baack, 2006; Rieseberg and Willis, 2007). Yet, interspecific hybridization has not been detected in *Cedrela*, and genetic differentiation or connectivity at inter- and intraspecific levels remains unclear. The geographic distribution of *Cedrela* from Mexico throughout Central America and South America in lowland and montane forest habitats provides an ideal case for a continent-wide phylogeographical analyses to evaluate the phylogeographic structure and speciation processes: If gene flow was restricted in the Andes since its orogeny in the Miocene through climatic zonation and topographic barriers, one would expect to find genetic specificity of taxa and clear geographic structures of genetic diversity. However, the production of wind-dispersed seeds may also promote long distance dispersal and gene flow. Dichogamy of *Cedrela* and unspecific animal-mediated pollination facilitate outcrossing. Gene flow at intraspecific levels enhances genetic variability, although interspecific hybridization might also obscure genetic delimitations among species. Here, we present a continent-wide phylogeographic analysis based on nuclear (ITS) and plastid (*psbA-trnH*) markers to evaluate the correlation between genetic and species diversity, and its implications for our understanding of mechanisms that trigger genetic diversity of 'Andean-centred' Neotropical trees.

3. 3. Material and Methods

Taxonomical Concept

We apply the concept of the operational taxonomic units (OTU's) as described in Koecke et al (2013). All specimens are listed in Appendix S3-4 (TABLES A and B), additional accenssions used for phylogenetic analyses are provided in Appendix S1-2 (legends of Figs. 1 and 2).

Isolation of DNA, amplification and sequencing

We used the DNeasy Plant Mini Kit (Quiagen, Hilden, Germany) to extract total genomic DNA from dried plant material following the manufacturer's protocol. The *psbA-trnH* spacer region is highly variable compared to other plastidial markers and has been widely used to study closely related taxa (Shaw et al., 2005). We therefore amplified this region for phylogenetic and network analyses using the primer pair psbA3′f/ trnHf (Sang, Crawford, and Stuessy, 1997). A 25 µl reaction mix for *psbA-trnH* amplification contained 0.5 - 2 µl DNA-isolate (20 -30 ng/µl), 21.5 - 23 µl ReddyMix PCR Master Mix (2.5 mmol MgCl2; Advanced Bioenzymes, Surrey, UK), 0.25 µl of each primer, 1 µl of DMSO, and 0.5 µl of BSA. The internal transcribed spacer (ITS) region was amplified using the primer pair AB101/AB102 (Douzery et al., 1999). A 25 µl PCR reaction mix for ITS amplification contained 0.5 - 2 µl DNA-isolate (20 -30 ng/µl), 20.5 - 22 µl ReddyMix PCR Master Mix (2.5 mmol MgCl2; Advanced Bioenzymes, Surrey, UK), 0.5 µl of each primer, 1 µl of dimethyl sulfoxide (DMSO), and 0.5 µl of bovine serum albumin (BSA 0.4%) as described in Muellner et al. (2003). PCR products were purified using the Quiaquick gel extraction kit (Quiagen) or directly purified using the Nucleospin Extract kit (Machery-Nagel, Düren, Germany). Cycling conditions for both markers were carried out following the protocol of Muellner et al. (2003). Purified PCR products were directly sequenced with the same primers used for PCR amplification and the BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Germany) and analysed on an ABI 3730 DNA analyser. We additionally amplified the rpS16 and rpL16 region of Cedreleae and their closest relatives, *Capuronianthus* J.-F. Leroy, *Carapa* Aubl, *Entandrophragma* C. DC., *Khaya* A. Juss., *Neobeguea* J.-F. Leroy, *Pseudocedrela* Harms, *Schmardaea* H. Karst., *Swietenia* Jacq., *Xylocarpus* Koen. and two accessions from *Lovoa* Harms. For PCR reactions we used the primer pairs rpS16F/rpS16R (Shaw et al., 2005) and rpL16F71/rpL16R1516 (Small et al., 1998). Amplification of *rpL16* was carried out following (Shaw et al., 2005) and amplification of *rpS16* was carried out using the following thermal cycle parameters: *rpL16* - 80°C, 5 min; 35x (94°C, 30 sec; 47°C, 30 sec; 72°C, 1 min), 72°C, 5 min.

Assembly of datasets

Forward and reverse sequences were assembled and edited in Geneious 5.1.7 (Biomatters Ltd., Auckland, New Zealand). An alignment was built using the Muscle algorithm with default settings as implemented in Geneious and subsequently edited mannually. Poly A-/T regions were excluded to avoid ambiguous sites in the alignment. For the network analyses, indels were coded as binary characters in either datasets by hand according to the "separate presence/absence characters" method (Simons & Ochoterena, 2000). Due to double peaks in some of the ITS electrophoretograms and the potential occurrences of pseudogenes within the alignment we performed cloning for 9 specimens. Amplified fragments of ITS were cloned using the pGEM-T Easy Vector System (Promega) following the manufacturer's protocol. Successfully transformed colonies (*Escherichia coli* JM-109) were selected for PCR by inoculating PCRs with transformed cells as template. PCR products were cleaned and sequenced as described above.

For the network analyses we excluded the cloned sequences from the ITS alignment, except for one sequence per OTU. The ITS dataset for the network analyses consisted of 68 specimens of *Cedrela* collected throughout its distributional range (Appendix S4, TABLE B). Alignment length was 621 bp of which 80 were variable and 59 potentially parsimony informative. The *psbA-trnH* dataset for the network analyses consisted of 74 individuals of *Cedrela* (Appendix S4, TABLE B). Length of the alignment was 413 bp of which 41 were variable and 27 potentially parsimony informative. The concatenated dataset of *rpL16* and *rpS16*, which was used for infrafamiliar relationships had a total length of 1677 sites of which 113 were variable and 49 potentially parsimony informative.

Phylogeny

Phylogenetic analyses were run using RAxML 7.4.2 with raxmlGUI, a graphical user interface (Stamatakis, 2006; Silvestro and Michalak, 2012) and MrBayes 3.2 (Ronquist et al., 2012). The best-fit model of nucleotide substitution was selected according to the Akaike Information Criterion (AIC) using jModeltest 0.1.1 (Posada,

2008). The general time-reversible model with rate variation (GTR+G) was chosen for the Bayesian and Maximum Likelihood analyses of both ITS and the plastid markers.

Statistical Parsimony

Processes of reticulate evolution, such as hybridization or horizontal gene transfer leading to homoplasy, often remain undetectable on phylogenetic trees (Posada and Crandall, 2001). The reason for this is the assumption that speciation proceeds in dichotomous splitting of lineages. Network analysis can help to understand relationships of a group from another perspective, in particular if genes undergo processes of recombination. Thus, a combination of both phylogenetic and network analyses may best contribute to understanding the evolutionary history of *Cedrela*. Unrooted phylogenetic relationships of haplotypes based on ITS and *psbA-trnH* were reconstructed using statistical parsimony as implemented in the programm TCS (Clement, Posada, and Crandall, 2000). The connection limit of species was set to 95% which corresponds to the maximum number of single substitutions between haplotypes, gaps were treated as missing data. Sequences differing only by missing or ambiguous characters were automatically clustered together.

Geographic regions

Six geographic regions were defined based on the distribution of *Cedrela* 1. Mexico 2. Central America 3. Caribbean Islands 4. Northern South America (Columbia, Ecuador) 5. Central South America (Peru, Brazil, French Guiana) and 6. Southern South America (Bolivia, Argentina, Paraguay).

3. 4. Results

Cloning of ITS

Cloning results showed intrainidivual polymorphism of ITS within *Cedrela cubensis*, *C.* aff. *odorata* (group II), *C. saltensis* Zapater & del Castillo and *C. salvadorensis* Standl., as previously found in C. fissilis (Garcia et al., 2011). Phylogenetic trees were built comprising sequences from all species of *Cedrela*, including the cloned sequences from Garcia et. al (2011). We did not find divergent intragenomic copies of ITS (Appendix S1). All cloned sequences clustered in lineage specific groups, except for one clone of *C. saltensis* (36029_l), which was recovered as sister lineage to the clade comprising the remaining ITS copies of this individual. Our results therefore indicate an advanced stage of concerted evolution within *Cedrela* which lowers potentially misleading signals from homoplasy (Feliner and Rosselló, 2007).

Phylogenetic relationships

Phylogenetic results based on ITS only (Appendix S1) indicated that the South American *C. fissilis* sensu lato comprises three well-supported subclades, thus corroborating previous findings from Garcia et al (2011), who found sublevels of genetic variability in this group. *Cedrela balansae* is nested within one of these clades together with *C. fissilis* from the Atlantic forest of Brazil. The '*angustifolia-montana*' complex surprisingly contains one specimen from Nicaragua, previously determined as *C. tonduzii*.

Analyses of intergeneric relationships from members of Swietenioideae based on plastidial markers (rpS16 and rpL16) corroborated previous studies from Muellner et al. (2003) showing a close relationship of the genera *Capuronianthus* and *Lovoa*, being together with *Entandrophragma* more closely related to Cedreleae (*Toona* and *Cedrela*) than the remaining genera of Swietenioideae (Appendix S2). The plastidial markers, rpS16 and rpL were not useful to resolve phylogenetic relationships within *Cedrela*.

psbA-trnH network analyses

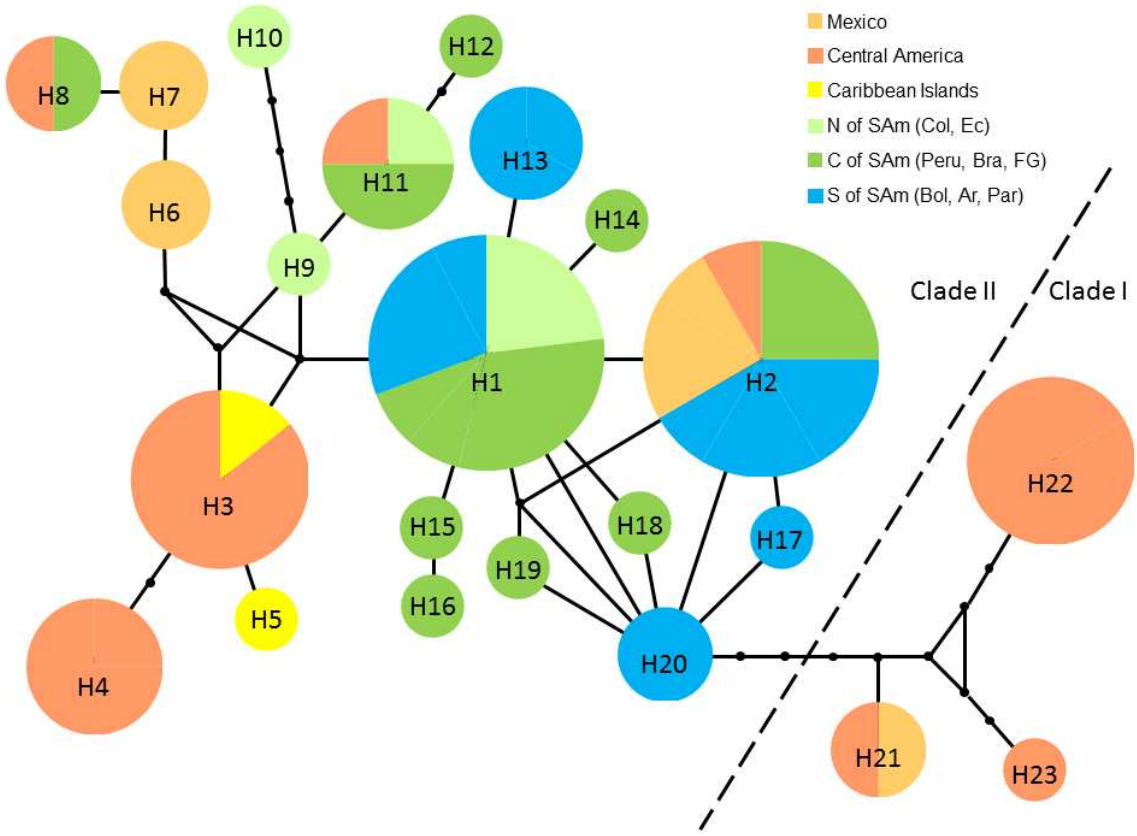
Network analyses based on *psbA-trnH* sequences from 74 individuals revealed 23 haplotypes including 11 singletons (Fig. 1). The network includes loops in five positions that could not be resolved unambiguously. Clades I (Central American species) and II (Central and South American species) were clearly separated. Haplotypes from clade I (H21-23) were more distinct among each other when compared to the haplotype diversity within clade II. We found only one true lineage specific haplotype (H22) comprising several accessions of *C. tonduzii* C. DC. The other haplotypes were singletons or were shared by different species. Haplotypes 1 and 2 (H1-2) at the centre of the network were most frequent and included most of the species. Haplotype 1 is distributed throughout South America in several species of *Cedrela* (see TABLE A in the Appendix S3). Haplotype 2 is geographically even more widespread. It was mainly found in South America (from Paraguay to Peru) but was also shared by a specimen of *C. odorata* s.s. from Belize three different species from Mexico (Fig. 1 and TABLE A). The widespread *C. fissilis* was found in both H1 (specimens from Brazil and Peru) and H2 (specimens from Bolivia), but also within the closely related haplotypes H17, H18, and H20. *Cedrela balansae* from Brazil had H19 whereas taxa from Argentina and Paraguay were integrated in H2. Haplotype 13 consisted of undetermined lowland specimens that were collected between the Andean Cordillera Oriental and the Yungas in tropical semideciduous forest and one specimen from Argentina. Two specimens of *C. angustifolia* with H1 were from La Paz in Bolivia. Haplotype 14 belongs to one specimen of *C. angustifolia* that was found close to Cuzco (Ollantaytambo, at 2700m) in Peru. The remaining specimens of *C. angustifolia* from central and northern Peru clustered together with *C. montana* from Ecuador and northern Peru and one specimen from Nicaragua. The new endemic taxon from Ecuador had a distinct haplotype (H10, for first mentioning of this taxon please see Cavers et al., 2013) which was most closely related to one specimen of *C. montana* from Ecuador (H9). Haplotypes 6-8 consisted of *Cedrela odorata* s.s. of which H6-7 occurred in Mexico whereas H8 belonged to specimens from Peru and El Salvador indicating a re-entry into Central and South America from the North. Haplotypes 3-5 include several Central American species from clade II (TABLE A). Specimens of *C.* aff. *odorata* (group II) possessed H3 and H4, although differing in

distributional ranges and phenology, which contributed to genetic differentiation within this group.

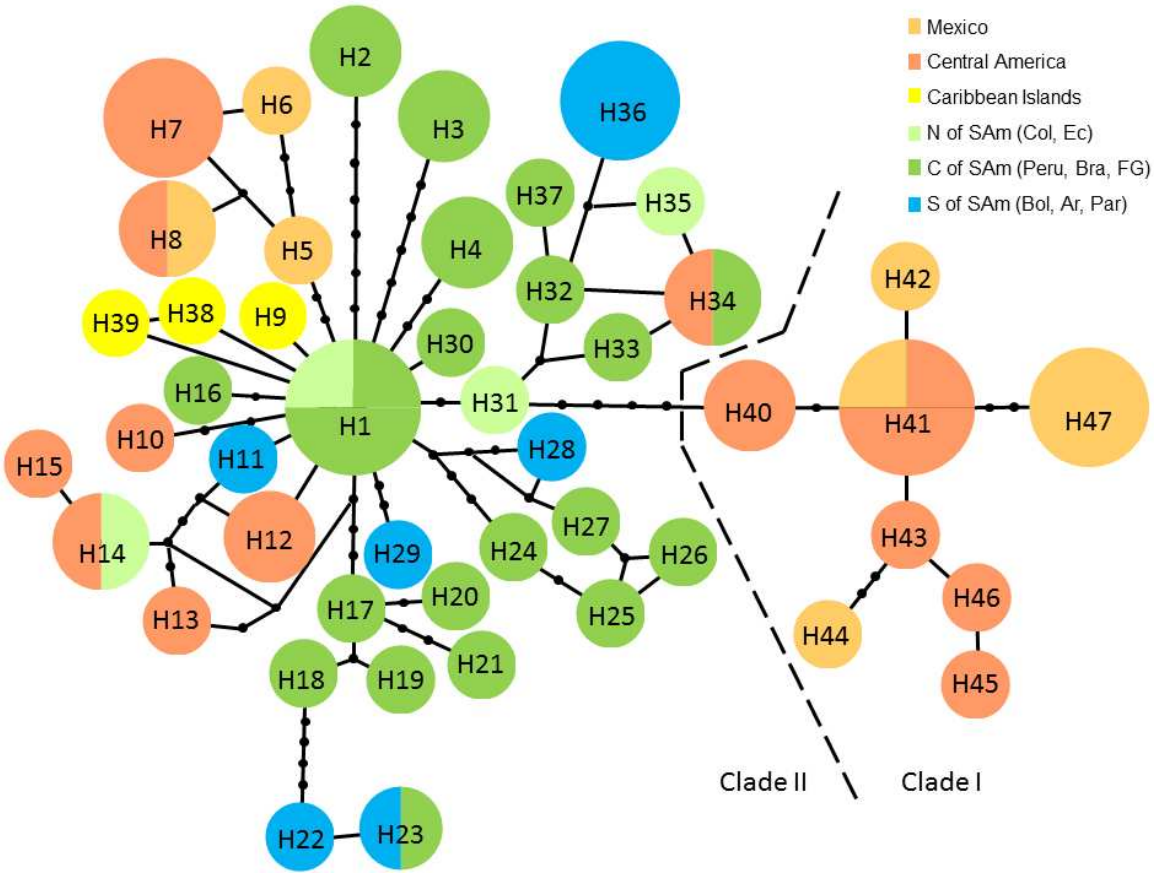
ITS network analyses

Network analyses based on ITS sequences from 68 individuals recovered 47 haplotypes, 33 of which were singletons (see Fig. 2 and TABLE B in the Appendix S4). Eight positions in the TCS network revealed loops that could not unambiguously be resolved. As in the plastid network, clades I and II were well separated, although the network structures are to some extent incongruent. Within clade I, haplotype variability was apparent in *C. tonduzii* (H41-43) and *C. salvadorensis* (H44-46) whereas *C. monroensis* T.D. Penn. had a lineage specific haplotype and *C. dugesii* S. Wats. and *C. oaxacensis* C. DC. (H47) grouped together. The haplotype structure within clade II was starlike with a central haplotype (H1) occurring in Ecuador and Brazil. We found lineage-specific haplotypes for *C.* spec. nov. from Panama and Costa Rica (Koecke et al. in prep.), *C. kuelapensis* T.D. Penn., *C. molinensis* T.D. Penn., and *C. weberbaueri* Harms (H2-4). All of these have restricted distributional ranges, the latter three are Peruvian endemics. The group of H5-8 represents *C. odorata* s.s. including *C. discolor* S.F. Blake. *Cedrela* aff. *odorata* (group II) had variable ITS haplotypes, H10 and H13-15 which were closely related to *C. saltensis* (H11 and H16) and the lineage specific H12 of *C.* sp. nov (Koecke et al. in prep.). Haplotypes 17-29 belonged to the broad *C. fissilis* complex, but H17-21 could be assigned to specimens from the western interior of Brazil, while H24-28 represents a group of specimens from the Atlantic range (Garcia et al. 2011). Haplotypes 22-23 were found in *C. balansae* from Argentina and Paraguay and in one specimen from the Brazilian Chiquitano dry forest from Garcia et al. (2011), probably also representing *C. balansae*. Haplotypes 31-36 were found in the endemic species from Ecuador (H31) and specimens of *C. angustifolia* and *C. montana* (H32-36). Interspecific sequence variability of the ITS region is rather low when compared to the variability present within species.

Figure 9. Haplotype networks



A. Haplotype network from *psbA-trnH* sequences of 74 individuals of *Cedrela*



B. Haplotype network from ITS sequences of 68 individuals of *Cedrela*

Comparison of haplotype structures based on psbA-trnH and ITS

Both haplotype networks revealed a clear geographical structure with lineages distributed mainly in South (clade I) or Central America (clade II) with some congruent single haplotypes. Not only similarities, but also differences were found: Within the ITS network the haplotypes of *C. tonduzii*, *C. oaxacensis* and *C. salvadorensis* clearly fell all together into the Central American clade I, whereas part of the same individuals harboured the otherwise South American haplotype H2 in the *psbA-trnH* network (but see TABLES A and B in the Appendix S3-4). A similar pattern was found among specimens of *C. angustifolia* and *C. montana* grouping together in the ITS network (TABLE B), but some having H1 within the plastidial network (TABLE A). The endemic species of Ecuador had a derived haplotype in the plastidial network (H10, Fig. 1) whereas it was found in a more central position within the ITS network (H31, Fig. 2). Many other species that were included in the centre (H1 and H2) of the

plastidial network (Fig. 1) had derived lineage specific haplotypes within the ITS network (Figure 9.B, such as *C. "fissilis"* (H29), *C. kuelapensis* (H2), *C. molinensis* (H3) and *C. balansae* (H22-23)). Our studies showed genetic variability within Bolivian taxa, which were perhaps wrongly determined as *C. fissilis*. Two of them, found in the Andes of the La Paz department between 1300 and 1500 m possessed H2 in the *psbA-trnH* network. They were unfortunately missing from our ITS dataset (due to repeated failure of amplification), but in the ITS network another specimen from an adjacent area which occurred at 2000m had a distinct haplotype (H29, Fig. 2) that was not associated with any of the other two '*fissilis*' clusters.

3. 5. Discussion

According to the coalescent theory most frequent haplotypes have a greater number of mutational connections, which also raises the probability of one allele being the oldest (Crandall and Templeton, 1993; Posada and Crandall, 2001). Hence, ancestral haplotypes often have central positions in haplotype networks and might thus indicate where groups of taxa originated. Within *Cedrela*, central and widespread haplotypes of *psbA-trnH* (H1 and H2) were most frequent in South America, however, extant species clearly descended from Northern/Central American lineages as evident from numerous fossil findings (Koecke et al., 2013). Within the *psbA-trnH* network (Fig. 1) H1 was shared by a total of nine South American species and H2 by six out of 21 OTU's of *Cedrela*. Most of the species sharing H1 and 2 (*C. odorata* s.s., *C.* aff. *odorata* (group II), *C. kuelapensis*, *C. molinensis*, *C. weberbaueri*, the *C. fissilis*-complex and *C. balansae*) exhibited distinct species-specific haplotypes within the starlike structure of the ITS network (Fig. 2) suggesting a radiation from a common South American ancestor. *Cedrela kuelapensis*, *C. molinensis* and *C. weberbaueri* are endemics of seasonally dry forests of distinct Andean valleys and presumably constitute ancient lineages that have been isolated from their closest relatives since the final Andean uplift. Our results thus corroborate the theory of older species compositions in interandean valleys (Pennington et al., 2010; Särkinen et al., 2012).

As evident from both the *psbA-trnH*- (H3-8, H11) and ITS network (H5-8, H10, H12-15, H34) Central America was re-colonized from the South. The higher number of mutational steps between the *psbA-trnH* haplotypes in clade I indicate the loss of haplotypes in northern latitudes and therefore stands in contrast to diversification in clade II. This scenario is corroborated by the historical biogeography and niche evolution of the genus, which suggested major climatic changes since the Eocene having led to step-wise extinction of ancient lineages of *Cedrela* in Northern and Central America and range shifts into the South (Muellner et al., 2010; Koecke et al., 2013). Among the species possessing H2, some Central American species were present which were otherwise found in clade I (*C. salvadorensis*, *C. tonduzii* and *C. oaxacensis*). This pattern can either be explained by ancestral chloroplast polymorphism (Jakob and Blattner, 2006; Modliszewski et al., 2006) or alternatively, by introgression through hybridization (Palme et al., 2004; Seehausen, 2004), in this case from clade I to clade II. If this pattern was indeed caused by hybridization, it would mean that geographically distant species were able to interbreed, suggesting incomplete reproductive isolation between clade I and II. The alternative explanation would imply that South American H2 was inherited from an ancient polymorphic Central American ancestor. *Cedrela salvadorensis*, *C. tonduzii* and *C. oaxacensis* still harbour H2 in addition to their specific haplotypes (H21-23). Haplotypes 1 and 2 constitute the most frequent haplotypes among South American species. Accordingly, migration from Central to South America might have led to a severe bottleneck-effect. It might also hint at ongoing gene flow among species that share H1 and 2 which shows potentially different, but not mutually exclusive explanations for the higher frequency of these haplotypes.

In contrast to the chloroplast homogeneity of several species, haplotype diversity is high within the *C.* '*fissilis*-complex' (H17-20) in the *psbA-trnH* and the ITS network. Similarly, phylogenetic studies (Garcia et al., 2011) have shown differentiation of at least two distinct lineages within the *C.* '*fissilis*-complex', one from the Atlantic range to the East of Brazil (H24-28) and another one corresponding to the western interior Chiquitano forest (H17-H21). These groups are geographically separated by the fire-prone Cerrado savanna, which constitutes an effective barrier to non-fire adapted plants. The ITS haplotype structure suggests that *C. balansae* was derived from the

group of the eastern Atlantic range of Brazil, which would also explain why Garcia et al. (2011) found “sublevels of genetic structure” within the clade of *C. fissilis* comprising sequences from several taxa of the eastern Atlantic. In fact, we found that some of the individuals of *C.* “*fissilis*” from the southeastern Paraná-Paraíba forest are conspecific with *C. balansae* inasmuch as their ITS sequences were identical. This indicates that *C. balansae* is nested within the eastern Atlantic clade of *C. fissilis* and thus might not only occur in Bolivia, Argentina and Paraguay as described in Pennington and Muellner (2010), but also southeastern Brazil. The holotype specimen of *C. fissilis* was collected in the Brazilian state of Minas Gerais. We therefore assume that the group from the Atlantic range represents *C. fissilis* sensu stricto whereas the ‘western-interior-lineage’ potentially is a new species. The differentiation between the eastern and western clades is phylogenetically well-supported and ecologically justified. Our studies further suggest that Bolivian specimens from higher altitudes (1200-1500m; H29, ITS and H2,17, *psbAtrnH*) which were determined as *C. fissilis*, might belong to an additional, but genetically yet unknown group, which is closely related, but not identical to the *C.* ‘*fissilis*-complex’.

The closely related species *C. montana* and *C. angustifolia* occur in the northern and southern Andes, respectively, occupying distinct climatic niches (Pennington and Muellner, 2010; Koecke et al., 2013). Their geographical ranges overlap in southern Ecuador and northern Peru where several specimens show intermediate characters of both species. We therefore expected to find a taxon-specific or geographically structured pattern of haplotypes (north vs. south) and eventually signals of interspecific hybridization. However, *psbA-trnH* haplotypes did not reflect a taxon-specific structure and were surprisingly polymorphic, suggesting that ancestral polymorphisms might have persisted through speciation, perhaps due to much earlier range expansions which were followed by range contractions and isolation of populations in distinct Andean areas. Alternatively, this pattern could be the result of chloroplast capture through repeated hybridization. It might be interesting to analyse ploidy levels of *C. montana* and *C. angustifolia* and test if these may have arisen through hybridization as differences in chromosome numbers within *Cedrela* exist (Styles and Vosa, 1971). Although ITS haplotypes were also polymorphic (H32-36), a geographical structure within this network was more apparent as Bolivian specimens

of *C. angustifolia* clustered together (H36). This strongly indicates local limitation to pollen dispersal and genetic differentiation of southern taxa. In the *psbA-trnH* network, one haplotype H13 belonged to a group of specimens from Bolivia and Argentina, which were previously determined as *C. angustifolia*, but this entity might represent a distinct yet unknown taxon.

Within *C.* aff. *odorata* (group II) we found two different haplotypes (H3 and H4) of *psbA-trnH* that correlate with different ecologies. Haplotype 3 was shared by *C. cubensis*, *C.* spec. nov. (from Panama and Costa Rica, Koecke et al. in prep.) and *C.* aff. *odorata* (group II), whereas H4 belonged to just one specific group of *C.* aff. *odorata* (group II), the latter only distributed on the Caribbean side of Panama where rainfall is much higher than on the more seasonal Pacific side. We refer to this lineage as 'lineage A'. Specimens harbouring H4 are geographically not restricted to the wet Caribbean side; we refer to them as 'lineage B'. Notably, lineage A (H4) and B (H3) differ in phenology and *C.* spec. nov. exhibits another (third) phenology. Our observations were made during our field work in April 2009 at the start of the rainy season: *Cedrela* spec. nov. was fully in leaf and flowering, lineage A was at an early stage of fruiting, lineage B was completely leafless with open mature fruits. We thus assume that differences in phenology could be triggers of reproductive isolation due to the fact that these three entities have overlapping areas of distribution in western Panama being a relatively small but geographically heterogeneous area. Reproductive isolation might have led to morphological differentiation of *Cedrela* spec. nov. (Koecke et al. in prep.) and genetic/ecological differentiation of linages A and B of *C.* aff. odorata (group II). Reproductive isolation through distinct timing of flowering might be particularly effective in conjunction with ecological differentiation (Lowry et al. 2008, Martin and Willis 2006, Widmer et al. 2009). Nevertheless, it has been previously disputed whether differences in phenology are indeed strong barriers against gene flow as in some cases they remain permeable (Karrenberg, Edwards, and Kollmann, 2002). However, there might also be selection for incomplete reproductive isolation if hybridization serves to enhance genetic variability between otherwise diverging populations (Rieseberg, 1995; Rieseberg and Willis, 2007).

Our continent-wide phylogeographical analysis of *Cedrela* demonstrated distinct levels of genetic variability in different groups of the genus. This has implications for

understanding genetic diversity and evolutionary processes within *Cedrela*. Although *Cedrela* is a relatively old genus dating back to the Eocene, interspecific variability of the ITS region is rather low when compared to the variability present within species. Bottle-neck effects during the colonization of South America and at the same time, loss of haplotypes in clade II due to extinction, could have led to a reduction of genetic diversity. Following the colonization of South America genetic diversity increased again, which most likely as a result of radiations into Andean valleys, adjacent lowland areas and re-colonization of Central America (as evident in the ITS network, Fig. 1). This is coherent with increased climatic niche evolution (during the Miocene/Pliocene) among Central and South American species of *Cedrela* in clade II (Koecke et al., 2013). It points to an important notion of Wiens et al. (2010), who stated that niche evolution might be impeded by gene flow. Niche evolution seems to be coupled to an initial bottle-neck effect in *Cedrela* and reduced gene-flow at perhaps extra-limital conditions, such as interandean valleys, where vicariance might have played a major role in speciation. On the contrary, gene flow at interspecific levels can also not be completely rejected among several other lineages of clade II, simply due to the fact that many species share widespread haplotypes. Thus, interspecific reproductive isolation seems to be incomplete, particularly across some of the South American species and stands in contrast to stronger geographical structures of genetic diversity within species of interandean valleys and the geographically structured *C.* '*fissilis*-complex'.

4. 6. Acknowledgements

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Chapter III

Cedrela ngobe Köcke, T. D. Penn. & Muellner-Riehl ined., A new species of *Cedrela* (Meliaceae) from Panama and Costa Rica

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Keywords. *Cedrela*, Costa Rica, cuticle, IUCN conservation assessment, Meliaceae, morphology, niche model, Panama, species, stomata, taxonomy.

4. 0. Declaration of contribution/ Erklärung über die jeweiligen Autorenanteile

Erklärung über Anteile an Kapitel 3 (**Chapter III**) der Promotionsarbeit.
Promovierende, VK, **Anteile in Prozent und in Klammern ( ) Prozentanteile der KoautorInnen und weiterer KollegInnen**

(1) Entwicklung und Planung
Promovierende, VK: 50% (50% AM-R und TP)

(2) Durchführung der einzelnen Untersuchungen/ Experimente
Promovierende, VK: 100 % der taxonomischen Revision von Herbarbelegen, die Feldarbeit wurde von VK durchgeführt in Kooperation mit Orlando Cáceres (Universidad Autónoma de Chiriqui-Panamá), Daniel Cáceres, Prof. Meike Piepenbring und Hauke Wessels; 100% mikroskopische Analysen; 100% Nischenmodel und Einordnung gemäß IUCN-Richtlinien.

(3) Generierung der Daten und Erstellung der Abbildungen
Promovierende, VK: 100% der georeferenzierten Verbreitungsdaten; 90% der Artbeschreibung entsprechend den Vorgaben von TP (10% TP); Feldbeobachtungen 50% (50% OC); 100% Unterscheidung von anderen Arten unter *Notes*; 100% der Abbildungen außer der Pflanzenillustration (das Plate wurde zu 100% von Rosemary Wise aus Kew gezeichnet); 100% Literaturrecherche.

(4) Analyse/ Interpretation der Daten
Promovierende, VK: 70% Analyse und Interpretation der taxonomischen Resultate (30% TP), 100% der Analyse über Verbreitung und Gefährdung.

(5) Übergeordnete Einleitung/Ergebnisse/Diskussion
Promovierende, VK: 100% Entwurf von Einleitung, Ergebnissen und Diskussion, 100% Überprüfung und Optimierung durch AM-R und TP.

#Bei 2, 3 und 4 bitte kurze inhaltliche Angaben der jeweiligen Anteile, bei 1 und 5 reichen prozentuale Angaben
*Mehrfacheintragungen möglich

4. 1. Abstract

We present a new species of Meliaceae, *Cedrela ngobe* from Panama and Costa Rica. A detailed description with illustrations, a distribution map and an identification key are provided. Based on distribution data and climatic niche modeling, the species is classified as Vulnerable according to IUCN Red List categories.

4. 2. Introduction

Cedrela P.Browne is a genus of economically important hardwood timber trees in the Neotropics. It is distributed from Mexico throughout Central and South America to northern Argentina. The latest systematic treatment by Pennington and Muellner (2010) comprises 17 species. These occur in seasonally deciduous or evergreen rain forests. *Cedrela* is closely related to the genus *Toona* (Endl.) M.Roemer which occupies similar habitats throughout the Indo-Australian Archipelago and China. *Cedrela* and *Toona* together form the monophyletic group of Cedreleae within Meliaceae subfamily Swietenioideae (Muellner et al., 2003). Although morphologically very similar, *Cedrela* and *Toona* differ in the structure of the androgynophore, the seed wing, and the point of attachment of the seed on the central columella of the fruit (Pennington and Muellner, 2010). Species distinctions in *Cedrela* are based on a combination of six morphological characters, viz.: number of leaflet pairs, amount and type of leaf indumentum, leaflet size, shape and venation, degree of union of sepals, adnation of the petal margins, and size of the capsule (Pennington and Muellner, 2010). We here describe a new species of *Cedrela* from Costa Rica and Panama which is distinct in most of these morphological traits. Molecular analyses also confirm that *C. ngobe* represents a new species which is distinct from other species of *Cedrela*, with a close relationship to the Andean *C. nebulosa* T.D.Penn. & A.Daza, *C. saltensis* Zapater & del Castillo and another genetic entity here designated *C. aff. odorata* L. from Central and northern South America (Koecke et al. 2013). *Cedrela* aff. *odorata* and *C. ngobe* are sympatrically distributed along the seasonal Pacific side of Central Panama to southern Costa Rica. In order to

test for suitable climatic niches and for better estimation of an IUCN criterion, we performed climatic niche modelling of the new species, *C. ngobe*.

Cuticular analyses are sometimes useful for identifying genus or species specific traits in addition to morphological characters. Stomatal architecture and type of indumentum can be taxon specific. Moreover, epidermal and cuticular traits may be preserved in fossil cuticles of carbonized leaves. Identification of fossils thus often depends on the identification of micro-characters. Leaf epidermal characters are therefore presented here together with the formal species description.

4. 3. Species Description

Cedrela ngobe Köcke, T. D. Penn. & Muellner-Riehl **sp.nov.** Type**.** Panama, Comarca Ngöbe-Buglé, foothills of Cerro Guánico, *A.V.Koecke, O. Cáceres, H. Wessels & M. Piepenbring* 180409/02 (holotype K, isotypes FR, PMA, UCH); **Plate 1.**

A speciebus aliis foliis 6--7 –jugis, infra breviter puberulis, petalis 6--6.5 mm longis, capsula 4--6 cm longa differt.

Young branches 4--5 mm diam., smooth, pale buff, with some elongate lenticels, glabrous. Leaves (petiole + rhachis) 40--50 cm long, leaflets opposite, 6--7 pairs, 8 x 4 -- 13 x 5 cm, ovate (apical pair), oblong-elliptic (middle pair) or elliptic (basal pair), apex acuminate, base asymmetric, rounded on one side, acute to obtuse on the other, chartaceous, subglabrous above, minutely puberulous on the midrib and secondary veins below, domatia present in the axils of secondary veins below; venation eucamptodromous, midrib not raised or sunken on the upper surface, secondaries (8--)12--14 pairs, ascending, slightly arcuate, parallel or slightly convergent, tertiaries mostly oblique and parallel. Petiole c. 10 cm long, finely and sparsely puberulous; petiolule c. 1 mm long. Inflorescence terminal, plant flowering when in leaf, 12--24 cm long, a broadly pyramidal panicle, lowest lateral branches up to 14 cm long, the ultimate branches often cymose, sparsely puberulous; pedicel 0.5--1 mm long. Calyx 2--3 mm long, cup-shaped, irregularly and shallowly lobed and split down one side to near the base, sparsely puberulous outside. Petals 6--6.5 mm long, 1--1.25 mm broad,

narrowly oblong, apex acute to obtuse, adnate to the androgynophore in the lower half, margins adnate for most of their length, pale tomentose outside, indumentum sparser inside. Staminal filaments (free portion), male: 1.5--2 mm long, glabrous, anthers c. 1 mm long, glabrous, female: filaments shorter, with smaller, shrunken anthers and no pollen, lower part of filaments fused to the androgynophore 1-1.5 mm long. Ovary borne at the top of the androgynophore, c. 2 mm long, glabrous, female: loculi with 10--11 ovules in 2 rows, style c. 2 mm long, with thick discoid style-head, male: pistillode longer and more slender with 5 small locules and vestigial ovules, style 2--3 mm long. Capsule pendulous, 4--6 cm long, c. 2.5 cm broad, ellipsoid, apex and base rounded, dark brown with pale lenticels, valves woody, 2--3 mm thick, columella 5-winged, seed attachment area 5--6 mm long. Seed 2.2--3.2 cm long (including the wing), brown.

Leaf Epidermal Characters (Figures. 10 A and B)

Epidermal cells of adaxial and abaxial leaf sides polygonal with straight to slightly undulated anticlines. Cuticle striated. Stomata on abaxial leaf sides, agglomerated on and along the leaf veins, oval-shaped, anomocytic, varying in length between 13-20 μm. Aperture spindle-shaped with slender I-pieces at the poles. Abaxial leaf surface densely covered with simple multicellular hairs.

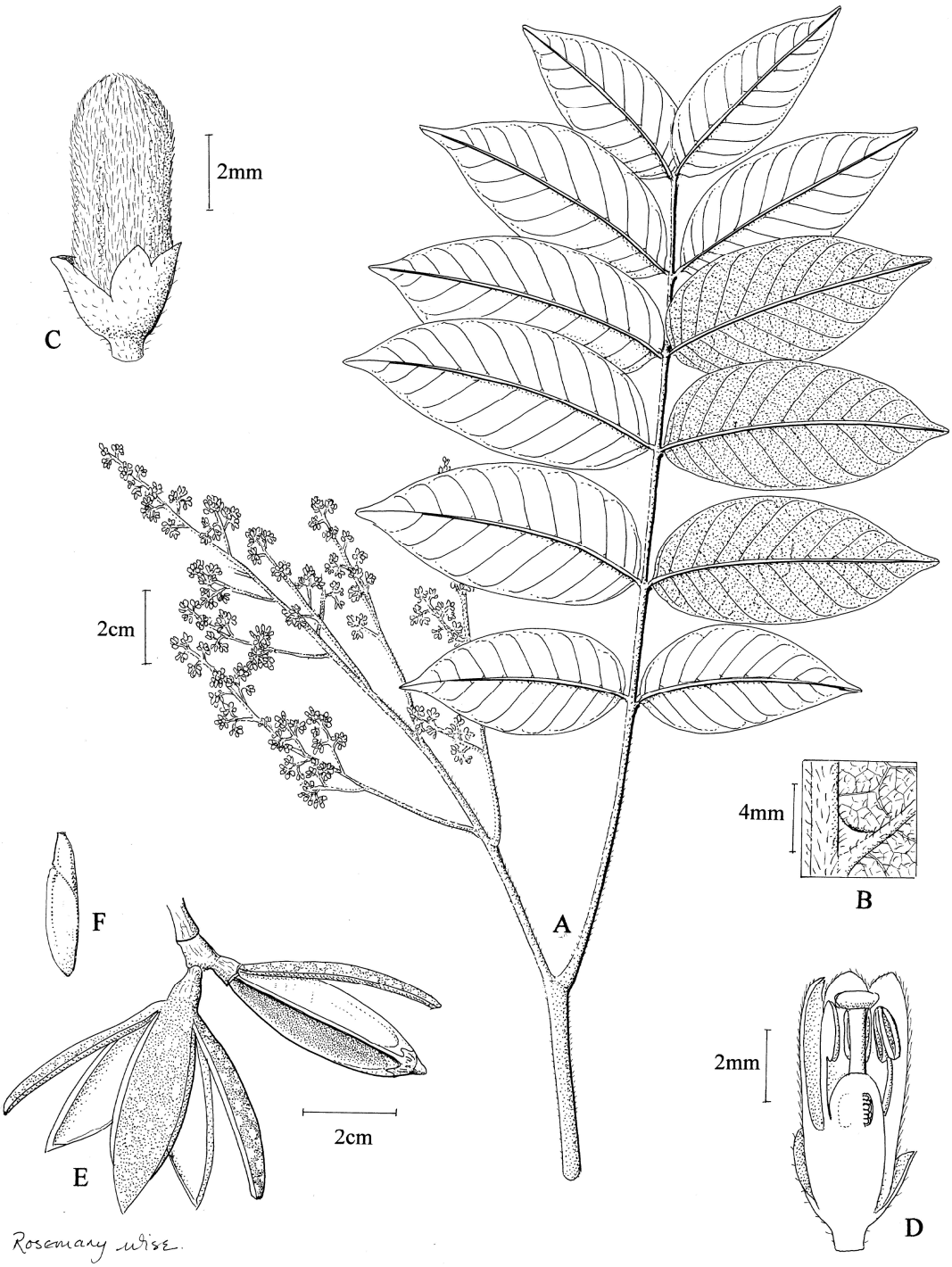
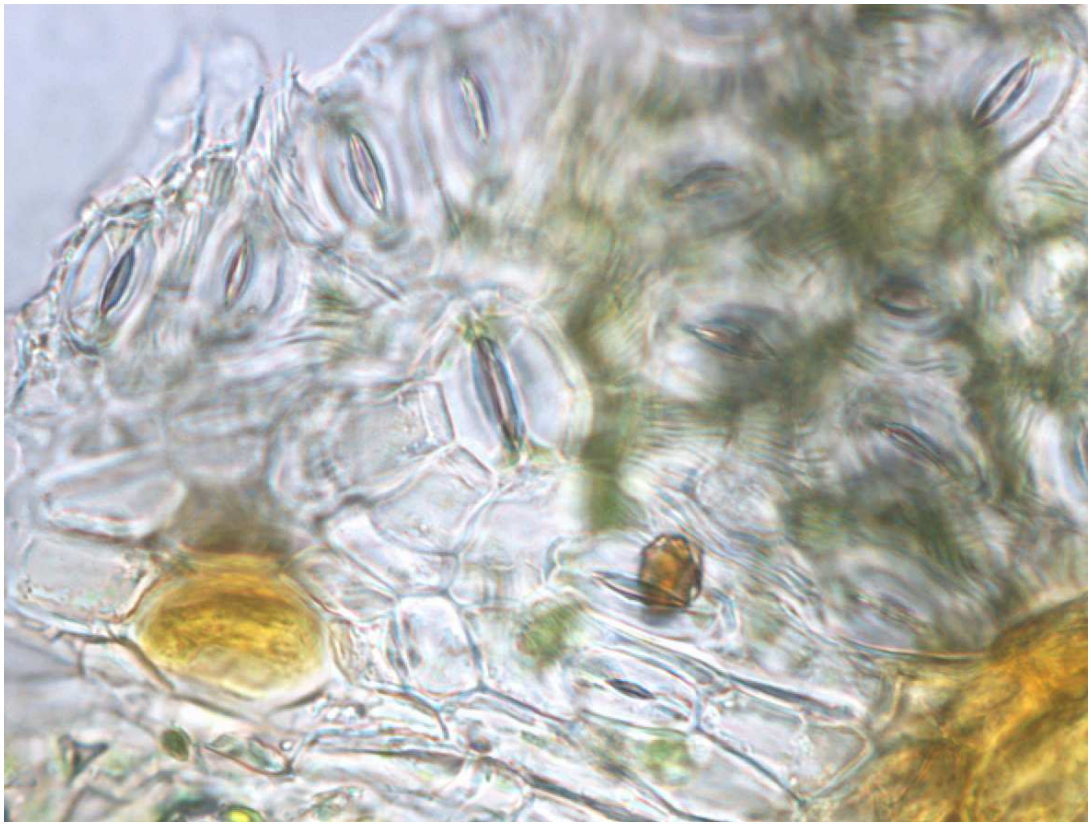


Plate 1. *Cedrela ngobe*, **A** Leaf and inflorescence; **B** Detail of leaf undersurface; **C** Flower; **D** Cross section of a male flower; **E** Capsules; **F** Seed (A-D, Koecke et al. 180409/02, E-F, Koecke et al. 180409/01).

Figure 10.



A. Stomata along vein on lower leaf side (Magnification 100x)

B. Base of a multicellular trichome (Magnification 100x)

Field Characters & Phenology

Tree to 30 m high and 50 cm diam., bole cylindrical, bark whitish grey, regularly longitudinally fissured. Deciduous, leafless at the beginning of the dry season (end of Dec.), in full leaf at the end of the dry/ early wet season (in April). The flower color varies from creamish white to green or yellow. Sterile specimens, especially from sucker shoots have much larger leaves with more numerous leaflets. Cut surfaces and crushed leaves have a strong onion like odour (pers. obs. A.V.K). Annotations on herbarium vouchers describe it as foul, bitter or garlic like. Flowering at the end of the dry season and early wet season (April to June), leaves present at flowering time; fruit maturing during the dry season.

Vernacular Name & Uses

Cedro de Sabana. The timber is used for furniture.

Distribution and Ecology

Extending from southeastern Costa Rica throughout the southern Pacific (seasonal) side of Panama to the Canal Zone and on the San José Islands of the Perlas Archipelago in the Gulf of Panama (Figure 11). It is a common species of (primary) semideciduous lowland forest (0-400 m).

Etymology. The new species is named *Cedrela ngobe*, in honour of the Ngöbe, an indigenous group in Panamá, who living in their traditional way within the Panamanian Comarca Ngöbe-Buglé, have contributed to preserving primary forest in Central and Western Panama.

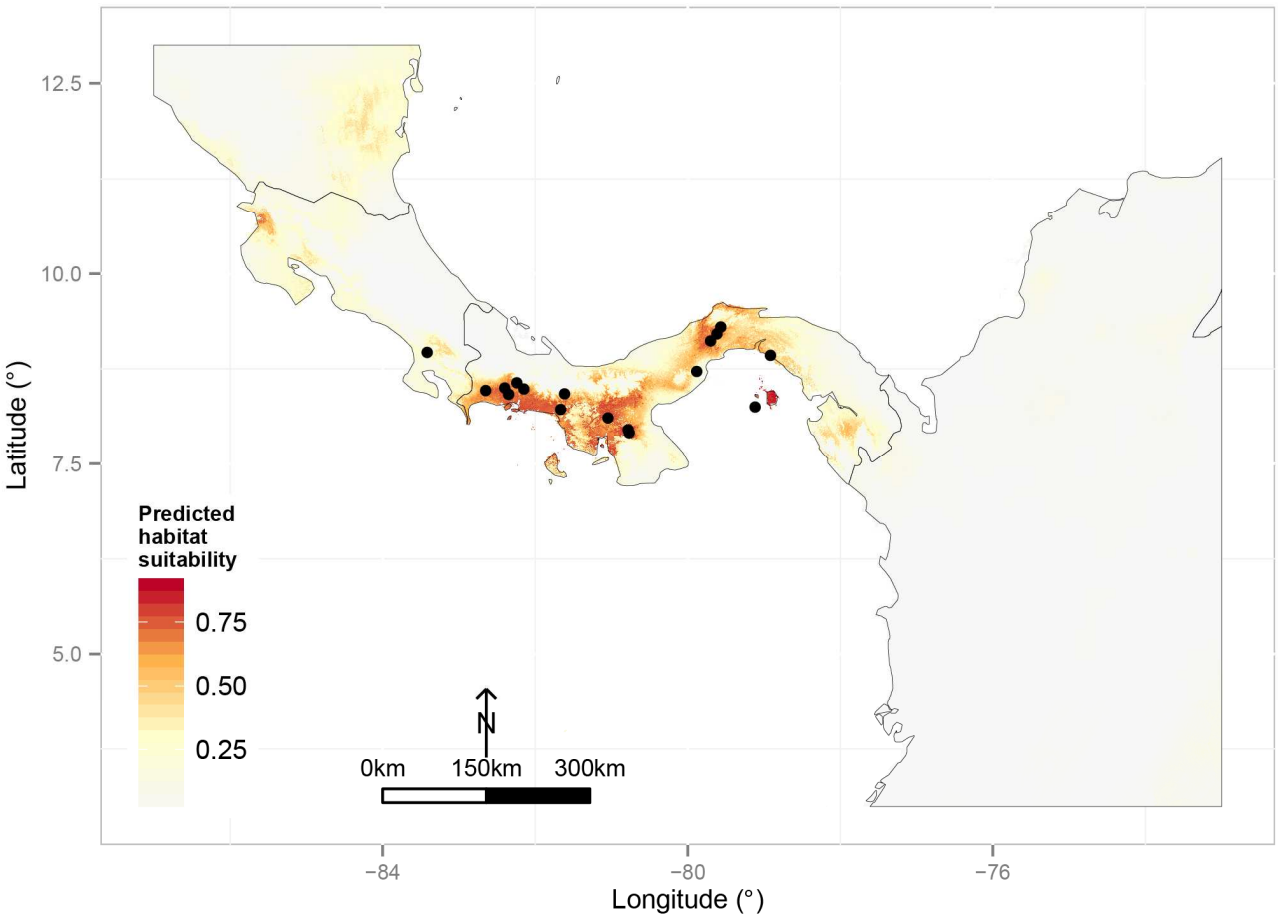


Figure 11. Map of distribution and climatic niche suitability of *C. ngobe* Köcke, T.D.Penn. & Muellner-Riehl. The value of each grid cell represents the mean (average value) of the 10 models. The color scale indicates the predicted suitability, low = 0 (light yellow), high = 1 (dark red).

Climatic Niche Modelling

To estimate the accuracy of the distribution map and detect potential areas of occurrence, we performed maximum entropy modelling of the geographic distribution using the program Maxent (v.3.3.3k; Phillips and Dudík, 2008) based on the collection sites of 17 individuals of the new species and 19 climatic variables (Bioclim, Hijmans et al., 2005). We used climatic layers with a resolution of 30s (ca. 1 km^2). The Maxent analyses were performed using 10 replicates. The average test AUC (Area Under the

receiver operating Curve) values of the models were above 0.9. Results from the Maximum Entropy modeling (as indicated by the color gradient in Figure 11) show that climatically suitable habitats of *C. ngobe* are only located in southern Costa Rica and throughout Panama within comparatively small scattered areas.

Specimens Examined

COSTA RICA: **Puntarenas,** Rio Grande de Terraba, vicinity of La Presa, about 3 miles above Palmar, *Allen* 5280 (A). Around **Alhajuela,** *Pittier* 3729 (NY-01239674).

PANAMA: **Chiriquí,** Ngöbe-Buglé: Nole Duime, Comunidad, de Llano Nopo, *Cáceres* 4003 (FR); along the interamerican highway between Chiriquí village and David, next to the bridge crossing Río Chiriquí, *Koecke et al.* 180409/01 (FR, K), *M. Piepenbring & O. Cáceres* 5195 (UCH); Ngöbe-Buglé, foothills of Cerro Guánico, *Koecke* et al. 180409/02 (FR, K, PMA, UCH); road to Gualaca, close to Bella Vista, *Koecke* et al. 220409/01 (FR, K); Los Algarrobos, *Munoz* 22 (MO-2901926); SE of La Tranca in pasture beside cliffs near Rio Caldera, *Schmalzel* 1560 (MO-5755198), (MO-5755199); pasture along road between David and El Hato, *Stern & Chambers* 103 (NY-01239675); **Herrera**, 10 mi S of Ocu, *Tyson* et al. 2827 (MO-1817269); vicinity of Ocu, *Allen* 4082 (MO-1600383); **Panama**, Canal Zone, *Blum* 680 (MO-1817743); between Santa Cruz and entrance to pipeline road, near Gamboa, near Canal zone, *Croat* 38307, (MO-2388650); Canal Zone, Madden forest, *Gentry* 2068 (MO-2692676); mouth of Rio Pasiga, *Gentry* 2205B (MO-2301774); Old Panama, *Getek* 3588 (MO-1138432); Arraiján, near the city, *Lao* 104 (FHO-00004467); on road to Cerro Campana, *Lazor* 5799 (MO-2593115); **Perlas Archipelago**, Gulf of Panama, San Jose Isl., *Johnston* 323 (MO-1590779); **Veraguas**, El Embalsadero, 8 miles west of Santiago, *Tyson* 6077 (MO-1973862); S of Santa Fe, *Nee* 8056 (FHO-00004477).

COLOMBIA: **La Guajira**, Via La Paz a Manaure, *Cuadros & Gentry* 3432 (MO-4007754).

4. 4. Phylogenetic Relationships

Molecular phylogenetics as well as network analyses based on the nuclear internal transcribed spacer region (ITS) and the plastid *psb*A-*trn*H region also confirm that *C. ngobe* represents a new species which is genetically distinct from other species of *Cedrela* (Cavers et al., 2013 and Koecke et al. in prep.; Koecke et al., 2013). Phylogenetic relationships show a close relationship to the Andean *C. nebulosa*, *C. saltensis* and another genetic entity of *Cedrela* which is designated as *C.* aff. *odorata* from Central and northern South America (Koecke et al., 2013). This corroborates previous phylogenetic and phylogeographic analyses, which uncovered the distinctness of some genetic entities within the broad species complex of *C. odorata* sensu lato (Muellner, Pennington, and Chase, 2009; Cavers et al., 2013; Koecke et al., 2013). From the current perspective, these entities comprise the newly described *C. ngobe*, *C. cubensis* Bisse (from Cuba and the Cayman Islands), *C. odorata* sensu stricto (basically from northern Central America with some occurrences in South America), and *C.* aff. *odorata* (from southern Central and South America).

4. 5. Notes

Sterile specimens of *Cedrela ngobe* were formerly associated with *C. fissilis* Vell. on account of the leaf indumentum, but the recent collection of flowering and fruiting specimens from Panama shows that it is morphologically distinct from this and all other species. The combination of characters that define *C. ngobe* is: 6--7 pairs of subsessile leaflets, –the lower surface of the leaflets puberulous on the midrib and secondary veins, petals 6--6.5 mm long, and capsule 4--6 cm long. *Cedrela fissilis*, which is not native in Central America, is clearly separable by its more numerous leaflets (10--17 pairs), longer petals (8--10.5 mm long), and much larger fruit (7--11 cm long). The sympatric *C. aff. odorata* differs in the subglabrous lower leaflet surface, generally longer petals and distinct phenology (*C. ngobe* flowers at the end of the dry season and early wet season, *C. aff. odorata* at end of wet season and early dry season).

Another species occurring in Panama is *C. tonduzii* C.DC., but this is clearly distinct in its more numerous, larger leaflets (mostly 8--12 pairs, 14--20.5 cm long, longer petals (8.5--10 mm long) and distinct ecology (wet montane forest at 1000--2500 m alt.).

4. 6. IUCN conservation assessment

Cedrela ngobe is threatened by deforestation throughout southwestern and central Panama. The provinces Chiriquí and Veraguas are among the most productive areas of crops and livestock farming and have thus undergone more extensive deforestation compared to other regions in Panama since the second half of the 20th century (Kaimowitz, 1996). The Comarca Ngöbe-Buglé has experienced the greatest decline in forest cover since 1992 (ANAM, 2003; Parker, Carrión, and Sasmudio, 2004; Fishman, 2009), and we predict that *C. ngobe* will suffer further decline. Its distribution is fragmented, single trees sometimes occur as remnants of previously forested areas along streets and pastures. The largest extent of predicted climatic niche suitability covers the lowland areas of the Chiriquí province, Veraguas province and southern regions of the Comarca Ngöbe Buglé. To the East, potentially suitable habitats of *C. ngobe* are found in the province of Panamá and on the Archipélago Las Perlas. Its occurrence further east could not be confirmed, except for one questionable herbarium specimen from Colombia. The total area of occupancy of *C. ngobe* is therefore estimated to be less than 2.000 km^2 and has probably undergone a population size reduction of ≥30% within the last decades, thereby meeting the IUCN criteria listed under A and B for Vulnerable (VU).

4. 7. Acknowledgements

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General Discussion

5. 1. Overview

I have investigated the spatio-temporal evolution of the Neotropical tree genus *Cedrela*. The first chapter was dedicated to studying the climatic niche evolution of the genus through time and space. My results showed that climate change, geotectonic movements and topography have had a huge impact on speciation and extinction, thus influencing distributional patterns of *Cedrela*. The second chapter comprised phylogenetic and network analyses of *Cedrela*, in order to unravel intra- and interspecific relationships and phylogeographical patterns. We found distinct levels of genetic variability in different groups of the genus. Bottle-neck effects through the colonization of South America probably led to interim reduction of genetic diversity. Genetic diversity possibly increased again after colonization of South America through parapatric speciation in Interandean Andean valleys, ecological differentiation in lowland areas and recolonization of Central America with subsequent recolonization of the Andes. The third chapter includes the description of the new species *C. ngobe* Köcke, Penn. Muellner-Riehl, a result of field studies and an investigation of approximately 500 herbarium specimens of northern South and Central America. Each study required various methods, ranging from state-of-the-art tools from evolutionary biology, such as molecular network-, phylogenetic- and post tree analyses, to species distribution modelling and analyses of morphological traits (classical taxonomy). The survey of geological and paleobotanical studies relevant to the fossil history of *Cedrela* and natural history of the Americas played an important role in interpreting the results of my work.

5. 2. Taxonomy

Species, being essential entities in biology, count as valid measures of biodiversity and evolutionary diversification (Mayr 1969, Rieseberg 1994). The variety of species concepts as well as numerous studies focusing on factors contributing to diversification have led to a broader notion of what modern taxonomy actually is. The 'integrative species concept' comprises complementary views and insights, from e.g. genetics, ecology and morphological variation (Dayrat, 2005; Weigand, Götze, and Jochum, 2012). Collectively, consideration of various aspects of diversity might well be useful not only for taxonomic classification, but also to identify and assess traits that are important to evolutionary diversification.

In *Cedrela*, morphological variation is limited, e.g. floral morphology is mostly unspecific and diagnostic characters are often overlapping among species (Pennington and Muellner, 2010). Conversely, genetic differentiation within the widespread species *C. odorata* was thought to be exceptionally high (Gillies et al., 1997) until subsequent phylogenetic analyses uncovered its paraphyly (Muellner, Pennington, and Chase, 2009). The absence of clearly distinctive morphological traits suggests that selective pressures rather act upon non-morphological traits, such as ecological preferences. We indeed found evidence for variable climatic tolerances and distinct phenologies among closely related species of *Cedrela*. Ecological differences might also include specific soil preferences, e.g. in Cuba the serpentine and limestone sites are inhabited by *C. cubensis*, whereas *C. odorata* occurs at wetter sites (Puentes, 2005).

The detection of cryptic species is common, particularly since modern taxonomy relies more and more on phylogenetic reconstructions from DNA data (Pfenninger and Schwenk, 2007) and thus, the question upon being confronted with real cryptic species, or having overseen morphological (and other) characteristics often remains open. Furthermore, it should also be considered that strict monophyly of species cannot always be expected, as paraphyly may also point at local speciation in an early stage (at least in plants, but see Rieseberg and Brouillet, 1994).

Phylogenetic analyses of an amplified dataset based on nuclear and plastid DNA sequences of *Cedrela* unveiled a total of 21 operational taxonomic units within two main clades (I and II as shown in Figures 6, 9.A-B and Tree Figures in the Appendix).

The OTU's comprised the previously described 17 species of *Cedrela* (Pennington and Muellner, 2010), as well as *C. cubensis* (Bisse, 1988), the newly described *C. ngobe* and additionally two paraphyletic genetic entities of *C. odorata* sensu lato. With respect to Rieseberg and Brouillet (1994), local speciation apparently is more common among plant organisms with smaller effective population sizes and lower levels of gene flow, whereas long-lived woody perennials, such as *Cedrela*, are characterized by higher effective population sizes and more efficient gene flow, which thus makes speciation through geographic subdivision more likely. In fact, the distinct genetic entities of *C. odorata* sensu lato could be related to different geographic ranges in Central and South America based on 236 collection sites. *Cedrela odorata* sensu stricto (group I) is mainly distributed in Central America, whereas *C.* aff. *odorata* (group II) occurs in southern Central and South America. The third genetic entity of *C. odorata* sensu lato probably is a rare endemic of western Ecuadorian lowland forest, which urgently needs further taxonomic revision and possibly conservational protection. Preliminary revision of *C.* aff. *odorata* (group II) unveiled characteristic morphological traits, such as very large, elliptic to lanceolate, subopposite paripinnate leaflets being different to the smaller leaflet sizes of *odorata* sensu stricto, which exhibits opposite leaflet arrangement and longer petiolules. The widespread distribution of both entities (group I and II) still requires inspection of more specimens in order to verify the distinctness of these morphological traits. At the current state of knowledge, it thus seems that morphological traits might indeed serve at distinguishing paraphyletic entities of *C. odorata* sensu lato.

The newly described species, *C. ngobe* can be easily identified by the indumentum of the few oval-shaped leaflets and the exceptionally tiny flowers, which appear at the end of the dry season. It was named after the indigenous group of people in western and central Panama, the Ngöbe, whose traditional way of life has contributed to preserving remnants of primary forests in their reservation. *Cedrela cubensis* Bisse is a species of Cuba and the Cayman islands that was previously not recognized within the monograph of *Cedrela* (Pennington and Muellner 2010), but based on morphology, ecology (Bisse, 1988) and genetic evidence it represents a distinct taxon.

Cedrela fissilis sensu lato is genetically diverse (Garcia et al., 2011 and Koecke et al. in prep.). It can be clearly subdivided into two groups, one from the eastern Atlantic and another one from the western interior (Figure 9.B, haplotypes 17-21 and 24-28,

respectively). The geographical range between these groups belongs to the Cerrado savanna, a fire dominated biome. *Cedrela balansae* is genetically clearly distinct and derived from the western interior group. Phylogenetic reconstructions unveiled that it was nested within *C. fissilis*. I thus propose to focus future taxonomic work on these groups of taxa, too.

Additional remarks

Infrafamiliar relationships in the outgroup of Cedreleae within the subfamily Swietenioideae were supported by phylogenetic results from additional plastid markers (*rps16* and *rpL16*). Previous results from Muellner et al. (2003) were completely corroborated, showing a close relationship of the genera *Capuronianthus* and *Lovoa*, *Carapa* and *Khaya*, respectively and *Entandrophragma* being closely related to Cedreleae (Appendix, Figure III).

5. 3. Climatic Niche Evolution

Methodological approach

Ancestral niche reconstructions required climatic niche models (i.e. species distribution models based on climatic layers) of extant species. Distribution data were based on more than 2500 georeferenced localities of *Cedrela*. The niche models are attached in the Appendix, Figure I a) – q). Ancestral environmental tolerances were estimated with a non-parametric approach using the dated maximum clade credibility tree. For each climatic variable, suitability scores were randomly selected and estimated for all interior nodes using a Maximum Likelihood reconstruction under the assumption of Brownian motion evolution. The niche reconstructions were subsequently compared to the paleoclimatic conditions of the fossil sites (Appendix, TABLES C-E). To my knowledge, this is the first botanical study comprising a direct comparison of paleoclimatic data from extensive fossil records and ancestral niche reconstructions based on a fossil-constrained dated phylogeny of extant species.

Notes on the origin of Cedrela and the deciduous habit

Cedrela probably originated in northern latitudes during the Early Eocene (Appendix, TABLES C-D). Early Eocene climates were characterized by the lack of ice on earth and smaller differences in temperature between the equator and the poles (Sluijs et al., 2006; Weijers et al., 2007). Thus, the original habitat type of *Cedrela* was probably not characterized by very dry seasons as supposed by Pennington and Muellner (2010), instead climatic conditions at northern latitudes were characterized by high and evenly distributed humidity (but see Appendix, TABLE C). Yet, Earths' inclination presumably caused seasonality in this biome, although not in terms of low temperatures (frost), nor pronounced periods of drought, but lower intensity and duration of light in winter (Wolfe, 1978, 1985; Jahren, 2007). This ancient biome of northern latitudes cannot be compared to any extant ecosystem. Changing day length and smooth climatic seasonality might have yet induced fall of leaves in Cedreleae. Although this idea is rather notional, the deciduous habit in particular might have enabled subsequent

adaptation of *Cedrela* to periods of drought. In a paper on the origin of the deciduous and evergreen habit, Axelrod (1966) conversely supposed that the deciduous habit of most hardwood trees did not evolve in response to photoperiodicity at northern latitudes. He instead suggested from the impression he had based on paleobotanical evidence that it must have originated in response to drought periods at lower latitudes during the Cretaceous. He further stated that the majority of deciduous trees must have then migrated into the North supplanting step by step the local relict Jurassic-type vegetation when climate changed. Since we do not know of any older than the Eocene fossils of Cedreleae, our study of niche evolutionary processes in *Cedrela* does not support Axelrod's hypothesis. There is, however, a fascinating aspect about his idea, inasmuch he presupposed common ecological origin within specific ancestral areas (common origin of deciduous trees), from where numerous lineages would evolve and shift into similar directions (niche tracking towards northern latitudes). This idea anticipated (in 1966) the nowadays common view on effects of niche conservatism. Wiens and Donoghue (Wiens and Donoghue, 2004) hypothesized that ancestral areas, when considered as ancestral niches, can determine the regions and habitats to which a species group would likely spread. For instance, the latitudinal gradient of diversity was explained by origination and diversification of most flowering plants in the old tropical biome (e.g. Wallace, 1878; Stephens and Wiens, 2003; Davis et al., 2005; Ricklefs, 2006), and Wiens and Graham (2005) stated tropical niche conservatism had thus maintained the disparity in species richness over time, except for a few biome shifts that allowed temperate species to overcome seasonal frost. This is corroborated by the observation that many temperate elements are nested within tropical clades (Mittelbach et al., 2007). In this context, fossil history and niche evolution of *Cedrela* demonstrate a case of northern origin and migration from north to south at first, as opposed to many other species origins at mid-latitudes, the ancestral area being part of an Eocene northern tropical biome that does not exist anymore.

However, an alternative explanation of the deciduous habit in *Cedrela* would be that it was an inherited trait of older Meliaceae predating the emergence of Cedreleae. Consequently, ancestors of Cedreleae might have theoretically had the ability to occupy a niche (strong seasonal drought) that was unavailable in the early Eocene. This example shows how difficult it can be to distinguish between the realized and the fundamental niche, which pinpoints at one of the major issues in evolutionary biology,

the temporal and spatial scales at which niche conservatism and niche evolution are investigated (Wiens et al., 2010).

Niche evolution and niche conservatism act on different spatial and temporal scales

In a seminal paper on the immediate global impact of climate change on species distributions, Parmesan and Yohe (2003) showed that the majority of species (75%-81% out of more than 1000 species) had moved into the predicted poleward direction since 1930. Wiens and Graham (2005) interpreted this as outcome of niche conservatism, which would thus be a more likely response to climate change than rapid evolution. Even at a time scale of several millions of years, niche conservatism leads to 'habitat tracking' in many groups of organisms as reviewed by Eldredge et al. (2005) and Wiens et al. (2010). Also Crisp et al. (2009) discovered phylogenetic biome conservatism has been prevalent during the radiations of plant lineages. However, Edwards and Donoghue (2013) argue that plants may well have stronger capabilities to adjust, but may have less opportunities for niche evolution due to physical disconnections between most biomes. Thus, they conclude, we still don't know enough about where lineages have existed in the past, the adjacency or connectivity of different biomes, their change through time and their effects on trait evolution. However, we have learned from evolutionary processes in *Cedrela* and other recent studies on speciation and niche evolution (e.g. Graham et al., 2004; Evans et al., 2009; Hoorn et al., 2010), that for instance mountain uplifts and climatic changes may trigger trait evolution, and that these evolutionary processes can be very heterogeneous within differnt groups of taxa.

In *Cedrela*, we can see both, niche conservatism and niche evolution of climatic tolerances acting at very different spatial and temporal scales: Climatic changes might have well been triggers of diversification at early evolutionary stages, e.g. *Cedrela* underwent a biome shift from wet to seasonally dry forest habitats shortly after the Eocene-Oligocene boundary (at ca. 31 My ago). In the Oligocene, global temperatures decreased, whereas seasonality increased causing periods of drought in western North America (Wolfe, 1994; Sheldon and Retallack, 2004; Eldrett et al., 2009). Niche reconstructions suggested increased niche evolution during severe climate changes,

which was shown through divergence of two basal lineages of *Cedrela* into different rainfall regimes. This pattern was also corroborated by fossil findings, which were assigned to seasonal climates of the early Oligocene (John Day Formation, Oregon). Niche conservatism of climatic tolerances then caused southward migration of *Cedrela* and extinction in northern latitudes, probably due to the stronger decline of temperatures and increasing probabilities of frost in winter from the late middle Miocene onwards (ca. 12-5 My ago, Wolfe, 1994; Zachos et al., 2001). The fundamental 'temperature niche' of *Cedrela* might have indeed remained relatively stable given, for instance, that all extant species occur in mostly frost-free areas (Appendix, TABLE B).

Climatic disparity and thus lability of climatic niches with respect to temperature and precipitation parameters was highest among the youngest Andean sister species, *C. nebulosa* and *C. saltensis*. Therefore, niche evolution along different climatic gradients may have recently triggered speciation. Since Janzen (1967) published his theory of greater climatic zonation on tropical mountain slopes as potential cause of higher species richness in tropical regions, most studies dealing with potential speciation along climatic gradients in tropical mountains have focused on animals (e.g. Graham et al., 2004; Kozak and Wiens, 2007; McCain, 2009; Cadena et al., 2012). From the current perspective, speciation processes in *Cedrela* did not occur through climatic separation of species along gradients of elevational axes, but along climatic gradients from north to south. The climatic niche of *C. saltensis* from the southern Andes is characterized by higher seasonality of temperature and precipitation, whereas *C. nebulosa* from the central Andes occurs in less seasonal climate. I hence propose that Janzen's theory of speciation along steep climatic gradients on tropical mountains should also be considered from a north-south perspective of seasonality versus non-seasonality.

Biome shifts towards seasonal frost are rare

Biome shifts from tropical into temperate regions are known to be rare due to the necessity of evolutionary adjustment to seasonal frost (Crisp and Cook, 2012 and references cited herein). Notably, all extant species of *Cedrela* are deciduous and have bud scales, which usually is one prerequisite for plants to survive frost. In *Cedrela*,

however, these traits probably represent adaptations to periods of drought. Therefore, survival of *Cedrela* at temperatures below 0°C is probably not hindered by the decline in transpiration and water-uptake. Frost might instead modify biochemical cell structures or affect regulatory mechanisms in the metabolism of *Cedrela* which could be a reason for its frost intolerance, e.g. hardy plants of temperate regions often alter the fluidity of their cell consistency to survive frost in winter. It is thus noteworthy that the Indo-Malayan genus *Toona* (which is the closest relative to *Cedrela*) is also native to temperate regions of continental climates and might have thus undergone evolutionary divergence of temperature tolerances. For instance, areas to the south of North Korea, eastern and central China constitute part of the widespread distribution of *T. sinensis* (Hua and Edmonds, 2008), which accordingly is the only species of Cedreleae that can cope with moderate frost (and also is the only species with serrate leaf margins, a typical character of temperate plants).

The integration of extant and paleobotanical data complements perspectives on evolutionary processes

It is not unlikely that Miocene diversity was already comparable to extant diversity of climatic niches and habitats, since fossil findings and paleoclimatic data showed that Miocene species of *Cedrela* occurred in a variety of distinct climates (Appendix, TABLES C-D). Fossils from the La Quinta Formation in southern Mexico were e.g. part of former subtropical or tropical rain forest, whereas fossils from the Buffallo Canyon constituted part of sclerophyll woodlands, and other ancestral taxa, such as from the Mascall and Latah Formation, occurred under humid Mediterranean climates with coolish winters (Appendix, TABLE C: 10-11, 13). However, niche reconstructions from extant phylogenetic data appeared to be contradictory to the results from fossil findings, suggesting initial conservatism of temperature tolerance and only increasing diversification from the late Miocene onwards. These patterns of diversification were mainly apparent among South American species of clade II, whereas clade I (Central American species) seemed to be more conserved (Figure 7.A-B). This result may be explained by the common occurrence of uncertainties in ancestral trait reconstructions (Schluter et al., 1997).

Schluter (1997) predicted that likelihood reconstructions of ancestral traits relying on phylogenies that are based on extant taxa would always include uncertainties, due to the fact that phylogenetic trees are never 100% complete (they miss extinct and non-sampled taxa) and therefore only include a (possibly) non-random subset of all the species of a clade. As mentioned by Fritz et al. (2013), reconstructions of ancestral traits from extant species alone can be particularly misleading if extinction rates have been high and directional selection acted against former trait variability. The descriptions of climatic niches of Miocene fossils of *Cedrela* refer to North and Central American taxa. These supposedly disappeared from northern latitudes after deterioration of temperatures in the late Miocene. Absence of extinct North and Central American lineages in phylogenetic trees of extant species of *Cedrela* could therefore explain why divergence patterns mainly reflected diversification of temperature tolerances among species of clade II and not among Central American species of clade I. Accordingly, uncertainties of likelihood reconstructions might be also lowered by a direct comparison of paleontological and neontological data. Consideration of both, neontological and paleotological data has contributed to our understanding on climatic niche dynamics of *Cedrela*. In retrospect, I believe that a more extensive sampling of *Cedrela* throughout its northernmost area of distribution in Mexico (Sinaloa, Durango, México, Nuevo Leon and Tamaulipas) and along the central mountain ranges to the east and west, would perhaps be interesting for further phylogenetic and climatic niche analyses inasmuch as these areas might harbour old (or even the oldest) lineages of *Cedrela*.

Pitfalls and possibilities

The combination of neontological and paleobotanical data in this study has provided a better understanding of geographical range dynamics of *Cedrela* and amplified our knowledge of ancestral climatic niches. Phylogenetic niche reconstructions of extant species, or interpretation of fossil findings alone, create an incomplete picture of the spatio-temporal evolution of *Cedrela*. Fossil data should be used critically. The reliability of plant fossils largely depends on the accuracy of determination, their

abundance in the fossil record and exact dating of the geological setting. Yet, when treated with care, fossil data can provide highly valuable information on e.g. morphological traits, climatic tolerances and ancestral distributions. Not only fossils bear pitfalls, also Maximum Likelihood analyses which are usually based on extant data can be misleading due to phylogenetic incompleteness (Schluter et al., 1997, as described in the previous section). Fritz et al. (2013) recently summarized how to overcome weaknesses of neontological and paleontological studies by integrating both disciplines into hypothetical interdisciplinary future research. Specifically, the incorporation of extinct taxa into phylogenies of extant species will contribute to identifying evolutionary changes through time more precisely. Furthermore, temporal dynamics of geographical ranges are supposed to be better understood by studying the distributional ranges and environmental conditions of extant species under consideration of fossil occurrences and paleoenvironmental data. If, for instance, more comparative botanical studies focusing on niche evolution were available, we would have a better picture of extinction and potential biome shifts of species groups in response to climate change. Based on the idea of common ecological ancestry (e.g. Axelrod, 1966; Ricklefs, 2006), these studies could shed light on future range dynamics of plant associations that might undergo similar changes. We might also be able to predict what will happen after common habitat loss and what types of species have more odds to survive under consideration of environmental changes (Petit, Hu, and Dick, 2008; Dietl and Flessa, 2011).

5. 4. Phylogeography of *Cedrela*

Methodological approach

Analyses of inter- and intrageneric phylogenetic relationships were based on one nuclear and five plastid markers. Cloning unveiled intraindivual polymorphisms of nuclear ITS, which represented phylogenetically non-divergent intragenomic copies. This indicated an advanced stage of concerted evolution within *Cedrela* - possibly lowering the risk of misleading signals from homoplasy (Feliner and Rosselló, 2007).

ITS sequence data were, hence, used for phylogenetic Bayesian and Maximum Likelihood reconstructions, as well as network analyses based on statistical parsimony. Maternally inherited relationships were reconstructed through network analyses based on *psbA-trnH* sequence data, which belong to the most variable plastid marker regions (Shaw et al., 2005 and references cited therein). For phylogeographic analyses, six major distribution areas were defined, based on species occurrences of *Cedrela*: 1. Mexico, 2. Central America, 3. Caribbean Islands, 4. Northern South America (Columbia, Ecuador), 5. Central South America (Peru, Brazil, French Guiana) and 6. Southern South America (Bolivia, Argentina, Paraguay).

Groups of species of Cedrela underwent different evolutionary histories

The genetic subdivision of *Cedrela* into two major clades, comprising a smaller group of Central American species (clade I) and a greater complex of Central and South American species (clade II), respectively, was consistently found in phylogenetic tree reconstructions (see Tree Figures in the Appendix) and network analyses (Figure 9.A and B). Both of these clades exhibit very different phylogeographic histories. Species within clade I remained in Central America and have more restricted range distributions. Conversely, species within clade II underwent more recent range expansions from South to Central America, back migration into the Andes and dispersal into South American lowland areas.

Migration from Central to South America apparently led to an initial bottleneck-effect and subsequently, genetic diversification must have had distinct causes. In clade II, haplotype homogeneity of some species stands in contrast to geographically structured genetic differentiation within species and genetic variability among species that is not geographically structured. Several species share the plastidial haplotypes H1 and H2. The latter also occurs in Central America, which might indicate that this haplotype was inherited from an ancient polymorphic Central American ancestor. The frequent occurrences of H1 and H2 could also reflect interspecific gene flow, and at least with respect to the climatically divergent sister species *C. saltensis* and *C. nebulosa*, recent speciation seems to predate differentiation of plastidial genetic variability among

species. A unique non-geographically organized variability of plastid haplotypes was found among the closely related species *C. angustifolia* and *C. montana*, which indicates another evolutionary history of genetic diversification. Contrarily, geographically structured genetic differentiation of e.g. *C. fissilis* sensu lato (Figures 9.A and B) and the occurrence of lineage-specific haplotypes of some Andean species suggest speciation subsequent to geographic isolation. For instance, *C. fissilis* sensu lato comprised a group of individuals from the eastern moist Atlantic forests and a second group from the western interior of Brazil that are separated by the Cerrado biome, the latter representing a very specific ecosystem that is frequently subject to fires in the dry season. This pattern of geographically structured genetic variability was already detected by Garcia et al. (2011), although they found evidence for recent genetic connectivity between the Atlantic and the western interior group through corridors of gallery forest in the Cerrado. *Cedrela balansae* was found to be nested within *C. fissilis* sensu lato and network analyses unveiled its descent from the western interior group and colonization of southernmost areas in Brazil and adjacent countries. Diversification of *C. fissilis* sensu lato was thus either correlated with vicariance due to separation of former continuous distribution ranges between the eastern Atlantic coast and the western interior, or by dispersal from the western interior into the east and south, respectively. Paleobotanical studies have shown that the moist Atlantic forest of Brazil was significantly reduced and replaced by cold-adapted taxa in the Pliocene and Pleistocene, and that Antarctic cold fronts must have been much stronger and more frequent than today (Behling, 2002). Accordingly, it seems again possible that niche conservatism with respect to frost intolerance was mainly responsible for the contraction of populations into northeastern and western regions in Brazil during the Pleistocene, where climatic conditions were probably milder. *Cedrela* is not adapted to regular fires, which obviously explains its absence from the Cerrado biome and hence, separation of populations to the east, west and south of this region. The phylogeographical structure of *Hymenaea stigonocarpa* (Fabaceae), which is a tree of the Cerrado savanna (Ramos et al., 2007) resembles the genetic structure that was found in *Cedrela*. The genetic division of *H. stigonocarpa* into three clusters (same as in *Cedrela*: east, west and south) was also explained by its extinction throughout the southern region of the present-day Cerrado due to colder climates in the Pleistocene, and subsequent recolonization from refugial areas to the north (Ramos et al., 2007).

In southern Central America, geographically structured genetic diversity of *Cedrela* was certainly not caused by vicariance. Instead, limitated gene flow among the closely related and sympatrically distributed species *C. ngobe* and *C.* aff. *odorata* (group II) might be (at least partly) sustained by deviating phenologies. *Cedrela ngobe* flowers at the end of the dry season and early rainy season, while *C.* aff. *odorata* (group II) still is completely leafless during this time. Most of the tropical trees usually flower at the beginning of the dry season (Janzen, 1967), and therefore the phenology of *C. ngobe* clearly deviates from the prevailing pattern. However, earlier studies on potential speciation through diversification of floral traits, e.g. in Gesneriaceae (Perret et al., 2007), did not recognize a tendency of sympatric taxa to diversify along separation of morphological and phenological traits. Phenologies can be also varying from year to year, due to naturally occurring climatic oscillations. It has been thus disputed whether differences in phenology in some plant groups were indeed barriers to gene flow, as in some cases they remain permeable (Karrenberg, Edwards, and Kollmann, 2002). However, selection for incomplete reproductive isolation could also contribute to enhancing genetic variability between otherwise diverging populations (Rieseberg, 1995; Rieseberg and Willis, 2007), and hence, divergence of populations through distinct timing of flowering might explain co-existence of closely related species.

Gene flow, genetic diversity, and speciesrichness in the Neotropics

In a review on studies of gene flow and genetic diversity of trees, phylogeographic differentiation was shown to be generally higher in the Neotropics when compared to trees of temperate climates, which was explained by the association of lower population densities, density dependent animal pollination and mixed mating systems in the Neotropics (referring to outcrossing and inbreeding, but see Dick et al., 2008). The same authors noted that despite higher degrees of geographic structure, long distance gene flow and incomplete reproductive barriers are common characteristics of several Neotropical trees, too (Dick et al., 2007; Dick and Heuertz, 2008; Cerón-Souza et al., 2010; Hardesty et al., 2010; Fuchs and Hamrick, 2011; Scotti-Saintagne et al., 2013). Relatedness of species of *Cedrela*, their heterogeneous genetic structures and

gene flow at interspecific levels thus fit the patterns of genetic diversity which are typically found in populations of Neotropical trees. A common consequence of generally lower population densities in the tropics is the eventual occurrence of inbreeding and genetic drift contributing to subsequent diversification, as stated by Dick et al. (2003). However, in *Cedrela*, dioecy and dichogamy are probably effective mechanisms that impede inbreeding and encourage outcrossing (Pennington and Muellner, 2010). The nectaries at the base of the androgynophore attract several unspecific short-tongued insects. In species-rich forests of the Neotropics, these two mechanisms, suppression of inbreeding and attraction of insects over large distances, might ensure gene flow which sustains genetic stability of low density populations and wide geographical distribution. Interestingly, the majority of tropical tree species are pollinated by animals and most of the insect-pollinated flowers are visited by various species (Dick et al., 2008). No analyses on real population densities or estimates of effective seed- and pollen dispersal have yet been carried out on species of *Cedrela*. Like *Ceiba* (Malvaceae) and *Cordia* (Boraginaceae, but see Dick et al., 2007; Rymer et al., 2013), *Cedrela* is a wind-dispersed and drought-tolerant genus, which suggests its success of dispersal may be similarly high.

Although niche evolution has apparently played an important role in diversification of *Cedrela*, the relatively low number of extant species shows that other intrinsic processes and species traits might be more effective triggers of species-richness in the Neotropics. Longevity and morphological stasis were found to promote slow diversification and fewer species numbers among different groups of trees, whereas transitions from woody to herbaceous growth forms were correlated with higher diversification rates (Petit and Hampe, 2006). For instance, short generation times and fleshy fruits were found to be potential reasons for the high species richness observed in the woody genus *Inga* (Fabaceae, Richardson et al., 2001). Likewise, the species-rich genus *Ocotea* (Lauraceae), which is a characteristic tree of tropical mountain habitats, probably owes much of its success to frugivorous birds that disperse their seeds (Gibson and Wheelwright, 1995). Diversification of Andean *Lupinus* (Fabaceae) followed a single colonization event from North America with subsequent radiations in high ‘island-type’ habitats due to the emergence of largely unoccupied habitats after the final Andean uplift (Hughes and Eastwood, 2006). In this example, steep

environmental gradients were found to enhance geographical isolation and higher net diversification rates were correlated with iteroparity in mountain habitats (Drummond et al., 2012b). Key innovations, such as e.g. the Crassulacean Acid Metabolism (CAM) and the tank habit triggered species-richness in Neotropical Bromelioideae (Silvestro, Zizka, and Schulte, 2014). CAM was found to increase speciation rates, whereas the tank habit enabled epiphytic growth forms on large canopy trees, which was correlated with lower extinction rates when compared to terrestrial lineages (Silvestro, Zizka, and Schulte, 2014). In situ evolution of adaptations to fire can also be seen as a key innovation that enabled species to occupy the Cerrado Biome relatively recently (Simon et al., 2009). Therefore, one important aspect and perhaps precondition of species-richness of genera in the Neotropics results from the combination of opportunity (e.g. use of a special habitat) and ability (e.g. evolution of a key trait). Absence of conspicuous morphological traits among species of *Cedrela*, its woody growth form with longer generation times, and extensive distributions with low densities are inconsistent with any traits that I have found to be correlated with species-richness in Neotropical plant lineages. However, gene flow among Neotropical trees might also have a homogenizing effect which potentially overwrites signatures of events that may have contributed to speciation in the past (Pennington and Dick, 2010). This might, altogether, explain the relative success of the genus *Cedrela* being prominently distributed throughout different biomes of most forested regions in the Neotropics, despite its comparatively lower species richness.

Bibliography

ACKERLY, D. D. 2003. Community Assembly, Niche Conservatism, and Adaptive Evolution in Changing Environments. *International Journal of Plant Sciences* 164: S165-184.

ÁLVAREZ, I., and J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417-434.

ANAM. 2003. Informe Final de Resultados de la Cobertura Boscosa y uso del Suelo de la Republica de Panamá: 1992–2000. La Autoridad Nacional para el Ambiente (ANAM) y The International Tropical Timber Organization Panamá.

ANTONELLI, A. 2008. Spatiotemporal evolution of Neotropical organisms: new insights into an old riddle. Department of Plant and Environmental Sciences; Institutionen för växt-och miljövetenskaper.

ANTONELLI, A., and I. SANMARTÍN. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60: 403-414.

ANTONELLI, A., J. A. A. NYLANDER, C. PERSSON, and I. SANMARTÍN. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749-9754.

AXELROD, D. I. 1966. Origin of deciduous and evergreen habits in temperate forests. *Evolution*: 1-15.

AXELROD, D. I. 1985. Miocene floras from the Middlegate Basin, west-central Nevada

University of California Press , Berkeley

AXELROD, D. I. 1991. The early Miocene Buffalo Canyon flora of western Nevada. University of California Press , Berkeley

_____. 1995. The Miocene Purple Mountain Flora of western Nevada. University of California Press, Berkeley.

AXELROD, D. I. 2000. A Miocene (10-12 Ma) Evergreen Laurel-Oak Forest from Carmel Valley, California. University of California Press (Berkeley).

BAHADUR, K. N. 1988. Monograph on the genus Toona (Meliaceae). Shiva Offset Press, Dehra Dun, India

BARTOLI, G., M. SARNTHEIN, M. WEINELT, H. ERLENKEUSER, D. GARBE-SCHÖNBERG, and D. W. LEA. 2005. Final closure of Panama and the onset of northern hemisphere glaciation. *Earth and Planetary Science Letters* 237: 33-44.

BEHLING, H. 2002. South and southeast Brazilian grasslands during Late Quaternary times: a synthesis. *Palaeogeography, Palaeoclimatology, Palaeoecology* 177: 19-27.

BESTLAND, E. A., M. S. FORBES, E. S. KRULL, and T. FREMD. 2008. Stratigraphy, paleopedology, and geochemistry of the middle Miocene

Mascall Formation (type area, central Oregon, USA). *PaleoBios* 28: 41-61.

BISSE, J. 1988. Árboles de Cuba. Editorial Científico-Técnica Ciudad de La Habana.

BURNHAM, R. J. 2008. Hide and Go Seek: What Does Presence Mean in the Fossil Record. *Annals of the Missouri Botanical Garden* 95: 51-71.

CADENA, C. D., K. H. KOZAK, J. P. GÓMEZ, J. L. PARRA, C. M. MCCAIN, R. C. K. BOWIE, A. C. CARNAVAL, et al. 2012. Latitude, elevational climatic zonation and speciation in New World vertebrates. *Proceedings of the Royal Society B: Biological Sciences* 279: 194-201.

CAVERS, S., C. NAVARRO, and A. J. LOWE. 2003a. Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica. *Molecular Ecology* 12: 1451-1460.

_____. 2003b. A combination of molecular markers identifies evolutionarily significant units in *Cedrela odorata* L. (Meliaceae) in Costa Rica. *Conservation Genetics* 4: 571-580.

CAVERS, S., A. TELFORD, F. ARENAL CRUZ, A. J. PÉREZ CASTAÑEDA, R. VALENCIA, C. NAVARRO, A. BUONAMICI, et al. 2013. Cryptic species and phylogeographical structure in the tree *Cedrela odorata* L. throughout the Neotropics. *Journal of Biogeography* 40: 732-746.

CERÓN-SOUZA, I., E. RIVERA-OCASIO, E. MEDINA, J. A. JIMÉNEZ, W. O. MCMILLAN, and E. BERMINGHAM. 2010. Hybridization and introgression in New World red

mangroves, Rhizophora (Rhizophoraceae). *American Journal of Botany* 97: 945-957.

CHANDLER, M. E. J. 1964. The lower tertiary floras of southern England. *Bulletin of the british Museum of Natural History. Geology* 12: 1-151.

CLEMENT, M., D. POSADA, and K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

CODY, S., J. E. RICHARDSON, V. RULL, C. ELLIS, and R. T. PENNINGTON. 2010. The Great American Biotic Interchange revisited. *Ecography* 33: 326-332.

COSTELLO, M. J., R. M. MAY, and N. E. STORK. 2013. Can We Name Earth's Species Before They Go Extinct? *Science* 339: 413-416.

CRANDALL, K. A., and A. R. TEMPLETON. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134: 959-969.

CRICK, F., L. BARNETT, S. BRENNER, and R. J. WATTS-TOBIN. 1961. General nature of the genetic code for proteins. Macmillan Journals Limited.

CRICK, F., S. BRENNER, A. KLUG, and G. PIECZENIK. 1976. A speculation on the origin of protein synthesis. *Origins of life* 7: 389-397.

CRICK, F. H. C. 1963. On the genetic code. *Science* 139: 461-464.

CRISP, M. D., and L. G. COOK. 2012. Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? *New Phytologist* 196: 681-694.

CRISP, M. D., S. A. TREWICK, and L. G. COOK. 2011. Hypothesis testing in biogeography. *Trends in Ecology & Evolution* 26: 66-72.

CRISP, M. D., M. T. K. ARROYO, L. G. COOK, M. A. GANDOLFO, G. J. JORDAN, M. S. MCGLONE, P. H. WESTON, et al. 2009. Phylogenetic biome conservatism on a global scale. *Nature* 458: 754-756.

CUENCA, A., C. B. ASMUSSEN-LANGE, and F. BORCHSENIUS. 2008. A dated phylogeny of the palm tribe Chamaedoreeae supports Eocene dispersal between Africa,

North and South America. *Molecular Phylogenetics and Evolution* 46: 760-775.

DALEY, B. 1972. Some problems concerning the Early Tertiary climate of southern Britain. *Palaeogeography, Palaeoclimatology, Palaeoecology* 11: 177-190.

DARWIN, C. 1852. The origin of species. 1872. Reprinted 1962. New York: Macmillan.

DAVIS, C. C., C. O. WEBB, K. J. WURDACK, C.-A. JARAMILLO, and M. J. DONOGHUE. 2005. Explosive Radiation of Malpighiales Supports a Mid-Cretaceous Origin of Modern Tropical Rain Forests. *The American Naturalist* 165: E36-E65.

DAVIS, M. A., M. K. CHEW, R. J. HOBBS, A. E. LUGO, J. J. EWEL, G. J. VERMEIJ, J. H. BROWN, et al. 2011. Don't judge species on their origins. *Nature* 474: 153-154.

DAVIS, S. D., V. H. HEYWOOD, O. HERRERA-MACBRYDE, J. VILLA-LOBOS, and A. C. HAMILTON. 1995. Centres of plant diversity: a guide and strategy for their conservation. Volume 3. The Americas. IUCN-The World Conservation Union, Publications Services Unit.

DAYRAT, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407-415.

DICK, C., O. HARDY, F. A. JONES, and R. PETIT. 2008. Spatial Scales of Pollen and Seed-Mediated Gene Flow in Tropical Rain Forest Trees. *Tropical Plant Biology* 1: 20-33.

DICK, C. W., and M. HEUERTZ. 2008. The complex biogeographic history of a widespread tropical tree species. *Evolution* 62: 2760-2774.

DICK, C. W., K. ABDUL-SALIM, and E. BERMINGHAM. 2003. Molecular Systematic Analysis Reveals Cryptic Tertiary Diversification of a Widespread Tropical Rain Forest Tree. *The American Naturalist* 162: 691-703.

DICK, C. W., E. BERMINGHAM, M. R. LEMES, and R. GRIBEL. 2007. Extreme long-distance dispersal of the lowland tropical rainforest tree Ceiba pentandra L. (Malvaceae) in Africa and the Neotropics. *Molecular Ecology* 16: 3039-3049.

DIETL, G. P., and K. W. FLESSA. 2011. Conservation paleobiology: putting the dead to work. *Trends in Ecology & Evolution* 26: 30-37.

DORIA, G., D. L. ROYER, A. P. WOLFE, A. FOX, J. A. WESTGATE, and D. J. BEERLING. 2011. Declining atmospheric CO2 during the late Middle Eocene climate transition. *American Journal of Science* 311: 63-75.

DOUZERY, E. J. P., A. M. PRIDGEON, P. KORES, H. P. LINDER, H. KURZWEIL, and M. W. CHASE. 1999. Molecular phylogenetics of Diseae (Orchidaceae): a contribution from nuclear ribosomal ITS sequences. *American Journal of Botany* 86: 887-899.

DRUMMOND, A., and A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

DRUMMOND, A. J., M. A. SUCHARD, D. XIE, and A. RAMBAUT. 2012a. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969-1973.

DRUMMOND, C. S., R. J. EASTWOOD, S. T. MIOTTO, and C. E. HUGHES. 2012b. Multiple continental radiations and correlates of diversification in Lupinus (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic Biology* 61: 443-460.

EDWARDS, E. J., and M. J. DONOGHUE. 2013. Is it easy to move and easy to evolve? Evolutionary accessibility and adaptation. *Journal of Experimental Botany* 64: 4047-4052.

ELDREDGE, N., J. N. THOMPSON, P. M. BRAKEFIELD, S. GAVRILETS, D. JABLONSKI, J. B. C. JACKSON, R. E. LENSKI, et al. 2005. The dynamics of evolutionary stasis. *Paleobiology* 31: 133-145.

ELDRETT, J. S., D. R. GREENWOOD, I. C. HARDING, and M. HUBER. 2009. Increased seasonality through the Eocene to Oligocene transition in northern high latitudes. *Nature* 459: 969-973.

ELITH, J., C. H. GRAHAM, R. P. ANDERSON, M. DUDÍK, S. FERRIER, A. GUISAN, R. J. HIJMANS, et al. 2006. Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29: 129-151.

ERDEI, B., L. HABLY, M. KÁZMÉR, T. UTESCHER, and A. A. BRUCH. 2007. Neogene flora and vegetation development of the Pannonian domain in relation to palaeoclimate and palaeogeography. *Palaeogeography, Palaeoclimatology, Palaeoecology* 253: 115-140.

ERKENS, R. H. J., L. W. CHATROU, J. W. MAAS, T. VAN DER NIET, and V. SAVOLAINEN. 2007. A rapid diversification of rainforest trees (Guatteria; Annonaceae) following dispersal from Central into South America. *Molecular Phylogenetics and Evolution* 44: 399-411.

EVANS, M. E. K., S. A. SMITH, R. S. FLYNN, and M. J. DONOGHUE. 2009. Climate, Niche Evolution, and Diversification of the "Bird-Cage" Evening Primroses (Oenothera, Sections Anogra and Kleinia). *The American Naturalist* Vol. 173: 225-240.

FARRIS, D. W., C. JARAMILLO, G. BAYONA, S. A. RESTREPO-MORENO, C. MONTES, A. CARDONA, A. MORA, et al. 2011. Fracturing of the Panamanian Isthmus during initial collision with South America. *Geology* 39: 1007-1010.

FELINER, G. N., and J. A. ROSSELLÓ. 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. *Molecular Phylogenetics and Evolution* 44: 911-919.

FINE, P. V. A., F. ZAPATA, D. C. DALY, I. MESONES, T. M. MISIEWICZ, H. F. COOPER, and C. E. A. BARBOSA. 2013. The importance of environmental heterogeneity and spatial distance in generating phylogeographic structure in edaphic specialist and generalist tree species of Protium (Burseraceae) across the Amazon Basin. *Journal of Biogeography* 40: 646-661.

FISHMAN, A. 2009. The Past and Present of Panamanian Deforestation. *Biol 134b: Restoration Ecology*.

FRITZ, S. A., J. SCHNITZLER, J. T. ERONEN, C. HOF, K. BÖHNING-GAESE, and C. H. GRAHAM. 2013. Diversity in time and space: wanted dead and alive. *Trends in Ecology & Evolution* 28: 509-516.

FUCHS, E. J., and J. HAMRICK. 2011. Mating system and pollen flow between remnant populations of the endangered tropical tree, Guaiacum sanctum (Zygophyllaceae). *Conservation Genetics* 12: 175-185.

GARCIA, M. G., R. S. SILVA, M. A. CARNIELLO, J. W. VELDMAN, A. A. B. ROSSI, and L. O. DE OLIVEIRA. 2011. Molecular evidence of cryptic speciation, historical range expansion, and recent intraspecific hybridization in the Neotropical seasonal forest tree Cedrela fissilis (Meliaceae). *Molecular Phylogenetics and Evolution* 61: 639-649.

GENTRY, A. H. 1982. Neotropical Floristic Diversity: Phytogeographical Connections Between Central and South America, Pleistocene Climatic Fluctuations, or an Accident of the Andean Orogeny? *Annals of the Missouri Botanical Garden* 69: 557-593.

GIBSON, J. P., and N. T. WHEELWRIGHT. 1995. Genetic structure in a population of a tropical tree Ocotea tenera (Lauraceae): influence of avian seed dispersal. *Oecologia* 103: 49-54.

GILLIES, A. C. M., J. P. CORNELIUS, A. C. NEWTON, C. NAVARRO, M. HERNÁNDEZ, and J. WILSON. 1997. Genetic variation in Costa Rican populations of the tropical timber species Cedrela odorata L., assessed using RAPDs. *Molecular Ecology* 6: 1133-1145.

GRAHAM, A. 1976. Studies in Neotropical Paleobotany. II. The Miocene Communities of Veracruz, Mexico. *Annals of the Missouri Botanical Garden* 63: 787-842.

_____. 1991a. Studies in Neotropical Paleobotany. IX. The Pliocene Communities of Panama-Angiosperms (Dicots). *Annals of the Missouri Botanical Garden* 78: 201-223.

_____. 1991b. Studies in Neotropical Paleobotany. X. The Pliocene Communities of Panama-- Composition, Numerical Representations, and Paleocommunity Paleoenvironmental Reconstructions. *Annals of the Missouri Botanical Garden* 78: 465-475.

_____. 1991c. Studies in Neotropical Paleobotany. X. The Pliocene communities of Panama - composition, numerical representations, and paleocommunity paleoenvironmental reconstructions. *Annals of the Missouri Botanical Garden* 78: 465-475.

_____. 1993. History of the vegetation: cretaceous (Maastrichtian)-Tertiary. *Flora of North America* 1: 57-70.

_____. 1999a. The Tertiary history of the northern temperate element in the northern Latin American biota. *American Journal of Botany* 86: 32-38.

_____. 1999b. Studies in Neotropical paleobotany. XIII. An Oligo-Miocene palynoflora from Simojovel (Chiapas, Mexico). *American Journal of Botany* 86: 17-31.

_____. 2010. Late Cretaceous and Cenozoic history of Latin American vegetation and terrestrial environments. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

GRAHAM, A., and D. L. DILCHER. 1998. Studies in Neotropical Paleobotany. XII. A Palynoflora from the Pliocene Rio Banano Formation of Costa Rica and the Neogene Vegetation of Mesoamerica. *American Journal of Botany* 85: 1426-1438.

GRAHAM, C. H., and P. V. A. FINE. 2008. Phylogenetic beta diversity: linking ecological and evolutionary processes across space in time. *Ecology Letters* 11: 1265-1277.

GRAHAM, C. H., S. R. RON, J. C. SANTOS, C. J. SCHNEIDER, and C. MORITZ. 2004. Integrating Phylogenetics and Environmental Niche Models to Explore Speciation Mechanisms in Dendrobatid Frogs. *Evolution* 58: 1781-1793.

HABLY, L. 2006. Catalogue of the Hungarian Cenozoic leaf, fruit and seed floras from 1856 to 2005. *Studia bot. hung.* 37: 41-129.

HARDESTY, B. D., C. W. DICK, J. L. HAMRICK, B. DEGEN, S. P. HUBBELL, and E. BERMINGHAM. 2010. Geographic influence on genetic structure in the widespread Neotropical tree Simarouba amara (Simaroubaceae). *Tropical Plant Biology* 3: 28-39.

HARMON, L. J., J. A. SCHULTE, A. LARSON, and J. B. LOSOS. 2003. Tempo and Mode of Evolutionary Radiation in Iguanian Lizards. *Science* 301: 961-964.

HARMON, L. J., J. T. WEIR, C. D. BROCK, R. E. GLOR, and W. CHALLENGER. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24: 129-131.

HARMON, L. J., J. B. LOSOS, T. JONATHAN DAVIES, R. G. GILLESPIE, J. L. GITTLEMAN, W. BRYAN JENNINGS, K. H. KOZAK, et al. 2010. Early bursts of body size and shape evolution are rare in comparative data. *Evolution* 64: 2385-2396.

HEIBL, C. 2011. Phyloclim: integrating phylogenetics and climatic niche modelling. R package.

HERNANDEZ, P. A., C. H. GRAHAM, L. L. MASTER, and D. L. ALBERT. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29: 773-785.

HEYWOOD, V. H. 1995. Global biodiversity assessment. Cambridge University Press.

HICKEY, L. J., and R. W. HODGES. 1975. Lepidopteran Leaf Mine from the Early Eocene Wind River Formation of Northwestern Wyoming. *Science* 189: 718-720.

HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, and A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978.

HOLT, R. 1996a. Demographic constraints in evolution: Towards unifying the evolutionary theories of senescence and niche conservatism. *Evolutionary Ecology* 10: 1-11.

HOLT, R. D. 1996b. Adaptive Evolution in Source-Sink Environments: Direct and Indirect Effects of Density-Dependence on Niche Evolution. *Oikos* 75: 182-192.

_____. 2009. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences* 106: 19659-19665.

HOORN, C., V. MOSBRUGGER, A. MULCH, and A. ANTONELLI. 2013. Biodiversity from mountain building. *Nature Geosci* 6: 154-154.

HOORN, C., F. P. WESSELINGH, H. TER STEEGE, M. A. BERMUDEZ, A. MORA, J. SEVINK, I. SANMARTÍN, et al. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science* 330: 927-931.

HREN, M. T., M. PAGANI, D. M. ERWIN, and M. BRANDON. 2010. Biomarker reconstruction of the early Eocene paleotopography and paleoclimate of the northern Sierra Nevada. *Geology* 38: 7-10.

HUA, P., and J. M. EDMONDS. 2008. *Toona* (Endlicher) M. Roemer. *Flora of China* 11: 112-115.

HUGHES, C., and R. EASTWOOD. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences* 103: 10334-10339.

HUTCHINSON, G. E. 1957. Concluding remarks. *Cold Spring Harbor Symp Quantitative Biology* 22: 415-427.

IGIC, B., L. BOHS, and J. R. KOHN. 2006. Ancient polymorphism reveals unidirectional breeding system shifts. *Proceedings of the National Academy of Sciences of the United States of America* 103: 1359-1363.

JAHREN, A. H. 2007. The Arctic forest of the middle Eocene. *Annu. Rev. Earth Planet. Sci.* 35: 509-540.

JAKOB, S. S., and F. R. BLATTNER. 2006. A Chloroplast Genealogy of Hordeum (Poaceae): Long-Term Persisting Haplotypes, Incomplete Lineage Sorting, Regional Extinction, and the Consequences for Phylogenetic Inference. *Molecular Biology and Evolution* 23: 1602-1612.

JANZEN, D. H. 1967. Why Mountain Passes are Higher in the Tropics. *The American Naturalist* 101: 233-249.

KAIMOWITZ, D. 1996. Livestock and deforestation in Central America in the 1980s and 1990s: a policy perspective, vol. 9. Cifor.

KARRENBERG, S., P. J. EDWARDS, and J. KOLLMANN. 2002. The life history of Salicaceae living in the active zone of floodplains. *Freshwater Biology* 47: 733-748.

KAWECKI, T. 1995. Demography of source—sink populations and the evolution of ecological niches. *Evolutionary Ecology* 9: 38-44.

KOECKE, A. V., A. N. MUELLNER-RIEHL, T. D. PENNINGTON, G. SCHORR, and J. SCHNITZLER. 2013. Niche evolution through time and across continents: The story of Neotropical Cedrela (Meliaceae). *American Journal of Botany* 100: 1800-1810.

KOZAK, K. H., and J. WIENS. 2006. Does niche conservatism promote speciation? A case study in North American salamanders. *Evolution* 60: 2604-2621.

KOZAK, K. H., and J. J. WIENS. 2007. Climatic zonation drives latitudinal variation in speciation mechanisms. *Proceedings of the Royal Society B: Biological Sciences* 274: 2995-3003.

LAMARCK, J. B. P. A. D. Monet de. 1809. Philosophie zoologique, ou, Exposition des considérations relative à l'histoire naturelle des animaux.

LEGRAND, P. 2003. Inventaire de la macroflore du Miocene superieur de la diatomite de Murat (Cantal, Masif Central, france). *Ann. Soc. Géol. du Nord* 2: 25-55.

LOSOS, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* 11: 995-1003.

_____. 2011. Seeing the Forest for the Trees: The Limitations of Phylogenies in Comparative Biology. *The American Naturalist* 177: 709-727.

LÖTSCHERT, W., and K. MÄDLER. 1975. Die plio-pleistozäne Flora aus dem Sisimico-Tal, El Salvador. Schweizerbart, Stuttgart, Germany.

MACGINITIE, H. D. 1941. A middle Eocene flora from the Central Sierra Nevada. *Carnegie Inst. Washington Publ.* 534: 1-94.

_____. 1974. An early middle Eocene flora from the Yellowstone-Absaroka volcanic province, northwestern Wind River Basin, Wyoming

University of California Press (Berkeley).

MANCHESTER, S. R., and W. C. MCINTOSH. 2007. Late Eocene silicified fruits and seeds from the John Day Formation near Post, Oregon. . *PaleoBios* 27: 7–17.

MAYR, E. 1996. What Is a Species, and What Is Not? *Philosophy of Science* 63: 262-277.

MCCAIN, C. M. 2009. Global analysis of bird elevational diversity. *Global Ecology and Biogeography* 18: 346-360.

MENDEL, G. 1866. Versuche über Pflanzenhybriden. *Verhandlungen des naturforschenden Vereines in Brunn 4: 3* 44.

MEYER, H. W., and S. R. MANCHESTER. 1997. The Oligocene Bridge Creek flora of the John Day Formation, Oregon. University of California Press (Berkeley).

MITTELBACH, G. G., D. W. SCHEMSKE, H. V. CORNELL, A. P. ALLEN, J. M. BROWN, M. B. BUSH, S. P. HARRISON, et al. 2007. Evolution and the latitudinal diversity

gradient: speciation, extinction and biogeography. *Ecology Letters* 10: 315-331.

MITTERMEIER, R. A., N. MYERS, J. B. THOMSEN, G. A. B. DA FONSECA, and S. OLIVIERI. 1998. Biodiversity Hotspots and Major Tropical Wilderness Areas: Approaches to Setting Conservation Priorities. *Conservation Biology* 12: 516-520.

MITTERMEIER, R. A., W. R. TURNER, F. W. LARSEN, T. M. BROOKS, and C. GASCON. 2011. Global biodiversity conservation: the critical role of hotspots, Biodiversity Hotspots, 3-22. Springer.

MODLISZEWSKI, J. L., D. T. THOMAS, C. FAN, D. J. CRAWFORD, C. W. DEPAMPHILIS, and Q.-Y. XIANG. 2006. Ancestral chloroplast polymorphism and historical secondary contact in a broad hybrid zone of Aesculus (Sapindaceae). *American Journal of Botany* 93: 377-388.

MONTES, C., A. CARDONA, R. MCFADDEN, S. E. MORÓN, C. A. SILVA, S. RESTREPO-MORENO, D. A. RAMÍREZ, et al. 2012. Evidence for middle Eocene and younger land emergence in central Panama: Implications for Isthmus closure. *Geological Society of America Bulletin* 124: 780-799.

MOORE, J. A. 1983. Thomas Hunt Morgan—The Geneticist. *American Zoologist* 23: 855-865.

MOSBRUGGER, V., T. UTESCHER, and D. L. DILCHER. 2005. Cenozoic continental climatic evolution of Central Europe. *Proceedings of the National Academy of Sciences of the United States of America* 102: 14964-14969.

MUELLNER, A. N., T. D. PENNINGTON, and M. W. CHASE. 2009. Molecular phylogenetics of Neotropical Cedreleae (mahogany family, Meliaceae) based on nuclear and plastid DNA sequences reveal multiple origins of “Cedrela odorata”. *Molecular Phylogenetics and Evolution* 52: 461-469.

MUELLNER, A. N., V. SAVOLAINEN, R. SAMUEL, and M. W. CHASE. 2006. The mahogany family “out-of-Africa”: Divergence time estimation, global biogeographic patterns inferred from plastid rbcL DNA sequences, extant, and fossil distribution of diversity. *Molecular Phylogenetics and Evolution* 40: 236-250.

MUELLNER, A. N., T. D. PENNINGTON, A. V. KOECKE, and S. S. RENNER. 2010. Biogeography of Cedrela (Meliaceae, Sapindales) in Central and South America. *American Journal of Botany* 97: 511-518.

MUELLNER, A. N., R. SAMUEL, M. W. CHASE, C. M. PANNELL, and H. GREGER. 2005. Aglaia (Meliaceae): an evaluation of taxonomic concepts based on DNA data and secondary metabolites. *American Journal of Botany* 92: 534-543.

MUELLNER, A. N., R. SAMUEL, S. A. JOHNSON, M. CHEEK, T. D. PENNINGTON, and M. W. CHASE. 2003. Molecular phylogenetics of Meliaceae (Sapindales) based on nuclear and plastid DNA sequences. *American Journal of Botany* 90: 471-480.

MULCH, A., C. E. UBA, M. R. STRECKER, R. SCHOENBERG, and C. P. CHAMBERLAIN. 2010. Late Miocene climate variability and surface elevation in the central Andes. *Earth and Planetary Science Letters* 290: 173-182.

MYERS, N. 1988. Threatened biotas: "Hot spots" in tropical forests. *Environmentalist* 8: 187-208.

_____. 1990. The biodiversity challenge: Expanded hot-spots analysis. *Environmentalist* 10: 243-256.

MYERS, N., R. A. MITTERMEIER, C. G. MITTERMEIER, G. A. B. DA FONSECA, and J. KENT. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

NOVICK, R. R., C. W. DICK, M. R. LEMES, C. NAVARRO, A. CACCONE, and E. BERMINGHAM. 2003. Genetic structure of Mesoamerican populations of Big-leaf mahogany (Swietenia macrophylla) inferred from microsatellite analysis. *Molecular Ecology* 12: 2885-2893.

PAGEL, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877-884.

PALME, A. E., Q. SU, S. PALSSON, and M. LASCOUX. 2004. Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among Betula pendula, B. pubescens and B. nana. *Molecular Ecology* 13: 167-178.

PARKER, T., J. CARRIÓN, and R. SASMUDIO. 2004. Tropical Forest Conservation, Protection, and Management in Panama: Assessment and Recommendations. *Section 118/119 Biodiversity and Tropical Forestry Assessment of the USAID/Panama Program. Submitted to USAID/Panama by Chemonics International Inc.*

PARMESAN, C., and G. YOHE. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37-42.

PEARMAN, P. B., A. GUISAN, O. BROENNIMANN, and C. F. RANDIN. 2008. Niche dynamics in space and time. *Trends in Ecology & Evolution* 23: 149-158.

PEARSON, R. G., and T. P. DAWSON. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12: 361-371.

PENNINGTON, R. T., and C. W. DICK. 2004. The role of immigrants in the assembly of the South American rainforest tree flora. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 1611-1622.

PENNINGTON, R. T., and J. A. RATTER. 2010. Neotropical savannas and seasonally dry forests: plant diversity, biogeography, and conservation. CRC Press.

PENNINGTON, R. T., and C. W. DICK. 2010. Diversification of the Amazonian flora and its relation to key geological and environmental events: a molecular perspective.

PENNINGTON, R. T., M. LAVIN, D. E. PRADO, C. A. PENDRY, S. K. PELL, and C. A. BUTTERWORTH. 2004. Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359: 515-538.

PENNINGTON, R. T., M. LAVIN, T. SÄRKINEN, G. P. LEWIS, B. B. KLITGAARD, and C. E. HUGHES. 2010. Contrasting plant diversification histories within the Andean biodiversity hotspot. *Proceedings of the National Academy of Sciences* 107: 13783-13787.

PENNINGTON, T., and B. T. STYLES. 1975. A generic monograph of the Meliaceae. *Blumea* 22: 419-540.

PENNINGTON, T. D., and A. N. MUELLNER. 2010. A monograph of Cedrela (Meliaceae). dh books, Sherborne, UK.

PERRET, M., A. CHAUTEMS, R. SPICHIGER, T. G. BARRACLOUGH, and V. SAVOLAINEN. 2007. THE GEOGRAPHICAL PATTERN OF SPECIATION AND FLORAL DIVERSIFICATION IN THE NEOTROPICS: THE TRIBE SINNINGIEAE (GESNERIACEAE) AS A CASE STUDY. *Evolution* 61: 1641-1660.

PETIT, R. J., and A. HAMPE. 2006. Some Evolutionary Consequences of Being a Tree. *Annual Review of Ecology, Evolution, and Systematics* 37: 187-214.

PETIT, R. J., F. S. HU, and C. W. DICK. 2008. Forests of the Past: A Window to Future Changes. *Science* 320: 1450-1452.

PFENNINGER, M., and K. SCHWENK. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology* 7: 121.

PHILLIPS, S. J., and M. DUDÍK. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* 31: 161-175.

PONS, D., and D. D. FRANCESCHI. 2007. Neogene woods from western Peruvian Amazon and palaeoenvironmental interpretation. *Bulletin of Geosciences* 82: 343–354.

POOLE, I. 2000. Fossil angiosperm wood: its role in the reconstruction of biodiversity and palaeoenvironment. *Botanical Journal of the Linnean Society* 134: 361-381.

POSADA, D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25: 1253-1256.

POSADA, D., and K. A. CRANDALL. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution* 16: 37-45.

PUENTES, D. A. 2005. Flora de la Republica de Cuba. A. R. Ganther Verlag KG, Liechtenstein.

PURVIS, A., and A. HECTOR. 2000. Getting the measure of biodiversity. *Nature* 405: 212-219.

R CORE TEAM. 2012. R: A Language and Environment for Statistical Computing. website: http://www.R-project.org.

RAMOS, A. C. S., J. P. LEMOS-FILHO, R. A. RIBEIRO, F. R. SANTOS, and M. B. LOVATO. 2007. Phylogeography of the tree Hymenaea stigonocarpa (Fabaceae: Caesalpinioideae) and the influence of Quaternary climate changes in the Brazilian Cerrado. *Annals of botany* 100: 1219-1228.

REID , E. M., and M. E. J. CHANDLER. 1933. The London Clay flora. British Museum (Natural History), London, UK.

REID, E. M., and M. E. J. CHANDLER. 1933. The London Clay flora. British Museum (Natural History), London, UK.

RETALLACK, G. J. 2004. Late Oligocene bunch grassland and early Miocene sod grassland paleosols from central Oregon, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology* 207: 203-237.

RETALLACK, G. J., and M. X. KIRBY. 2007. Middle Miocene global change and paleogeography of Panama. *PALAIOS* 22: 667-679.

RETALLACK, J., E. A. BESTLAND, and T. J. FREMD. 2000. Eocene and Oligocene Paleosols of Central Oregon. The Geological Society of America, Boulder, Colorado.

RICHARDSON, J. E., R. T. PENNINGTON, T. D. PENNINGTON, and P. M. HOLLINGSWORTH. 2001. Rapid Diversification of a Species-Rich Genus of Neotropical Rain Forest Trees. *Science* 293: 2242-2245.

RICKLEFS, R. E. 2006. Evolutionary diversification and the origin of the diversity-environment relationship. *Ecology* 87: S3-S13.

RIESEBERG, L. H. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany* 82: 944-953.

RIESEBERG, L. H., and L. BROUILLET. 1994. Are Many Plant Species Paraphyletic? *Taxon* 43: 21-32.

RIESEBERG, L. H., and J. H. WILLIS. 2007. Plant Speciation. *Science* 317: 910-914.

RIESEBERG, L. H., T. E. WOOD, and E. J. BAACK. 2006. The nature of plant species. *Nature* 440: 524-527.

ROIRON, P. 1991. La macroflore d'age Miocene superieur des Diatomites de Murat (Cantal, France) - Implications Paleoclimatiques, Paleontographica Abt B, 169-203. 1991 E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D. L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, et al. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61: 539-542.

RYMER, P. D., C. W. DICK, G. G. VENDRAMIN, A. BUONAMICI, and D. BOSHIER. 2013. Recent phylogeographic structure in a widespread 'weedy' Neotropical tree species, Cordia alliodora (Boraginaceae). *Journal of Biogeography* 40: 693-706.

SANG, T., D. J. CRAWFORD, and T. F. STUESSY. 1997. Chloroplast DNA Phylogeny, Reticulate Evolution, and Biogeography of Paeonia (Paeoniaceae). *American Journal of Botany* 84: 1120-1136.

SÄRKINEN, T., R. T. PENNINGTON, M. LAVIN, M. F. SIMON, and C. E. HUGHES. 2012. Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests. *Journal of Biogeography* 39: 884-900.

SCHLUTER, D., T. PRICE, A. Ø. MOOERS, and D. LUDWIG. 1997. Likelihood of Ancestor States in Adaptive Radiation. *Evolution* 51: 1699-1711.

SCOTTI-SAINTAGNE, C., C. W. DICK, H. CARON, G. G. VENDRAMIN, E. GUICHOUX, A. BUONAMICI, C. DURET, et al. 2013. Phylogeography of a species complex of lowland Neotropical rain forest trees (Carapa, Meliaceae). *Journal of Biogeography* 40: 676-692.

SEEHAUSEN, O. 2004. Hybridization and adaptive radiation. *Trends in Ecology & Evolution* 19: 198-207.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. LIU, J. MILLER, K. C. SIRIPUN, et al. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

SHELDON, NATHAN D., and GREGORY J. RETALLACK. 2004. Regional Paleoprecipitation Records from the Late Eocene and Oligocene of North America. *The Journal of Geology* 112: 487-494.

SILVESTRO, D., and I. MICHALAK. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335-337.

SILVESTRO, D., G. ZIZKA, and K. SCHULTE. 2014. DISENTANGLING THE EFFECTS OF KEY INNOVATIONS ON THE DIVERSIFICATION OF BROMELIOIDEAE (BROMELIACEAE). *Evolution* 68: 163-175.

SIMON, M. F., R. GRETHER, L. P. DE QUEIROZ, C. SKEMA, R. T. PENNINGTON, and C. E. HUGHES. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proceedings of the National Academy of Sciences* 106: 20359-20364.

SIMPSON, G. G. 1951. The Species Concept. *Evolution* 5: 285-298.

SLUIJS, A., S. SCHOUTEN, M. PAGANI, M. WOLTERING, H. BRINKHUIS, J. S. S. DAMSTÉ, G. R. DICKENS, et al. 2006. Subtropical Arctic Ocean temperatures during the Palaeocene/Eocene thermal maximum. *Nature* 441: 610-613.

SMALL, R. L., J. A. RYBURN, R. C. CRONN, T. SEELANAN, and J. F. WENDEL. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear Adh sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* 85: 1301-1315.

SOBERÓN, J., and A. T. PETERSON. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics* 2: 1-10.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

STEPHENS, PATRICK R., and JOHN J. WIENS. 2003. Explaining Species Richness from Continents to Communities: The Time-for-Speciation Effect in Emydid Turtles. *The American Naturalist* 161: 112-128.

STYLES, B. T., and C. G. VOSA. 1971. Chromosome Numbers in the Meliaceae. *Taxon* 20: 485-500.

TANAI, T. 1970. The Oligocene Floras of the Kushiro Coal Field. *Journal of the Faculty of Science, Hokkaido University* Series 4, Geology and Mineralogy 383-514.

TANAI, T., and N. SUZUKI. 1963. Miocene floras of southwestern Hokkaido, Japan. Collaborative Association to Commemorate the 80th Anniversary of the Geological Survey of Japan, Tokyo, Japan.

TEODORIDIS, V., P. MAZOUCH, R. A. SPICER, and D. UHL. 2011. Refining CLAMP — Investigations towards improving the Climate Leaf Analysis Multivariate Program. *Palaeogeography, Palaeoclimatology, Palaeoecology* 299: 39-48.

THUILLER, W. 2007. Biodiversity: Climate change and the ecologist. *Nature* 448: 550-552.

WALLACE, A. R. 1878. Tropical Nature: And Other Essays. Macmillan and Company.

WARREN, D. L., R. E. GLOR, and M. TURELLI. 2008. Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution* 62: 2868-2883.

WEIGAND, A. M., M.-C. GÖTZE, and A. JOCHUM. 2012. Outdated but established?! Conchologically driven species delineations in microgastropods (Carychiidae, Carychium). *Organisms Diversity & Evolution* 12: 377-386.

WEIJERS, J. W. H., S. SCHOUTEN, A. SLUIJS, H. BRINKHUIS, and J. S. SINNINGHE DAMSTÉ. 2007. Warm arctic continents during the Palaeocene–Eocene thermal maximum. *Earth and Planetary Science Letters* 261: 230-238.

WIENS, J. J., and M. J. DONOGHUE. 2004. Historical biogeography, ecology and species richness. *Trends in Ecology & Evolution* 19: 639-644.

WIENS, J. J., and C. H. GRAHAM. 2005. Niche Conservatism: Integrating Evolution, Ecology, and Conservation Biology. *Annual Review of Ecology, Evolution, and Systematics* 36: 519-539.

WIENS, J. J., D. D. ACKERLY, A. P. ALLEN, B. L. ANACKER, L. B. BUCKLEY, H. V. CORNELL, E. I. DAMSCHEN, et al. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters* 13: 1310-1324.

WILEY, E. O. 1978. The Evolutionary Species Concept Reconsidered. *Systematic Biology* 27: 17-26.

WING, S. L., and D. R. GREENWOOD. 1993. Fossils and Fossil Climate: The Case for Equable Continental Interiors in the Eocene. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 341: 243-252.

WING, S. L., T. M. BROWN, and J. D. OBRADOVICH. 1991. Early Eocene Biotic and Climate-Change in Interior Western North-America. *Geology* 19: 1189-1192.

WOLFE, J. A. 1977. Paleogene Floras from the Gulf of Alaska Region, 1-99. U.S. Govt. Print. Off. (Washington)

_____. 1978. A Paleobotanical Interpretation of Tertiary Climates in the Northern Hemisphere: Data from fossil plants make it possible to reconstruct Tertiary climatic changes, which may be correlated with changes in the inclination of the earth's rotational axis. *American Scientist* 66: 694-703.

_____. 1985. Distribution of Major Vegetational Types during the Tertiary, 357-375. Eric T. Sundquist,Wallace S. Broecker, Editors.

_____. 1994. Tertiary climatic changes at middle latitudes of western North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 108: 195-205.

ZACHOS, J., M. PAGANI, L. SLOAN, E. THOMAS, and K. BILLUPS. 2001. Trends, Rhythms, and Aberrations in Global Climate 65 Ma to Present. *Science* 292: 686-693.

ZACHOS, J. C., G. R. DICKENS, and R. E. ZEEBE. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* 451: 279-283.

ZANAZZI, A., M. J. KOHN, B. J. MACFADDEN, and D. O. TERRY. 2007. Large temperature drop across the Eocene-Oligocene transition in central North America. *Nature* 445: 639-642.

ZIZKA, G., I. MICHALAK, S. HELLER, and M. SCHMIDT. 2013. Ohne die klassische Taxonomie ist nichts zu machen. *Natur, Forschung, Museum* 143: 236-241.

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Appendix

TABLE A. Species included in Chapter I with their author names, voucher information, geographic origin, and GenBank accession numbers.

Taxon	Voucher information	Origin	GenBank accessions			
			ITS	*psbA-trnH*	*trnS-G*	*psbB-T-N*
Cedrela angustifolia C.DC.	Muellner 2054 (K)	Peru	KC155957	KC155970		
	Wood et al. 19222 (K)	Bolivia			FJ462508	FJ462540
Cedrela balansae C.DC.	Soria 2114 (K)	Paraguay		KC155981		
	Zapater & Castillo 2406 (K)	Argentina	FJ462473		FJ462505	FJ462537
Cedrela cubensis Bisse	Cuba excursion 462, Goethe University Frankfurt/Main (FR)	Cuba	KC155961	KC155978	—	—
Cedrela dugesii S. Watson	Styles A33/79 (Cavers, DNA-isolate 153841)	Mexico	KC155955			
	Germán et al. 450 (FHO)			KC155965	FJ462513	FJ462545
Cedrela fissilis Vell.	Kegler 321 (Z)	Brazil	KC155960	KC155980		
	Muellner 2064 (K)	Peru			FJ462507	FJ462539

Cedrela kuelapensis T.D.Penn. & Daza	Pennington et al. 17596 (K)	Peru		KC155979		
	Pennington et al., 17583 (K)		FJ462470		FJ462502	FJ462534
Cedrela molinensis T.D.Penn. & Reynel	Daza 1551 (K)	Peru		KC155975		
	Pennington et al. 17765 (K)		FJ462466		FJ462498	FJ462530
Cedrela monroensis T.D.Penn.	Monroe & Alexander 3081 (K)	El Salvador	FJ462486	KC155966	FJ462516	FJ462548
Cedrela montana Moritz ex Turczaninov	Pennington et al. 17623 (K)	Peru	FJ462480	KC155972	FJ462510	FJ462542
Cedrela nebulosa T.D.Penn. & Daza	Muellner 2056 (K)	Peru	FJ462461	KC155971	FJ462493	FJ462525
Cedrela oaxancensis C.DC. & Rose	Styles A33/79 (Cavers, DNA-isolate, 153862)	Mexico		KC155968		
	Mendoza et al., 291 (K)		FJ462482		FJ462512	
	Cedillo et al., 880 (FHO)					FJ462544

Cedrela odorata L. s.str.	Lott 3568 (K)	Mexico		KC155977		
	Pratt 0010 (K)	West Indies, Antigua	GU338247			
	Villacorta & Berendsohn 271 (K)	El Salvador			FJ462500	FJ462532
Cedrela cf. *odorata* (group II)	Koecke et al. 280409-03 (FR)	Panama		KC155976		
	Muellner 2056 (K)	Peru	FJ462461		FJ462493	FJ462525
Cedrela saltensis M.A. Zapater & del Castillo	Pennington 17635 (K)	Peru	KC155959			
	Pennington 17750 (K)	Peru		KC155973		
	Zapater 2348 (K)	Argentina			FJ462526	GU295815
Cedrela salvadorensis Standley	Breedlove 26871 (MO)	Mexico	KC155958			
				KC155967		
	Formoso 2 (K)	Costa Rica			FJ462514	FJ462546
Cedrela sp. nov.	Koecke et al. 180409-01 (FR)	Panama	KC155962	KC155982		
Cedrela tonduzii C.DC.	German 832 (FHO)	Mexico	KC155956	KC155969		
	Styles 82 (K)	Costa Rica			FJ462547	GU295830

Cedrela weberbaueri Harms	Pennington et al. 17901 (K)	Peru		KC155974		
	Daza 4012 (K)		FJ462472		FJ462536	GU295822
Toona calantas Merr. & Rolfe	Cabrera JFC-2012-099RP (FR)	Philippines	KC155953	—	—	—
Toona ciliata M. Roem.	Edmonds T 61 (DNA-isolate, FR)	USA, Hawaii		KC155963		
	PIF25085, AQ606666 (K)	Australia	FJ462488		FJ462517	FJ462549
Toona sinensis M. Roem	Wan & Chow 79175 (K)	China	FJ462490	—	FJ462518	FJ462551
Toona sureni (Blume) Merr.	Edmonds T17 (DNA-isolate, FR)	Malaysia	KC155954	KC155964	—	—
Swietenia macrophylla King	Chase 250 (NCU)	USA	DQ861609	—	FJ462521	FJ462554

TABLE B. Modeled climatic tolerances in *Cedrela* (weighted mean values for each of the seven bioclimatic variables that were used in Maxent).

	SC1								SC2									
	Central America					South America, Andees			South America					Central- and South America				
	monr	*dug*	*ton*	*salv*	*oax*	group C	*ang*	*mont*	*kue*	*fis*	*mol*	*bal*	*web*	odo	*cub*	sp. nov	group B	*salt*	*neb*
BIO2	12.0	15.4	11.4	12.4	13.7	10.6	13.3	11.0	12.5	11.5	12.7	11.8	14.3	11.3	9.8	-	10.1	14.2	11.4
BIO4	8.5	21.9	10.1	13.5	14.4	4.2	21.74	3.1	5.6	15.9	12.4	34.1	9.7	15.6	14.77	-	8.8	27.6	3.6
BIO6:	15.6	5.5	13.4	13.5	9.9	16.1	6.7	9.9	6.6	13.8	13.2	10.2	5.7	15.0	16.8	-	17.8	4.4	11.4
BIO14	1.2	0	22.0	8.9	2.1	73.0	11.8	51.0	25.7	39.0	0	41.2	11.2	35.5	34.0	-	52.0	1.9	68.3
BIO15	85.6	97.1	71.5	84.0	91.8	44.9	71.4	42.5	46.3	55.5	105.0	50.0	60.1	62.4	55.2	-	53.2	78.3	39.2
BIO18	259.0	282.2	448.0	352.0	300.7	458.0	345.6	377.2	312.2	427.4	242.7	433.8	285.6	437.3	422.1	-	415.2	361.8	453.7
BIO19	166.0	3.0	221.0	146.6	19.3	458.3	42.0	296.9	59.5	217.2	0	158.0	29.6	221.0	139.3	-	521.6	1.6	363.3

Abbreviations of species names are the same as in Fig. 6 (Chapter I). In addition: mol = molinensis, web = weberbaueri. Mean diurnal Temperature *T* is given in °C, Precipitation *P* is given in mm. Abbreviations are: species names *monr = monroensis*, *dug = dugesii*, *ton = tonduzii*, *salv = salvadorensis*, *oax = oaxacensis*, *ang = angustifolia*, *mont = montana*, *kue = kuelapensis*, *fis = fissilis*, *mol = molinensis*, *bal = balansae*, *web = weberbaueri*, *cub = cubensis*, *salt = saltensis*, *neb = nebulosa*; SC1 = Subclade 1; SC2 = Subclade 2.

TABLE C. Fossil record of *Cedrela* from North and Central America.

(Abbreviations: mya = million years ago; MAT = mean annual temperature, CMMT = coldest monthly mean temperature, WMMT = warmest mean monthly temperature, MART = mean annual range of temperatures, MAP = mean annual precipitation)

	Geologic Time, mya	Formation	Vegetation	Climate	Literature
1	Early Eocene, 51-50	Wind River, NW Wyoming	?	Frost-free, humid, subtropical climatic conditions, MAT 16-18°C	Hickey and Hodges (1975), Wing (1991)
2	Early Eocene, 52-49	Chalk Bluffs, Sierra Nevada	Angiosperm-dominated subtropical forest with little to no grasses or gymnosperms, high elevation (>2000 m) range with moderate to low relief.	Very humid, MAT 22-24°C	MacGinitie (1941), Hren et al. (2010)
3	Late Middle Eocene 40.4-37.2	Samovar Hills, Kulthieth Formation, Gulf of Alaska	Paratropical or submontane rainforest of the paleotropical region, strong relationship to London Clay.	Subtropical MAT 19.4°C, CMMT 12.6 °C, WMMT 26.2°C, MART 13.6, abundant precipitation (>1500 mm), high atmospheric humidity, no pronounced dry season	Wolfe (1977), Greenwood et al. (2010)
4	Late Eocene, 36.21	Oregon John Day, near Post	Warm temperate forest of thermophilic character.	?	Manchester (2007)

5	Eocene-Oligocene boundary, 34	Florissant, Colorado	Subtropical flora at 900-1000m elevation to more temperate flora on volcanic highlands up to 2700m to the west.	MAT 24°C, MAP 525 mm	Manchester (2001), Graham (1993)
6	Early Oligocene, 33.6	Oregon John Day/Iron Mountain	Temperate mixed mesophytic forest, deciduous, affinities to E Asia and E North America, low to moderate relief from 500-1000m, surrounded by higher elevations from the Blue Mountains uplift.	MAT 8-12°C, probability of frost	Meyer and Manchester (1997)
7	Early Oligocene, 33.6-32.1	Oregon John Day/Cove Creek	"	"	"
8	Early Oligocene, 32.5	Oregon John Day/Fossil	"	"	"
9	Early Oligocene, 32.1	Oregon John Day/Crooked River and Lost Creek	"	"	"
10	Early Miocene 23-16	La Quinta, Simojovel, Chiapas (Mexico)	Tropical or subtropical forest.	Temperatures ≥24°C, MAP 2500 mm, evenly distributed	Graham (1999a)
11	Early Miocene, 18	Buffalo Canyon, W Nevada	Sclerophyll woodland.	MAT 10°C, mild winters, MAP 890-1000 mm	Axelrod (1991)
12	Early Miocene, 18.5	Middlegate Basin West, Central Nevada	Evergreen sclerophyll forest, partially also conifer hardwood forest; transition zone between humid-temperate region to the N and subtropical subhumid to the S.	MAT 12°C, temperatures ranging relativly equable from 5-20°C, MAP 760-890 mm, moist mild winters with little snow, summer rainfall	Axelrod (1985)

13	Early Miocene, 17	Carmel Valley, California	Oak-palm-laurel woodland and subtropical shrub on low elevation, about 4 km from the Pacific sea side.	MAT 14.5C°, no frost, MAP 640 mm (summer rain)	Axelrod (2000)
14	Late early Miocene, 16	Mascall and Latah, John Day basin	Humid, temperate climate (humid Mediterranean) with dry warm summers and cool winters, lowland deciduous forest with affinities to E and W North America and Szechuan, China.	Middle Miocene Climatic Optimum	Bestland et al. (2008)
15	Middle Miocene, 15	Temblor, California	Deciduous elements and few broad-leafed evergreens, near the coast at sea level	MAT 14-15°C, MAP 635 mm	Axelrod (1995, 2000)
16	Middle to Late Miocene 11.8-8.6	Gatún (Panama)	Lower to upper montane wet forest and/or tropical dry forest. First evidence of montane habitats in southern Central America.	MAT 27°C, MAP 1900-3600 mm, sharp dry season	Graham (1991a and b, 1992, 1997, 2010)
17	Middle Pliocene 3.6	Paraje Solo, Veracruz (Mexico)	High evergreen/semi-evergreen forest of restricted distribution.	MAT <25°C, MAP >1800 mm, seasonality, but no pronounced dry season, greater or uniformly distributed rainfall	Graham (1976,1999b)
18	Pliocene	Iquitos region (Peru)	?	seasonality with dry periods	Pons and Franceschi (2007)
19	Early Pleistocene 2-3	Cuscatlán, Sisimico Valley (El Salvador)	Tropical wet evergreen forest of the lowland 0-800 m.	MAT 22-27°C, no pronounced dry season, MAP >1700 mm	Lötschert and Mädler (1975)

TABLE D. Fossil record of *Cedrela* from Europe.

(Abbreviations: Cont. = continuing numbers, mya = million years ago; MAT = mean annual temperature, CMMT = coldest monthly mean temperature, WMMT = warmest mean monthly temperature, MART = mean annual range of temperatures, MAP = mean annual precipitation)

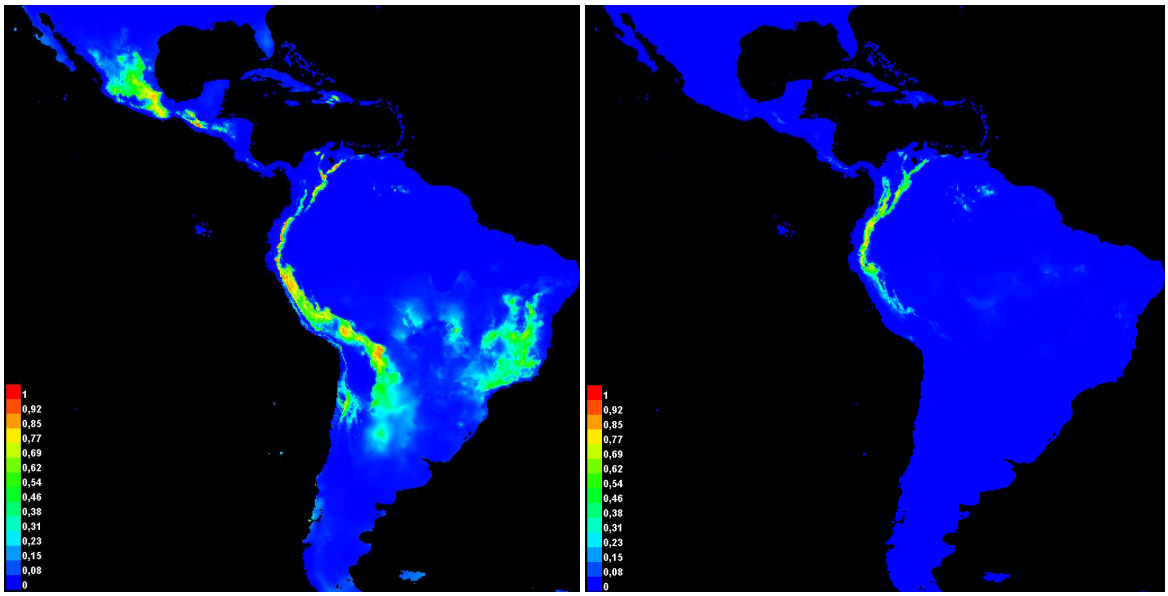
Cont.	Geologic Time, mya	Formation	Vegetation	Climate	Literature
20	Early Eocene	London Tard Clay, United Kingdom	Boreotropical flora, paratropical, no strong seasonality, but probably alternating pluvial and drier climatic periods. Indo-Malaysian affinities.	MART 20-27°C, mainly frostless	Reid and Chandler (1933), Chandler (1964) Daley (1972) Poole (2000)
21	Late Oligocene 28-24	Eger Wind, Vértesszölös and Kesztölc, Hungary	Warm-temperate to subtropical conditions with deciduous and evergreen plants, humid, affinities to Eastern Asia.	MAT 16-17°C, MAP 979-1250 mm	Hably (2006), Erdei et al. (2007)
22	Late Miocene	Murat, Cantal, France	(Temperate?) Mixed mesophytic forest with gymnosperms and many temperate elements. Constant humidity and temperate to mild winters.	MAT 10-13°C	Roiron (1991) Legrand (2003)

TABLE E. Fossil record of *Toona* (*Cedrela*) from Asia.

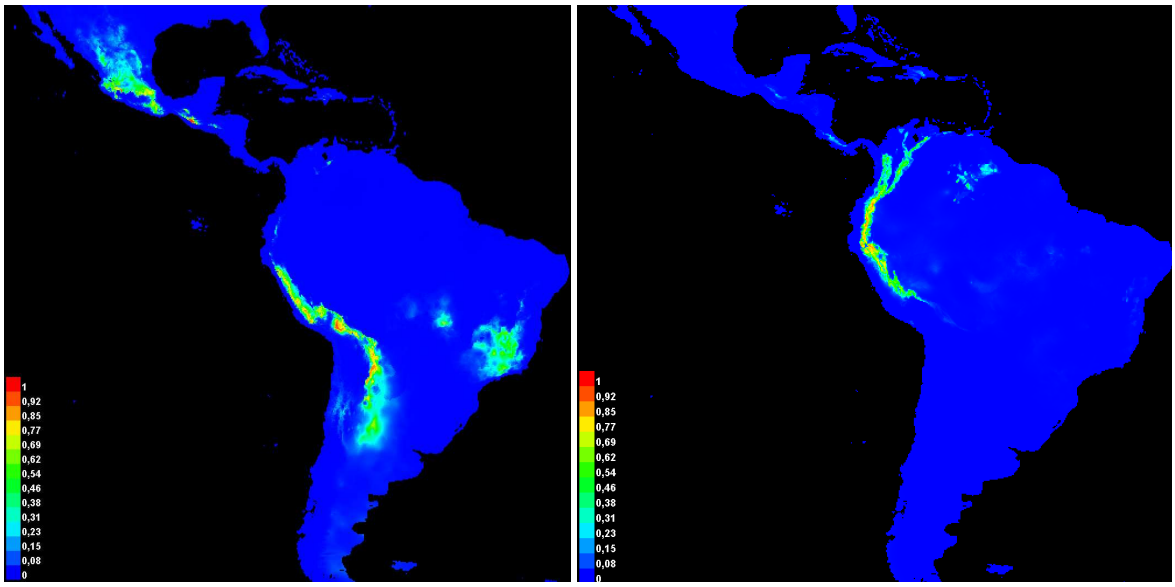
(Abbreviations: cont. = continuing numbers, mya = million years ago; MAT = mean annual temperature)

Cont.	Geologic Time, mya	Formation	Vegetation	Climate	Literature
23	Early Late Eocene 37.2-33.9	Kushiro Coal Field, Hokkaido, Japan	Warm-temperate forest, broad-leafed evergreen and deciduous hardwood trees in the lowlands with Arcto-Tertiary members - closely similar to the mixed mesophytic forest of Central China. Modern plants of East Asia and eastern North America.	MAT 13°C	Tanai (1970) Wolfe (1985)
24	Early Miocene 23-15.9	Yoshioka Formation, southwestern Hokkaido, 25Japan	Warm-temperate forest, mild maritime conditions, many deciduous hardwood trees, some evergreen taxa. Similarity to *Toona sinensis* (leaves) and *Cedrela sarmatica* (seed).	?	Tanai and Suzuki (1972)

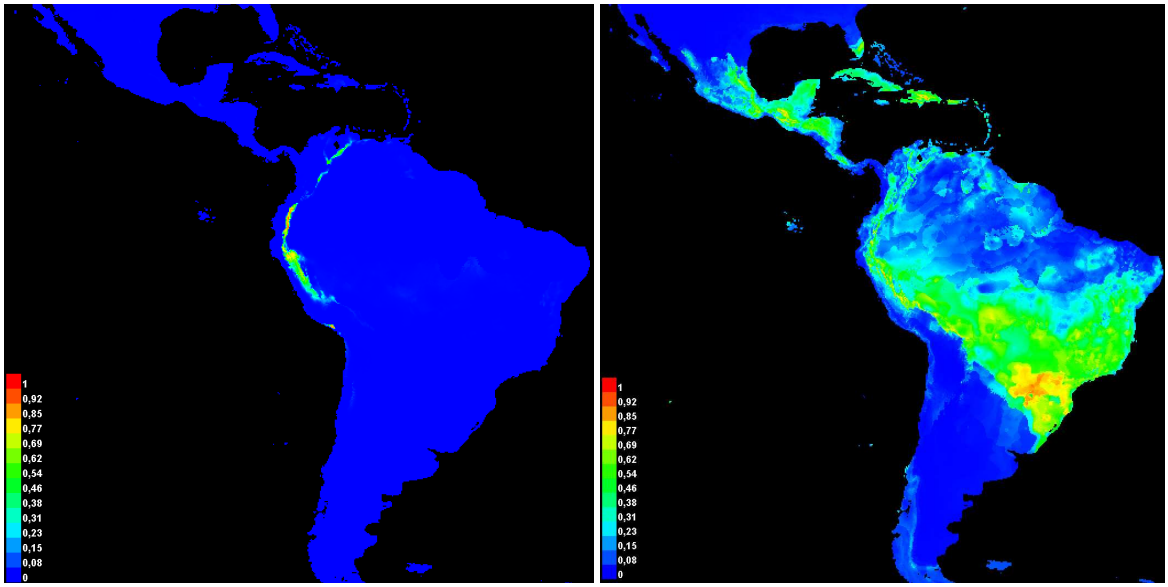
FIGURE I. Climatic niche models of 17 species of *Cedrela* (Maxent results), point-wise mean of the 10 output grids. Climatic suitability: low = 0 (blue), high = 1 (red).



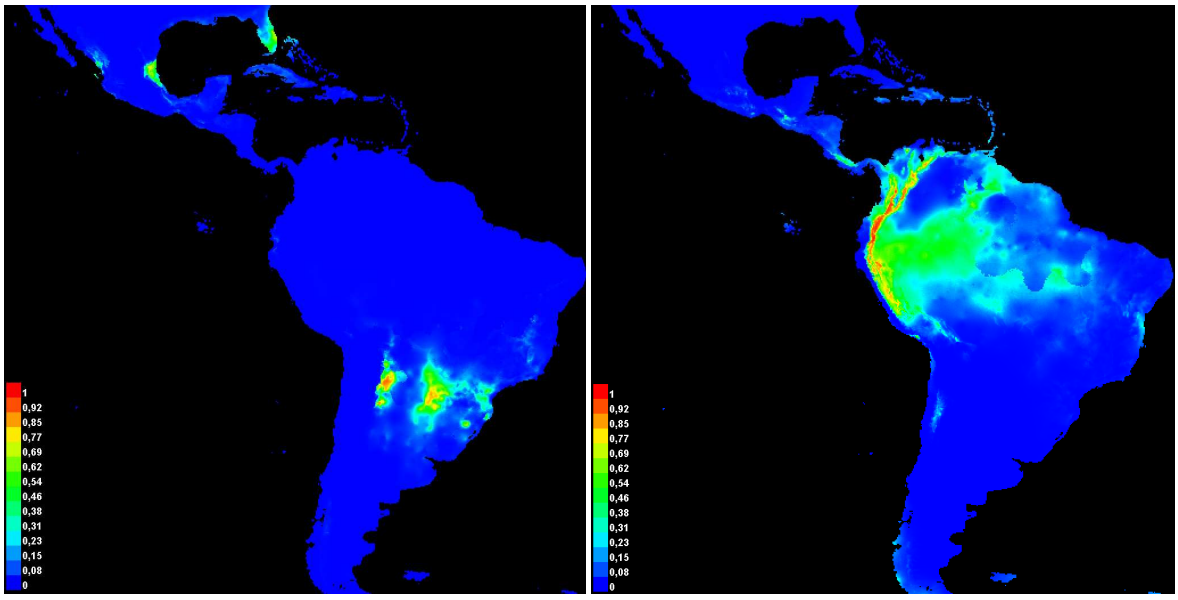
a) *C. angustifolia* (upper left) and **b)** *C. montana* (upper right), closely related Andean species



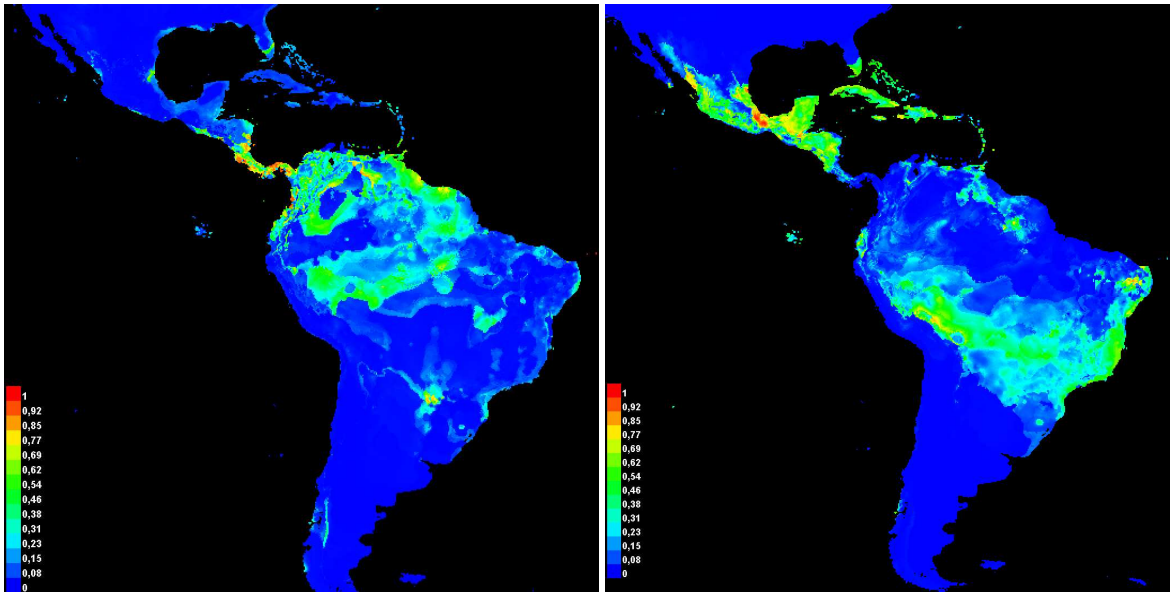
c) *C. saltensis* (lower left) and **d)** *C. nebulosa* (lower right), closely related Andean species



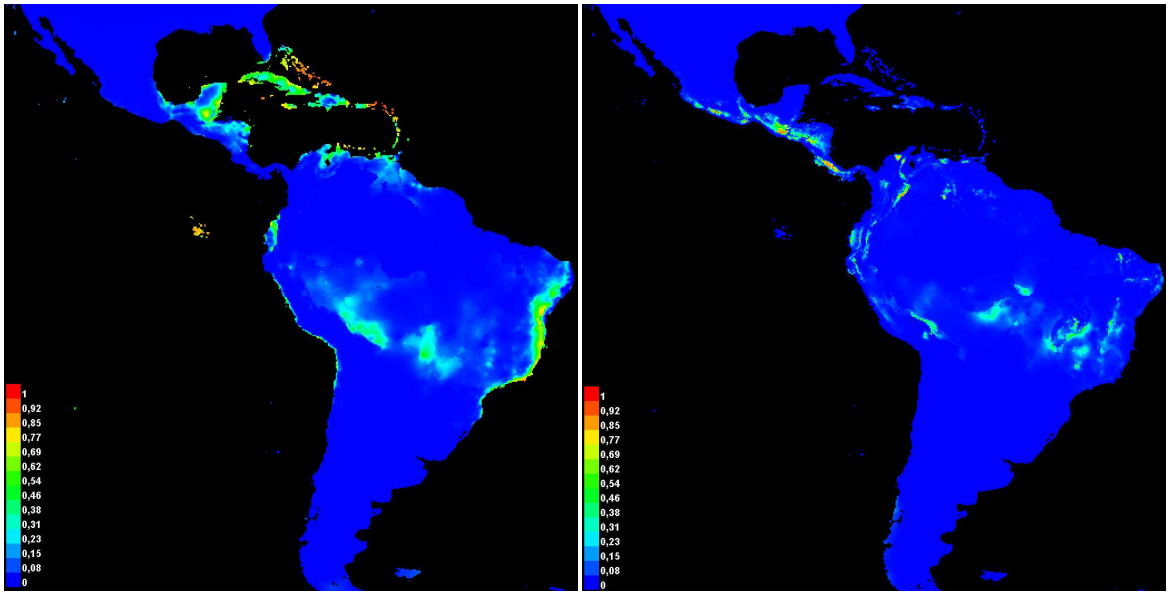
e) *C. kuelapensis* (upper left) and **f)** *C. fissilis* (upper right)



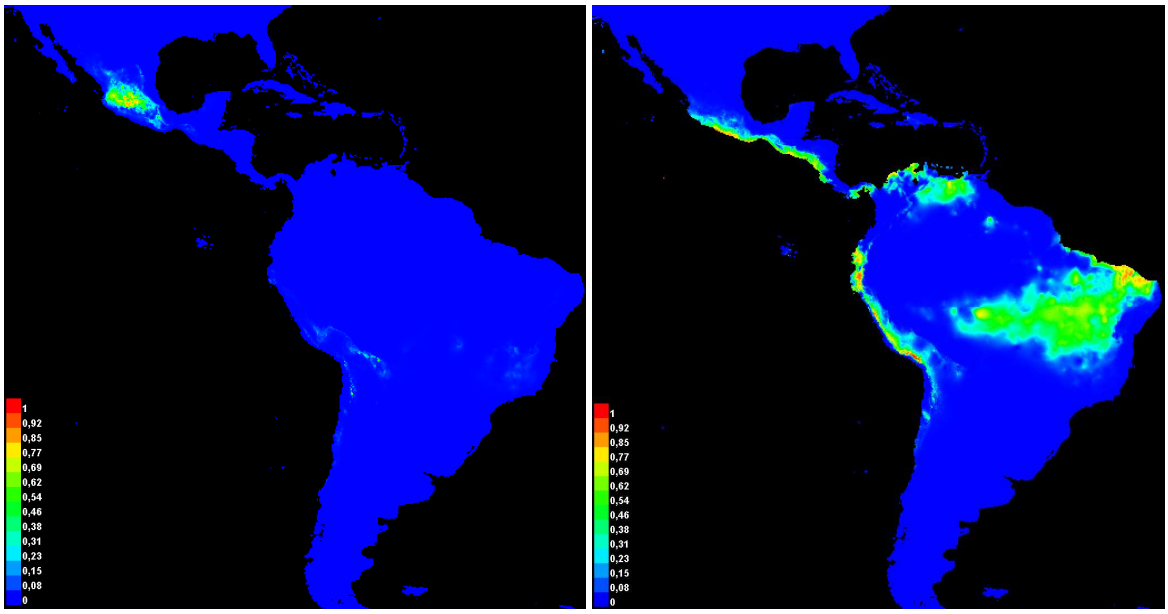
g) *C. balansae* (lower left) and **h)** *C.* aff. *odorata* group III (lower right)



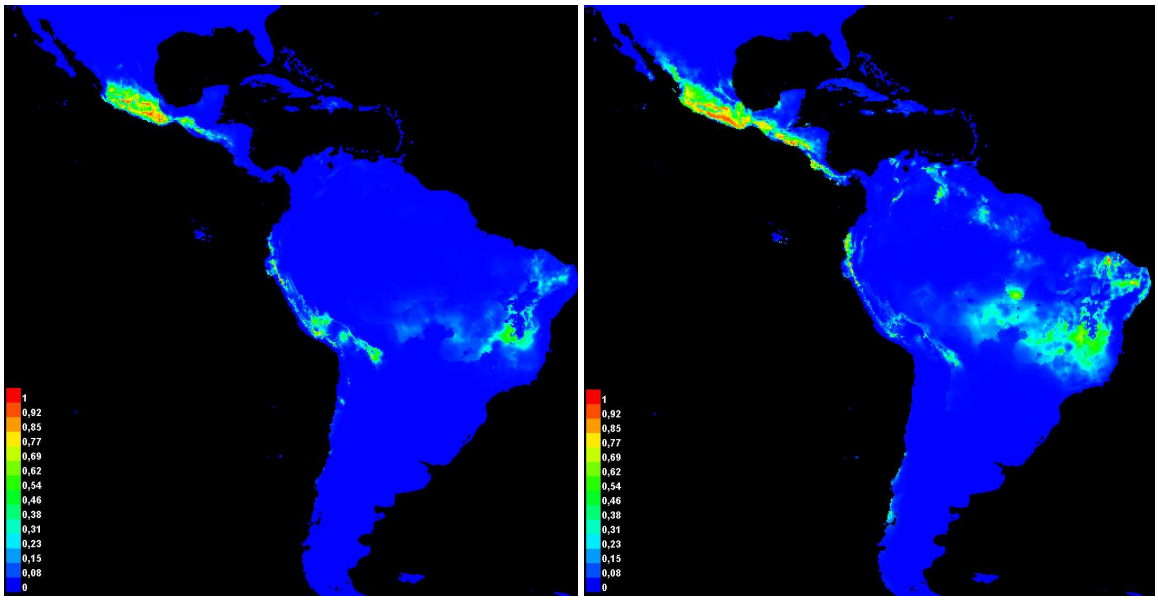
i) *C.* aff. *odorata* group II (upper left) and **j)** *C. odorata* senso stricto (upper right)



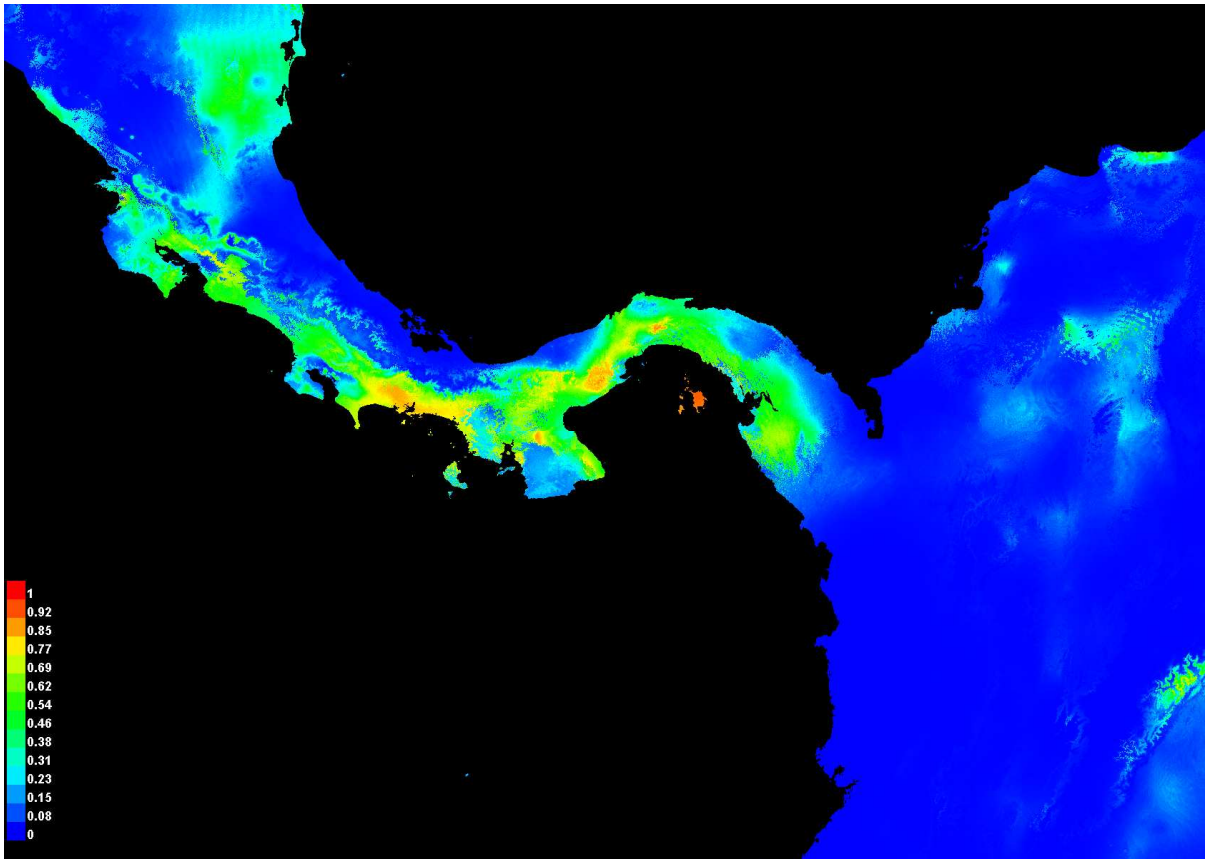
k) *C. cubensis* (lower left) and **l)** *C. tonduzii* (lower right)



m) *C. dugesii* (upper left) and **n)** *C. monroensis* (upper right)



o) *C. oaxacensis* (lower left) and **p)** *C. salvadorensis* (lower right)



q) Climatic niche model of *C. ngobe* Köcke, Penn. & Muellner-Riehl, based on the same seven variables that were used for the other models and niche reconstructions in Chapter I.

TABLE D. Haplotype list of *psbA-trnH* sequences.

H1	H2	H3	H4
C. aff. *odorata* (group II), Neill et al. 6230 , Ecuador	*C*. *balansae*, Zapater & Castillo 2406, Argentina	*C*. *cubensis*, Cuba excursion 412, Goethe University Frankfurt/Main (FR), Cuba	*C*. aff. *odorata* (group II), Koecke et al. 230406, Panama
C. aff. *fissilis*, Agra et al. 5014, Brazil	*C*. *balansae*, Soria 2114, Paraguay	*C*. aff. *odorata* (group II), Gentry, A. & Mejia, M. 50718, Dominican Republic	*C*. aff. *odorata* (group II), Neill, D. 2269, Nicaragua
C. *angustifolia*, Wood et al. 19222, Bolivia	*C*. *fissilis*, Beck 22420, Bolivia	*C*. aff. *odorata* (group II), Koecke et al. 280403, Panama	*C*. aff. *odorata* (group II), Koecke et al. 220402, Panama
C. *angustifolia*, Steudel BS402, Bolivia	*C*. *fissilis*, Steudel BS469, Bolivia	*C*. aff. *odorata* (group II), Koecke et al. 190405, Panama	*C*. aff. *odorata* (group II), Koecke et al. 230401, Panama
C. *angustifolia*, Steudel BS401, Bolivia	*C*. *molinensis*, Pennington, T.D. et al. 17765, Peru	*C*. aff. *odorata* (group II), Koecke et al. 230407, Panama	
C. *fissilis*, Muellner 2064, Peru	*C*. *oaxacensis*, Mendoza et al. 291, Mexico	*C*. sp. nov., Koecke et al. 220401, Panama	
C. *kuelapensis*, Pennington, et al. 17596, Peru	*C*. *odorata* s.s., Gentry, A. 8268, Belize	*C*. sp. nov., Koecke et al. 180401, Panama	
C. *longipetiolulata*, Foster 9914, Peru	*C*. *odorata* s.s., Pennington et al. 17901, Peru	*C*. sp. nov., Koecke et al. 180402, Panama	
C. *montana*, Idrobo, J.M. & R. Jaramillo 11145, Colombia	*C*. aff. *odorata*, Styles A16/93 FIII, Brazil		
C. *nebulosa*, SW0077, Ecuador	*C*. *paraguariensis*, Zardini, E. & Velázquez, H. 23167, Paraguay		
C. *odorata* s.s., Mori et al. 19208, French Guiana	*C*. *salvadorensis,* Breedlove, D.E. 26871, Mexico		

C. saltensis, Pennington, T.D. 17635, Peru *C. saltensis*, Beck et al. 9689, Bolivia *C. saltensis*, Zapater 2348, Argentina	*C. tonduzii*, Germán, M.T. 832, Mexico *C. weberbaueri*, Daza 4012, Peru		
H5	**H6**	**H7**	**H8**
C. cubensis, Cuba excursion 462, Goethe University Frankfurt/Main (FR), Cuba	*C. odorata* s.s, Lott 3568, Mexico (West) *C. discolor*, Palmer E. 184, Mexico (West)	*C. odorata* s.s, Pennington & Sarukhan 9653, Mexico (East) *C. odorata* s.s, Leonti 98, Mexico (East)	*C. odorata* s.s, Muellner, A.N. 2059, Peru *C. odorata* s.s, Villacorta, R. & Berendson, W. 271, El Salvador
H9	**H10**	**H11**	**H12**
C. montana, Palacios & Iguago 3046, Ecuador	*C.* sp. nov. 2, Piepenbring et al. MPE22, Ecuador	*C. angustifolia*, Muellner 2054, Peru *C. montana*, Piepenbring et al. MPE23, Ecuador *C. montana*, Pennington et al. 17623, Peru *C.* aff. *tonduzii*, Stevens, W.D. 22539, Nicaragua	*C. angustifolia*, Pennington et al. 1150, Peru

H13	H14	H15	H16
C. sp., Steudel BS434, Bolivia *C*. sp*.,* Steudel BS435, Bolivia *C*. sp*.,* 7292, Argentina	*C*. *angustifolia*, Muellner 2061, Peru	*C*. *nebulosa,* Pennington et al.17646	*C*. *odorata* s.s, Pratt, C.D. 0010, West Indies (Antigua)
H17	**H18**	**H19**	**H20**
C. *fissilis*, Steudel BS464, Bolivia	*C*. *fissilis*, Kegler 321, Brazil (Southeast)	*C*. aff. *balansae*, Oliveira, F.C.A. et al. 1182, Brazil (East)	*C*. *fissilis*, Steudel BS444, Bolivia *C*. *fissilis*, Steudel BS443, Bolivia
H21	**H22**	**H23**	
C. *monroensis,* Monroe & Alexander 308, El Salvador *C*. *dugesii*, Germán, M.T. et al. 450, Mexico	*C*. *tonduzii*, Styles 82, Costa Rica *C*. *tonduzii*, Koecke et al. 280406, Panama *C*. *tonduzii*, Cáceres 4056, Panama *C*. *tonduzii*, Cáceres 4114A, Panama *C*. *tonduzii*, Cáceres 4103, Panama	*C*. *salvadorensis*, Boshier 65, Honduras	

	C. tonduzii, Koecke et al. 270404, Panama		

TABLE E. Haplotype list of ITS sequences.

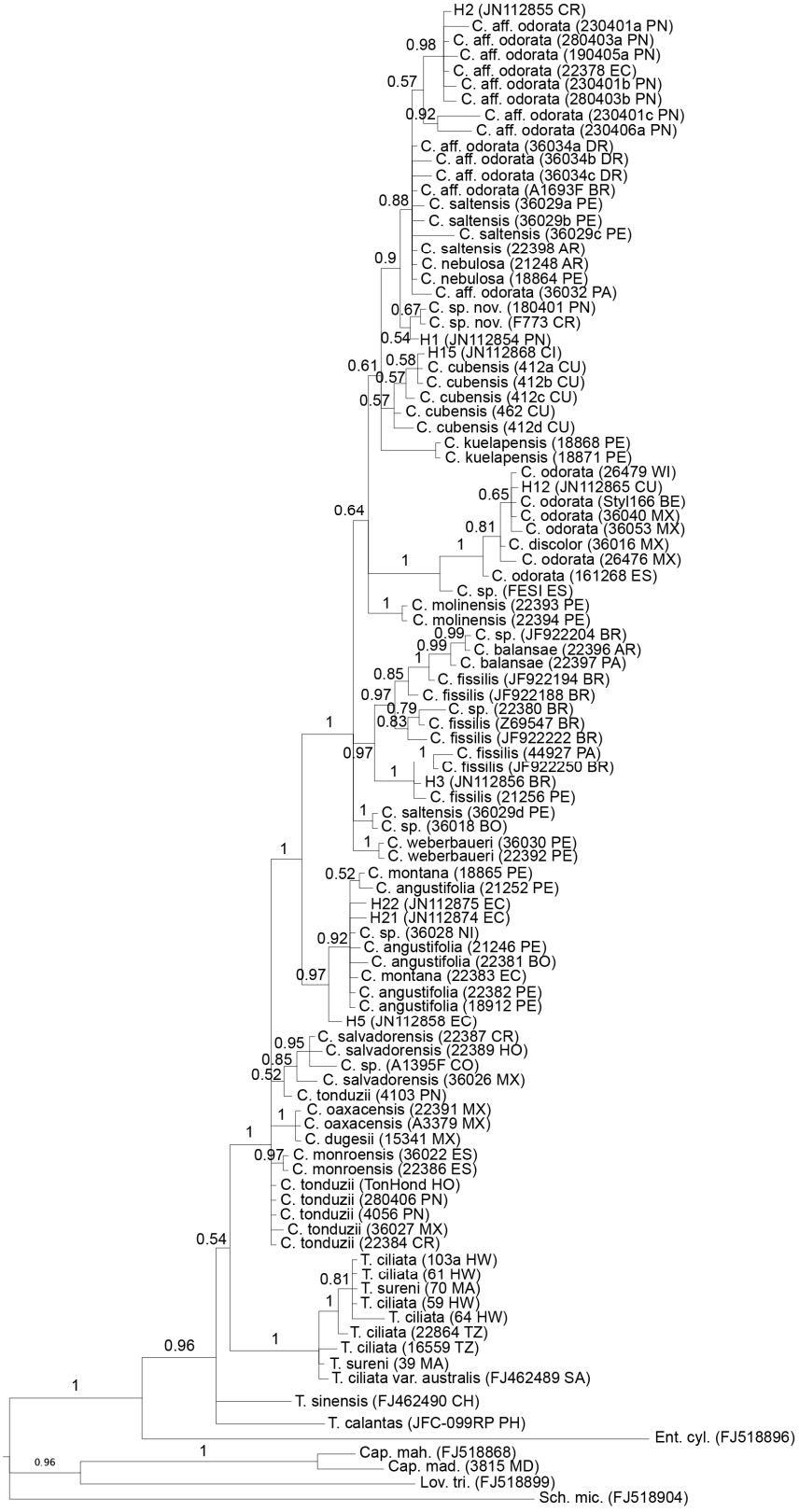
H1	H2	H3	H4
C. montana, Piepenbring et al. MPE23, Ecuador	*C. kuelapensis*, Pennington, et al. 17596, Peru	*C. molinensis*, Pennington, T.D. et al. 17765, Peru	*C. weberbaueri*, Daza 4012, Peru
C. nebulosa, Pennington et al. 17646, Peru *C. nebulosa*, Muellner, A.N. 2056 K, Peru *C.* aff. *odorata*, Styles A16/93 F, Brazil	*C. kuelapensis*, Pennington, et al. 17583, Peru	*C. molinensis*, Daza 1551, Peru	*C. weberbaueri*, Pennington et al. 17901, Peru
H5	**H6**	**H7**	**H8**
C. odorata s.s, Pennington & Sarukhan 9653, Mexico (East)	*C. odorata* s.s, Leonti 98, Mexico (East)	*C.* aff. *odorata* (group II), Neill, D. 2269, Nicaragua *C. odorata*, Styles 166, Belize *C. odorata* s.s, Pratt, C.D. 0010, West Indies (Antigua)	*C. odorata* s.s, Lott 3568, Mexico (West) *C. discolor*, Palmer E. 184, Mexico (West)

H9	H10	H11	H12
C. aff. *odorata* (group II), Gentry, A. & Mejia, M. 50718, Dominican Republic	*C*. aff. *odorata* (group II), Koecke et al. 230406, Panama	*C. saltensis*, Zapater 2348, Argentina	*C*. sp. nov., Koecke et al. 180401, Panama *C*. sp. nov., Koecke et al. 180402, Panama *C*. sp. nov., Cavers F7/73, Costa Rica
H13	**H14**	**H15**	**H16**
C. aff. *odorata* (group II), Koecke et al. 230401, Panama	*C*. aff. *odorata* (group II), Neill et al. 6230, Ecuador *C*. aff. *odorata* (group II), Koecke et al. 280403, Panama	*C*. aff. *odorata* (group II), Koecke et al. 190405, Panama *C. nebulosa,* Pennington et al.17646	*C. saltensis*, Pennington, T.D. 17635, Peru
H17	**H18**	**H19**	**H20**
C. fissilis, genbank accession JF922214, Brazil	*C. fissilis*, genbank accession JF922188, Brazil	*C. fissilis*, genbank accession JF922200, Brazil	*C. fissilis*, Agra et al. 5014, Brazil
H21	**H22**	**H23**	**H24**
C. fissilis, genbank accession JF922222, Brazil	*C. balansae*, Zapater & Castillo 2406, Argentina	*C. balansae*, Soria 2114, Paraguay *C. fissilis*, genbank accession JF922204, Brazil	*C. fissilis*, Muellner 2064, Peru

H25	H26	H27	H28
C. fissilis, genbank accession JF922229, Brazil	*C. fissilis*, Steudel BS464, Bolivia	*C. fissilis*, genbank accession JF922250, Brazil	*C. fissilis*, Zardini and Cardozo 44927, Paraguay
H29	**H30**	**H31**	**H32**
C. fissilis, Lewis 40673, Bolivia	*C.* aff. *odorata* (paraguariensis), Zardini, E. & Velázquez, H. 23167, Paraguay	*C.* sp. nov. 2, Piepenbring et al. MPE22, Ecuador	*C. montana*, Pennington et al. 17623, Peru
H33	**H34**	**H35**	**H36**
C. angustifolia, Pennington et al. 1150, Peru	*C. angustifolia*, Muellner 2054, Peru *C.* aff. *tonduzii*, Stevens, W.D. 22539, Nicaragua	*C. montana*, Palacios & Iguago 3046, Ecuador	*C. angustifolia*, Wood et al. 19222, Bolivia *C. fissilis*, Steudel BS401, Bolivia *C. fissilis*, Steudel BS402, Bolivia
H37	**H38**	**H39**	**H40**
C. cubensis, Cuba excursion 412, Goethe University Frankfurt/Main (FR)	*C. cubensis*, Cuba excursion 462, Goethe University Frankfurt/Main (FR)	*C. monroensis,* Monroe & Alexander 308, El Salvador *C. monroensis,* Witsberger 773, El Salvador	*C. tonduzii*, Styles 82, Costa Rica *C. tonduzii*, Koecke et al. 280406, Panama

			C. tonduzii, Cáceres 4056, Panama *C. tonduzii*, Cavers TonHond, Honduras
H41	**H42**	**H43**	**H44**
C. tonduzii, German 832, Mexico	*C. tonduzii*, Cáceres 4103, Panama	*C. salvadorensis,* Breedlove, D.E. 26871, Mexico	*C. salvadorensis*, Boshier 65, Honduras
H45	**H46**		
C. salvadorensis, Formoso 2, Costa Rica	*C. oaxacensis*, Mendoza et al. 291 *C. oaxacensis*, Styles A33/79, Mexico *C. dugesii*, Germán, M.T. et al. 450, Mexico		

APPENDIX TREE FILES, FIGURE II.

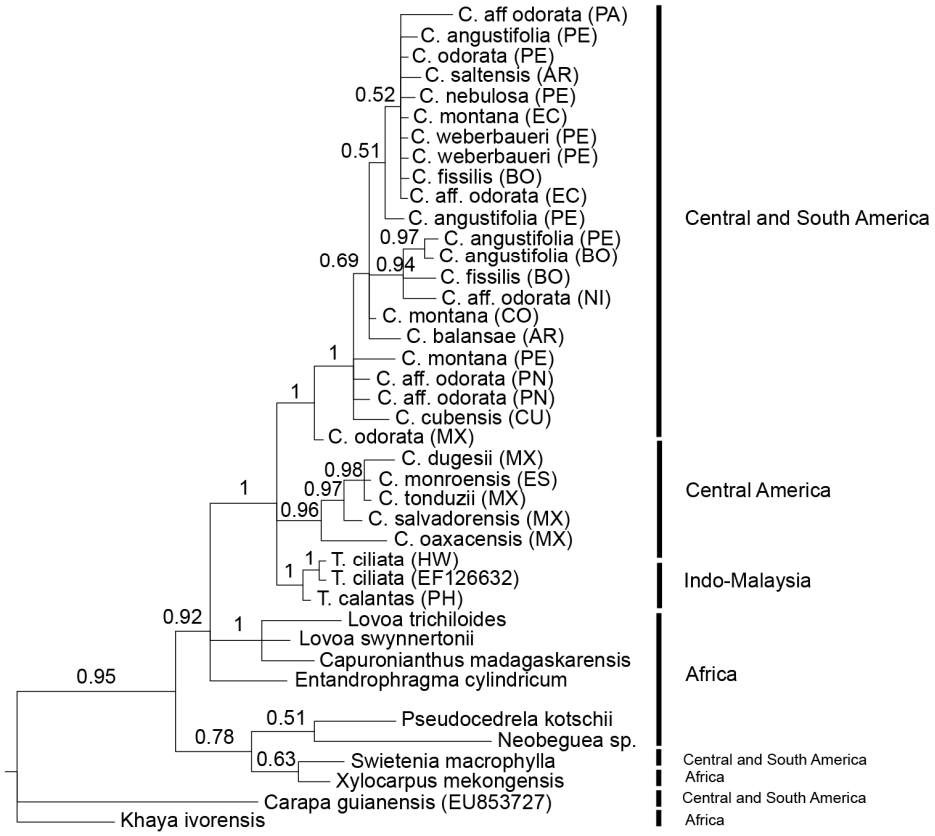


ITS tree of neotropical *Cedrela*, indomalayan *Toona* and closely related genera →

→ Bayesian analysis based on ITS, tree tips are labelled as follows: Genus and species name, or upper case H and haplotype number representing different genetic entities in the study of Cavers et. al (2013); in brackets: genbank numbers (in many cases still DNA numbers) and country of origin.

Abbreviations: *C.* = *Cedrela*, aff. = affine, *T.* = *Toona*, *Ent. cyl.* = *Entandrophragma cylindricum*, *Cap. mah./ mad.* = *Capuronianthus mahafalensis*/ *madagascarensis*, *Lov. tri.* = *Lovoa trichiloides*, *Sch. mic.* = *Schmardaea microphylla*; countries: AR = Argentina, BE = Belize, BO = Bolivia, BR = Brazil, CH = China, CI = Cayman Islands, CO = Colombia, CR = Costa Rica, CU = Cuba, DR = Dominican Republic, EC = Ecuador, ES = El Salvador, FG = French Guiana, HO = Honduras, HW = Hawaii, MA = Malaysia, MD, Madagaskar, MX = Mexico, NI = Nicaragua, PA = Paraguay, PE = Peru, PH = Philippines, PN = Panama, SA = South Africa, TZ = Tanzania (in cult.), WI = West Indies.

FIGURE III.



Bayesian Analysis showing infrageneric relationships of the tribus Swietenioideae →

→ Bayesian tree based on a concatenated dataset of rpS16 and rpL16

Abbreviations: *C.* = *Cedrela*, aff. = affine, *T.* = *Toona*, countries: AR = Argentina, BO = Bolivia, CO = Colombia, CU = Cuba, EC = Ecuador, ES = El Salvador, HO = Honduras, HW = Hawaii, MX = Mexico, NI = Nicaragua, PA = Paraguay, PE = Peru, PH = Philippines, PN = Panama.

Sources (herbarium, collectors numbers or genbank accession numbers):

Khaya ivorensis (K, coll. Kisseado 61), *Carapa guianensis* (genbank nr.), *Xylocarpus mekongensis* (K, col. Sandom 40), *Swietenia macrophylla* (FHO, coll. Kao 7627), *Neobeguea* sp. (K, coll. Cheek et al. 3-25-1), *Pseudocedrela kotschyi* (FHO, coll. Fay 8081), *Entandrophragma cylindricum* (FHO, coll. Kisseado 56), *Capuronianthus madagaskarensis* (K, coll. Cheek et. al 3-25-4), *Lovoa swynnertonii* (K, Faden and Evans 70/122), *Lovoa trichiloides* (FHO, Chapman 3269), *Toona calantas* (FR, Cabrera JFC-2012-099RP), *Toona ciliata* (EF126632), *T. ciliata*, (leaf material: coll. Edmonds 103), *Cedrela oaxacensis* (K, coll. Mendoza et al. 291), *C. salvadorensis* (K, coll. Breedlove 26871), *C. tonduzii* (K, coll. Germán 832), *C. monroensis* (K, coll. Witsberger 773), *C. dugesii* (K, coll. Germán et al. 450), *C. odorata* s.s. (K, coll. Lott 3568), *C. cubensis* (FR, Cuba excursion 462, Goethe University Frankfurt/Main), *C.* aff. *odorata*, group II (FR, coll. Koecke et al. 190405), *C.* aff. *odorata*, group II (FR, coll. Koecke et al. 230406), *C. montana* (K, coll. Pennington et al. 18865), *C. montana* (K, coll. Idrobo and Jaramillo 11.143), *C. balansae* (K, coll. Zapater and Castillo 2406), *C.* aff. *odorata*, group II (K, coll. Neill 2269), *C.* sp. from Bolivia (FR, Steudel BS444), *C. angustifolia* (FR, coll. Steudel BS 434), *C. angustifolia* (K, coll. Pennington et al. 17584), *C. angustifolia* (K, coll. unknown 7292), *C. angustifolia* (K, coll. Muellner and Pennington 17723), *C.* aff. *odorata*, group II (K, Neill et al. 6230), *C. fissilis* from Bolivia (FR, Steudel BS464), *C. weberbaueri* (K, coll. Daza 4012), *C. weberbaueri* (K, coll. Pennington et al. 17901), *C. montana* (FR, coll, Piepembring MPE23), *C. nebulosa* (K, Pennington et al. 17646), *C. saltensis* (K, coll. Zapater 2348), *C. odorata* s.s. (K, coll. Pennington et al. 17635), *C.* aff. *odorata* (paraguariensis) (K, Zardini, E. & Velázquez, H. 23167).