

ALEXANDER HUAMÁN-MERA

**EVOLUTIONARY HISTORY OF *Cedrela* (MELIACEAE)  
IN CENTRAL BRAZIL**

Thesis submitted to the Botany  
Graduate Program of the  
Universidade Federal de Viçosa, as  
part of the requirements to obtain the  
title of Doctor Scientiae.

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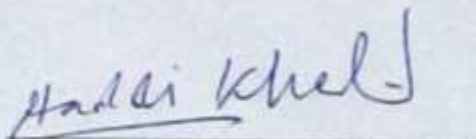
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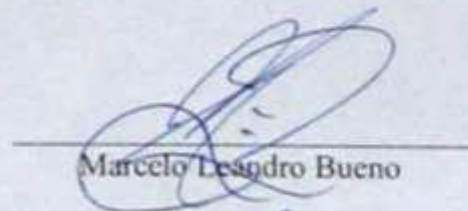
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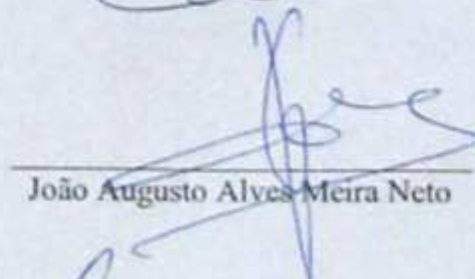
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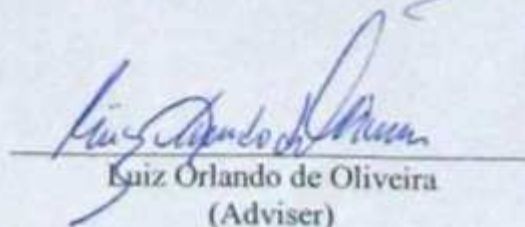
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To my parents, Mardóneo and Yolanda  
and my family in my country, Peru,  
to my own family, Yuriko and Akira,  
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## **BIOGRAPHY**

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## ABSTRACT

HUAMÁN-MERA, Alexander, D.Sc., Universidade Federal de Viçosa, June, 2018. **Evolutionary History of Cedrela (Meliaceae) in Central Brazil**. Adviser: Luiz Orlando de Oliveira.

The genus *Cedrela* in Meliaceae is monophyletic and currently it comprises 18 species. *Cedrela* is distributed across the Neotropics and is associated with closed-canopy forests — seasonal forests and moist forests — and ecotones that are adjacent to seasonal forests. The morphological delimitation of the *Cedrela* species is in fact extremely complicated. Biodiversity hotspots are regions known to have especially high numbers of endemic species. Two of the largest biodiversity hotspots are located in Brazil: the Cerrado and the Atlantic forest. The Cerrado is so ancient that it might pre-date the time South America split from Africa. The climatic fluctuations affected the Brazilian environments during the Late Neogene/Quaternary boundary. Thus, the center and Northeast Cerrado were likely displaced by Caatinga expansions, but the South and West Cerrado probably remaining as refugia. Refugia could directly relate to conservatism of niche. Niche phylogenetic conservatism refers to the retention of ecological traits, despite time, among related species. The concept is being used to explain latitudinal gradients of diversity, and other spatial patterns of species distribution. The long-term environmental dynamics of paleoclimates and paleosols affected the evolution of *Cedrela* in temporal and climatic scales. Currently paleoclimatic data from the Quaternary is being used for elucidate the differences in the distribution among populations of the same species. Species Distribution Models (SDM) is widely used in Ecology and conservation research.

## RESUMO

HUAMÁN-MERA, Alexander, D.Sc., Universidade Federal de Viçosa, junho de 2018. **História Evolutiva de Cedrela (Meliaceae) no Brasil central.** Orientador: Luiz Orlando de Oliveira.

O gênero *Cedrela* em *Meliaceae* é monofilético e atualmente compreende 18 espécies. *Cedrela* é distribuída pelos Neotrópicos e está associada a florestas de copas fechadas - florestas sazonais e florestas úmidas - e ecótonos adjacentes a florestas sazonais. A delimitação morfológica das espécies de *Cedrela* é, na verdade, extremamente complicada. Os hotspots de biodiversidade são regiões que possuem um número especialmente alto de espécies endêmicas. Dois dos maiores hotspots de biodiversidade estão localizados no Brasil: o Cerrado e a Mata Atlântica. O Cerrado é tão antigo que pode ser anterior ao tempo em que a América do Sul se separou da África. As flutuações climáticas afetaram estruturalmente os ambientes brasileiros durante o limite do Neogeno tardio com o Quaternário. Assim, o centro e o nordeste do Cerrado foram provavelmente desalojados pelas expansões da Caatinga, mas o Sul e Oeste do Cerrado provavelmente permanecem como refúgios. Refúgio poderia relacionar-se diretamente ao conservadorismo de nicho. O conservadorismo filogenético de nicho refere-se à retenção de características ecológicas, apesar do tempo, entre espécies relacionadas. O conceito está sendo usado para explicar os gradientes latitudinais de diversidade e outros padrões espaciais de distribuição de espécies. A dinâmica ambiental em longo prazo de paleoclimas e paleossolos afetou a evolução de *Cedrela* nas escalas temporal e climática. Dados paleoclimáticos atuais do Quaternário estão sendo usados para elucidar as diferenças na distribuição entre populações da mesma espécie. Modelos de Distribuição de Espécies (SDM) são amplamente utilizados em pesquisas de ecologia e conservação.

## **I. GENERAL INTRODUCTION**

### **1.1 The central Brazil**

The central Brazil is an important geographical region that is part of the central-west political region of Brazil, which groups the states of Goiás, Mato Grosso and Mato Grosso do Sul. The central Brazil is part of wide and long orogenic system known as the Tocantins Province, which includes the Brasília Belt (Pimentel, 2016). The plate tectonic movements located the central Brazil in an elevated and continuous plateau (Pimentel, 2016). The plateau of central Brazil was extensively covered by predominant vegetation of a woody savanna with a physiognomy varying from closed woodland to open grassland. This vegetal cover was prior to the human disturbances (Oliveira-Filho and Ratter, 1995)

### **1.2 The Neotropical genus *Cedrela* (Meliaceae)**

The genus *Cedrela* (Browne, 1756) belongs to Meliaceae, order Sapindales (APG IV, 2016). The genus is monophyletic; currently it comprises 18 species (Pennington and Muellner, 2010; Köcke et al., 2015). *Cedrela* is distributed across the Neotropics, Northern Mexico to Northwestern Argentina to the Atlantic Forest of Eastern Brazil (Pennington and Muellner, 2010; Styles, 1981). Environmental humidity shapes the distribution of species of *Cedrela*, so they grow over well-drained soils to soils with high amounts of humidity. More likely, this trait would be associated to niche conservatism and the ability to spread out over environments that detains a wide range of humidity. Thus the changes in precipitations during the Miocene would be related to the diversification of the genus. The main range of diversification was located in the central Andes hotspot (Pennington and Muellner, 2010; Muellner et al., 2009; Koepke et al., 2013.). *Cedrela* is phylogenetically closed-related to *Toona* (Endl.) M. Roem., an Asian-Australian genus. The time divergence of both *Cedrela* and *Toona* was in Oligocene (~48 Mya) (Muellner et al., 2010). *Cedrela* is associated with closed-canopy forests — seasonal forests and moist forests — and ecotones that are adjacent to seasonal forests (Styles, 1981; Pennington and Muellner, 2010). *Cedrela* is characterized by monopodial trees that measure from three to 50 meters in height when they are adults. Species of *Cedrela* have annual reproduction. They present composite leaves, unisexual flowers, protogynous, with presence of well-developed rudiments of the opposite sex in the same flower. Plants of *Cedrela* have male and female flowers, the male ones have pistiloides and

rudimentary ovules, and the female ones have anthers without pollen on shorter filaments. All flowers present an androgynophore with functions of nectary and where the true or rudimentary ovary is established according to the type of flower and the petals are adnate by a central keel for at least half their length. *Cedrela* presents fruits of the capsule-type septícida with winged seeds (Gouvêa et al., 2008; Muellner et al., 2009; Pennington and Muellner, 2010). The morphological delimitation of the *Cedrela* species is in fact extremely complicated. The morphological analysis uses widely variable and juxtaposed characters, such as simple and multicellular non-branched hair, number of leaflets, number of secondary veins, type of phenology at the beginning of flowering in young plants, division of the calyx and capsule size (Muellner et al., 2009; Pennington and Muellner, 2010).

### **1.3 The hotspot Cerrado, Neotropical Forests, and tropical refuges**

Biodiversity hotspots are regions known to have especially high numbers of endemic and threatened species. Remnant habitats reach just over two percent of the dry surface of the earth (Myers et al., 2000). Thirty-five regions have been declared as biodiversity hotspots around the world which nine regions occur in the new world. Two of the largest biodiversity hotspots of the new world are located in Brazil: the Cerrado and the Atlantic forest (Biodiversity hotspots revisited, conservation international, 2011). “Cerrado” is the Portuguese denomination for the plateau of central Brazil of woodlands, savannas, grasslands, and Neotropical forests such as – dry, gallery, and ciliar forests – (Klink and Machado, 2005). The Cerrado is the second largest biome in Neotropics, exceeded only by Amazonia; it includes a great part of central Brazil, but its extension reach parts of northeastern Paraguay and eastern Bolivia (Ab'Saber, 1977). According some authors the origin of the Cerrado biome is so ancient that it might pre-date the time South America split from Africa. In such a scenario, there was a prototypic form of Cerrado when the Tertiary began (~65 Mya B.P.) (Ratter et al., 1997). However, it seems probably that Cerrado biome itself as part of the South America savannas originated between 25–28 Mya B.P.; and it expanded during the Miocene-Pliocene boundary (25–2 Mya B.P.) (Gottsberger and Silberbauer-Gottsberger, 2009). The Cerrado is an important biodiversity hotspot and it is part of the open diagonal formations together with the Caatinga and the Chaco (Werneck et al., 2012). The Cerrado hotspot is largely threaten by extractive human activities such as farming, agriculture, charcoal extraction, etc (Ratter et al., 1997).

On the other hand, in the same scenario of origin of South America savannas, tropical forests already existed and had increased their expansion. Thus, during the Paleogene period (~65 Mya B.P.), climatic conditions was warm and humid and this favored the enormously expansion of tropical rainforests, such conditions was similar than current. Later in the Paleocene/Eocene boundary (~56 Mya B.P.), the presence of warmer and humid environments permitted the increase of Angiosperm biodiversity as well as the presence of humid forests in South America (Gottsberger and Silberbauer-Gottsberger, 2009). Probably in the Eocene/Oligocene boundary (~33 Mya B.P.), the humid forests were continue and widespread from Pacific Ocean to Atlantic Ocean that means they covered a region twice as large as current. In Brazil, with the beginning of the drier and cooler climate of the Neogene period (~23 Mya B.P.), the Neotropical forests reduced in their geographic extension and were also partially displaced. At the same time, the expansion of savannic areas had began and Neotropical forests were restricted to some specific geographical ranges such as small tributaries of main rivers (Gottsberger and Silberbauer-Gottsberger, 2009; Antonelli and Sanmartin, 2011). All those factors likely took to Neotropical forest taxons to isolate in environments with more favorable climates for life. As result, the Cerrado as a large-scale biome became established in Pliocene period. With all these scenarios the distribution of species changed considerably and the extinction or establishment of new taxons took place (Hewitt, 2000).

The term refuge is often used to define relict areas of lowland forest. Refuge theory has been proposed as priority geographical places for biological conservation under historical climate reversals (Prance, 1982). The distribution of vegetation suffered displacements following the Pleistocene climatic fluctuations, which brought about glacial and warm periods and this caused the fragmentation of species ranges and the isolation of portions of the respective vegetation in ecological refuges. Their role in the conservation is due to the capacity to favor survival of biota under unfavorable conditions (Haffer, 1982; Prance, 1982; Davis et al., 2013). Main evidences for refuge model are identify in plants such as occurrences of areas of endemism within the rainforest, the presence of xerophytic plants as relicts of drier climatic periods within humid forest areas, and morphological variation of some widespread species (Prance, 1982). In the Late Neogene/Quaternary, the Brazilian environments suffered climatic fluctuation. Thus, the center and Northeast Cerrado were likely displaced by Caatinga expansions, but the South and West Cerrado (e.g. Paraná river Basin, Brasilia and Chapada dos Veadeiros) probably remaining establish and compound a unique and extensive refuge (Ab' Sáber, 1983). The savannas extended until reach the current

territories of Bolivia where was identified an extensive and unique refuge during the Pleistocene. The presence of climatic stability in the past allowed remaining as an ecological refuge (Werneck et al., 2012).

#### **1.4 Brazilian seasonal Forest**

In Brazil, the Atlantic forest is widely threatened (FUNDAÇÃO SOS Mata Atlântica; INPE 2010). The main ecosystems of the Atlantic forest are the seasonal forests. They are characterized by the dual climatic seasonality. Seasonal forests present a period of rainy season followed by a long dry period (reaching more than five consecutive months). In areas where there is no dry season, the intense winter cold, with average temperatures below 15° C, causes physiological drought (Oliveira-Filho and Fontes, 2000; Veloso et al., 1991). In South America, about 60% of the forests are highly destroyed. The causes range from climate change, habitat fragmentation, fire, population density and agriculture (Miles et al., 2006). These forests also represent a widely threatened ecosystem in countries such as Bolivia (Steininger et al., 2001). The agriculture is highly intensive in this ecosystems because of seasonal forests occur in soils highly fertiles (Oliveira-Filho et al., 1994). In the last 20 years, there has been an increase in the interest in seasonal forests, since they have been diagnosed as areas of endemism (Prado, 2000; Pennington et al., 2004).

Currently, many studies test hypotheses from shelter areas based on modeling analyzes of species distribution at multiple temporal and spatial scales with the purpose of explaining species richness and the implication of these (Wiens and Donoghue, 2004). Since climate is a major contributor to evolutionary changes in biodiversity, there are many climate models that try to infer potential new areas of distribution or point out those areas where they were distributed in the very ancient past (Erwin, 2009). In Brazil, paleoclimatic models have shown the presence of a historical forest refuge in the Atlantic forest, as well as the spatial variation of the forests along the Pleistocene (Carnaval and Moritz, 2008).

#### **1.5 Niche phylogenetic conservatism of *Cedrela* and Species Distribution Modeling (SDM)**

Niche phylogenetic conservatism refers to the retention of ecological traits, despite time, among related species and they assembled forming different communities that usually share common spatial distribution. The environmental gradients influence considerably in the

differentiation and distribution of conservatism across taxonomic levels. The palaeoenvironmental conditions during the radiation of the phylogenetic lineages could corroborate the current differences among environmental gradients. The concept is being used to explain latitudinal gradients of diversity, and other spatial patterns of species distribution. (Prinzing et al., 2001; Wiens and Graham, 2005; Pearman et al., 2007; Cavender-Bares et al. 2009; Wiens et al., 2010; Antonelli and Sanmartín, 2011). In *Cedrela*, the long-term environmental dynamics of paleoclimates and paleosols affected the evolution of this species in temporal and climatic scales Koecke et al. (2013). While the conservatism of the niche would explain the extinction of species of *Cedrela* that in the past were distributed in the northern hemisphere when these areas constituted tropical forests and today only present fossils of *Cedrela* (Muellner et al. 2010). Therefore, current conditions could also explain the restricted distribution of species in more humid environments such as those distributed in tropical forests. On the other hand, the species differed tolerating climatic shifts would be explained with events of recent speciation, showing overlapped distribution in nearby and concomitant areas.

Currently paleoclimatic data from the Quaternary is being used for elucidate the differences in the distribution among populations of the same species. Species Distribution Models (SDMs) is widely used in Ecology and conservation research. It is the process of using computer algorithms to predict geographic distributions of species which based on correlative occurrences between known records and the environmental conditions at geographic localities (Phillips et al., 2006; Elith and Leathwick, 2009). SDMs contribute to know about niche displaces over time or space, for instance those displacements that occur between populations separated in geographic spaces (Pearman et al., 2007).

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**CHAPTER I: CLIMATIC FLUCTUATIONS OF TERTIARY/QUATERNARY BOUNDARY AS THE DRIVERS OF NEW LINEAGES OF *Cedrela* (MELIACEAE) IN THE CENTRAL BRAZIL**

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## ABSTRACT

Climatic fluctuations are alternate climatic events that the Earth experimented in past geological epochs. The Tertiary and Quaternary were the most important epochs that influenced strongly in the origin, abundance, and distribution of biodiversity. Contrast periods of warmer and humid climate and cooler and arid climate played an important role of driving biodiversity in the past. These climatic fluctuations would have influenced in the shape of current diversity of plants and especially of *Cedrele* in central Brazil. To assess our hypothesis we carried out both molecular analyses (microsatellite markers and DNA sequencing) and species distribution modeling (SDM) of *Cedrele* species. Microsatellite analysis exhibited six genetic groups ( $K = 6$ ) of *Cedrele* within Brazil, two groups previously known and the remaining four were new. Our results suggested the remaining four groups were new and distinct genetic entities within *Cedrele*. Three of those four groups are phylogenetically more closely-related to clade *Cedrele fissilis* and the last one is more related to clade *Cedrele odorata*. The time of divergence of new groups of *Cedrele* took place between 7–2 Mya B.P. The SDM analysis showed a big dynamics of niche displacement during the Quaternary, but these would not explain the new entities. Likely, the origin of the biodiversity and endemism resulted during the Last Tertiary when the South America's climate was drier and cooler than present.

**KEY WORDS:** Cedreleae; Forest gallery; Riverine Forests; Cerrado; Seasonal Forests; Phylogeography; Refugia.

## I. INTRODUCTION

Historical climatic fluctuations are alternate climatic events that the Earth experimented in past geological periods. The Tertiary and Quaternary (65 – 1.8 Mya) were the most important epochs that influenced strongly in the origin, abundance, and distribution of biodiversity (Hewitt, 2000; Pennington et al., 2004; Gottsberger and Silberbauer-Gottsberger, 2009). Long-term periods of warm and humid tropical climate dominated almost most of the Paleogene and Eocene, and during these periods Neotropical rainforest expanded enormously in all South America. In those periods several plant species raised and dominated Neotropics (Pennington et al., 2004; Gottsberger and Silberbauer-Gottsberger, 2009). However, with the return of the Antarctic continent to the circle pole (45 – 40 Mya) and the uplift of the Andes during the Pangaea drift the Antarctic glaciations increased. In the beginning of the Oligocene onwards and overall in the Neogene, cooler to arid climates began to dominate Neotropics affecting the atmospheric circulation in the Southern Hemisphere (Gregory-Wodzicki, 2000; Antonelli et al., 2006; Gottsberger and Silberbauer-Gottsberger, 2009; Lavina and Fauth, 2011). The lowering of the global temperatures and precipitations in Neotropics favored the expansion of more xeric vegetation in large parts of several central and northern parts of South America including Brazil (Gottsberger and Silberbauer-Gottsberger, 2009). During this scenario, the drier and cooler climate of the Neogene reduced and partly displaced the Neotropical rainforests from central Brazil. Additionally in south of South America had the disappearance of tropical flora and decreasing in subtropical species (Parrish, 1993; Gottsberger and Silberbauer-Gottsberger, 2009; Lavina and Fauth, 2011; Le Roux, 2012). Thus, the xeric vegetation was expanded as a corridor of open vegetation formations called “the diagonal of open formations” (Vanzolini, 1963). Between 25 – 2 Mya, dry forests and more open vegetation were widespread in central Brazil. At the same time, Neotropical forests were reduced to some humid stable areas such as the small tributaries of large rivers (Paraná and Sao Francisco rivers) which for that geologic time they were already existed. In that scenario, probably the widespread Neotropical forests that dominated in the Paleocene and Eocene in central Brazil were retracted as riverine forests, which were ubiquitous in water bodies throughout the growing like-savannas regions. Currently nearly all water bodies of the region are fringed by forests and are called gallery forests (Ab’ Saber, 2000; Pennington et al., 2000; Oliveira-Filho and Ratter, 2002; Gottsberger and Silberbauer-Gottsberger, 2009; Lavina and Fauth, 2011). In the next 3 – 2 Mya (Tertiary/Quaternary period), the climatic oscillations were more frequent. However, is a matter of controversial between researchers (Gottsberger

and Silberbauer-Gottsberger 2009). The influence of Quaternary glacial/interglacial cycles in the origin and distribution of plant species on earth is evident (Hewitt, 2000). Pollen records from tropical South America provided a general big picture of the climatic conditions during the late Quaternary. Thus, pollen records of species that compound current gallery forests suggested stable delimited areas that fringed water bodies in Quaternary periods where central Brazilian vegetation exhibited changes in their distribution, which experimented expansions and retractions (van der Hammen, 1991; Barberi et al., 2000; Bueno et al., 2017).

The Neotropics are the most biodiversity regions on earth and also harbor the highest plant biodiversity in the world (Myers et al., 2000). The Neotropical forests that assemble in central Brazil are the called gallery forests and deciduous and semideciduous forests (Oliveira-Filho and Ratter, 1995). Here, we have special attention for the gallery forest since they are part of the Neotropical seasonal forests that served as connections between Amazonian and Atlantic vegetation when the xeric vegetation expanded within central Brazil (Ab' Saber, 2000; Pennington et al., 2000; Oliveira-Filho and Ratter, 1995; Oliveira-Filho and Ratter, 2002; Gottsberger and Silberbauer-Gottsberger, 2009). Likely these connection areas of gallery forests were harboring populations of species that were distributed in widespread areas before began the cooler and drier climate of the Tertiary. With the retraction of vegetation the populations of species confined to these areas were acquiring and accumulating genetic and morphological differences (Hewitt, 2000; Pennington et al., 2000; Oliveira-Filho and Ratter, 2002; Gottsberger and Silberbauer-Gottsberger, 2009). Currently the endemism of plant species in gallery forests would be explained by the climatic oscillations of recent geologic period as the Quaternary and not by the Tertiary. Seemingly the lack of proper fossil records made that literature does not report specific areas of endemism for species of gallery forest from the Tertiary origin (Hooghiemstra and van der Hammen, 1998; Pennington et al., 2004). Thus, the scenario that researchers use to explained high levels of endemism and biodiversity is thanks to the climatic oscillations during the Quaternary in Brazilian vegetations (Cordeiro et al., 2017). Even it was postulated a theory of refugia for the Neotropics that was supported by Paleobotany, Geomorphology, and Geology studies, and currently even by molecular, Ecology, and climatic modeling studies (Haffer, 1982; Ramírez-Barahona and Eguiarte, 2013; Sosa and Loera, 2017). However not at all Brazilian species richness of plants had their origin in the Quaternary. Many plant species had their origin even before the beginning of the Tertiary period. It is highly probable that the main driver for the formation of plant diversity in Brazilian biomes such as Atlantic Forest was the

sympatric speciation because of the absent of considerable geographic barriers (Pennington et al., 2004; Gastauer et al., 2015).

The genus *Cedrela* is compound for two widespread accepted species, *Cedrela odorata* L. and *Cedrela fissilis* Vell. in Brazil (Pennington and Muellner, 2010). However, only *C. fissilis* received significant and deep attention about significant differences that populations of *C. fissilis* exhibit along Brazil. Studies developed in *C. fissilis* with molecular approaches suggested two filogenetic lineages, the Chiquitano and Atlantic, which clearly were separated by the Cerrado biome (Garcia et al., 2011; Mangaravite et al., 2016). The outcomes also suggested that *C. fissilis* was not monophyletic. Thus, specimens of *C. balansae* e *C. odorata* grouped together with specimens belong to the Atlantic lineage of *C. fissilis* clade (Garcia et al., 2011). The divergence time of both Chiquitano and Atlantic lineages took place in the Early Pliocene and Late Miocene and this is in agreement with the intensification of diversification in the central Andes (Muellner et al., 2010, Garcia et al., 2011). An unpublished study in populations of *Cedrela* from the upper Parana River basin using microsatellite markers suggested three new phylogenetic lineages of *Cedrela* (Huamán-Mera, 2014.). These lineages would be differente from those reported by Garcia et al. (2011). At least one o these lineagens was deeply studied and showed a new genetic entity for central Brazil that was treated as *Cedrela brachystachya*, a specialized species to wetland environments (Huamán-Mera et al., submitted). This especie was previously synonymized with widespread *Cedrela odorata* (Pennington and Muellner, 2010). Recently a new study of population genetics of a natural population of *Cedrela fissilis* in Northern Minas Gerais – Brazil suggested that the genetic status of this natural population would be a novel lineage. So it needs being treated with molecular and taxonomical approaches for determining its real status within *Cedrela* (Díaz-Soto et al., 2018).

In this study, we explored the role of the climatic fluctuations of Tertiary/Quaternary boundary in shaping the evolutionary history of diversity of lineages of *Cedrela* from central Brazil, as well as to evaluate the contemporary patterns of genetic and morphological diversity. In addition to assess the distribution of *Cedrela* lineages throughout central Brazilian environments based on historical climate modeling and the study of molecular markers. Firstly, we used microsatellite data from representative populations of either the Atlantic range or the Chiquitano range to determine the genealogical placement of *Cedrela* lineages sampled from central Brazil. Subsequently, we obtained DNA sequences of

representative individuals of different lineages of populations from central Brazil to investigate the phylogeny placement of lineages within *Cedrela* and the time of divergence of the same lineages. Finally, we used the Global Position System (GPS) records of strategic individuals of different *Cedrela* lineages which are distributed along central Brazil to infer current and past climatic models, as well as determine possible climatic stable areas with potential conservation purpose. This study addressed the following five questions: (1) How is the genetic variability of microsatellite data of *Cedrela* lineages from central Brazil, the genetic material is different and unique, is sharing with lineages previously studied? (2) How the lineages of *Cedrela* from central Brazil are related with lineages previously established (Cedrinho, Chiquitano, and Atlantic)? (3) How the lineages of *Cedrela* from central Brazil are phylogenetically related to the entire genus? (4) Is there correspondence between the lineages of *Cedrela* from central Brazil and their morphological diversity? (5) Is there evidence of ancestral refugia for species of *Cedrela* within the ecosystems of central Brazil? We also present the implications of our results for the genetic conservation of *Cedrela* species and for the debate about the origins and distribution of Neotropical biodiversity in Tertiary/Quaternary boundary periods.

## **II. MATERIALS AND METHODS**

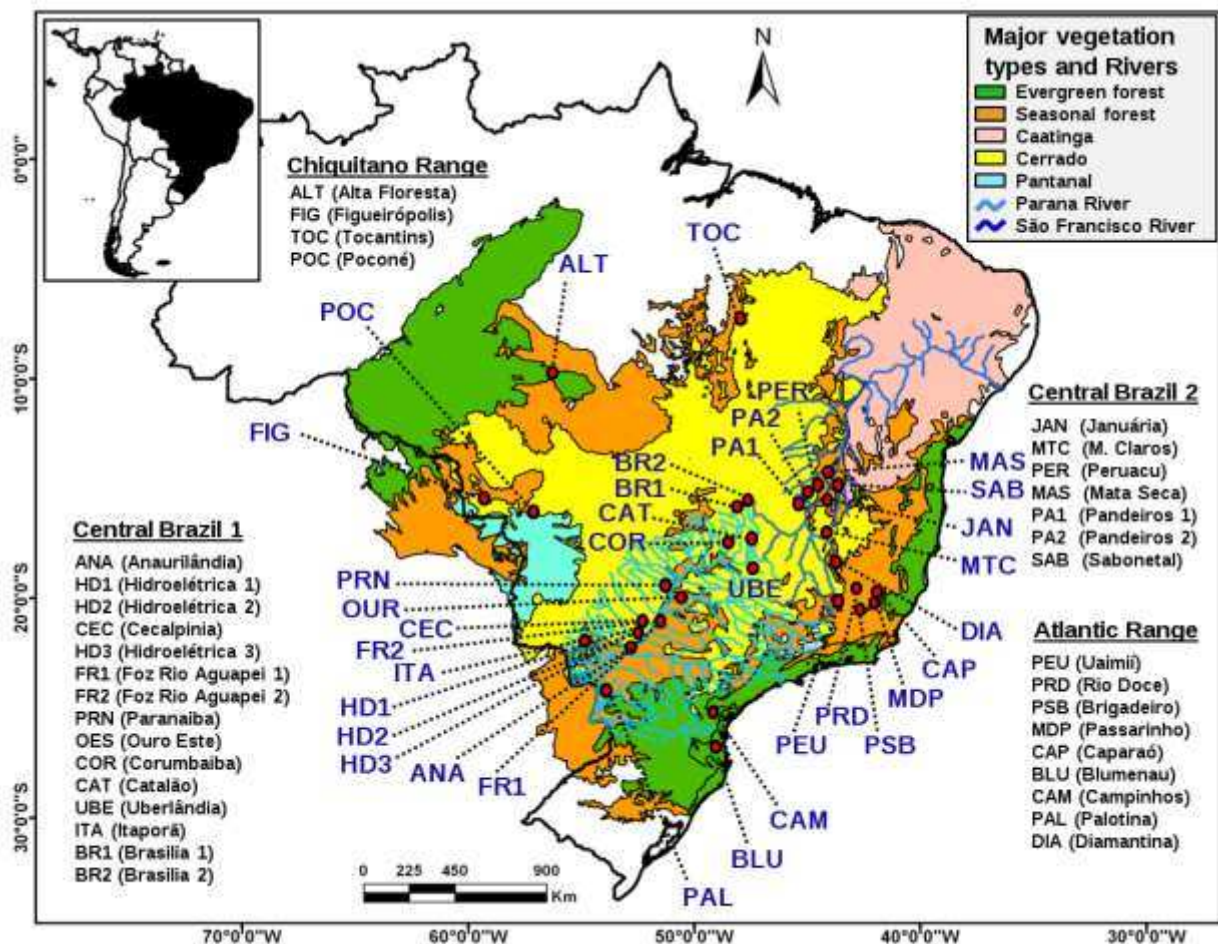
### **2.1 Sampling Strategy and DNA extraction**

We sampled 104 specimens of *Cedrela* in present study. We collected leaves samples; they were dried using silica gel and kept at room temperature until further use. We focused strategic points of sampling in central Brazil. The sites were located within the Cerrado biome and also in ecotones sites among Cerrado, Atlantic Rainforest and Caatinga biomes in Brazil. Collecting trips took place in 2015, 2016 and 2017; we recorded their location using a Global Positioning System (GPS) receiver. The sites of collection were from eight: Montes Claros (MTC), Peruaçu (PER), Mata Seca (MAS), Pandeiros (two populations, PA1 and PA2), Sabonetal (SAB), and Brasilia (two populations, BR1 and BR2) (Figure 1, Table 1). We used the Terrestrial Ecoregions of the World database from the World Wildlife Fund (Olson et al., 2001) to define the associated vegetation formations that surrounded the sampling sites. Voucher specimens resulting from fieldworks were deposited in Herbarium Viçosense (VIC).



Additionally we used 386 specimens that were obtained from previous studies. These specimens belong to: (1) *Cedrela fissilis* (Mangaravite et al., 2016; Huamán-Mera et. al. Submitted), which distribution were located alongside Brazilian Cerrado into the previously established as Chiquitano and Atlantic ranges (García et al., 2011); (2) *Cedrela brachystachya* (Huamán-Mera et. al., submitted) from central Brazilian wetlands, and (3) *Cedrela* spp. from central Brazil also (Huamán-Mera, 2014) (Figure 1 and Table 1).

In total, we evaluated 490 specimens of genus *Cedrela* from 35 localities in Brazil (Figure 1 and Table 1). The sampling sites were chosen to cover most of the geographical range of the genus *Cedrela*.



**Figure 1.** Locations of populations of genus *Cedrela* included in the present study and their major vegetation types and Rivers. Populations were organized into three groups according to the geographic distribution: the novel Central Brazil group, and the two previously known groups, Chiquitano and Atlantic ranges that represented *Cedrela fissilis*). The populations of central Brazil were grouped again into two sub-groups, Central Brazil 1 with 15 populations (ANA, HD1, HD2, CEC, HD3, FR1, FR2, PRN, OES, COR, CAT, UBE, ITA, BR1, and BR2), and Central Brazil 2 with seven populations (JAN, MTC, PER, MAS, PA1, PA2, and SAB); the Chiquitano range with four populations (TOC, ALT, FIG, and POC) and the Atlantic range with nine populations (PEU, PRD, PSB, MDP, CAP, BLU, CAM, PAL, and DIA).

**Table 1.** Sampled populations of *Cedrela*, with sample size (N) and geographic coordinates. Eight populations were collected for this work. Additional dataset of *Cedrela* used in this work were taken from previous studies: 11 populations from Mangaravite et al. (2016), eight populations from Huamán-Mera et al. 2018 (submitted), and eight populations from Huamán-Mera (2014). Every population has information about their respective distribution range, locality and population code, sample size, and geographic coordinates.

<b>Distribution Range</b>	<b>Locality (Population code)</b>	<b>N</b>	<b>Coordinate (Latitude/Longitude)</b>
<b>Chiquitano</b>	Alta Floresta (ALT)*	21	-09° 53' 56,97" / -56° 09' 16,12"
	Figueirópolis D'Oeste (FIG)*	22	-15° 26' 43,80" / -58° 44' 25,73"
	Tocantins (TOC)*	9	-07° 12' 26,92" / -47° 45' 49,04"
	Poconé (POC)*	22	-16° 19' 08,50" / -56° 31' 52,50"
<b>Central</b>	Anaurilândia/ Nova Londrina/ Teodoro Sampaio (ANA)**	24	-22° 12' 51.10" / -52° 50' 37.20"
	Hidroeletrica1 (HD1)***	13	-21° 45' 52.40" / -51° 15' 47.70"
	Hidroeletrica2 (HD2)***	7	-21° 44' 52.10" / -52° 15' 41.80"
	Cecalpina (CEC)***	7	-21° 15' 26.80" / -51° 58' 24.20"
	Hidroeletrica3 (HD3)***	7	-21° 11' 07.70" / -51° 51' 51.40"
	Foz Rio Aguapei1 (FR1)***	11	-21° 08' 10.00" / -51° 47' 27.60"
	Foz Rio Aguapei2 (FR2)***	5	-21° 07' 25.10" / -51° 44' 29.70"
	Ouro Este (OES)***	18	-20° 00' 40.90" / -50° 21' 45.60"
	Paranaíba (PRN)***	15	-19° 43' 00.70" / -51° 05' 37.10"
	Corumbaíba (COR)**	11	-18° 06' 43.00" / -48° 37' 00.00"
	Catalão (CAT)**	16	-18° 11' 45.00" / -47° 57' 21.00"
	Uberlândia (UBE)**	19	-18° 58' 45.00" / -48° 02' 12.00"
	Itaporã (ITA)*	9	-22° 00' 09,68" / -54° 42' 52,59"
	Januária (JAN)*	9	-15° 11' 22,56" / -44° 12' 24,48"
	Montes Claros (MTC)	9	-16° 38' 26.05" / -43° 52' 55.16"
	Peruaçu (PER)	17	-15° 06' 59.30" / -44° 14' 29.90"
	Mata Seca (MAS)	15	-14° 50' 59.25" / -43° 59' 32.54"
	Brasília 1 (BR1)	18	-15° 44' 23.00" / -47° 55' 49.00"
	Brasília 2 (BR2)	9	-15° 42' 53.00" / -47° 55' 24.00"
	Pandeiros 1 (PA1)	17	-15° 33' 41.95" / -44° 48' 18.77"
Pandeiros 2 (PA2)	15	-15° 41' 03.90" / -44° 35' 14.74"	
Sabonetal (SAB)	4	-15° 20' 15.76" / -44° 03' 46.01"	

<b>Atlantic</b>	Parque Estadual Uaimií (PEU)**	17	-20° 14' 57.41" / -43° 34' 16.76"
	Parque Estadual Rio Doce (PRD)**	3	-19° 42' 48.05" / -42° 43' 55.30"
	Parque Serra Do Brigadeiro/MG (PSB)**	10	-20° 41' 01.00" / -42° 26' 41.00"
	Mata do Passarinho (MDP)**	7	-20° 27' 38.86" / -41° 50' 14.70"
	Caparaó (CAP)*	36	-20° 31' 45,60" / -41° 55' 14,10"
	Blumenau (BLU)*	18	-26° 55' 07,51" / -49° 03' 57,69"
	Campinhos (CAM)*	7	-25° 02' 17,00" / -49° 05' 26,00"
	Palotina (PAL)*	28	-24° 18' 34,29" / -53° 54' 32,15"
	Diamantina (DIA)*	13	-18° 24' 33,60" / -43° 29' 24,70"
<b>TOTAL</b>		<b>490</b>	

Legend: Additional dataset from Mangaravite et al. (2016) (\*), from Huamán-Mera et al. 2018 (Submitted) (\*\*), and from Huamán-Mera (2014) (unpublis. data) (\*\*\*)

Total genomic DNA was extracted from dried leaf samples following a protocol described by Mangaravite et al. (2016), but with some modifications that were done in this study (Appendix A). The genomic DNA from each specimen has been archived in the Phylogeography and Molecular Biology laboratory at the Federal University of Viçosa, Brazil.

## 2.2 Microsatellite analyses

We used a genotyping approach. The genotyping approach was carried out using 11 microsatellite loci: Ced2, Ced18, Ced41, Ced44, Ced54, Ced65, Ced95, Ced131, CF26, CF66A, and CF66B. Each forward primer was labeled with either 6-FAM, HEX (MWG-Biotech, Ebersberg, Germany), or NED (Applied Biosystems, São Paulo, Brazil) fluorescence (Table 2, Figure Supplementary 1). The conditions of the polymerase chain reaction (PCR) used during genotyping were essentially those described previously by Mangaravite et al. (2016) and slightly modified by the authors of this study. We used the following PCR program: 96°C for 2 minutes; 29 cycles of denaturation temperature 94°C for 1 minute, annealing temperature 55°C for 1 minute and extension temperature 72°C for 1 minute; then one cycle of 94°C for 1 minute, 55°C for 1 minute and 72°C for 20 minutes. With this genotyping approach, we obtained 104 microsatellite data for specimens of *Cedrela* from eight populations: MTC, PER, MAS, PAN1, PAN2, SAB, BR1 and BR2.

**Table 2.** Primers used for microsatellite analyses, with locus identification.

<b>Locus</b>	<b>Primer Sequences (5'→3')</b>	<b>Array</b>	<b>Dye Set</b>	<b>N<sub>A</sub></b>	<b>Allele size (pb)</b>	<b>Reference</b>
<b>Ced2</b>	F: TTTGCTTTGAGAAACCTTGT* R: AACTTTTCGAATTGGTTAAGG	(GA) <sub>20</sub>	6-FAM	28	131-241	Hernandez, et al. (2008)
<b>Ced18</b>	F: CAAAGACCAAGATTTGATGC* R: ACTATGGGTGGCACAACACTAC	(GA) <sub>23</sub>	HEX	22	113-161	Hernandez, et al. (2008)
<b>Ced41</b>	F: TCATTCTTGGATCCTGCTAT* R: GTGGGAAAGATTGTGAAGAA	(TC) <sub>18</sub>	HEX	22	110-158	Hernandez, et al. (2008)
<b>Ced44</b>	F: ACTCCATTAAGTCCATGAA* R: ATTTTCATTCCCTTTTAGCC	(TG) <sub>14</sub> (AG) <sub>17</sub>	6-FAM	29	162-224	Hernandez, et al. (2008)
<b>Ced54</b>	F: GATCTCACCCACTTGAAAAA* R: GCTCATATTTGAGAGGCATT	(GA) <sub>15</sub> (AG) <sub>6</sub> G(GA) <sub>5</sub>	6-FAM	17	172-218	Hernandez, et al. (2008)
<b>Ced65</b>	F: GAGTGAGAAGAAGAATCGTGATAGC* R: GAGGTTTCGATCAGGTCTTGG	(GA) <sub>7</sub> (CA) <sub>14</sub>	HEX	15	157-193	Hernandez, et al. (2008)
<b>Ced95</b>	F:ATTTTCATTCCCTTTTAGCC* R:TTATCATCTCCCTCACTCCA	(CT) <sub>17</sub> (AC) <sub>13</sub>	NED	27	80-120	Hernandez, et al. (2008)
<b>Ced131</b>	F: CTCGTAATAATCCCATCCA* R: GGAGATATTTTTGGGGTTTT	(CT) <sub>16</sub>	NED	24	66-128	Hernandez, et al. (2008)
<b>CF26</b>	F: CCAAATCCAGAGGAGAG* R: GTTCTGCTTCATCGAAGG	(AG/TC) <sub>13</sub>	6-FAM	26	139-187	Gandara (2009)
<b>CF66A</b>	F: CAGCAGTTCTGAAACAGTAA* R: ATTCAGCAACTTGAGAGC	(AG/TC) <sub>13</sub>	6-FAM	28	113-175	Gandara (2009)
<b>CF66B</b>	F: CAGCAGTTCTGAAACAGTAA* R: ATTCAGCAACTTGAGAGC	(AG/TC) <sub>13</sub>	6-FAM	23	199-253	Gandara (2009)

Note: (\*) show primers fluorescently labeling. N<sub>A</sub>, number of alleles per locus, bp, base pairs

Raw microsatellite data from present study were combined with raw data that had been obtained for *C. fissilis*: 76 specimens from four populations of the Chiquitano range (ALT, FIG, TOC, and POC), 64 specimens from five populations from central Brazil (ITA, JAN, COR, CAT and UBE), and 139 specimens from nine populations of the Atlantic range (BLU, CAM, CAP, PAL, DIA, PRD, PEU, PSB, and MDP) (Mangaravite et al., 2016; Huamán-Mera et al., submitted); also were combined with raw data previously obtained for *C. brachystachya*: 24 specimens from ANA population of central Brazil (Huamán-Mera et al., submitted) and raw data obtained for *Cedrela* spp.: 83 specimens from eight populations from central Brazil (HD1, HD2, HD3, CEC, FR1, FR2, OUR, PRN) (Huamán-Mera, 2014). The sizes of population samples varied from 4 to 36 (Table 1). Those additional specimens from both previous studies had been genotyped with the same 11 microsatellite loci; thus, the two datasets could be combined for a joint analysis. In the combined dataset, the fragments were scored using GeneMapper version 4.0 (Applied Biosystems, São Paulo, Brazil).

We used the total 490 specimens of *Cedrela*. Then, the Bayesian clustering approach of Structure, version 2.3.4 (Pritchard et al., 2000; Hubisz et al., 2009) inferred the number of Bayesian groups using the Monte Carlo Markov Chain (MCMC) approach. We set runs with a burn-in period of 250,000 steps followed by 750,000 steps, with 15 independent replications. In Structure, we set K from 1–15, following previous study Huamán-Mera et al. (submitted). We used the  $\Delta K$  method of Evanno et al. (2005), as implemented in Structure Harvester (Earl and VonHoldt, 2011) to find the best K (the number of Bayesian groups that best fit the data). Then, we used the software Clumpp (Jakobsson and Rosenberg, 2007) to converge the data of the 15 interactions in the best K and the software Distruct (Rosenberg, 2004) to graphically display the results. For each population, we followed Mangaravite et al. (2016) and summed the membership coefficients for all samples to obtain a diagram depicting the relative contribution of each Bayesian group we had found (best K).

### 2.3 cpDNA and ITS datasets Sequencing

The previous microsatellite analyses showed clearly strong different genetic groups among the populations of *Cedrela* in central Brazil. With this previous result, we carried out properly phylogenetic analysis. We conducted Polymerase chain reactions (PCR) and DNA sequencing using methods and primers described in Garcia et al. (2011). We sequenced from the chloroplast genome (cpDNA) the following four gene regions: AB (the intergenic spacer

between trnT and the 5' exon of trnL), CD (the intron of trnL), SG (the trnS–trnG intergenic spacer), and BF (the psbB–psbT–psbN genes) (Taberlet et al., 1991; Hamilton, 1999). From the nuclear genome, we sequenced the entire internal transcribed spacer (ITS) region of the nuclear 18S–26S ribosomal RNA genes, which included the 5.8S ribosomal gene. PCR primer sequences used to amplify these genes are shown in Table 3.

Finally we used the BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to compare the identity of sequences of present study with those deposited in the Genbank for *Cedrela*.

**Table 3.** PCR primers with oligonucleotide sequences, optimal annealing temperatures, and expected amplicon sizes.

<b>Target region</b>	<b>Primer pairs</b>	<b>Ta (°C)</b>	<b>Amplicon size (bp)</b>	<b>Reference</b>
ITS	ITS4 (5'-TCCTCCGCTTATTGATATGC-3')	58	644	White et al. 1990
	ITS.LEU (5'-GTCCACTGAACCTTATCATTTAG-3')			Baum et al. 1998
trnS-G	trnS (5'-GCCGCTTTAGTCCACTCAGC-3')	54	716	Hamilton 1999
	trnG (5'-GAACGAATCACACTTTTACCAC-3')			Hamilton 1999
psbB-T-N	psbB (5'-GTTTACTTTTGGGCATGCTTCG-3')	54	684	Hamilton 1999
	psbF (5'-CGCAGTTCGTCTTGGACCAG-3')			Hamilton 1999
trnT-L	A2 (5'-CAAATGCGATGCTCTAACCT-3')	58	918	Cronn et al. 2002
	Ac (5'-CGTAGCGTCTACCGATTTTCG-3')			Taberlet et al. 1991
	C (5'-CGAAATCGGTAGACGCTACG-3')	59	598	Taberlet et al. 1991
	D (5'-GGGGATAGAGGGACTTGAAC-3')			Taberlet et al. 1991



## 2.4 Supplementary information from GenBank

To we can infer the phylogenetic relationships of *Cedrela* from central Brazil and congeners, we supplemented our set of sequences with 20 additionally sequences available from the GenBank to represent 12 congeners that were sampled across the genus. Those sequences represented information from: (a) the two previously genealogical lineages of *C. fissilis* found in Brazil; (b) one species from the central Brazil: *Cedrela brachystachya*; (c) two species from the Argentinian Yungas: *Cedrela saltensis* and *C. balansae*; (d) two species from the southern Peruvian Andes: *C. weberbaueri*, *C. angustifolia*; (e) three species from the northern Peruvian Andes: *C. nebulosa*, *C. kuelapensis*, *C. molinensis*; (f) two species from the Ecuadorian Andes: *C. montana* and *C. odorata*; and (g) *C. tonduzii* from Costa Rica Table 4.

**Table 4.** Taxa, codes, origins, and GenBank accession numbers for specimens used in phylogenetic analyses.

Taxon	Code	Origen	GenBank Accessions			
			ITS	trnS-G	psbB-T-N	trnT-L
Cedrela balansae C.DC	-	Argentina	FJ462473			
Cedrela balansae C.DC	-	Paraguay	FJ462474			
Cedrela balansae C.DC	CBRS2	Argentina		KT384358	KT384361	KT384356
Cedrela brachystachya (C.DC.) C.DC.	1123	Brazil	KT316318	KT316295	KT369046	KT310249
Cedrela brachystachya (C.DC.) C.DC.	1126	Brazil	KT316319	KT316296	KT369047	KT310250
Cedrela brachystachya (C.DC.) C.DC.	1145	Brazil	KT316325	KT316302	KT369053	KT310256
Cedrela brachystachya (C.DC.) C.DC.	1170	Brazil	KT316331	KT316308	KT369059	KT310262
Cedrela brachystachya (C.DC.) C.DC.	1185	Brazil	KT316335	KT316311	KT369062	KT310265
Cedrela fissilis Vell	AFL28	Brazil	KT316337	KT316312	KT369063	KT310266
Cedrela fissilis Vell	CMO296	Brazil	KT316338	KT316313	KT369064	KT310267
Cedrela fissilis Vell	RBR423	Brazil	KT316340	KT316315	KT369066	KT310269
Cedrela fissilis Vell	VIC_177	Brazil	JF922199	JF922266	JF922162	JF922135
Cedrela fissilis Vell	VNI_37	Brazil	JF922184	JF922262	JF922158	JF922131
Cedrela fissilis Vell	PLA_277	Brazil	JF922179	JF922282	JF922179	JF922152
Cedrela fissilis Vell	COL_268	Brazil	JF922233			
Cedrela fissilis Vell	SMA73	Brazil	JF922213			
Cedrela kuelapensis T.D. Penn. & A. Daza		Peru	FJ462469			
Cedrela angustifolia DC.		Peru	FJ462479			
Cedrela molinensis T.D. Penn. & Reynel		Peru	FJ462465			
Cedrela montana Moritz ex Turczaninov	MN3	Ecuador	KT316339	KT316314	KT369065	KT310268
Cedrela nebulosa T.D. Penn. & A. Daza		Peru	FJ462460			
Cedrela odorata L.		Ecuador	FJ462464			
Cedrela saltensis Zapater & del Castillo	SSA8	Argentina	FJ462462			
Cedrela weberbaueri Harms		Peru	FJ462472			
Cedrela tonduzii C.DC.		Costa Rica	FJ462485			
Toona ciliata		Australia	FJ462488			

## 2.5 Bayesian phylogenetic analyses

To develop this part of our study we used the previous information generated in microsatellite analysis. We chose appropriate number of sequences for each different group generated in previous microsatellite analysis. So, two distinct datasets were assembled to carry out Bayesian phylogenetic analyses. Dataset A (N=86; 652pb) contained sequences of the ITS region only, while dataset B (N=71; 2922 pb) consisted of the concatenation of four gene regions (AB, CD, SG, and BF) of the cpDNA. Each dataset was input independently to the software MRMODELTEST v2.3 (Nylander, 2004). The Akaike Information Criterion indicated HKY+I+G (for dataset A) and GTR+I+G (for dataset B) as the best fit models among the 24 models of molecular evolution. For each dataset, Bayesian analyses were performed in MRBAYES v3.1.2 (Ronquist and Huelsenbeck, 2003). The ITS dataset and the cpDNA dataset had *Toona ciliata* and *Cedrela tonduzi* as outgroups, respectively. Each analysis was carried out using two simultaneous runs of five million generations each, with one cold and three heated chains in each run; average standard deviation of split frequencies at the end of each run was near 0.01; trees were sampled once every 1,000 generations; the first 250 trees were discarded as burn-in samples. For each dataset, a 50%-majority-rule consensus tree of the two independent runs was obtained with posterior probabilities that were equal to bipartition frequencies. Final trees were visualized and edited for publication with the help of the program FigTree (Rambaut, 2009).

## 2.6 Divergence dating

To estimate the time of divergence among the different genetic groups of *Cedrela* in Brazil, we used the relaxed clock method as implemented in BEAST v1.8.4 (Drummond and Rambaut, 2007). The BEAST analysis assumes evolutionary models and for chose the best evolutionary model we followed as implemented by Garcia et al. 2011. For unrevealing the position in the phylogeny of *Cedrela* of our different genetic groups, we merged part of our data (ITS dataset A) with interspecific data from Genbank accessions (Appendix B). We chose one sequence for each different genetic group, one sequence for *Cedrela brachystachya*, and four sequences from congeners obtained in this study (*Cedrela angustifolia*, *C. kuelapensis*, *C. molinensis* and *C. montana*). Then, we combined our data with the ITS dataset of *Cedrela* congeners. Our final dataset used in BEAST analyses contained a total of 36 sequences: 34 for the tribe Cedreleae (31 sequences of *Cedrela* and three of *Toona*) and one

sequence of each of the two outgroups, *Khaya anthotheca* and *Swietenia macrophylla*. The Akaike Information Criteria (Akaike, 1973) in MrModeltest 2.3 (Nylander, 2004) indicated GTR+I+G to be the best-fit model of molecular evolution. We performed in BEAUTi v1.8.4 software (Drummond and Rambaut, 2007) the conversion of nexus file obtained previously from our ITS data into \*.xml input file used in BEAST. For BEAUTi parameters we followed as Garcia et al. (2011), the molecular clock model was chosen the uncorrelated log-normal relaxed clock option. Yule process speciation was used as the tree prior. We calibrated the BEAST analysis with the following normally distributed priors: the date of the most recent common ancestor (MRCA) of Cedreleae was set to 48.6 million years ago (Mya) (Reid and Chandler 1933; Chandler 1964), with an SD of 1; the date of the MRCA of Cedrela set to 33.62 Mya (Meyer and Manchester, 1997), with an SD of 1; and the date of the MRCA of *Khaya* and *Swietenia* was set to 22.5 Mya (Castañeda-Posadas and Cevallos-Ferriz, 2007), with an SD of 1. The analysis was run for 10 million generations, with samples taken every 2000 generations. These settings ensured that both model parameters and time estimates were sampled adequately (Effective Sample Size, ESS, values were well above 500 for all statistics in Tracer 1.5).

## 2.7 Species Distribution Modeling (SDM)

Additionally, we carried out species distribution models (SDMs) analyses for different groups of genus *Cedrela* in central Brazil. We use occurrences of *Cedrela* across central Brazil. Some of those occurrences were obtained in present study and others took from Huamán-Mera (2014). At least five to ten points are enough to predict a SDM in MAXENT when dealing with small sites occurrences (Hernandez et al., 2006). Then we built models of distribution for current conditions (0 kya pre-industrial), midHolocene (6 kyr BP), LGM (21 kyr BP), and Last Interglacial (LIG, 120-140 kyr BP) periods.

The suitable areas for the current potential distribution were modeled with Maxent v.3.4.1 (Phillips and Dudik, 2008). Environmental data were obtained for all geographical coordinates (19 standard BIOCLIM variables; Hijmans et al., 2005). We used climatic layers with a resolution of 30s (1 km spatial resolution) and cropped to the range of Brazil. To avoid overparameterization of SDM due to redundant variables (Dormann et al., 2013), the correlations between bioclimatic variables were assessed and those with presumed reduced biological relevance ( $r > .9$ ) (Werneck et al., 2012; Bueno et al., 2017). Palaeoclimatic data represent downscaled climate data from simulations with Global Climate Models (GCMs)

based on the Coupled Model Intercomparison Project Phase 5 (CMIP5; Taylor et al., 2012). For the LIG model, the Otto-Bliesner et al. (2006) approach was used, and for LGM and Holocene, the Community Climate System Model—CCSM4 was employed (Gent et al., 2011). All geographic information system (GIS) analyses were performed in ArcGIS v.10 (ESRI, 2011). All bioclimatic layers are available in the WorldClim website ([www.worldclim.org](http://www.worldclim.org)). The final selected variables were 13 (TableS2).

The models were evaluated randomly separating the occurrence points into training (75% of data) and testing (25% of data) data sets and then constructed each model ten times and averaged the output to produce the final results. Then we performed an analysis that evaluates the performance of the model using a single value, the area under the curve (AUC) (Phillips et al., 2006).

To infer potential areas of climatic stability for each of the four groups of *Cedrela* from central Brazil during the Quaternary, the four climatic projections were summed using the raster calculator tool in ArcGIS v.10 (ESRI, 2011) and then the resulting layer was reclassified to show only the areas of high ecological stability. These combined maps depicted historically stable areas, which we considered to be potential refugia.

### **III. RESULTS**

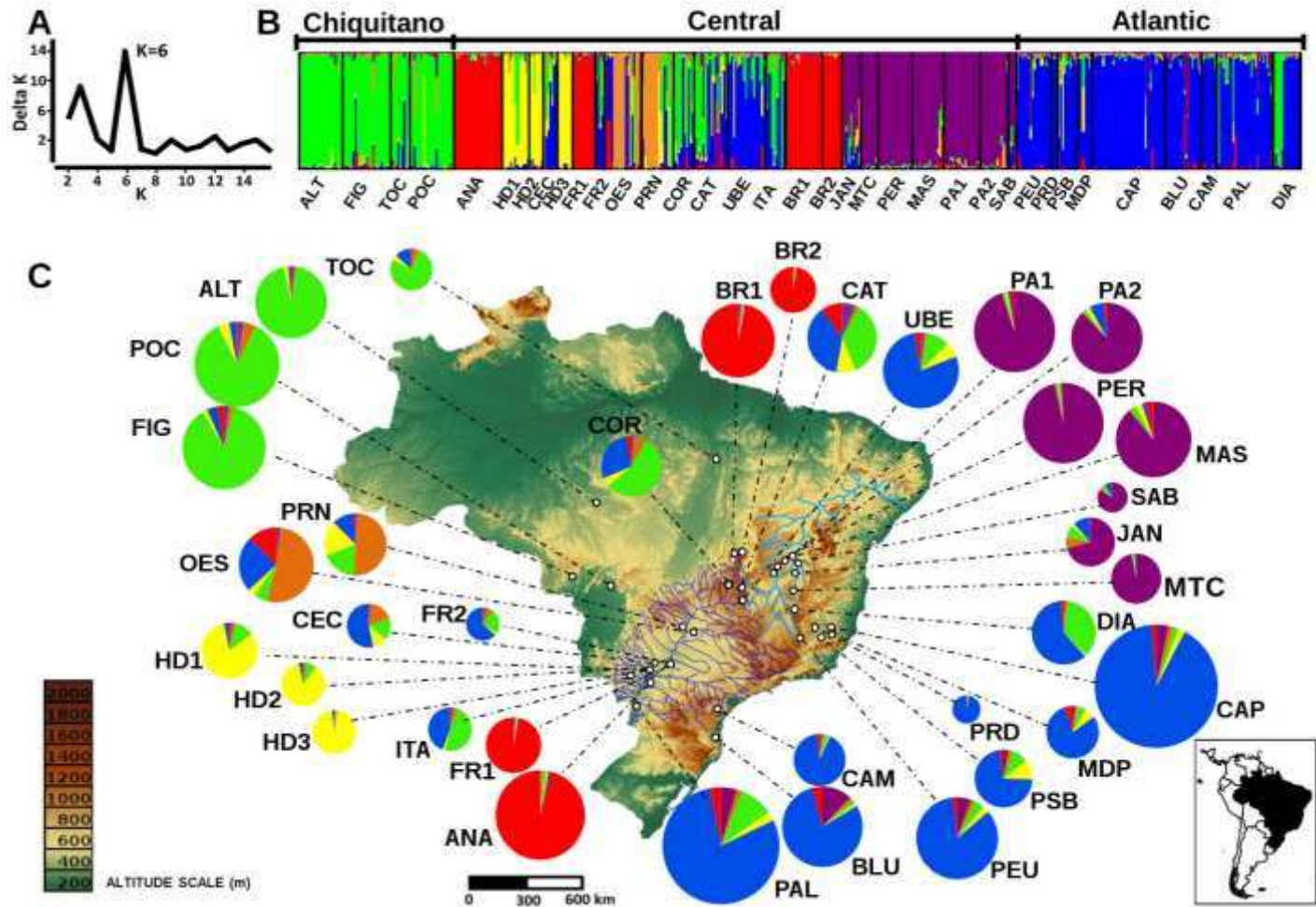
#### **3.1 Analyses of microsatellite loci**

We assessed the Bayesian analysis of population structure and it showed the presence of six genetically different groups (best  $K=6$ ) (Figure 2). Two groups split according to the geographic placement previously known as East lineage or Chiquitano range (green in color) and West lineage or Atlantic range (blue in color) (Garcia et al., 2011). The remaining four outstanding groups were denominated as follow: the red group corresponded to *Cedrela brachystachya* samples, an ecologically specialized and taxonomically revalidated species of *Cedrela* from upper Parana River Basin (Aneurilândia/MG, ANA) (Huamán-Mera et al., submitted). In present study, we discovered more places of distribution for this species (Populations FR1, BR1 and BR2), afterwards we denominated as “Cedrinho” group. The yellow group corresponded to populations distributed in a place that we denominated as “Hidroelétrica” group with three populations (HD1, HD2 and HD3). The orange group was denominated as “Iturama” group; this group has two populations (Paranaíba/MS, PRN and Ouro Este/SP, OES). The populations PRN and OES showed individuals with only admixture genetic information from the others groups, just a bit more than 50% of individuals showed an

assignment proportion over 98% of the genetic information of the orange group *strictu sensu*. The last genetic group, the purple group, had populations located in Northern Minas Gerais state so we denominated as “Norminas” group. The populations that grouped inside this group were JAN, MTC, PER, MAS, PA1, PA2 and SAB. With our new microsatellite data we covered a larger sampling area and we established the current bordering of *Cedrela* groups within central Brazil.

All populations of the six lineages sharing genetic information among them, they showed high assignment proportion in each peculiar population. For instance, the four populations from Chiquitano range showed an assignment proportion over 86% in the first group, depicted in green, and the remaining 14% was sharing among red, yellow, orange, purple and blue lineages; the nine populations from Atlantic range showed an assignment proportion over 81% in the second group, depicted in blue, and over 18% of assignment proportion was sharing in red, yellow, orange, purple and green groups (Figure 2 B and C). In the other hand, in central Brazil, we observed the four remaining groups highly structured and others populations sharing proportional genetic information from all the six groups found in present study. For instance, the four populations (ANA, FR1, BR1 & BR2) from “Cedrinho” group showed an assignment proportion over 96.7%, depicted in red, and the remaining 3.3% was sharing among the other groups. The three populations (HD1, HD2 & HD3) from “Hidroelétrica” group showed an assignment proportion 88%, depicted in yellow, and the remaining 12% was sharing among the other groups. The “Iturama” group is compound by individuals inside the populations PRN and OES, this group is compound by 33 individuals, but only 17 individuals formed the fifth group, ten individuals in PRN population and seven individuals in OES population. The 17 individuals joined together showed an assignment proportion over 95%, depicted in orange, and the remaining 5% was sharing among blue, green and yellow groups. The remaining 16 individuals of the Iturama group showed admixture assignment proportion from the other groups. The seven populations from the “Norminas” group showed an assignment proportion over 88%, depicted in purple, and the remaining 12% was sharing among the other five groups. Moreover, in the central range, we observed populations sharing proportional genetic information from the two major groups (Chiquitano and Atlantic), six populations (CEC, FR2, COR, CAT, UBE & ITA) showed over 31.6% of assignment proportion corresponding to Chiquitano range information and over 50.5% corresponding to Atlantic lineage information, the remaining 17.9% of assignment proportion corresponding to the others groups (red, yellow, orange and purple in color). Finally, we found less proportion of genetic signature of each four groups in all populations of

present study, for instance, the less proportion of genetic signature of “Cedrinho” group in the remaining populations ranged from 0.2% (MTC) to 10% (CAT) of assignment proportion, the less proportion of genetic signature of “Hidroelétrica” group in the other populations ranged from 0.4% (FR1 and BR1) to 11% (CEC and PSB), the less proportion of genetic signature of “Iturama” group in the other populations ranged from 0.4% (HD3, BR2, MTC, PA1) to 18% (CEC) and the less proportion of genetic signature of “Norminas” group in the other populations ranged from 0.3% (ANA) to 12% (BLU).



**Figure 2.** Clustering analyses and the geographic distribution of populations of *Cedrela*. (A) The best K ( $K=6$ ) was calculated according to the  $\Delta K$  method (Evanno et al. 2005). (B) Plot of the clustering analysis in the STRUCTURE software (Pritchard et al. 2000), showing the two lineages of *Cedrela fissilis* (west lineage, green; east lineage, blue), the population ANA that belongs to *Cedrela brachystachya* (coded red) plus the populations FR1, BR1, and BR2 that depicted in coded red also, the lineage Hidroelétrica (coded Yellow), the lineage Iturama (coded Orange), and the lineage Norminas (coded purple). Along the x-axis, each vertical bar represents a sampled individual; along the y-axis, membership coefficient of a sample for a lineage represents the fraction of its genome that has ancestry in that lineage. (C) The geographical origin of each population and lineage contribution to contemporary gene pools. Each pie diagram represents the sum of membership coefficients for all samples from that population. Circle size is proportional to population sizes. (Refer to Figure 1 and Table 1 for population codes).

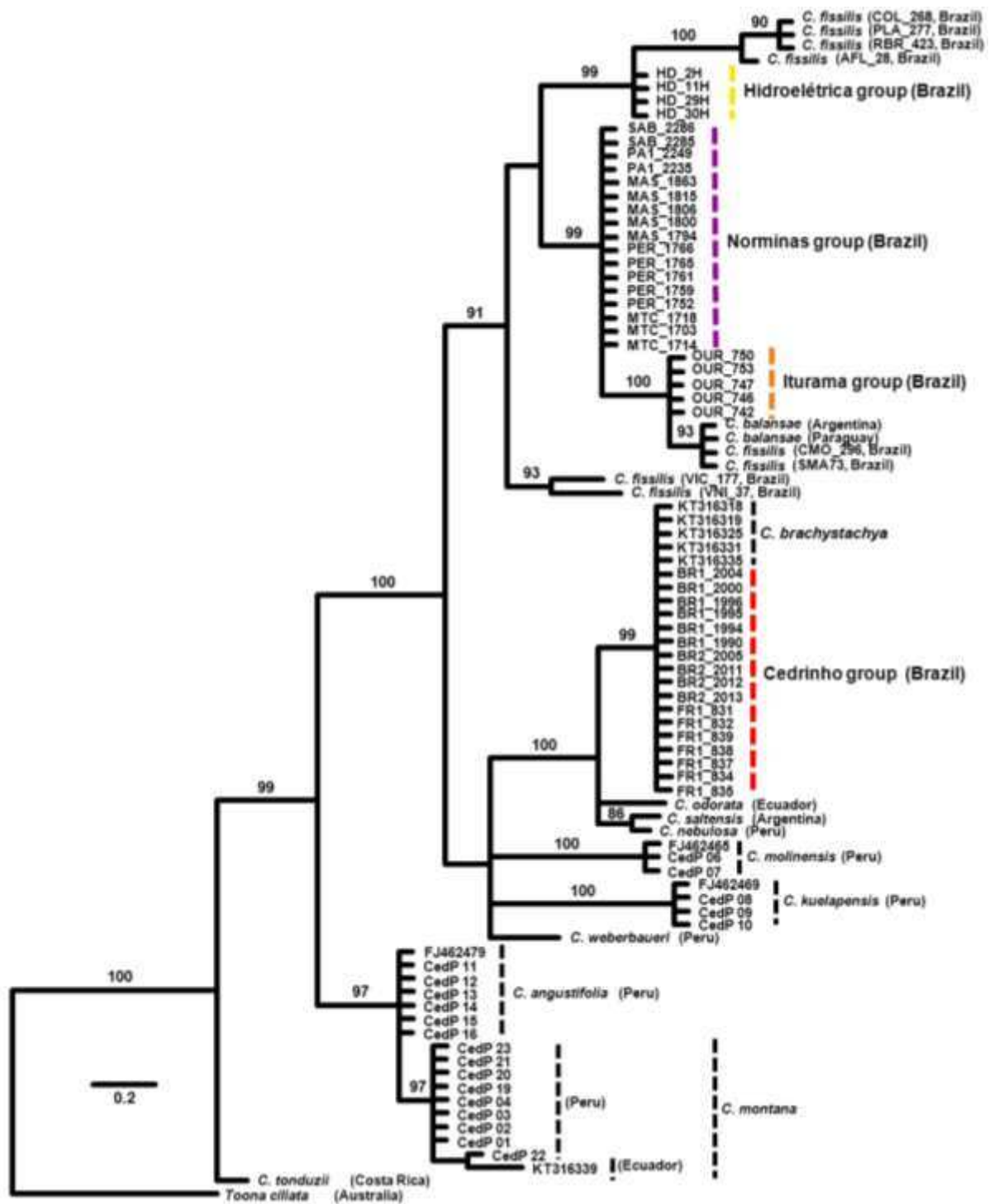


### 3.2 Bayesian phylogenetic analyses

We obtained 88 sequences from the specimens distributed along central Brazil and use to evaluate phylogenetic relationships. For ITS region, we obtained 17 sequences from the “Cedrinho” group, four sequences from the “Hidroelétrica” group, five sequences from the “Iturama” group, and 17 sequences from the “Norminas” group. For cpDNA dataset, we obtained 15 sequences from the “Cedrinho” group, five sequences from the “Hidroelétrica” group, five sequences from the “Iturama” group, and 20 sequences from the “Norminas” group. Also we obtained 30 sequences for four species of *Cedrela* from the northern and southern Peruvian Andes. 19 ITS sequences for: (a) six specimens of *Cedrela angustifolia*; (b) three specimens of *C. kuelapensis*; (c) two specimens of *C. molinensis* and (d) eight specimens of *C. montana* (Table 4). And 11 cpDNA dataset sequences for the same four species of *Cedrela* described above: (a) two specimens of *Cedrela angustifolia*; (b) two specimens of *C. kuelapensis*; (c) one specimens of *C. molinensis* and (d) six specimens of *C. montana*.

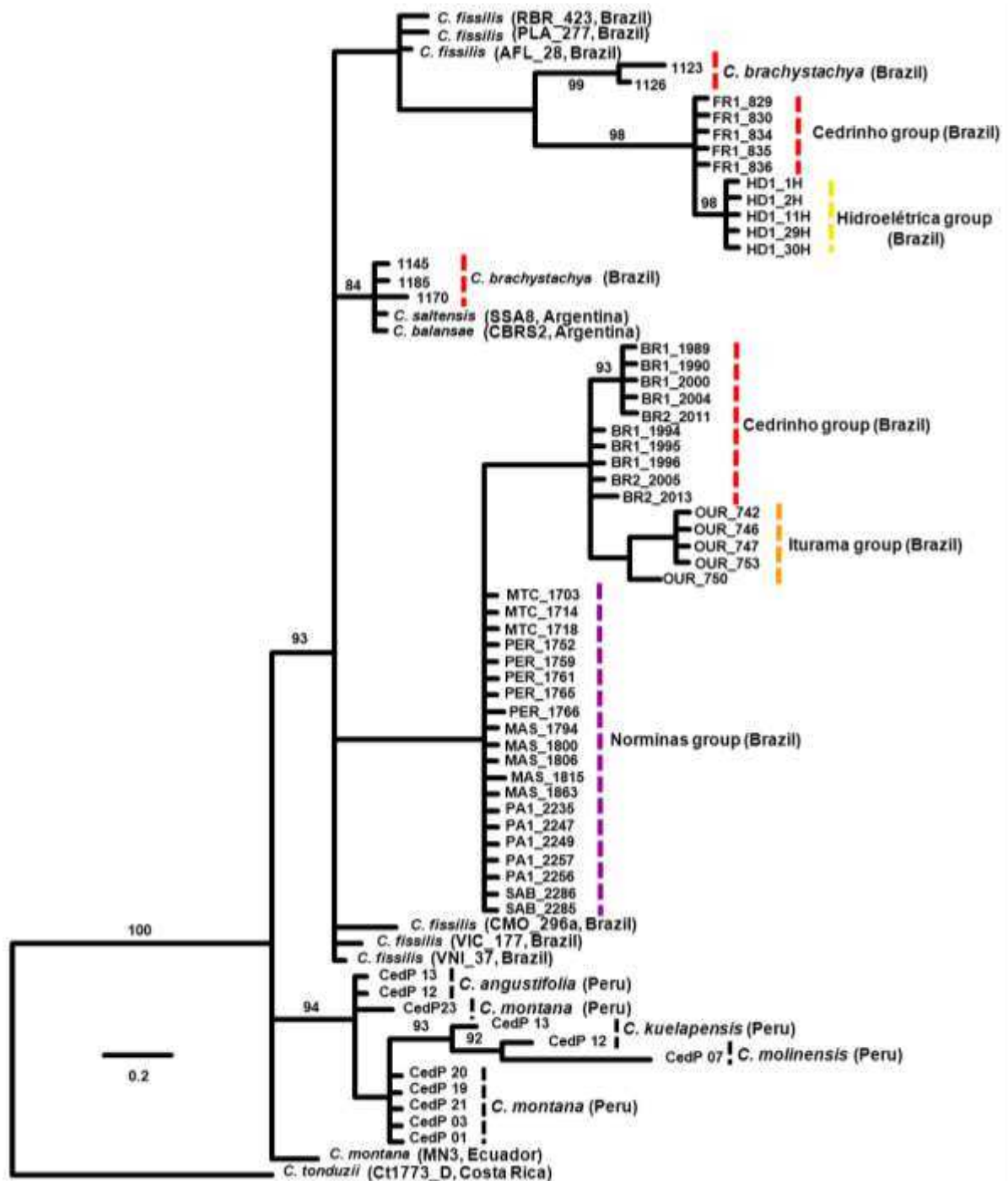
We recovered Bayesian phylogenetic trees for *Cedrela* from central Brazil (Figure 3, for the ITS region; Figure 4 for the cpDNA dataset) that showed both well-supported nodes (PP > 90% on most of the nodes). The Bayesian tree for the ITS region (Figure 3) split *Toona ciliata* from *Cedrela*; within *Cedrela*, *C. tonduzii* (from Central America distribution) and *C. angustifolia* and *C. montana* (from Northern South America distribution) occupied the most basal positions. The “Cedrinho” group formed, all 17 specimens, a tip clade together with ITS sequences of *Cedrela brachystachya* (PP= 100%), this was not a surprise because our BLAST analysis showed an identity of 100% with accessions range from KT316316 to KT316336, which belong to *Cedrela brachystachya* (Huamán-Mera et al., submitted), also the “Cedrinho group” grouped together with *C. saltensis*, *C. nebulosa*, and *C. odorata* to form a highly supported sub-clade (PP=100%). The “Iturama” group grouped with sequences of *C. fissilis* from Atlantic range (Accessions CMO\_296 and SMA73) and *C. balansae* from Argentina and Paraguay to form a well-supported sub-clade (PP=100%). Our BLAST analysis showed an identity of 99% with Genbank accessions of *C. fissilis*, KT316338 and *C. balansae*, FJ462473. All four specimens of “Iturama” group formed a tip clade (PP=100%). The Norminas group grouped with the sub-clade formed by specimens of Iturama group, *C. fissilis* from the Atlantic range (Accessions CMO\_296 and SMA73) and *C. balansae*, a well-supported sub-clade (PP=98%). Our BLAST analysis showed an identity of 99% with Genbank accessions of *C. fissilis* from Atlantic range such as JF922189 and JF922188. The last group, the

“Hidroelétrica” group grouped together with specimens of *C. fissilis* from Chiquitano range to form a well-supported sub-clade (PP=99%). Our BLAST analysis showed an identity of 99% with accessions of *C. fissilis* such as JF922245 or JF922241.



**Figure 3.** Bayesian phylogeny (consensus tree) resulting from the ITS dataset, showing the relationships between the four new groups of *Cedrela* from central Brazil and 12 congeners, with *Toona ciliata* used as outgroup. Also we are included new sequences of Andean *Cedrela* (*Cedrela angustifolia*, *C. kuelapensis*, *C. molinensis*, and *C. montana*). Branch lengths are drawn to scale; nodal support values are given as posterior probabilities (%) above the branches (when > 80%). Scale bar corresponds to the expected number of substitutions per site. See Table 4 for the additional information about the accession numbers.

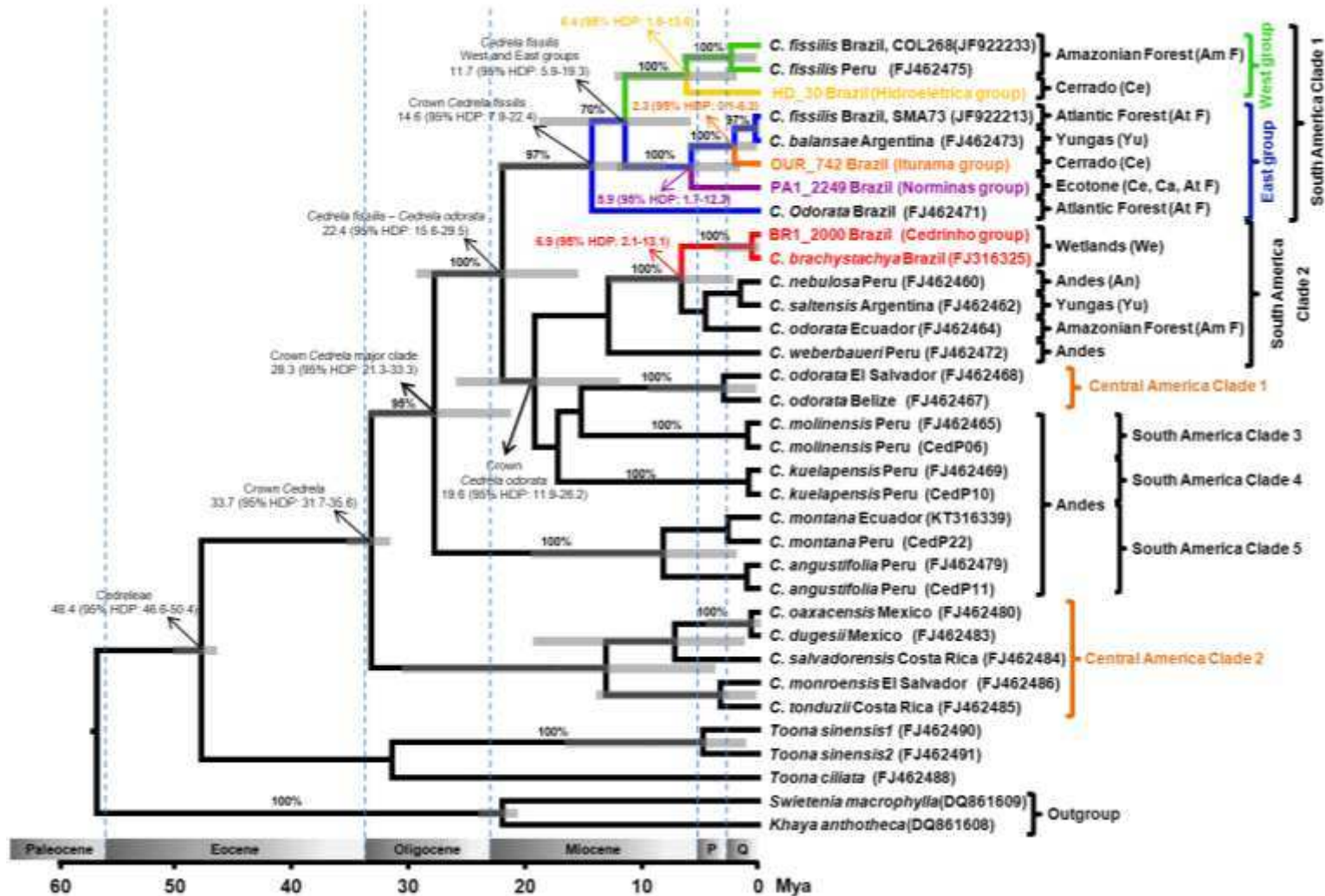
The Bayesian tree based on cpDNA dataset (Figure 4) exhibited additional details about the phylogenetic relationships of specimens of different genetic groups of *Cedrela* from central Brazil and its congeners. Specimens from the “Cedrinho” group grouped in different clades, but the groups that formed were from the same geographic distribution. For instance, specimens from FR1 population formed a sub-clade with the sub-clade where two specimens of *C. brachystachya* (1123 and 1126) and at the same time these two sub-clades formed other sub-clade related to specimens of *C. fissilis* from Chiquitano range. On the other hand specimens of BR1 and BR2 populations grouped in the clade where specimens of Iturama group formed a sub-clade. Others specimens of *C. brachystachya* (1145, 1170 and 1185) grouped together with *C. balansae* and *C. saltensis*. Specimens of “Hidroelétrica” group formed a tip clade and were related to specimens of FR1 population from the “Cedrinho” group. They altogether formed a sub-clade which was related to specimens of *C. brachystachya* (1123 and 1126). Specimens of “Iturama” group, mentioned above, formed a sub-clade related to specimens of BR1 and BR2 populations. Specimens of the last group “Norminas” group grouped all together and formed a sub-clade with the sub-clade formed between specimens of BR1 and BR2 and specimens of “Iturama” group.



**Figure 4.** Bayesian phylogeny (consensus tree) resulting from the cpDNA dataset, showing the relationships between the four new groups of Cedrela from central Brazil and 9 congeners, with Cedrela tonduzii used as outgroup. Also we are included new sequences of Andean Cedrela (Cedrela angustifolia, C. kuelapensis, C. molinensis, and C. montana). Branch lengths are drawn to scale; nodal support values are given as posterior probabilities (%) above the branches (when > 80%). Scale bar corresponds to the expected number of substitutions per site. See Table 4 for the additional information about the accession numbers.

### 3.3 Estimated date of divergence

The BEAST analysis output a maximum clade credibility tree (Figure 5). We chose five sequences from our new different groups (BR1\_2000 and *C. brachystachya* 1145 from the “Cedrinho” group; HD-30H from the “Hidroelétrica” group; OUR\_742 from the “Iturama” group; and PA1\_2249 from the “Norminas” group) for BEAST analysis, which were more representative among all sequences. The BEAST analysis grouped the sequences of *Cedrela* species into seven greatest groups, two Central America clades and five South America clades. Current distribution of South America clades ranges randomly from Andes in the West to Atlantic forest in the East. Also our maximum clade credibility tree showed an important clade, the denominated *Cedrela fissilis*-*Cedrela odorata* crown (PP = 100%). These major crown split into two minor Crowns, the *Cedrela odorata* crown and the *Cedrela fissilis* crown, and its divergence time took place about 22.4 Mya. The divergence time for *Cedrela odorata* crown took place about 19.6 Mya and is compound by sequences of *Cedrela* species with current different geographic distribution from Central America to South America, mostly in Andes distribution. The sequences BR1\_2000 and FJ316325 that belong to *Cedrela brachystachya* grouped into the *Cedrela odorata* crown and formed a strongly supported sub-clade (PP= 100%) together with *C. nebulosa*, *C. saltensis* and *C. odorata* from Ecuador. The divergence time between the sequence BR1\_2000 and *C. brachystachya*, and congeners from this sub-clade took place about 6.9 Mya. The crown *Cedrela fissilis* formed a strongly supported sub-clade (PP=97%), its divergence time took place about 14.6 Mya and is compound by sequences of *Cedrela* species distributed from Amazonian to Atlantic forest, including the Cerrado domain. The sequences PA1\_2249, OUR\_742 and HD\_30 grouped within the crown *Cedrela fissilis*, in the previously denominated *Cedrela fissilis* West and East lineages (Garcia et al., 2011), two strongly supported sub-clades (PP = 100%) which divergence time took place about 11.7 Mya. The sequences OUR\_742 and PA1\_2249 grouped within the East group together with *C. fissilis* (SMA73) and *C. balansae* (FJ462473) from Argentina. The sequence PA1\_2249 took the basal placement in this sub-clade and its time divergence took place about 5.9 Mya, on the other hand the divergence time of the sequence OUR\_742 took place about 2.3 Mya. The sequence HD\_30 grouped within the West group together with two sequences of *C. fissilis* (JF922233 from Brazil and FJ462475 from Peru), the Beast analysis suggested that the divergence time of sequence HD\_30 took place about 6.4 Mya.



**Figure 5.** Maximum clade credibility tree for the nuclear ITS region across Cedreleae, estimated with BEAST. GenBank accession numbers are listed in Appendix B; nodal support values are given as posterior probabilities (%) above the branches (shown when >90%). Grey bars at nodes are the 95% highest probability density (HPD) for the age of that node (shown along x axis). Colour code: blue, East group; green, West group; red, Cedrinho group; purple, Norminas group; orange, Iturama group; yellow, Hidroelétrica group. Geographical distribution is as represented only for South America clades. Mean ages in millions of years (Mya) and the corresponding 95% HPD for Cedreleae, Cedreleae, Cedrinho group, Norminas group, Iturama group, Hidroelétrica group, and *C. fissilis* West and East group are as shown.

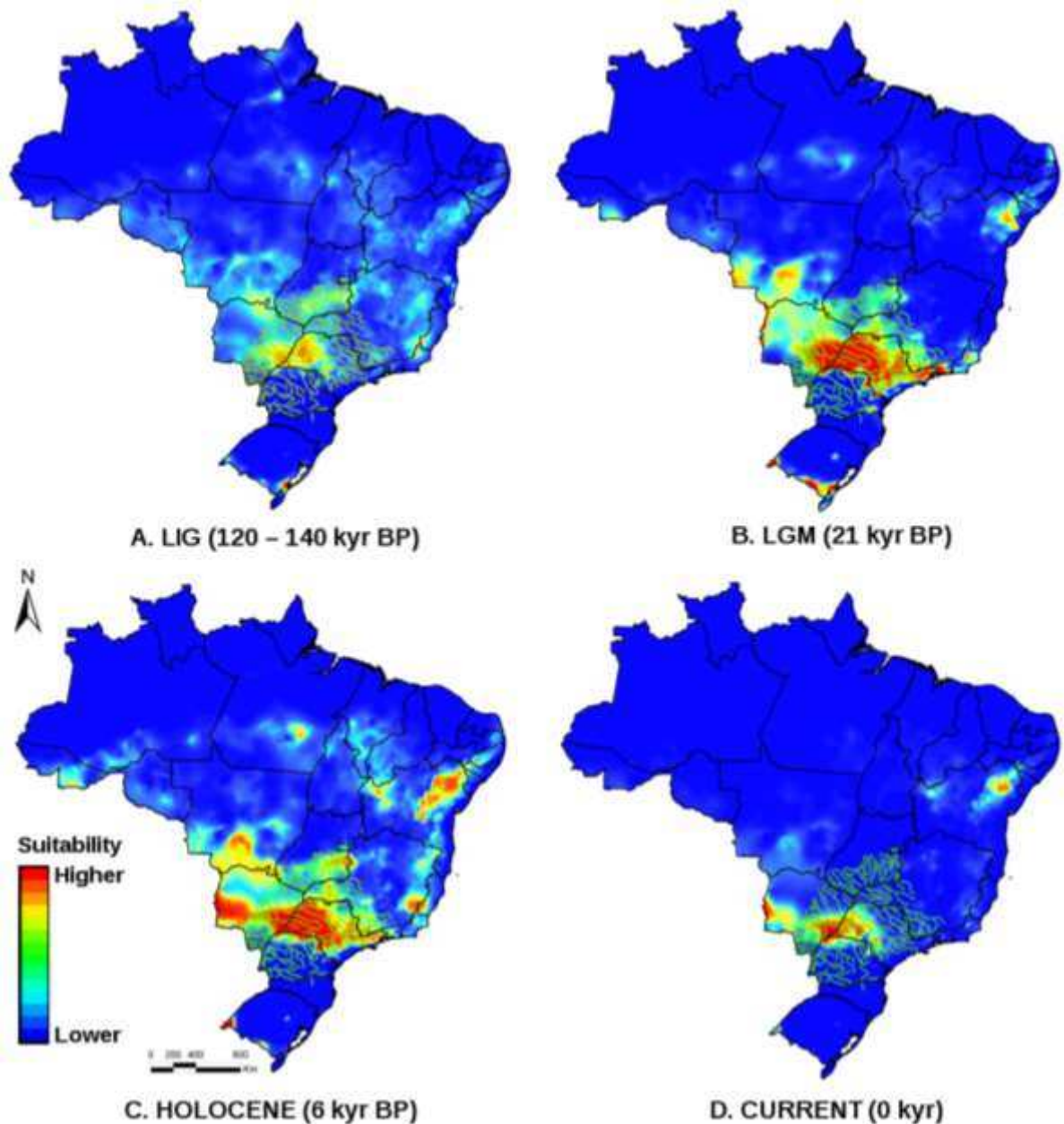
### 3.4 Species Distribution Modeling (SDM)

The Species Distribution Modeling (SDM) suggested significant changes in the suitability and distribution ranges of three groups of *Cedrela* from central Brazil across the Quaternary. The average test AUC (Area Under the receiver operating Curve) values of the models were above 0.9, so the quality of the models showed that samples and background predictions generated by MaxEnt were in agreement. We considered records for the “Cedrinho” group and *C. brachystachya* as the same group (24 occurrences in total), for the “Hidroelétrica” group (29 occurrences), for the “Iturama” group (16 occurrences), and for the “Norminas” group (103 occurrences).

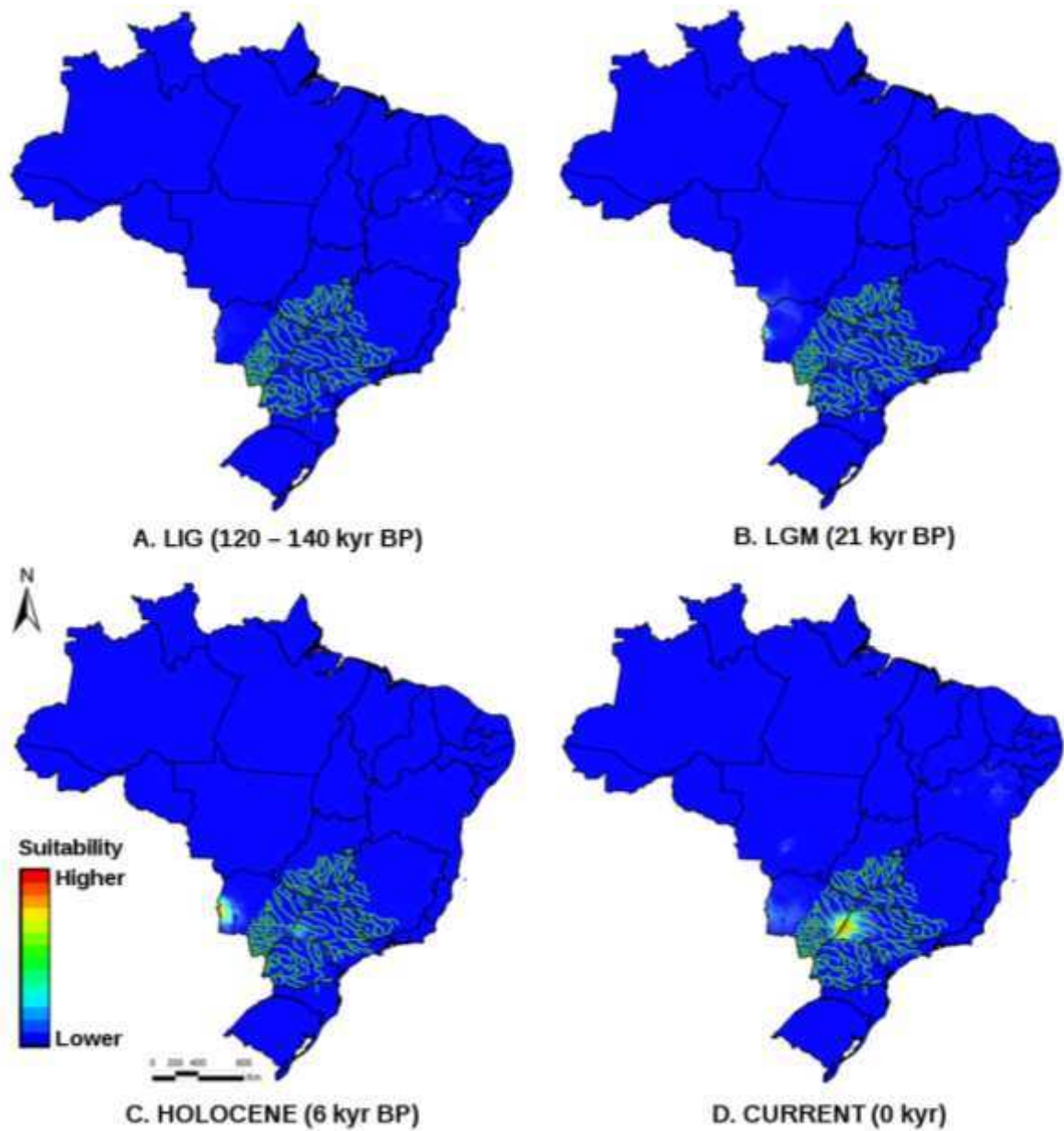
The dynamic of niche displacements are related to expansions and contractions of niche distribution for each of the new four groups of *Cedrela* from central Brazil. Thus, the maximum contraction for “Cedrinho” group occurred during the warm periods, in the LIG and Current periods (Figure 6A & 6D). During the LIG the distribution of “Cedrinho” group almost disappeared when compare with the distribution of Current period; it would be considering a partial retraction. In contrast, the models suggest important expansions of the distribution of “Cedrinho” group during the LGM and Mid-Hol (Figure 6B and 6C). This expansion would have reached new populations of “Cedrinho” group discovered in present study, such as FR1, BR1 and BR2 populations. The dynamic contraction-expansions of “Cedrinho” group distribution across Quaternary are mostly restricted to central-southern Cerrado biome. The raster calculator tool of ArcGis software predicted the isolated and climatically stable areas with high suitability for “Cedrinho” group which showed until three multiple potential refugia for this species. The three refugia are: Central-southern Cerrado; near Pantanal Biome; and west-Northern Bahia state inside Caatinga Biome. The most important was the first area because showed a considerable geographic extension (Figure 10A).

Thee Hidroelétrica group, exhibited gradually retraction of its current distribution to LIG period and also displacement from current position in central Brazil to west until reach the southern Pantanal biome in Brazil (Figure 7 A-D). The raster calculator tool of ArcGis software displayed two potential refugia for Hidroelétrica group, one in central-Southern Cerrado biome and this overlapped with one of the three potencial refugia of “Cedrinho” group. The last one is located in the Southern Pantanal Biome in border with Paraguay country (Figure 10B).





**Figure 6.** Predicted occupancy of suitable areas of “Cedrinho” group in central Brazil during past and current environmental conditions: A. the Last Interglacial (LIG, 120–140 kya BP); B. the Last Glacial Maximum (LGM, 21 kya BP); C. the Mid-Holocene (6 kya BP); and under Current climate (0 kya, pre-industrial)]. Predictions were based on ecological niche models of climatic preferences using the MaxEnt algorithm (Phillips et al. 2006). Black lines represent the borders of Brazilian states. With more quantity of occurrences, the model exhibited similar occupancy to previous study (Huamán-Mera et al. Submitted)

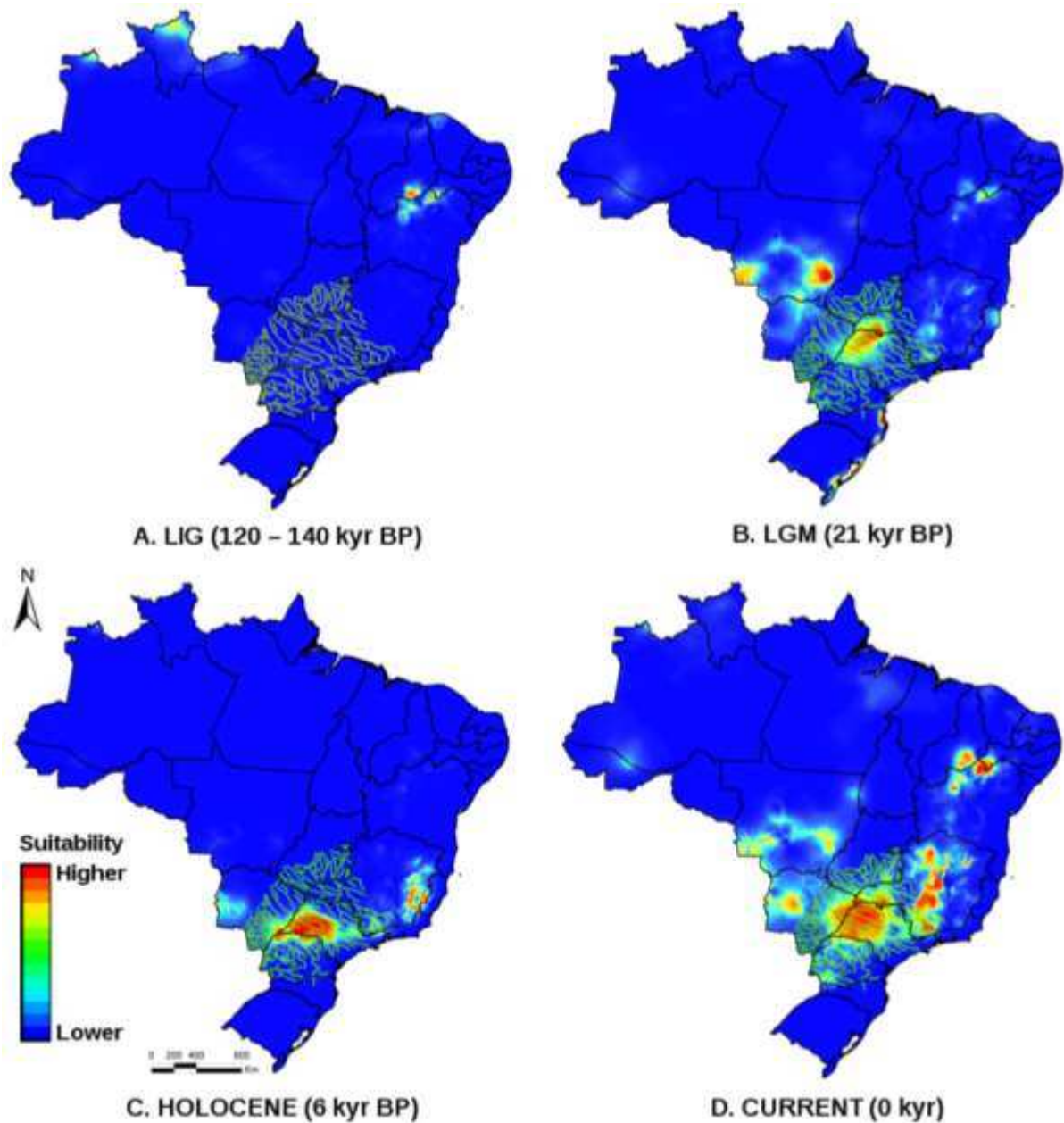


**Figure 7.** Predicted occupancy of suitable areas of “Hidrolétrica” group in central Brazil during past and current environmental conditions: A. the Last Interglacial (LIG, 120–140 kya BP); B. the Last Glacial Maximum (LGM, 21 kya BP); C. the Mid-Holocene (6 kya BP); and under Current climate (0 kya, pre-industrial)]. Predictions were based on ecological niche models of climatic preferences using the MaxEnt algorithm (Phillips et al. 2006). Black lines represent the borders of Brazilian states.

The “Iturama” group, showed the maximum coverage of distribution in the current period in contrast with the periods in the past. In the past, the distribution of Iturama group exhibited a reduction in the LIG period, in this period the niche disappeared completely if we compared with the more recent periods. In the LIG period only a reduced area was observed in Northeastern Brazil (Figure 8 A-D). The raster calculator tool of ArcGis software displayed a large potential refugium for Iturama group and a small refugium distributed surrounded the large. The large potential refugium is located in the Southeastern Brazil in the border of Sao Paulo state with Mato Grosso do Sul, Parana and Minas Gerais states (Figure 10C).

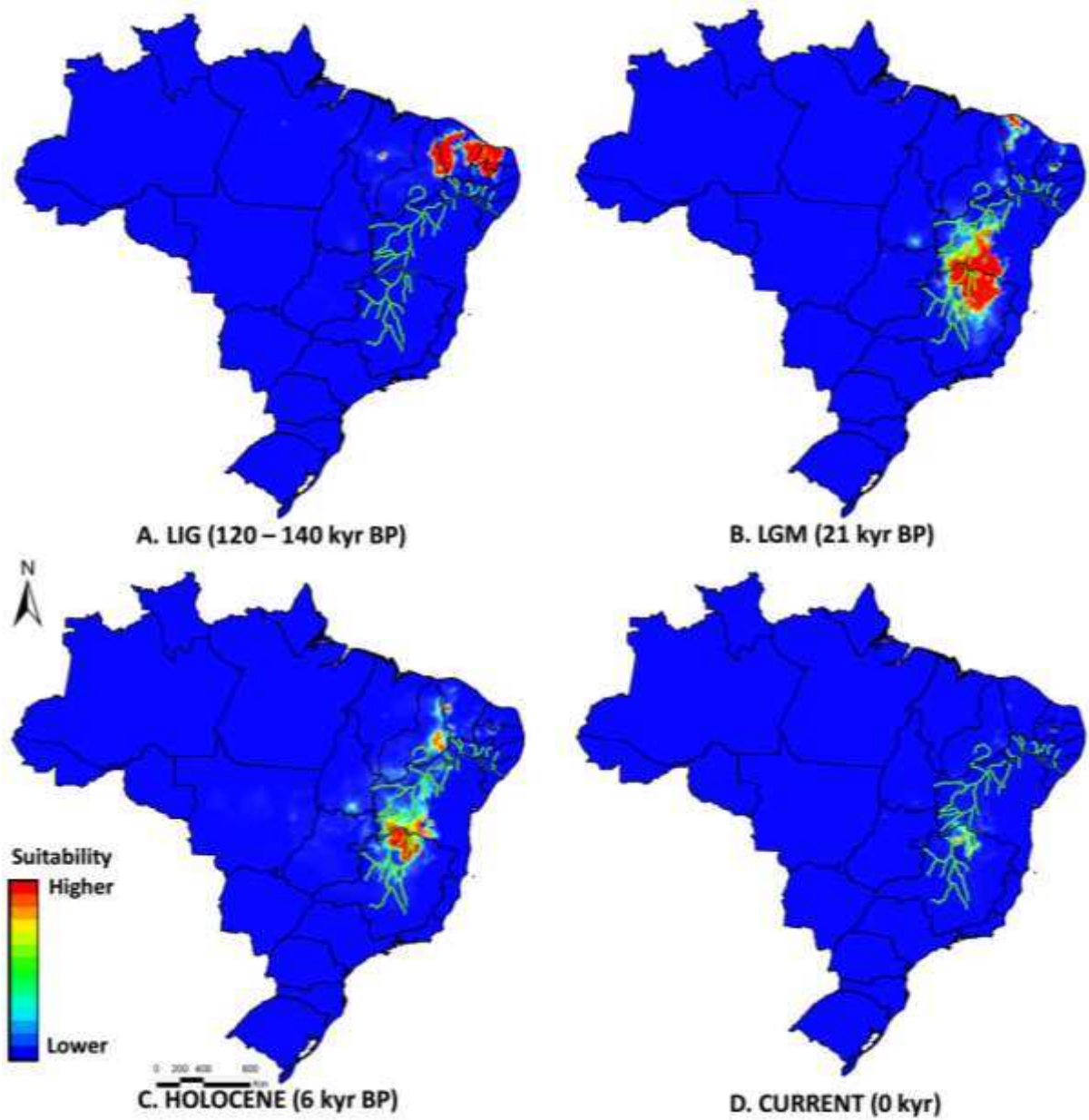
The “Norminas” group exhibited an interested dynamic of distribution across Quaternary. For instance; in current period the population of “Norminas” group showed a restricted distribution and limited to the geographic coordinates taken in our fieldwork (Figure 9D); in past periods such as Holocene and LGM, the distribution of “Norminas” group displayed an increase of the area of geographic distribution (Figure 9 B-C), even the distribution also displaced to Northeastern Brazil in smaller proportion. The most interesting situation occurred when the MaxEnt software modeled the LIG period, the distribution of “Norminas” group was totally displacement from current geographic distribution to the Northeastern Brazil. In the LIG period the geographic distribution was displacement and restricted to Piauí, Ceará, Rio Grande do Norte and Paraíba states in Brazil. The current geographic circumscription of “Norminas” group was totally absent in LIG period (Figure 9A). However the raster calculator tool performed in ArcGis software displayed one potential refugium for “Norminas” group, which is limited to its current distribution, in Northern Minas Gerais State in Brazil and always associated to Sao Francisco River (Figure 10D).

It is important to notice that the dynamics of distribution of *Cedrela* from central Brazil during Quaternary are strictly related to the Parana River Basin and Sao Francisco River Basin, in this point specifically related to Pandeiros and Peruacu rivers.

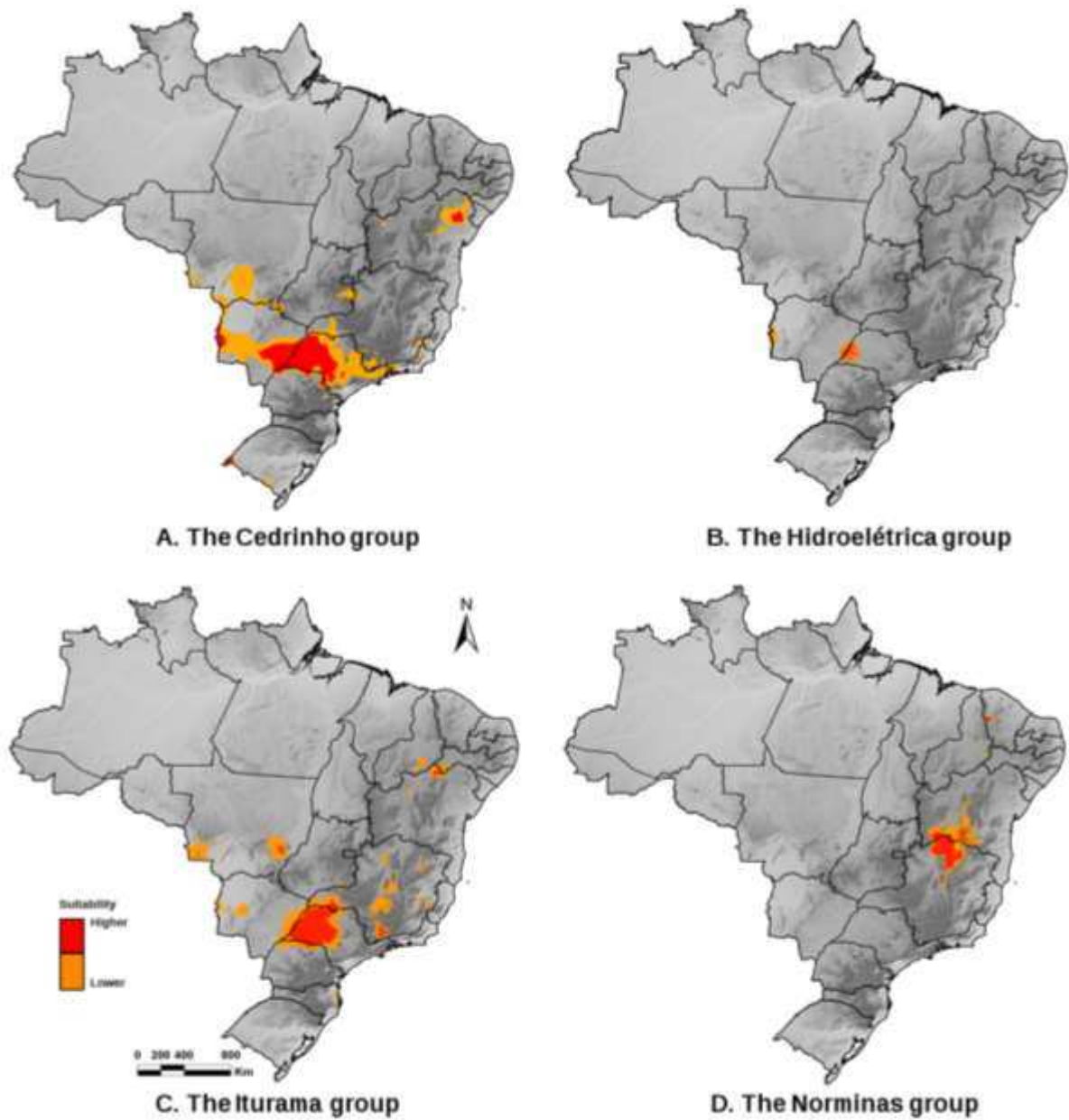


**Figure 8.** Predicted occupancy of suitable areas of “Iturama” group in central Brazil during past and current environmental conditions: A. the Last Interglacial (LIG, 120–140 kya BP); B. the Last Glacial Maximum (LGM, 21 kya BP); C. the Mid-Holocene (6 kya BP); and under Current climate (0 kya, pre-industrial)]. Predictions were based on ecological niche models of climatic preferences using the MaxEnt algorithm (Phillips et al. 2006). Black lines represent the borders of Brazilian states.





**Figure 9.** Predicted occupancy of suitable areas of “Norminas” group in central Brazil during past and current environmental conditions: A. the Last Interglacial (LIG, 120–140 kya BP); B. the Last Glacial Maximum (LGM, 21 kya BP); C. the Mid-Holocene (6 kya BP); and under Current climate (0 kya, pre-industrial)]. Predictions were based on ecological niche models of climatic preferences using the MaxEnt algorithm (Phillips et al. 2006). Black lines represent the borders of Brazilian states.



**Figure 10.** Predicted regions of historical climatic stability for the fourth groups of genus *Cedrela* in the central Brazil across the Quaternary, based on summing the predicted occupancy of suitable areas of the fourth groups. Maps are given for (A) the Cedrinho group, (B) the Hidroelétrica group, (C) the Iturama group, and (D) the Norminas group. Areas in red are those where the groups of *Cedrela* are predicted to occur at all four time periods, and represent postulated refugial areas. Black lines represent the borders of current Brazilian states.

## IV. DISCUSSION

### 4.1 New lineages restricted to central Brazil

This study investigated restricted populations of *Cedrela*, which are located within riverine forests such as gallery forests, ecotonal areas (Atlantic forest, Cerrado, and Caatinga) and wetland environments, all of them kind of semideciduous forests that are located towards central Brazil. In previous studies developed by Garcia et al. 2011 and Mangaravite et al. 2016 raised an initial hypothesis by which populations of *Cedrela* distributed in small blocks of seasonal forest within central Brazil would exhibit only hybrid individuals. These hybrid individuals would be grouped into small blocks that would be product of the admixture of historical events of reconnection of both West and East lineages of *C. fissilis*. However our results suggested that the small blocks of seasonal forests assembled in riverine forests sheltered populations with different genetic pools from *C. fissilis* lineages. There also were individuals which were product of the admixture of both West and East lineages of *C. fissilis*. We unveiled three new lineages of *Cedrela* and in addition we discovered more populations of the previous revalidated *Cedrela brachysthacya* (Huamán-Mera et al., Submitted), all of them from central Brazil (Figure 1 and 2).

### 4.2 Different historical origins of lineages of *Cedrela*

The time of divergence of species of *Cedrela* was annotated in the Oligocene/Early Miocene with and intensification in the Late Miocene and Early Pliocene (Pennington and Muellner, 2010; Muellner et al., 2009). In Brazil, the time of divergence of *Cedrela fissilis* into West and East lineages took place about 10 Mya (Late Miocene/Early Pliocene), this overlaps with the historical period in which the diversification of *Cedrela* intensified (Muellner et al., 2009; García et al., 2011). But, it would probably have two different historical events had driven this diversification of *Cedrela* in Neotropics. The first one, the influence of the Andes uplift would be directly the responsible for the diversification of *Cedrela* in Central America and northern South America. Diverse effects of the Andes uplift such as – source of new high-elevation habitats, a vicariate barrier or as generator of new environmental conditions – was involved in *Cedrela* diversification and was demonstrated in significant previous studies with others genera (Antonelli et al., 2006; Pennington et al., 2010; Antonelli and Sanmartín, 2011; Luebert and Weigend, 2014). The second one, the

combinations of both the Andes uplift and the climatic fluctuations of the Neogene would be responsible for the split of *C. fissilis* into West and East groups. When central Brazil became drier and cooler the xeric vegetation began dominating the landscapes and the widespread seasonal forests were restricted to alongside current-like Cerrado and within them populations of *C. fissilis* which would have diverged (Wolfe, 1971; Gottsberger and Silberbauer-Gottsberger, 2009). It is in this second scenario that present new lineages discovered in central Brazil fit in. The time of divergence of new lineages of *Cedrela* from central Brazil (Figure 5), match with the hypothesis of intensification of divergence of Neotropical *Cedrela* of Muellner et al. (2009) and it is likely that sympatric speciation forces were linked to the divergences of *Cedrela* in central Brazil (Gastauer et al., 2015).

#### 4.3 Recent wave of colonization of *Cedrela brachystachya* in central Brazilian plateau

A recent study focused on the ecological specialization of a riparian species, *Cedrela brachystachya*, turned special attention on the mechanisms of speciation during historical climatic fluctuations of the Quaternary and additionally it was unrevealed the restricted distribution of *C. brachystachya* (Huamán-Mera et al., Submitted). However in present study we unrevealed that currently the population ANA which belongs to *C. brachystachya* (Huamán-Mera et al., Submitted) grouped together with populations such as FR1, BR1, and BR2 in microsatellite analysis (Figure 2, see Table 1 for name population). There is also a morphological correspondence among them (See chapter II). The populations FR1, BR1, and BR2 are geographically so distant from population ANA, but all together grouped. No other populations grouped together with these populations spite of we sampled other populations which were geographically located between the populations described above. For instance, ANA and FR1 populations are separated by 200 km, FR1 and BR1-BR2 by 700 km, and ANA and BR1-BR2 by 900 km (Figure 1). It was so surprise to know that the populations BR1 and BR2 settled down in Brasilia DF, in central Brazilian plateau were related to populations ANA and FR1. Moreover, our SDM (Species Distribution Modeling) analysis for the current period showed no niche linkage between populations (Figure 6D). However, when we modeled the past climatic periods we found a recent connection during the Quaternary climatic fluctuations between populations (Figure 6A-C) and it was reflected in the divergence of time between representative individuals of populations ANA and BR1 (~1 Mya B.P.) (Figure 5). Probably populations were connected when the Cerrado biome contracted its niche distribution and allowed the expansion of Seasonal forests in central Brazil (Bueno et al.,



2017). Our results agreed with the hypothesis that the Quaternary climatic fluctuations contributed to shaping current distribution of either Neotropical or tropical plant species (Hmeljevski et al., 2017; Buzatti et al., 2017; Ordonez and Svenning, 2017; Liang et al., 2018; Silva et al., 2018). Finally the three populations (FR1, BR1, and BR2) were new records of *C. brachystachya* in central Brazilian plateau.

#### 4.4 Convergence of hybridization zones of *Cedrela fissilis* and others *Cedrela* lineages ranges.

Natural hybridization plays an important role in the evolution of many taxonomic groups. In presence of historical events such as climatic fluctuations, the mechanism how natural hybridization acted is by the creation of recombinants from interspecific mating between divergent parental taxa when they come into geographic contact (Soltis and Soltis 2009; Whitney et al., 2010; Taylor et al., 2015). As a previous study exhibited, there are some small blocks of neotropical seasonal forest within central Brazil where some populations would detain gene pools entirely from parental source of both West and East ranges of *C. fissilis* by which they form hybridization zones of *C. fissilis* (Garcia et al., 2011; Mangaravite et al., 2016; Oliveira et al., Submitted) identified three possible locals of hybridization for *Cedrela* in Brazil. The possible admixture took place when both lineages of *C. fissilis* reconnected likely when the contraction of the Cerrado occurred and seasonal forests expanded reconnecting both Amazon and Atlantic forests during the Quaternary climatic fluctuations (Garcia et al., 2011; Sobral-Souza et al., 2015; Mangaravite et al., 2016; Bueno et al., 2017; Buzatti et al., 2017). Our results suggested that there is a convergence of admixture of gene pools within the individuals regardless of their grouping in populations in central Brazil. In most cases, individuals detained the most proportion assignment of both lineages of *C. fissilis* in the same individual (Hybrid individuals from populations COR, CAT, UBE, ITA, FR2) and in less proportion the remaining four lineages in other cases (Figure 2). In this geographical scenario we found the four new lineages converging together with hybrid individuals. Moreover the populations of the novel lineages existed long time ago in the geological time in this geographical scenario (Figure 5). On the contrary the zones where we found hybrid individual of *C. fissilis* probably allowed the reconnections of populations from the West and East group which joined in a common point of central Brazil in a recent past from nowadays (Figure 1 and 5). On the other hand the quaternary climatic fluctuations did not alter the genetic signature of *C. brachystachya* because of conservatism of niche which maintained its crucial

and current characteristics. *C. brachystachya* could develop an ecological specialization to different and antagonistic environments which is surrounded by a big one (Huamán-Mera et al. (Submitted). This fact had completed millions years ago before the onset of the climatic fluctuations of the Quaternary period (Figure 5) (Wiens et al., 2010).

#### 4.5 Displacement of niche of the Lineage from Northern Minas Gerais

In a previous study a population with 18 individuals randomly sampled from blend of ecosystems of Northern Minas Gerais and denominated as “Pandeiros” (PAN) exhibited a different genealogical placement of what was expected. It did not fit within the genetic variation expected for either the east lineage or the west lineage of *C. fissilis* (Días-Soto et al., 2018). With that premise we increased the sample size for Northern Minas Gerais areas (Figure 1) and confirmed the different genealogical placement established previously ((Días-Soto et al., 2018). As well as our results of molecular data, our SDM analysis showed unexpected outcomes contrary to the others lineages of *Cedrela* from central Brazil. Lineage “Norminas” exhibited a displacement of niche throughout the Quaternary climatic fluctuations (Figure 12 A-D). This event was not observed in the SDM analysis of others lineages. The niche of “Norminas” displaced from Northeast in the LIG period (Figure 12A) to Southwest until reach the Current period (Figure 12D), though the stable climatic areas for “Norminas” lineage remained established within the current distribution (Figure 13). Probably the niche behaved like this because of the displacement of largest biomes such as the Atlantic forest Cerrado and Caatinga at the same time what Quaternary climatic oscillations beginning (Carnaval and Moritz, 2008; Bueno et al., 2017; Kock et al., 2017). The initial hypothesis about the presence of hybrid zones product of the admixture of both west and East lineages of *C. fissilis* within small blocks of seasonal forest in central Brazil (Garcia et al., 2011; Mangaravite et al., 2016). It had being extended to the Northeastern Brazil, in states of Paraíba, Pernambuco, and Piauí, where also were identified a hybrid zone of *C. fissilis* (Oliveira et al., submitted). In the beginning, the presence of this hybrid zone wanted to explain the origin and displacement of “Norminas” lineage, however the event of speciation of this lineage took place in the late Miocene (Figure 5) and not during the quaternary climatic oscillations where Hybrid zone would tale origin (Oliveira et al., submitted).

#### 4.6 Conservation implications of current distribution of *Cedrela* lineages

Spite of three of four new lineages partakes of current geographic distribution (Figure 6D, 8D, and 10D) they do not share genetic information (Figure 2), as well as with the remaining fourth lineages “Norminas” group (Figure 2 and 9D). Notwithstanding stability of Quaternary climate could supply the accumulation and maintenance of diversity in *Cedrela* lineages as it happened with other groups (Carnaval et al., 2009, Werneck et al., 2012). The lack of admixture of genetic signatures among new lineages would explain by the different overlapped niche that each lineage developed before the beginning of called climatic stability. In most of lineages, current overlapped distribution would respond to the stability of overlapped niches (Haffer, 1982), which would shape stable areas of distribution of *Cedrela* throughout Quaternary displacement of central Brazilian vegetation (Bueno et al., 2017). Unfortunately according to our results the SDM climatically stable areas are mostly outside the current Brazilian protected areas (Figures 7, 9, 11, and 13). We identified only some exceptions, for instance in the “Cedrinho” group only the populations BR1 and BR2 are located in the Federal District (at National Park of Brasilia). Other exception is the “Norminas” group from Northern Minas Gerais which most of the populations are located inside protected areas such as Pandeiros River Basin Environmental Preservation Area, Peruaçu Caves National Park, and Mata Seca State Park.

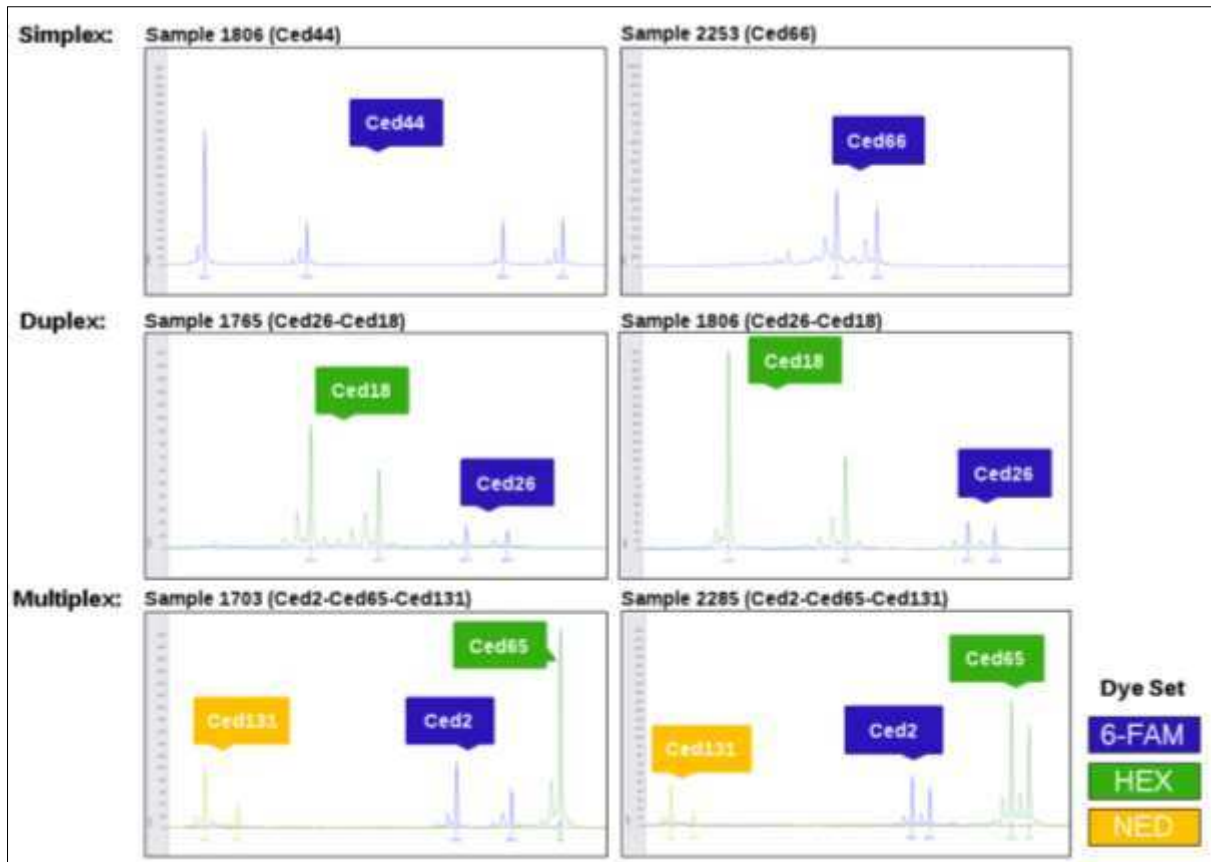
#### 4.7 Morphological correspondence of new *Cedrela* lineages

The morphological distinction of species in *Cedrela* is extremely complicated (Muellner et al., 2009). The complete distinction is validated by the analysis of morphological characters that in *Cedrela* need to be overlapping to reach a valid taxonomical determination (Muellner et al., 2009; Pennington and Muellner, 2010). There are until eight overlapping morphological characters used in *Cedrela* (see chapter II) (Muellner et al., 2009; Pennington and Muellner, 2010). Although *Cedrela fissilis* showed cryptic behavior within its populations in both west and east groups (García et al., 2011), the specimens of *Cedrela* could be treated morphologically with the supporting of molecular resolution and give us a good determination for new taxa within *Cedrela*. The new lineages have high correspondence between morphology and molecular features and it was represented in the description of new taxa for *Cedrela* (See chapter II for more information).

## V. CONCLUSION

- ✓ The genetic pool that exhibited populations of *Cedrela* lineages from central Brazil is different and unique. We found three new lineages of *Cedrela*, and confirmed more populations of Cedrinho group (*Cedrela brachystachya*) in central Brazilian plateau.
- ✓ There are some geographical sites within riverine forests where some individuals are sharing genetic information with lineages previously studied (Cedrinho, Chiquitano, and Atlantic). Also we showed that there are places in central Brazil that exhibit hybrid zones. Populations of COR, CAT, UBE, and ITA showed individuals with mostly admixture of Chiquitano and Atlantic lineages.
- ✓ Nuclear and Chloroplast sequences suggested that new *Cedrela* lineages either they originated during independent speciation events or derivate within a phylogenetic cluster. *Cedrela brachystachya* placed within the crown *Cedrela odorata* and the remaining three lineages placed within the crown *Cedrela fissilis*. Lineage “Hidroelétrica” placed within the west group and lineages “Iturama”, and “Norminas” within the East group of the crown *C. fissilis*. The events of speciation occurred between 7 – 2 Mya B.P.
- ✓ There is correspondence between the morphology of lineages and the genetic identity i.e. each lineages exhibited particular morphology features which were different each other. *Cedrela brachystachya* was morphologically treated previously (Huamán-Mera et al., submitted). Only two of the remaining three lineages were morphologically treated in chapter two of present thesis.
- ✓ Probably there is ancestral refugia for lineages of *Cedrela* in central Brazil, which had originated in the riverine forests of Tertiary and remain stable and isolated from other blocks of forests during Quaternary climatic fluctuations.

## Supplementary Material



**Figure Supplementary 1.** Representative Imagens of scoring microsatellite peaks in different systems of Polymerase Chain Reaction (PCR). We showed the combination of PCR systems and primers [Simplex (Ced 44; Ced66), Duplex (Ced26-Ced18), and Multiplex (Ced2-Ced65-Ced131)]. Were chosen the samples more representatives for present figure. The fluorescence dye set is represented on the right.

TableS1. 13 bioclimatic variables selected by MaxEnt in present study.

Code	Bioclimatic variables
Bio 1	Annual mean temperature
Bio 2	Mean temperature diurnal range
Bio 3	Isothermality
Bio 4	Temperature seasonality
Bio 5	Max temperature of warmest month
Bio 7	Temperature annual range
Bio 8	Mean temperature of wettest quarter
Bio 12	Annual precipitation
Bio 13	Precipitation of wettest month
Bio 14	Precipitation of driest month
Bio 15	Precipitation seasonality
Bio 17	Precipitation of driest quarter
Bio 19	Precipitation of coldest

TableS2. Taxa, codes, origins, and GenBank accession numbers for specimens used in time of divergence.

<b>Taxon</b>	<b>Code</b>	<b>Origen</b>	<b>GenBank number ITS Accessions</b>
<i>Cedrela balansae</i> C.DC	-	Argentina	FJ462473
<i>Cedrela brachystachya</i> (C.DC.) C.DC.	1145	Brazil	KT316325
<i>Cedrela dugesii</i>	-	Mexico	FJ462483
<i>Cedrela fissilis</i> Vell	COL_268	Brazil	JF922233
<i>Cedrela fissilis</i> Vell	SMA73	Brazil	JF922213
<i>Cedrela fissilis</i> Vell	-	Peru	FJ462475
<i>Cedrela kuelapensis</i> T.D. Penn. & A. Daza	-	Peru	FJ462469
<i>Cedrela angustifolia</i> DC.	-	Peru	FJ462479
<i>Cedrela molinensis</i> T.D. Penn. & Reynel	-	Peru	FJ462465
<i>Cedrela moroensis</i> T.D. Penn.	-	El Salvador	FJ462486
<i>Cedrela montana</i> Moritz ex Turczaninov	MN3	Ecuador	KT316339
<i>Cedrela nebulosa</i> T.D. Penn. & A. Daza	-	Peru	FJ462460
<i>Cedrela odorata</i> L.	-	Belize	FJ462467
<i>Cedrela odorata</i> L.	-	Brazil	FJ462471
<i>Cedrela odorata</i> L.	-	Ecuador	FJ462464
<i>Cedrela odorata</i> L.	-	El Salvador	FJ462468
<i>Cedrela oaxacensis</i> C.DC. & Rose	-	Mexico	FJ462480
<i>Cedrela saltensis</i> Zapater & del Castillo	SSA8	Argentina	FJ462462
<i>Cedrela salvadorensis</i> Standl.	-	Costa Rica	FJ462484
<i>Cedrela weberbaueri</i> Harms	-	Peru	FJ462472
<i>Cedrela tonduzii</i> C.DC.	-	Costa Rica	FJ462485
<i>Toona ciliata</i>	-	Australia	FJ462488
<i>Toona sinensis</i> 1	-	-	FJ462490
<i>Toona sinensis</i> 2	-	-	FJ462491
<i>Swietenia macrophylla</i>	-	-	DQ861609
<i>Khaya anthotheca</i>	-	-	DQ861608

## Appendix section

**Appendix A.** Modified protocol for extraction of nucleic acids from Herbarium samples. Modified from Arbeláez-Cortes et. al (2007) and Doyle & Doyle (1990).

1. Prepare buffer CTAB 2X (without Proteinase K, neither B-mercaptoetanol) in a recipient resistant to high temperatures; then heating until 60°C using a water bath, considering that is used 1 ml of buffer in each sample.
  - 1.1 Proteinase K is inactive at temperatures above 37°C.
2. For macerating, take a microbute of 2 ml and add the leaf tissue (100-200 mg) inside, then add 0,0175g of PPV together with two metal beats for each sample.

After maceration takes out the metal beats to avoid oxidation.
3. From the initial preparation of buffer CTAB 2X (without Proteinase K, neither B-mercaptoetanol), take 600 µl and add to the microtubes where the leaf tissue were macerated, then leave the microtubes in a water bath at 60° C for 15 min.
4. Lower the temperature of the water bath to 37 °C and add the remaining 400 µl of buffer CTAB 2X (herein, with Proteinasa K and B-mercaptoetanol) to the microtubes, leave in water bath at 37°C during 40 min. Shake gently during five minutes every 10 min.
5. Now, add 800 µl of cloroformo:alcohol isoamilico (24:1), in a shaker mix gently for 15 min and then centrifuge at 12000 rpm for 15 min.
6. Transfer the supernatant to a new 1.5 ml microtube, add cold isopropanol equivalent to 2/3 of the recovering volume of supernatant, also Acetato de sodio (3M pH5.2) equivalent to 1/10 of the recovering volume of supernatant. Mix and leave precipitating in freezer at -4°C for an hour.
7. Centrifuge to 12000 rpm for 15 min and discard the supernatant, the microtubes must leave drying up in a paper towel.
8. For washing the pellets, add 500 µl of cold ethanol 70% to the dry microtubes and then centrifuge at 12000 rpm for five min.
9. Repeat previous step, in this time using cold etanol 95%. After the last step drying up the microtubes in a paper towel.
10. Prepare TE (10mM Tris:1mM EDTA:RNAsa 40 ug/ml), then add 50–100 µl of TE in each microtube.
  - 10.1 Incubate the samples at 37°C for 35 min, and then keep in a freezer at -20°C.
11. For visualization of the quality of extracted DNA, it is recommended to use a gel of agarose 1.0%. Run at 90-100V.

11.1 Proportion of recommended cocktail: 2 $\mu$ l of ADN + 1,25 $\mu$ l of Blue 6X + 4,25 $\mu$ l of water.

Composition of the protocol

Buffer CTAB 2X (1000ml)	
CTAB	2g
B-mercaptoetanol	0,1%
Proteinasa K	60 $\mu$ g/ml
NaCl	1,4M
EDTA	20mM
TRIS-Hcl pH 8,0	100mM



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**CHAPTER II: TWO NEW SPECIES AND NEW OCCURRENCES OF *Cedrela*  
(MELIACEAE) FROM CENTRAL BRAZIL**

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## ABSTRACT

The genus *Cedrela* is an arboreal genus with 18 accepted species in the Meliaceae family. *Cedrela* is different from other genera in Meliaceae by – Presence of an androgynophore; petals adnates to the androgynophore; and Free staminal filaments. The distribution of the genus is Neotropical and is associated with seasonal forest. In the last decades, *Cedrela* was object of different studies with the aim of unraveling the quantity of circumscribed species to the genus. The 18 morphological species are recognized and accepted on the basis of the combination of eight overlapping morphological characters. *Cedrela* has supplied the most important timber on local and international markets. *Cedrela* is compound by two accepted species (*Cedrela odorata* and *C. fissilis*) in Brazil. However new insights of molecular studies supported the presences of new species which need to be described. Herein, we investigated a taxonomical approach of two genetic entities discovered in first chapter of present thesis and new occurrences of *Cedrela brachystachya*, a recent ecological specialized species. We carried out morphometric analysis of specimens of *Cedrela* from central Brazil. Our results suggested two new species (*Cedrela microanthus* and *Cedrela pandeirensis*) both from central Brazil and new populations of *Cedrela brachystachya*. The new findings corroborate that the genus *Cedrela* is exceptionally diverse within Meliaceae

**KEY WORDS:** Cedreleae; Sapindales; Speciation; Ecological specialization.



## I. INTRODUCTION

The genus *Cedrela* (Browne, 1756) is an arboreal genus with 18 accepted species (Pennington and Muellner, 2010; Köcke et al., 2015) and a new revalidated name of species (Huamán-Mera et al., Submitted) in the Meliaceae family (Styles, 1981). *Cedrela* is different from other genera in Meliaceae by – Presence of an androgynophore; petals adnates to the androgynophore; and Free staminal filaments. The remaining other genera in the family have the filaments compressed and with gradual fusion into a staminal tube (Pennington and Muellner, 2010). The distribution of the genus is from Northern Mexico in North America to Northwestern Argentina to the Atlantic Forest of Eastern Brazil in South America. Mostly species of *Cedrela* are associated with seasonal forest (Styles, 1981; Garcia et al., 2011). Except for one species, which in a recent study was determined that is associated with wetlands in central Brazil (Huamán-Mera et al., Submitted).

In the last decades, *Cedrela* was object of different studies with the aim of unraveling the quantity of circumscribed species to the genus. Thus, in his revision of *Cedrela*, Smith (1960) used the morphology, biogeography and ecology approaches, recognized six accepted species; *Cedrela fissilis*, *C. odorata*, *C. angustifolia*, *C. lilloi*, *C. oaxacensis*, and *C. montana*. In that study, *Cedrela weberbaueri* already appeared, but was not included as part of the taxonomical key because of lack of better specimens. Later, in his *Flora Neotropica*, Styles (1981) recognized seven species (*Cedrela fissilis*, *C. lilloi*, *C. montana*, *C. oaxacensis*, *C. odorata*, *C. salvadorensis*, and *C. tonduzii*), and four imperfectly known species (*Cedrela angustifolia*, *C. discolor*, *C. imparipinnata*, *C. weberbaueri*), the identity of these imperfect species will be confirmed, but one of them, *C. imparipinnata*, was synonymized with *C. odorata* by Pennington and Muellner (2010). In the last revision of *Cedrela* were recognized 17 species, six species from the seven of Styles (1981) were confirmed. The remaining species, *C. lilloi*, was synonymized with *C. angustifolia*. Moreover three of the four imperfectly known species also were confirmed. Also in this revision were included three species (*C. balansae*, *C. dugesii*, *C. longipetiolulata*) which were synonymized with the two widespread species *C. odorata* and *C. fissilis* by Styles, and a new species from Argentinean Yungas, *C. saltensis* (Zapater et al., 2004; Pennington and Muellner, 2010). In addition, four new species were discovered; *C. monroensis*, *C. molinesis*, *C. kuelapensis*, and *C. nebulosa* (Pennington and Muellner, 2010). A recent study indicated the discovery of a new species, *Cedrela ngobe* from southeastern Costa Rica to Panamanian semideciduous lowland forest (Köcke et al., 2015). In a previous work we suggested the taxonomical revalidation of *C. brachystachya*, a species

from the riparian ecosystems of central Brazil (Huamán-Mera et al., submitted), which was synonymized with *C. odorata* by Pennington and Muellner (2010). Currently, a total of 18 morphological species are now recognized and accepted (Pennington and Muellner, 2010; Köcke et al., 2015) on the basis of the combination of eight overlapping morphological characters: number of pair of leaflets; leaflet size; type and amount of leaf indumentum; shape and number of secondary veins on the leaflet; flowering from young wood when the tree is in leaf or older wood when the tree is leafless; adnation of the petal margins; degree of division of the calyx; and capsule size (Muellner et al., 2009; Pennington and Muellner, 2010).

From the Indias, the ancient name of the discovered America, species of *Cedrela* such as *Cedrela odorata* has supplied the most important timber on local and international markets (Pennington and Muellner, 2010). As we move forward in time, after the establishment of Spanish colony, several of the support architecture and all interior panelling and decoration of the buildings such as churches, cathedrals, governments Palaces, and others important official statements of Central America and South America were made of *Cedrela* timber. The durability of the timber is observed until nowadays (Smith, 1960; Pennington and Muellner, 2010). The demand of the lumber of *Cedrela* species in Latin America was strong until last century and was observed in all the countries where *Cedrela* are distributed (FAO, 1997). With this demand, species of *Cedrela* has been highly depredated and today they are within the red list of threatened species. For instance, the two widespread species of *Cedrela*, *Cedrela odorata* and *C. fissilis* are “vulnerable A3bcd+4bcd” and “endangered A1acd+2cd” respectively (IUCN, 2017).

*Cedrela* was represented firstly by three accepted species (*Cedrela balansae*, *C. fissilis* and *C. odorata*) in recent revisions of the genus in Brazil (Styles, 1981; Cervi et al., 2008), and then by two accepted species (*Cedrela odorata* and *C. fissilis*) (Pennington and Muellner, 2010). However a posterior study suggested that records of specimens of *C. odorata* grouped together with *C. fissilis* clade (Garcia et al., 2011). On the other hand, molecular markers suggested that two separate phylogenetic lineages within *C. fissilis* that corresponded to populations located in separate geographic regions (Garcia et al., 2011, Mangaravite et al., 2016). Moreover, an investigation of genetic structure of *Cedrela* in central Brazil suggested three new genetic identities of *Cedrela*, one of them was recently studied deeply and probably the number of accepted species of *Cedrela* would be increased in Brazil (Huamán-Mera, 2014; Huamán-Mera et al., Submitted). Additionally one large population denominated as Northern Minas Gerais was confirmed as new genetic identity from was recently confirmed as unique and different from others until now studied (Díaz-Soto et al., 2018). Independently of the

taxonomical and molecular treatment of *Cedrela* in Brazil, the species have also been overexploited together with other species that compound the Brazilian seasonal forest (FUNDAÇÃO SOS Mata Atlântica; INPE, 2010).

Taxonomists have often experienced difficulties in the circumscription of species using morphological characters as the single source of data, so as seen above, these professionals have turned their attention to molecular markers as complement in taxonomical studies as a means of yielding robust results (Cavers et al., 2003; Muellner et al., 2009; Garcia et al., 2011).

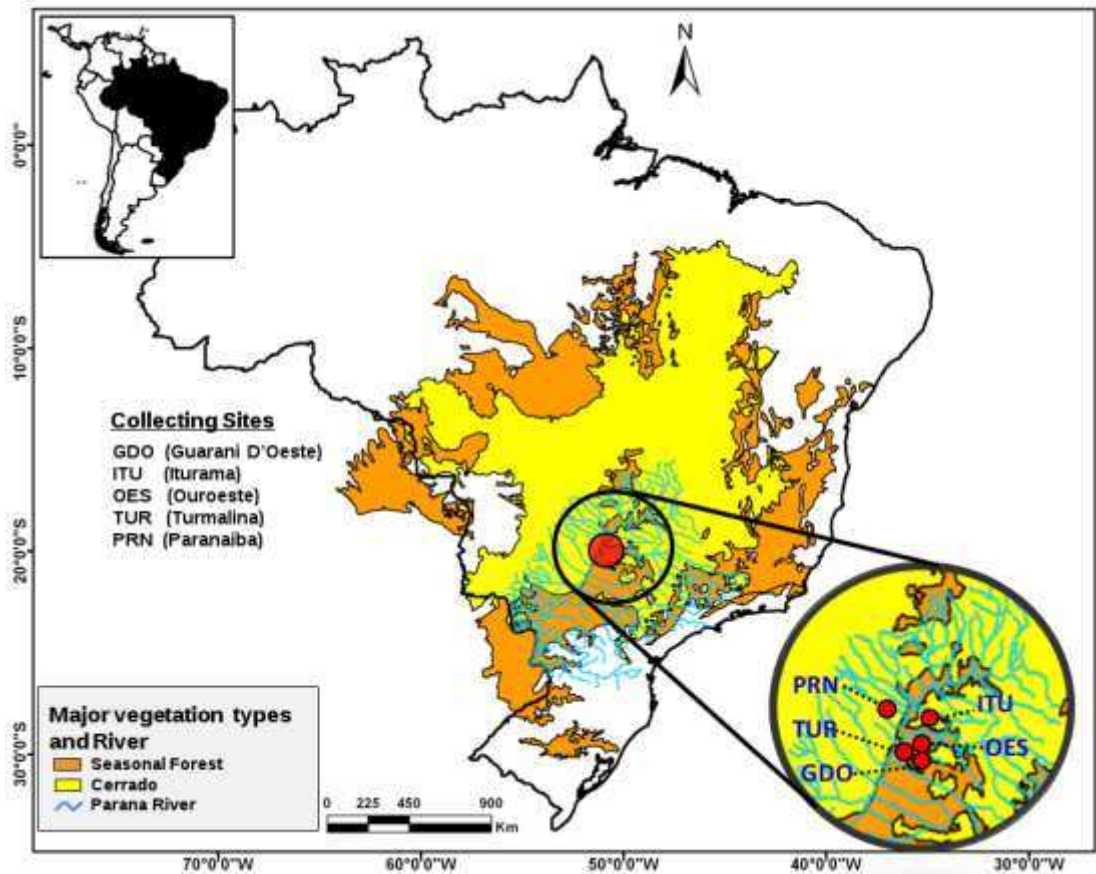
In this second chapter we investigated a taxonomical approach of two of genetic entities discovered in first chapter of present thesis. The lack of appropriated botanical samples of others enteties made impossible that we did not treat them. So we formally described two genetic entities as new species, the first one from Iturama locality, and the second one from the Northern Minas Gerais state, both from central Brazil. Additionally we also used the results of the first chapter for confirming and reporting two new occurrences of a recent revalidated species, *Cedrela brachystachya*, also from central Brazil.

The new findings corroborate that the genus *Cedrela* is exceptionally diverse within Meliaceae.

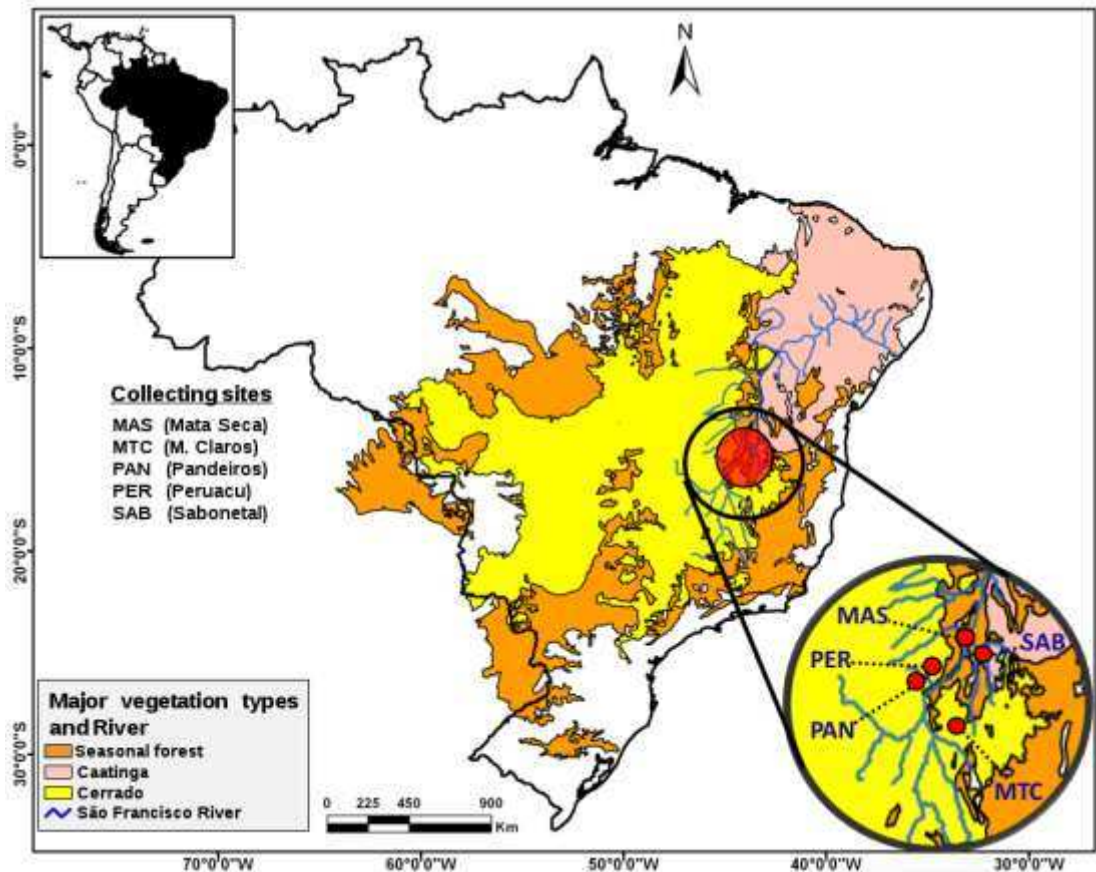
## **II. MATERIALS AND METHODS**

### **2.1 Sampling**

For the description of the first new species (from Iturama locality or Iturama group), afterwards *Cedrela* sp.1, we collected nearby Iturama county in Minas Gerais state, Brazil; collecting trips took place in January 2017 (Figure 1). For the description of the second species (from Northern Minas Gerais or Norminas group), afterwards *Cedrela* sp.2, we collected along Northern Minas Gerais, in small tributaries of Sao Francisco River such as Pandeiros and Peruacu minor rivers; collecting trips took place in 2015, 2016, and 2017 (Figure 2).

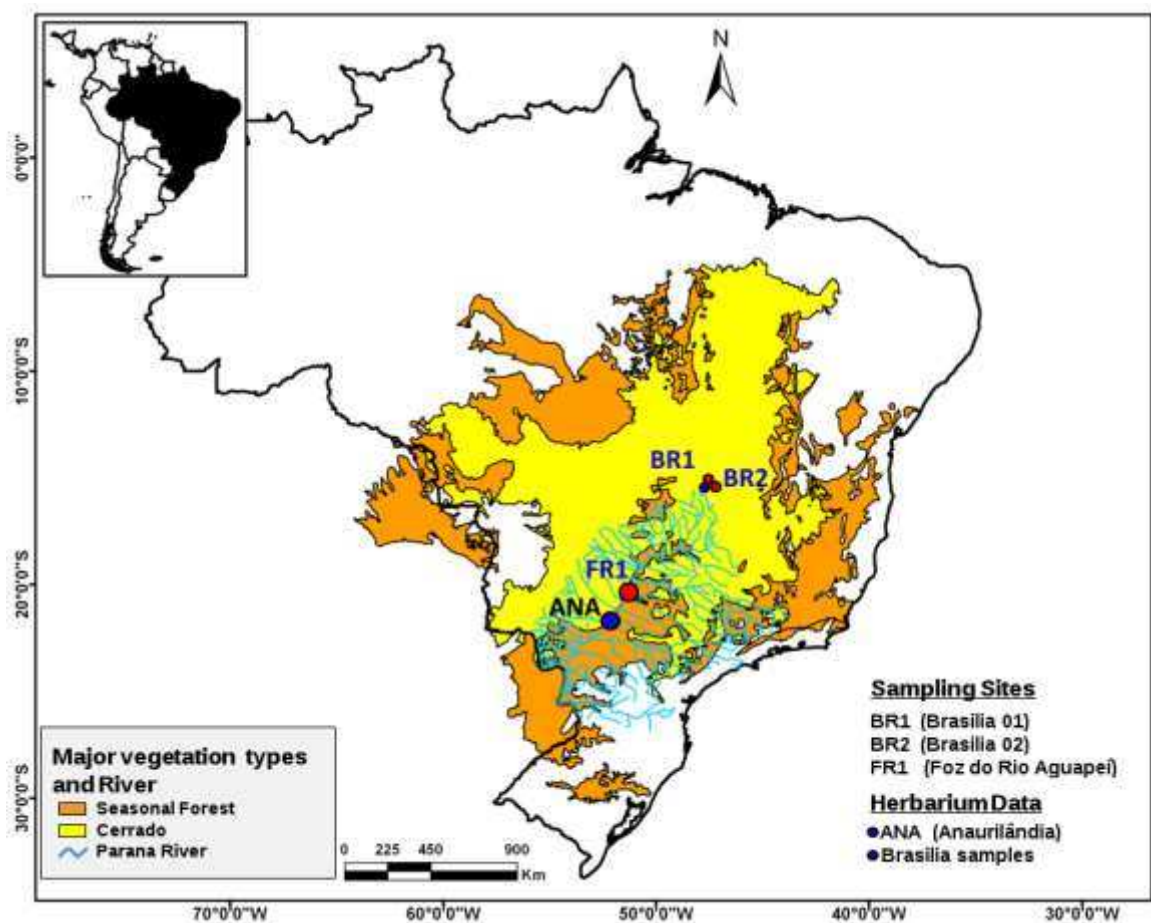


**Figure 1.** Collecting sites of *Cedrela* sp.1 along localities of the counties Ouroeste (GDO, OES, and TUR) Iturama (ITU), and Paranaíba (PRN). Major vegetation covering and Parana River are also depicted.



**Figure 2.** Collecting sites of *Cedrela* sp.2 along micro basins of São Francisco River such as Pandeiros and Peruacu rivers in Northern Minas Gerais, and major vegetation covering.

For analysis of new occurrences of *Cedrela brachystachya* we considered populations from Foz do Rio Aguapeí (FR1 population) and Brasilia DF (BR1 and BR2 populations) because in previous molecular analysis they grouped in the same genetic group that *C. brachystachya*. For FR1 population we only collected few botanical samples; collecting trips took place in 2013. For BR1 and BR2 populations we used both the botanical samples that we opportunely collected and records that we reviewed in Herbarium CEN (Brasilia DF) that were used partially in recent study (Huamán-Mera et al., Submitted); collecting trips took place in 2015 (Figure 3).



**Figure 3.** Collecting sites of new occurrences and Herbaria data of *Cedrela brachystachya* along Paraná upper river basins, and major vegetation covering.

For morphometric analysis of *Cedrela* sp.1, we sampled seven specimens of *Cedrela* from Guarani D'Oeste (SP), Iturama (MG), Ouroeste (SP), and Turmalina (SP). We also reported occurrences of specimens of *Cedrela* sp.1 in Paranaíba (MS), but we did not collect for present study because they were deforested currently (Figure 1). For morphometric analysis of *Cedrela* sp.2, we sampled 20 specimens of *Cedrela* from Itacarambi, Mata Seca, Montes Claros, Pandeiros, and Peruacu, in Northern Minas Gerais state (Figure 2). Voucher

specimens resulting from fieldworks were deposited in Herbarium Viçosense (VIC). In the future we will be deposited in Botanical Garden of Rio de Janeiro Herbarium (RB); Herbarium Molinensis (MOL), Herbarium Selva Central–Oxapampa (HOXA), Herbarium Universidad Nacional Mayor de San Marcos (USM) and Missouri Botanical Garden (MO). We recorded the geographic coordinates of each site using a Global Positioning System (GPS) receiver.

## 2.2 Morphometric analyses

We carried out morphometric analyses in a number of characteristics of leaves and inflorescences in specimens of *Cedrela* sp.1 and *Cedrela* sp.2. While choosing diagnostic characters for distinguishing amongst species of *Cedrela*, we follow the recommendations of Muellner et al. (2009) and Pennington and Muellner (2010). In leaves: number of leaflets, pedicel length, and leaf length. In leaflets: leaflets blade length, leaflets blade width, and number of secondary veins. In inflorescences: length, petiole length, flower length, corolla length, calyx tube length, ovary length, style length, androgynophore length, and capsule size.

For specimens of populations FR1, BR1, and BR2, we compared the vouchers of *Cedrela brachystachya* deposited in Herbarium VIC (Universidade Federal de Vicosa) with specimens of populations FR1, BR1, and BR2.

## III. RESULTS

### 3.1 Taxonomic treatment

**According the morphometric assessment, *Cedrela* sp.1 from Iturama locality was described and taxonomically treated as following:**

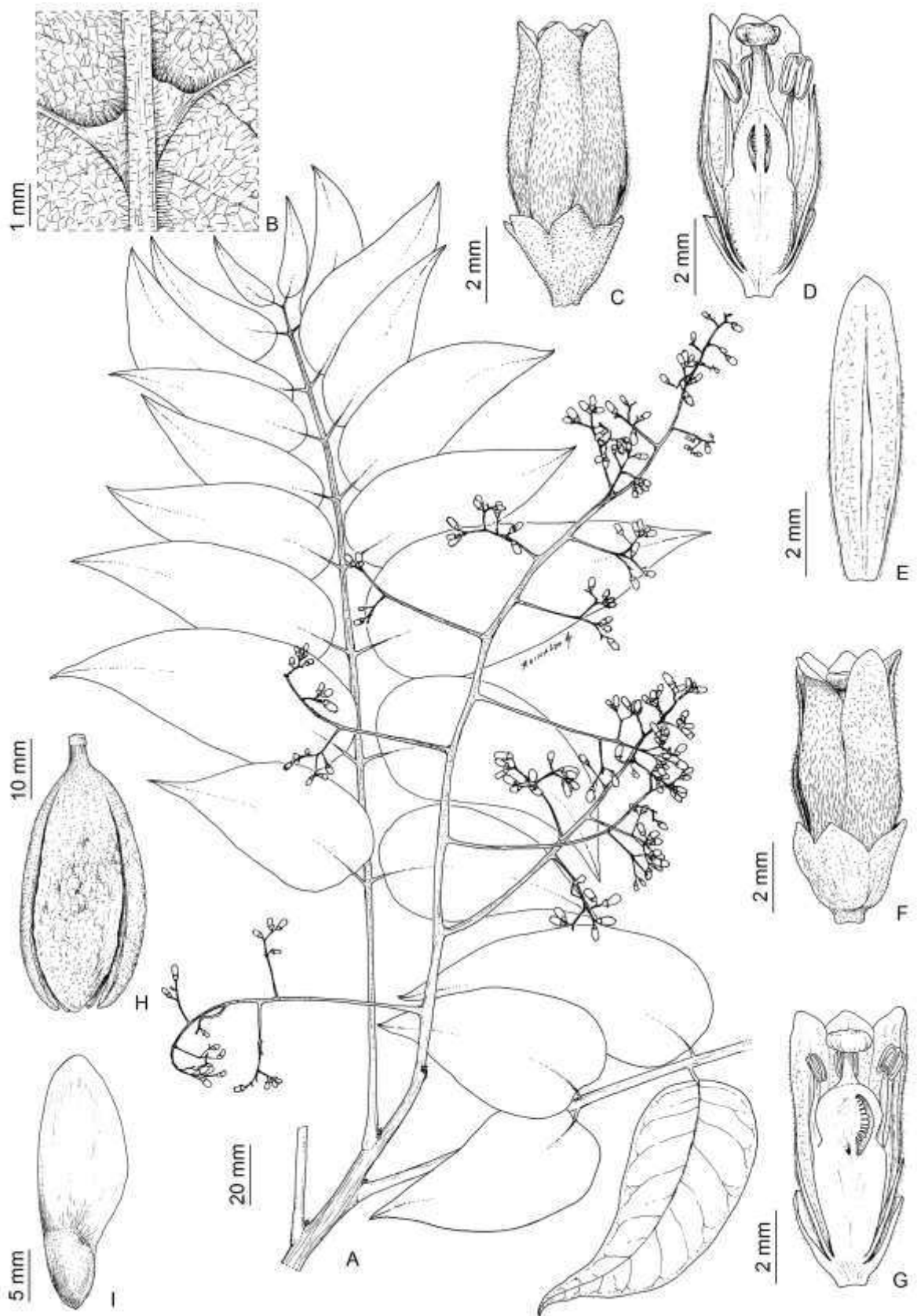
**3.1.1 *Cedrela microanthus*** Huamán-Mera A. and Oliveira LO, sp. nov. Type: Brazil, São Paulo, Turmalina, 20° 3'48.69"S, 50°27'11.79"W, 470 m, 10 January 2017, L.O. Oliveira 754, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (holotype VIC, isotype VIC). Figure 4 A–I. Figure 5.

Floribus quam in typo brevius.

Young branches smooth, 0.45–0.8 cm diam., pale greyish-brown, with small pale lenticels. Leaves (petiole + rhachis) 16–48 cm long; **leaflets** subopposite, sometimes opposite

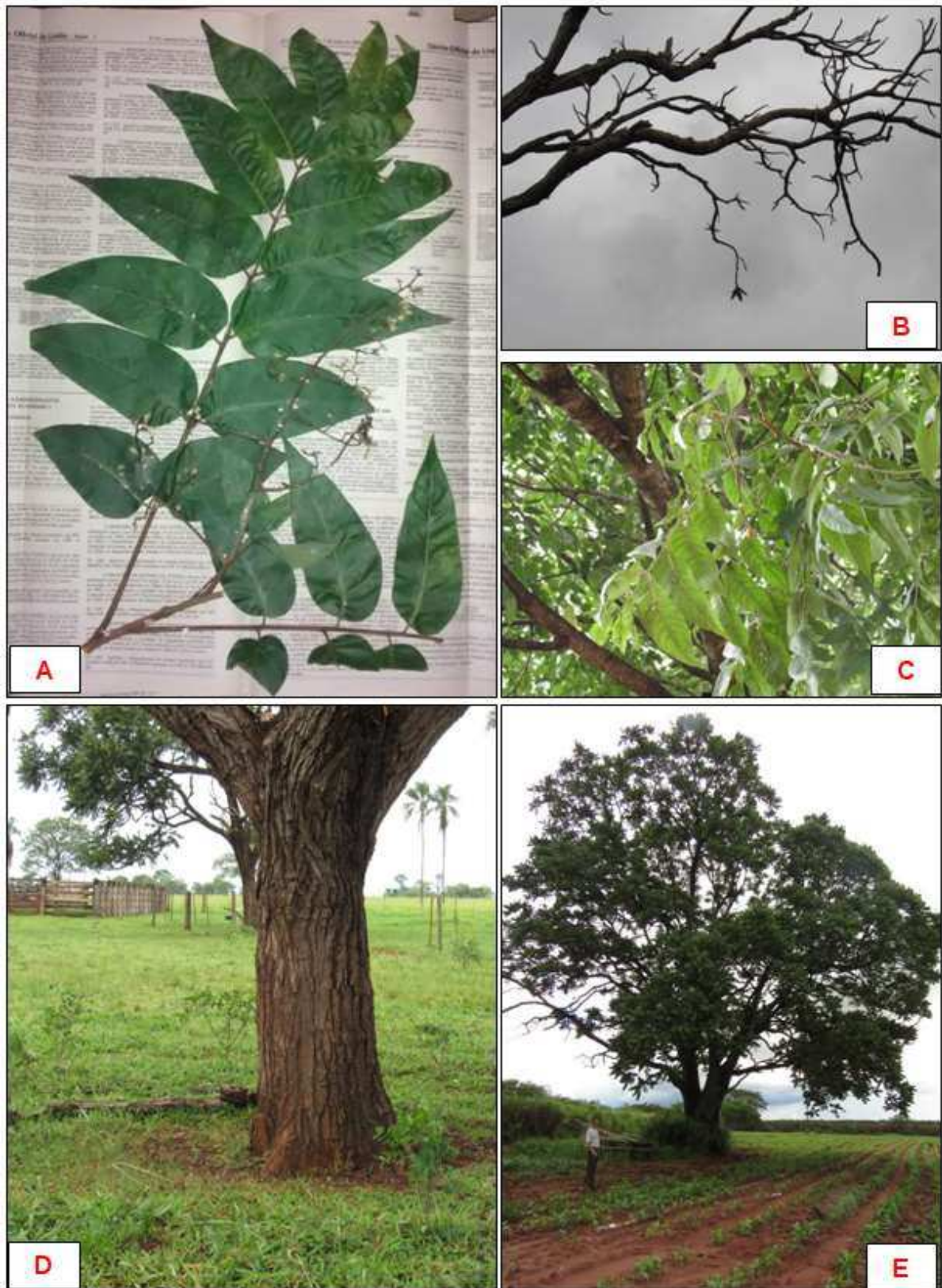
and opposite and subopposite in the same leaf, **(5)6–8(9) pairs**, 4.5–15 x 2.5–5.5 cm, usually ovate–lanceolate, slightly falcate, apex acute to slightly cuspidate, base usually slightly asymmetric and sometimes oblique, adaxial surfaces generally glabrous, sometimes presence of sparsely pubescent in the main vein, abaxial surface **sparsely pubescent**, mainly in the main and secondary veins, domatia present in the axils of secondary veins in abaxial surface, concentrate from the basis to the higher third leaflets; venation eucamptodromous, midrib flat on upper surface, **secondary veins (8–)9–12 pairs**, ascending, convergent, reticulate, slightly thin and elevated below. Petiole (4–)5–10(–11) cm long, slightly pubescent; petiolule (3–)4–7(–8) mm long, minutely puberulous. Inflorescence terminal, **plant flowering when in leaf**, 25–48 cm long, pendulous cymose, scarcely puberulous, without leaves in developing as appendixes in some branches; pedicel (0.2–0.25) mm long. Calyx gamosepalous 1.5–2(–2.5) mm long, **cup-shaped, lobed**, apex obtuse to acute, puberulous to tomentose outside and glabrous inside. Petals (5–)5.5–6 mm long, 1–1.5 mm wide, strap-shaped, apex obtuse to acute, outside in the lower half densely pubescent and in the higher half puberulous, slightly pubescence inside, petals adnate to the androgynophore in the lower third, margins adnate, only free in the bottom of the petals. Androgynophore 2–3.2 mm long, orangish-red brightly coloured. Stamens, male flower: filaments (free portion) 1.5–1.7 mm long, glabrous, anthers 0.8–1 mm long, glabrous, female flower: filaments (free portion) 1.5–1.7 mm long, anthers 0.5–0.7 mm long, shrunken, without pollen, lower part of filaments fused to the androgynophore. Ovary 5-locular, borne at the top of the androgynophore, 1.3–1.6 mm long, glabrous, with presence of lenticels, female flower: loculi **with 5–6 ovules** in 2 rows, style 1.2–1.4 mm long with a thick discoid style-head, male flower: pistillode 1–2.5 mm long, puberulous, with reduced loculi and vestigial ovules, style 1.7–2mm long with a thin style-head. **Capsule pendulous, 4–4.5 cm long**, oblong to obovoid, apex and base rounded, dark gray with few, small pale brown lenticels, valves woody, 1.5–1.7 mm thick, columella 5-winged. Seed 1.8–2.5 cm long (including the wing), pale brown.





**Figure 4.** *Cedrela microanthus*. A, flowering branch; B, detail of leaflet undersurface; C, male flower; D, male half-flower; E, detail of petal insertion; F, female flower; G, female half-flower; H, capsule; I, seed.





**Figure 5.** A. Flowering branch of *Cedrela microanthus* B. Single capsule of *Cedrela microanthus* C. Fruiting branch of *Cedrela microanthus* D. Trunk of a specimen of *Cedrela microanthus* E. Habit of *Cedrela microanthus*.

### 3.1.1.1 Distribution, habitat, and etymology

*Cedrela microanthus* is known from areas of Cerrado and seasonal Forest in the confluence of Grande and Paranaina Rivers minor basins, in confluence of the states of Mato Grosso do Sul, São Paulo, and Minas Gerais (Triângulo mineiro); all specimens included in this study were collected from this part of the range. The species grows between 340–500 meters above sea level, in areas in which was not possible to find other genera because of overexploited. *Cedrela microanthus* is vernacularly called “*Cedro*”. The epitetus of the species is related to the short flowers, no other species of *Cedrela* has flowers shorter than *C. microanthus*.

### 3.1.1.2 Field characters

Trees up to 10(–20) m high and 120–130(–145) cm diam. Bole cylindrical, up to 5–10 m high to the first branch, with fissured dark gray to grayish-brown bark. Presence of garlic odour. The flowers are greenish-cream to pale. The fruits are green with small and few lenticels. Flowering occurs mostly between October and January, densely flowering. The capsule matures between May and August.

The distinctive morphometric characters of *Cedrela microanthus* are shown as mean trait values compared with traits values of different congeners such as; the two widespread (*Cedrela fissilis* and *C. odorata*) and *C. balansae*. The combination of characters such as; the leaf length 42 cm, 5–8(9) pairs of broad leaflets, leaflet size 8–3 x 13–5 cm, usually ovate–lanceolate, slightly falcate, apex acute to slightly cuspidate, base usually slightly asymmetric and sometimes oblique, secondary veins 8–12 pairs, inflorescence length 35 cm, flowers length 5–6 mm, stamens length 1.5–2 mm, pistil length 1–2.5 mm, with capsule pendulous 3.5–4.5 cm long, oblong to obovoid, apex and base rounded.

### 3.1.1.3 Additional specimens examined

**BRAZIL. São Paulo:** Ouroeste, road to Ouroeste town, 19°58'26.05"S, 50°23'9.97"W, 479 m, 10 January 2017, L.O. Oliveira 742, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Ouroeste, road to Ouroeste town, 19°58'30.67"S, 50°23'10.53"W, 487 m, 10 January 2017, L.O. Oliveira 743, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Ouroeste, road to Ouroeste town, 19°58'31.78"S, 50°23'11.67"W, 487 m, 10 January 2017, L.O.

Oliveira 744, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Ouroeste, road to Ouroeste town, 19°58'32.59"S, 50°23'9.86"W, 489 m, 10 January 2017, L.O. Oliveira 745, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Guarani D'Oeste, 20°4'27.51"S, 50°19'43.56"W, 507 m, 10 January 2017, L.O. Oliveira 747, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Road to Turmalina, 1 km to Pov. so Sol, 20°0'15.75"S, 50°27'33.69"W, 451 m, 10 January 2017, L.O. Oliveira 753, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC). **Mato Grosso do Sul:** Paranaíba, 19°43'00.70"S, 51°05'37.10"W, 369 m, 10 January 2017, L.O. Oliveira 760,761, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC); Paranaíba, 19°42'42.40"S, 51°05'51.80"W, 344 m, 10 January 2017, L.O. Oliveira 763, 764, 765, 766, 767, A. Huamán-Mera, T. Ferreira & J.M. Díaz-Soto (VIC).

**According the morphometric assessment, *Cedrela* sp.2 from Northern Minas Gerais localities was described and taxonomically treated as following:**

**3.1.2 *Cedrela pandeirensis*** Huamán-Mera A. and Oliveira LO, sp. nov. Type: Brazil, Minas Gerais, Januária, Pandeiros community, Area de Protecao Ambiental Pandeiros, - 15.473 S, -44.75 W, 500 m, 12 December 2017, fl., L.O. Oliveira 2344 & A. Huamán-Mera (holotype VIC, isotype VIC). Figure 6 A–I. Figure7.

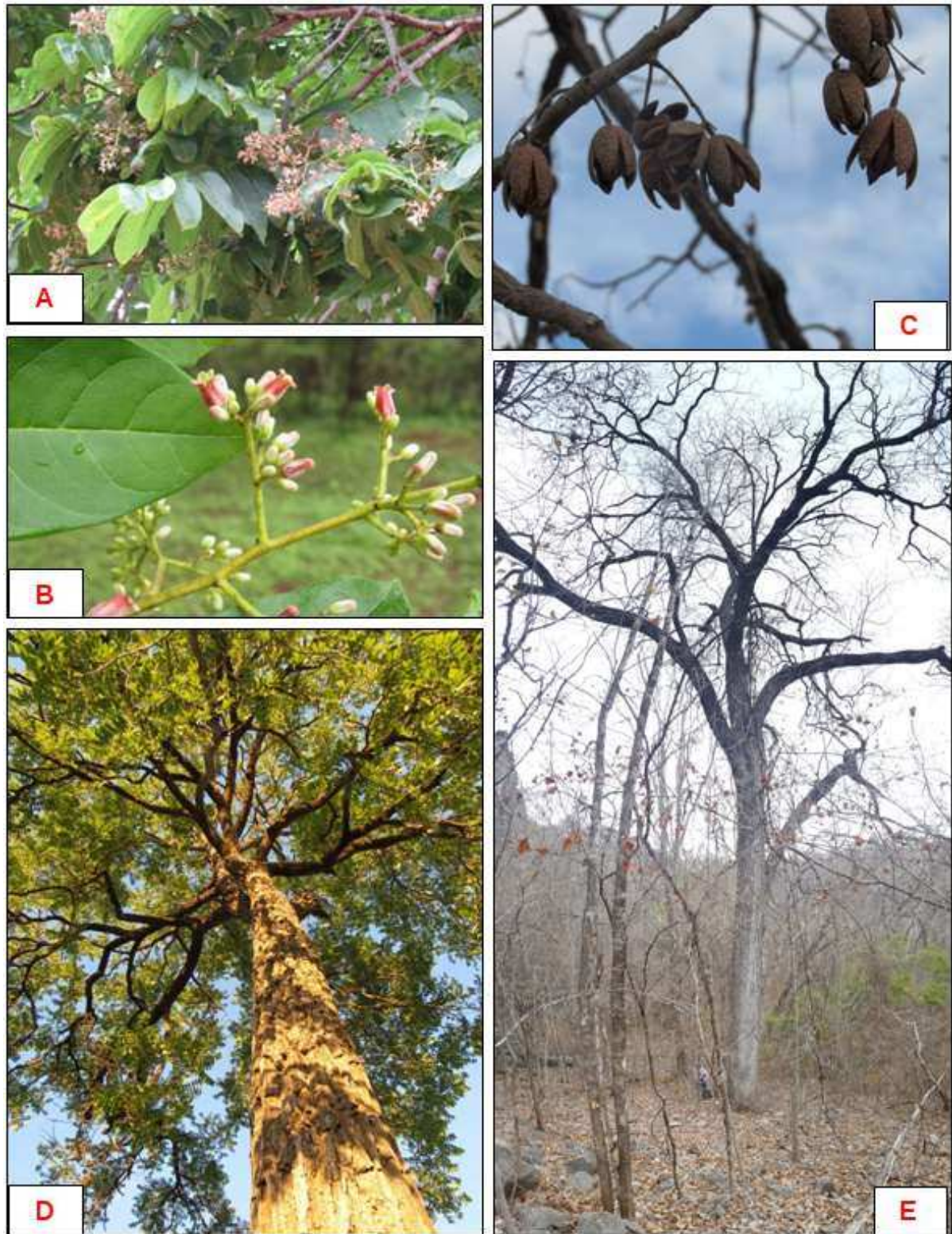
Young branches smooth, 0.46–0.6 cm diam., pale greyish-brown, with small pale lenticels. Leaves (petiole + rhachis) 23.5–49.5 cm long; **leaflets** opposite or subopposite, mostly opposite, **(5)6–9 pairs**, 7–11.6 x 2.8–4 cm, usually ovate–lanceolate, slightly falcate, apex obtuse to acute or to cuspidate, base usually slightly asymmetric and oblique, **sometimes thinly coriaceous** adaxial surfaces slightly pubescent, abaxial surface **sparsely pubescent**, mainly in the main and secondary veins, domatia present in the axils of secondary veins below, concentrate from the basis to the higher third leaflets; venation eucamptodromous, midrib flat on upper surface, **secondary veins 11–14(–15) pairs**, ascending, convergent, reticulate, slightly thin and elevated below. Petiole (4.7–)5.5–7.5(–8.5) cm long, glabrous; petiolule 1–4 mm long, minutely puberulous. Inflorescence terminal, **plant flowering when in leaf**, 18–30 cm long, pendulous cymose, scarcely puberulous, with leaves in developing with 1–3 leaflets pairs as appendixes in some branches; pedicel 0.3–0.4 mm long in female flowers and 1 mm long in male flowers; male flowers aestivation and female flowers Quincuncial aestivation. Calyx 2–3.5 mm long, gamosepalous, **cup-shaped, lobed**, apex obtuse to acute, hairy pubescent outside and glabrous inside. Petals 7–8.2 mm long, 2–2.6 mm

wide, strap-shaped, apex acute, heavy pubescence outside, heavy pubescence inside restricted to the higher part in touch to stamens area, from creamish red to pinkish red sometimes from greenish cream, petals adnate to the androgynophore from the lower half to the higher part, margins adnate, only free in the bottom of the petals. Androgynophore 2.5–5 mm long, cream coloured. Stamens, male flower: filaments (free portion) 1.8–3 mm long, anthers 1–1.3 mm long, glabrous, female flower: filaments (free portion) 2.4–3.2 mm long, anthers 0.8–1 mm long, shrunken, without pollen, lower part of filaments fused to the androgynophore. Ovary 5-locular, borne at the top of the androgynophore, 1.5 mm long, glabrous, female flower: loculi with (5–)6–7 ovules in 2 rows, style 2 mm long with a thick discoid style-head, male flower: pistilloide 3–4 mm long, glabrous, with reduced loculi and 6–7 vestigial ovules in 2 rows, style 2mm long with a thin style-head, the ovary located a half below to the insertion of filaments. **Capsule pendulous, 4.5–6 cm long**, oblong to obovoid, apex and base rounded, blackish brown with many irregular pale brown lenticels, valves woody, 2–3 mm thick, columella 5-winged. Seeds 3–4 cm long (including the wing), dark brown.



**Figure 6.** *Cedrela pandeirensis*. A, flowering branch; B, detail of leaflet undersurface; C, female flower; D, female half-flower; E, male flower; F, male half-flower; G, detail of petal insertion; H, capsule; I, seed.





**Figure 7.** A. Flowering branch of *Cedrela panderensis* B. Thyrses of flowers of *Cedrela panderensis* C. Capsules organized in an infructescence D. Trunk of a specimen of *Cedrela panderensis* E. Habit of *Cedrela panderensis*.

### 3.1.2.1 Distribution, habitat, and etymology

*Cedrela pandeirensis* sp. nova is distributed from Pandeiros River Basin Environmental Preservation Area, Peruaçu Caves National Park, and Mata Seca State Park to Sabonetal locality in both flanks of Sao Francisco River in Northern Minas Gerais State, Brazil. All specimens included in this study were collected from this part of the range. The species occurs in Ecotone habitats associated to Cerrado, Caatinga and Atlantic Forest, and associated with ranges of seasonally humidity located near body streams (Figure 2). The species grows between 400–700 meters above sea level, in areas in which other genus such as *Anadenanthera*, *Dimorphandra*, *Ceiba*, *Spondias*, *Cabranea* and herbaceous specimens also occur. *Cedrela pandeirensis* is vernacularly called “*Cedro*”, because as the same way their widespread Brazilian congener was over exploited for human populations settled in those places. The epithet of the species is named in honor of the Pandeiros community, who lived in the micro basin of the Pandeiros River.

### 3.1.2.2 Field characters

Trees up to 20(–30) m high and 170–190(–200) cm diam. Bole cylindrical, up to 10–20 m high to the first branch, with fissured dark gray to grayish-brown bark. Presence of garlic odour. The flowers are greenish-cream to pale; sometimes the entire flowers or the petals which are exposed to the sun are reddish. The fruits are green with small and few lenticels. Flowering occurs mostly between October and March; trees are densely flowering. The capsule matures between June and September.

The distinctive morphometric characters of *Cedrela pandeirensis* are shown as mean trait values compared with traits values of different congeners such as; the two widespread *Cedrela fissilis* and *Cedrela odorata* and *C. balansae*. The combination of characters of *Cedrela pandeirensis*, such as; the leaf length 23.5–49.5 cm, (5)6–9 pairs of narrow leaflets, leaflet size 7–11.6 x 2.8–4 cm, usually ovate–lanceolate, slightly falcate, apex obtuse to acute or to cuspidate, base usually slightly asymmetric and oblique, secondary veins 11–14(–15) pairs, inflorescence length 18–30 cm, flowers length 7–9 mm, stamens length 1.8–3.2 mm, pistil length 3.5–4.5 mm, with capsule pendulous 4.5–6 cm long, oblong to obovoid, apex and base rounded.

### 3.1.2.3 Additional specimens examined

**BRAZIL. Minas Gerais:** Januária, Estrema, 15°21'49.23"S, 44°15'21.17"W, 422 m, 12 December 2017, L.O. Oliveira 1711 & A. Huamán-Mera (VIC); Januária, Fabiao I, 15°10'41.28"S, 44°12'44.59"W, 485 m, 12 December 2017, L.O. Oliveira 1734 & A. Huamán-Mera (VIC); Itacarambi, Fabiao II, 15°9'33.24"S, 44° 9'31.74"W, 473 m, 12 December 2017, L.O. Oliveira 1865 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, 15°33'23.51"S, 44°42'50.78"W, 477 m, 13 December 2017, L.O. Oliveira 2234 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, 15°33'25.96"S, 44°42'48.20"W, 480 m, 13 December 2017, L.O. Oliveira 2237 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, 15°29'7.51"S, 44°45'1.95"W, 523 m, 13 December 2017, L.O. Oliveira 2243 & A. Huamán-Mera (VIC); Itacarambi, Rancharia, 14°57'6.56"S, 44°3'29.23"W, 473 m, 12 December 2017, L.O. Oliveira 2285 & A. Huamán-Mera (VIC); Itacarambi, Rancharia, 14°57'47.47"S, 44° 1'53.17"W, 463 m, 31 May 2016, L.O. Oliveira 2286 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, Raizana, 15°28'21.34"S, 44°44'58.70"W, 535 m, 13 December 2017, L.O. Oliveira 2344 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, Raizana, 15°32'6.00"S, 44°43'26.40"W, 499 m, 13 December 2017, L.O. Oliveira 2346 & A. Huamán-Mera (VIC); Januária, Pandeiros Community, Raizana, 15°32'6.00"S, 44°43'26.40"W, 499 m, 13 December 2017, L.O. Oliveira 2347 & A. Huamán-Mera (VIC) Januária, Pandeiros Community, Remanso, 15°41'3.97"S, 44°35'11.24"W, 477 m, 06 April 2017, L.O. Oliveira 2354, A. Huamán-Mera & J.M. Díaz-Soto (VIC); Januária, Pandeiros Community, Remanso, 15°41'4.18"S, 44°35'3.72"W, 479 m, 06 April 2017, L.O. Oliveira 2355, A. Huamán-Mera & J.M. Díaz-Soto (VIC).

### 3.2 New occurrences of *Cedrela brachystachya* in central Brazil

According with our previous complementary of original description of *Cedrela brachystachya* (Huamán-Mera et al., Submitted), the populations of FR1, BR1 and BR2 would belong to *C. brachystachya* and they are distributed in central Brazil. In presente study *C. brachystachya* is represented by ANA population and some herbarium records from National Park of Brasilia (Figure 3).

Some of the population FR1 (Foz do Rio Aguapeí 01) are distributed in: **Brazil. São Paulo:** Castilho, Reserva Particular do Patrimônio Natural (RPPN) Foz do Rio Aguapeí,



21°09'16,5''S, 51°47'30,0''W, 272 m, 28 November 2012, L.O. Oliveira 830 (VIC); Castilho, Reserva Particular do Patrimônio Natural (RPPN) Foz do Rio Aguapeí, 21°09'07,8''S, 51°47'30,0''W, 274 m, 28 November 2012, L.O. Oliveira 831 (VIC).

Populations of BR1 and BR2 (Brasilia 1 and 2) are distributed in: **Brazil. Brasilia DF:** Parque Nacional de Brasília, Mata de galeria do Córrego do Acampamento, 15°44'23''S, 47°55'49''W, 1045 m, 20 October 2015, L. Oliveira 1988 & A. Huamán (VIC); Parque Nacional de Brasília, Mata de galeria do Córrego do Acampamento, 15°44'23''S, 47°55'49''W, 1045 m, 20 October 2015, L. Oliveira 1989 & A. Huamán (VIC); Parque Nacional de Brasília, Mata de galeria do Córrego do Acampamento, 15°44'23''S, 47°55'49''W, 1045 m, 20 October 2015, L. Oliveira 1993 & A. Huamán (VIC); Parque Nacional de Brasília, Mata de galeria do Córrego do Acampamento, 15°44'23''S, 47°55'49''W, 1045 m, 21 October 2015, L. Oliveira 1997 & A. Huamán (VIC); Parque Nacional de Brasília, Mata de galeria do Ribeirão Bananal, 15°42'53''S, 47°55'24''W, 1025 m, 21 October 2015, L. Oliveira 2011 & A. Huamán (VIC); Parque Nacional de Brasília, Mata de galeria do Ribeirão Bananal, 15°42'53''S, 47°55'24''W, 1025 m, 21 October 2015, L. Oliveira 2012 & A. Huamán (VIC).

Additional vouchers were taken from previous study (Huamán-Mera et al., Submitted) and the distribution of *Cedrela brachystachya* increased its range. **Brazil. Brasilia DF:** Parque Nacional de Brasília; Mata do acampamento, 15°35'S, 48°10'W, 1045 m, 06 October 1998, A.B. Sampaio 255 (CEN); Mata do acampamento, Parque Nacional de Brasília, 06 September 1998, A.B. Sampaio 250 (CEN); Samambaia, Parque Boca da Mata, 15°52'S, 48°03'W, 13 August 1996, Joao Marcelo de Rezende 556 (CEN); Mata de galeria do Córrego da Onça, Fazenda Água Limpa, 15°57'S, 47°55'W, 1060 m, 22 September 1994, B.M.T. Walter, S.P. Cordovil & A.S. Amaral 2235 (CEN); Córrego Landim, 15°34'33''S, 47°52'50''W, 950 m, 17 March 1966, H.S. Irwin, J.W. Grear Jr., R. Souza, & R. Reis dos Santos 14046 (NY).

## IV. DISCUSSION

### 4.1 Morphometric analyses of *Cedrela microanthus*

The combination of these characters was not diagnostic in others species of *Cedrela*, overall the length of flowers, this feature would be inherent to *Cedrela microanthus*. According to our phylogenetic analysis using ITS, *C. microanthus* was close-related to both *C.*

*fissilis* from Atlantic range and *C. balansae* from Argentina and Paraguay. However, the traits values that exhibited these close species with *C. microanthus* were different. *C. fissilis* exhibited more number of leaflets (10–17) than *C. microanthus*, spite of both species have similar length leaves (42 cm), only that *C. fissilis* showed less leaflet blade width (2.5–5 cm) than *C. microanthus* and this increased the space to insert more leaflets per leaf in the same length of the rhachis. Other important difference between *C. fissilis* and *C. microanthus* is the number of secondary veins per leaflet, *C. fissilis* showed 12–17 and *C. microanthus* 8–12. The presence of a short inflorescence in *C. fissilis* (Mean = 22 cm) and a long one in *C. microflora* (mean = 35 cm) was also evidence for a different treatment. Moreover, the size of flowers in *C. fissilis* is almost two times the size of *C. microanthus* flowers, as well as the size of capsules showed the same situation. On the other hand the mean values of *C. balansae* are so close to *C. microanthus* mainly in vegetative characteristics, in reproductive features they are broadly different; for instance the inflorescence length in *C. balansae* is larger than observed in *C. microanthus*, also the size of flowers of *C. balansae* is larger than *C. microanthus*, other feature that offer insights on the differences between both congeners is the size of capsules, in *C. balansae*, they are in mean 3.75 cm and in *C. microanthus* is 4.2 cm. Therefore *Cedrela microflora* will form a morphologically distinct unit from others (Table 1).

#### **4.2 Morphometric analyses of *Cedrela pandeirensis***

The combination of these traits was not diagnostic in others species of *Cedrela*, but there is a slightly similarity with *C. fissilis*, however they are not completely the same morphological entity. *Cedrela pandeirensis* exhibited shorter vegetative characteristics than *C. fissilis*. The vegetative features more remarkable were the number of pair of leaflets per leaf, 4–9, and the length and width blade. Sometimes, these vegetative characteristics increase their size when we observed in farms where the presence of nitrogenous is evident. The reproductive characteristics also showed slightly reduced size, the stamens either male or female were many shorter than those observed in *C. fissilis*, as well as the size of capsules (4.5–6 cm, mean=5.56 cm) was shorter than *C. fissilis*. According to our phylogenetic analysis, *C. pandeirensis* also was also related to *C. balansae* from Argentina and Paraguay. However, the traits values that exhibited *C. pandeirensis* were slightly different of *C. balansae*. For instance, the vegetative characteristics that *C. pandeirensis* exhibited were shorter than *C. pandeirensis* showed, except in the number of secondary veins, in this feature *C. pandeirensis* showed more secondary veins than *C. balansae*; on the other hand, when we

compared the reproductive characteristics between both species (*C. pandeirensis* and *C. balansae*), we noticed that the size of vegetative features of *C. pandeirensis* were larger than the features of *C. balansae*, except in the length of the inflorescence, the inflorescence of *C. pandeirensis* (18–30cm, mean=24.7 cm) is shorter than the inflorescence of *C. balansae* (36–53 cm, mean=44.5 cm). Therefore *Cedrela pandeirensis* will form a morphologically distinct unit from others congeners (Table 1).

**Table 1.** Mean trait values for *Cedrela microanthus*, *Cedrela pandeirensis* and morphologically related species. Standard error of mean and the number of organs analyzed (n) are annotated for *C. microanthus* and *Cedrela pandeirensis*. Minimum and maximum measured values per species per traits are indicated below each mean for all species. Values of related species were taken from Pennington & Muellner (2010).

Traits/Species	<i>Cedrela microanthus</i>	<i>Cedrela pandeirensis</i>	<i>Cedrela brachystachya</i>	<i>Cedrela fissilis</i>	<i>Cedrela odorata</i>
<b>Leaf (petiole + rhachis) length (cm)</b>	42.33 +/- 2.08 (n=20) min = 22, max = 54	34.35 +/- 1.34 (n=50) min = 24, max = 50	18.43 +/- 1.27 (n = 26) min = 7, max = 35	mean = 42.5 min = 25, max = 60	mean = 37.5 min = 25, max = 50
<b>Number of pair of leaflets per leaf</b>	7.12 +/- 0.23 (n = 20) min = 5, max = 9	7 +/- 0.32 (n = 100) min = 4, max = 9	6.65 +/- 0.39 (n = 23) min = 3, max = 9	mean = 13.5 min = 10, max = 17	mean = 9 min = 7, max = 11
<b>Leaflet blade length (cm)</b>	11.31 +/- 0.26 (n = 100) min = 8.38, max = 13.26	9.87 +/- 0.23 (n = 100) min = 6.94, max = 11.57	5.12 +/- 0.45 (n = 23) min = 2, max = 10	mean = 11.5 min = 8, max = 15	mean = 11.5 min = 8, max = 15
<b>Leaflet blade width (cm)</b>	4.21 +/- 0.10 (n = 100) min = 3, max = 5	3.47 +/- 0.05 (n = 100) min = 2.83, max = 4.12	2.43 +/- 0.17 (n = 23) min = 1.3, max = 4.5	mean = 3.7 min = 2.5, max = 5	mean = 3.5 min = 2.5, max = 4.5
<b>Petiolule Length (mm)</b>	5.44 +/- 0.19 (n = 100) min = 3.8, max = 7.3	2.02 +/- 0.19 (n = 100) min = 1, max = 4.11	-- --	-- --	-- --
<b>Number of secondary veins</b>	10.75 +/- 0.19 (n = 100) min = 8, max = 12	13.5 +/- 0.15 (n = 100) min = 11, max = 15	7 +/- 0.35 (n = 27) min = 4, max = 10	mean = 14.5 min = 12, max = 17	mean = 11.5 min = 9, max = 14
<b>Inflorescence length (cm)</b>	35.4 +/- 1.63 (n = 20) min = 28, max = 48	24.71 +/- 1.01 (n = 50) min = 18, max = 32	17 +/- 0.68 (n = 21) min = 12.3, max = 24	mean = 22.5 min = 15, max = 30	mean = 27.5 min = 15, max = 40
<b>Corolla length (mm)</b>	5.45 +/- 0.05 (n = 50) min = 5, max = 6	8 +/- 0.15 (n = 50) min = 7, max = 9	7.5 +/- 0.19 (n = 21) min = 7, max = 9	mean = 9.25 min = 8, max = 10.5	mean = 8 min = 6, max = 10
<b>Calyx tube length (mm)</b>	2.07 +/- 0.06 (n = 50) min = 1.5, max = 2.5	3 +/- 0.0 (n = 50) min = 2, max = 3.5	2.26 +/- 0.14 (n = 21) min = 1, max = 3	mean = 2.25 min = 2, max = 2.5	mean = 2 min = 1.5, max = 2.5
<b>Androgynophore length (mm)</b>	2.57 +/- 0.04 (n = 50) min = 2, max = 3.2	3.83 +/- 0.26 (n = 50) min = 2, max = 5	2.78 +/- 0.04 (n = 20) min = 2.5, max = 3	--	mean = 3.25 min = 2.5, max = 4
<b>Stamens, male flower length (mm)</b>	1.96 +/- 0.01 (n = 100) min = 1.9, max = 2	2.38 +/- 0.01 (n = 100) min = 1.8, max = 3	3.86 +/- 0.04 (n = 26) min = 3.5, max = 4.3	mean = 3.63 min = 3.25, max = 4	mean = 3.05 min = 2.3, max = 3.8
<b>Stamens, female flower length (mm)</b>	1.87 +/- 0.02 (n = 100) min = 1.5, max = 2	2.63 +/- 0.01 (n = 100) min = 2.4, max = 3.2	2.58 +/- 0.03 (n = 21) min = 2.4, max = 2.9	mean = 4.25 min = 3.5, max = 5	--
<b>Pistillode length (mm)</b>	2.88 +/- 0.05 (n = 50) min = 2.5, max = 3	3.33 +/- 0.11 (n = 50) min = 3, max = 4	2.85 +/- 0.03 (n = 26) min = 2.7, max = 3	--	mean = 4 min = 3, max = 5
<b>Pistil length (mm)</b>	1.94 +/- 0.11 (n = 50) min = 1, max = 2.5	3.61 +/- 0.11 (n = 50) min = 3.5, max = 4.5	3.2 +/- 0.06 (n = 25) min = 2.5, max = 3.7	mean = 3.5 min = 3, max = 4	mean = 2.25 min = 2, max = 2.5
<b>Capsule size (cm)</b>	4.2 +/- 0.08 (n = 50) min = 3.5, max = 4.5	5.56 +/- 0.09 (n = 50) min = 4.5, max = 6.1	3.2 +/- 0.06 (n = 19) min = 2.5, max = 3.5	mean = 9 min = 7, max = 11	mean = 4 min = 3, max = 5

### 4.3 Conservation status of new taxa of *Cedrela*

The forests are highly destroyed and they still are under threat throughout Brazil (Sparovek et al., 2012) as well as in all South America. In present study we specially referred to seasonal forest. The causes of seasonal forest destruction range from climate change, habitat fragmentation, fire, population density and agriculture (Miles et al., 2006). The agriculture is highly intensive in seasonal forests because of they occur in soils highly fertiles (Oliveira-Filho et al., 1994). However Brazilian government had developed success efforts to control and reduce the agents that threaten them through National conservation areas which are managed by ICMBio (Chico Mendes Institute for Biodiversity Conservation). Populations of *Cedrela microanthus* would be in extreme danger of extinction because the populations where were sampled are distributed outside of conservation areas. It is greatful to mention that almost all populations of *Cedrela pandeirensis* are inside a good system of conservation areas from Northern Minas Gerais such as Pandeiros River Basin Environmental Preservation Area, Peruaçu Caves National Park, and Mata Seca State Park. On the other hand we were worried about the conservation status of *Cedrela brachystachya* because of the initial populations studied by Huamán-Mera et al. (Submitted) were distributed also outside of conservation areas, however in present work we confirmed the presence of populations (BR1 and BR2) of *C. brachystachya* within the National Park of Brasilia. All these conservation efforts would be possible the conservation of genetic pools of new taxa of *Cedrela*.

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