ALEXANDER HUAMÁN MERA

STRUCTURE AND GENETIC DIVERSITY OF Cedrela (MELIACEAE) ON THE UPPER PARANA RIVER

Dissertation submitted to the Universidade Federal de Viçosa, as part of the requirements of the Post Graduate Course in Botany for obtention of the title Magister Scientiae.

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Approved: February 24, 2014.

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João Augusto Alves Meira Neto	Christina Cléo Vinson

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Luiz Orlando de Oliveira
(Adviser)

To my parents, Mardóneo and Yolanda,

to my family, to my country, Peru,

to my new family in Brazil

And To Scientia amabilis

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BIOGRAPHY

ALEXANDER HUAMÁN MERA, son of Mardóneo Huamán Chávez and Yolanda Mera Cayao, born in Chomza Alta, La Peca, Bagua, Amazonas – Peru, 25th July 1983.

He completed his primary education in 1996 and high school in 1999 at the "Juan Pablo Guzmán y Vizcardo Zea" high school in La Victoria - Chiclayo - Peru.

He graduated from the Faculty of Biological Sciences, at "Pedro Ruiz Gallo" National University, Lambayeque - Peru, in the specialization of Botany in October 2006 when he received the grade of bachelor in Biology and in July 2011 he obtained the Professional title in Biology - Botany.

In March 2012, he began to study in the Master Program in Botany, in the Federal University of Viçosa, acting in the area of Systematic of Phanerogams.

SUMMARY

RESUMO

HUAMÁN MERA, Alexander. M.Sc. Universidade Federal de Viçosa, Fevereiro de 2014. **Estrutura e diversidade genética de Cedrela (Meliaceae) no alto do rio Paraná**. Orientador: Luiz Orlando de Oliveira.

Cedrela (Meliaceae) é um gênero monofilético que inclui 17 espécies florestais no Neotropico. A sua diversificação se intensificou no Mioceno tardio e Plioceno cedo. Muitas espécies de Cedrela possuem distribuição restrita, entretanto Cedrela fissilis Vell. e amplamente distribuída. No Brasil C. fissilis Vell. acontece geralmente associada a florestas úmidas e estacionais. Esta espécie esta considerada "em perigo" pela lista vermelha da IUCN. É formada por duas linhagens filogenéticas que não são um clado monofilético, Cedrela fissilis Vell. e Cedrela odorata L., contendo além, sequências de C. balansae, chamando-se de complexo C. fissilis. O rio Paraná se espalha pelo Cerrado, um importante hotspot de biodiversidade; provavelmente manteve-se estável e formaram um único grande refúgio durante o final do Pleistoceno, onde ocorreram mudanças climáticas importantes. Ferramentas moleculares irão contribuir para a compreensão do processo histórico de dispersão de genes. Florestas estacionais são fortes ecossistemas ameaçados, a pesar de ser consideradas áreas de endemismo. O objetivo deste estudo foi caracterizar a diversidade genética dentro e entre populações de Cedrela ao longo do alto do Rio Paraná. Dez microssatélites foram utilizados para a genotipagem de 192 indivíduos; obtiveram-se valores de H_O=0,65 e H_E=0,78 e valores positivos da F_{IS} (F_{IS}=0,18). Para AMOVA, 84,93 % da variação estavam dentro das populações e 15,07% estavam entre as populações, com valores de divergência genética (F_{ST}=0,151). O grupo da Cedrela fissilis linhagem 1 mostrou-se mais diverso, com 22 dos 54 alelos privados encontrados. Ele apresentou valor F_{ST} (F_{ST} = 0,111) menor do que as populações do grupo Cedrela sp. (F_{ST}=0,188). Verificou-se forte estrutura das populações, com cinco linhagens de Cedrela e com espécimes com menos admixtured nunca antes amostradas, sendo maior do que o número de linhagens conhecidas com base em sequências de ADN e do que os resultados do estudo do complexo C. fissilis usando SSR. Três destes cinco grupos são novas linhagens. Isto sugere, de acordo com nossos resultados, a presença de refúgios na bacia do alto rio Paraná, além de uma nova unidade geneticamente distinta e mais dois para análise botânica. Além disso, algumas populações mostraram relevância na conservação do habitat devido à presença de material genético estranho e processo histórico.

ABSTRACT

HUAMÁN MERA, Alexander. M.Sc. Universidade Federal de Viçosa, February, 2014. **Structure and genetic diversity of Cedrela (Meliaceae) on the upper Parana river**. Adviser: Luiz Orlando de Oliveira.

Cedrela (Meliaceae) is a monophyletic genus and includes 17 tree species in the Neotropics. Its diversification intensified in the Late Miocene and Early Pliocene. Most Cedrela species have restricted distribution ranges, however Cedrela fissilis Vell. is widespread. In Brazil C. fissilis Vell. occurs associated with Seasonal and Moist Forest. This species is "endangered" on the red list of the IUCN. Cedrela fissilis is formed by Cedrela fissilis Vell. and Cedrela odorata L., two phylogenetic lineages that are not a monophyletic clade, comprising moreover sequences of C. balansae, called C. fissilis complex. The upper Paraná River spreads into the Cerrado, an important biodiversity hotspot; it is probably remained stable and formed a single large Cerrado refugium during the late Pleistocene, where occurred important climatic changes that could influence in the spread of species. Molecular tools will contribute for understanding the historical process of gene dispersal since the Pleistocene. Seasonal forests are strong threatened ecosystems. The knowledge about seasonal forest has been increased since they are considered as areas of endemism. The aim of this study was to characterize the genetic diversity within and among populations of Cedrela along upper Parana River. Ten microsatellites were used to genotype 192 individuals, then it was obtained high values of heterozygosis (H_O=0.65, H_E=0.78), with positive values of F_{IS} (F_{IS}=0.18). For AMOVA, 84.93% of the variation were within populations and 15.07% were among populations which also reflected into the values of genetic divergence (F_{ST}=0.151). The Cedrela fissilis lineage 1 group showed more diverse, with 22 of 54 private alleles found. It presented F_{ST} value (F_{ST}=0.111) lower than the populations of Cedrela sp. group (F_{ST}=0.188). It has been found strong population structure showing a number of groups equal to five and being greater than the number of known phylogenetic lineages based on DNA sequences and also greater than the results of study of the C. fissilis complex using SSR markers. Three of these five groups are new lineages that no was found in previously works. This suggests, according our evidence, the presence of refugia in the upper Paraná River basin, moreover of a new genetically distinct unit and two more for botanical review. Furthermore, some populations showed conservation relevance of habitat due to the presence of peculiar genetic material and historical process.

INTRODUCTION

Cedrela (Meliaceae) is a monophyletic genus and includes 17 species with a distribution range in the Neotropics, from Central to South America (PENNINGTON & MUELLNER, 2010). The diversification started in the Oligocene and Early Miocene and intensified in the Late Miocene and Early Pliocene (MUELLNER et al., 2010). Most Cedrela species have restricted distribution ranges, however to species of Cedrela, Cedrela fissilis Vell. and C. odorata L., they are widespread (Colombia to Brazil), occur in diverse forest types from semideciduos forests to gallery forest and Cerrado vegetation in Brazil (GARCIA et al., 2011, MUELLNER et al., 2010). Cedrela fissilis occurs throughout South America (PENNINGTON, 1981), but its presence in Central America (Costa Rica and Panamá) is uncertain (PENNINGTON and MUELLNER, 2010). Cedrela fissilis shows an alternative characteristic, heliophilous or deciduous, these characteristics were important for colonization of seasonal forest (LORENZI, 2002). Cedrela fissilis behaves as an initial secondary or late secondary colonizer species in forests; it occur either in primary forest, mainly in the forest edge or in open spaces, or in secondary forest (CARVALHO, 2003). With habitat loss added to the high economic value of the timber, C. fissilis is listed as being 'Endangered A1acd+2cd' in the IUCN Red List of species (International Union for Conservation of Nature) (IUCN, 2013). Cedrela odorata has provided one of the most important timber resources for Latin America and the international market today, showing a species to be considered within the red list of 'Vulnerable A1cd +2cd', according to the International Union for Conservation of Nature (IUCN, 2013).

Recent phylogenetic and phylogeographic studies done in Brazilian seasonal forests about the relationships in the genus Cedrela, where were used sequences of the internal transcribed spacer regions (ITS), plastid regions (trnS-trnG, psbB-psbT-psbN, trnT-trnL) and SSR markers found that "*C. fissilis"* is not a monophyletic group, but comprises several evolutionary lineages. Furthermore, this complex comprises two genealogically distinct lines, which are separated by the Cerrado, one located in the West side and the other in the East side of this phytophysionomy (GARCIA et al. 2011; MANGARAVITE, 2012). In Brazil specimens of Cedrela are assigned to either C. fissilis or C. odorata based on few taxonomic characters. However, Garcia et al., (2012) showed that regardless of the specimen's identity (C. fissilis or C. odorata) they cluster according to geography but to according to species. According to the phylogeny

presented in Garcia et al (2011), Brazilian specimens identified as C. odorata group together with specimens identified as C. fissilis; both form a derived clade. Meanwhile, C. odorata from the Caribbean region and from Northern South America form a distant, basal clade. Therefore, the fact that clustering of Brazilian specimens takes place according to geography brings doubts about the true identity of these specimens and support Garcia's suggestion that a species complex exist in Brazil (GARCÍA et. al., 2011). Herein, we will follow GARCIA et al. (2011) and referred to the species "C. *fissilis"* as the Cedrela fissilis complex.

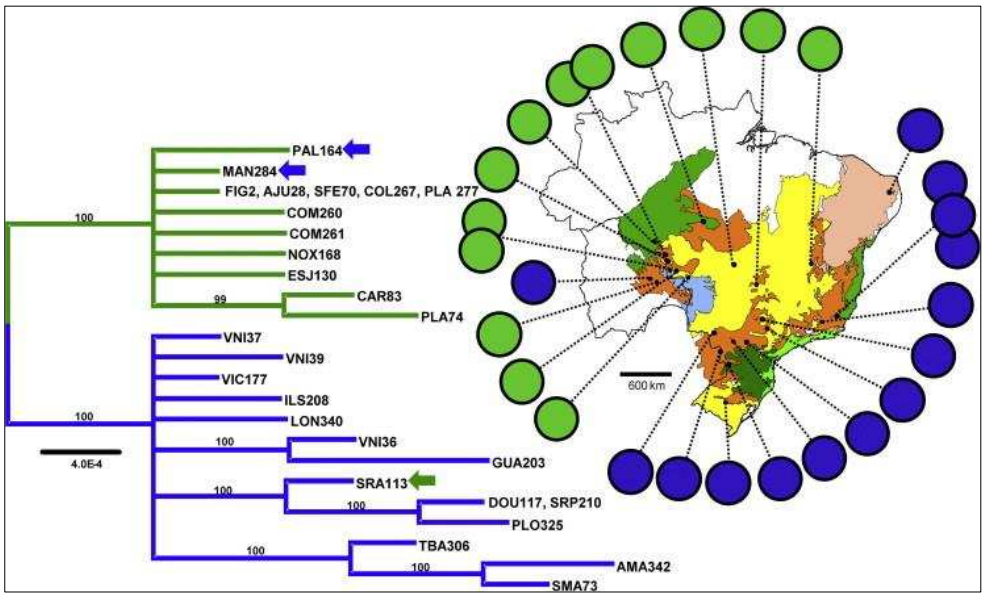

Figure 1. Geographical distribution of the main two lineages of Cedrela fissilis complex split by Cerrado. Chiquitano (green) and Atlantico (Blue). (Garcia et al, 2010)

The upper Paraná River is located inside two ecoregions, the Paraná-paraníba interior forest and the Cerrado (OLSON et. al., 2001). The Cerrado is the second largest Neotropical biome and is an important biodiversity hotspot; it is a 'diagonal of open formations' (WERNECK et al., 2012). During Quaternary climatic and vegetation fluctuations the central and north-eastern Cerrado were probably disturbed by Caatinga expansions and, in contrast, the southern and western (e.g. Upper Paraná basin, Brasilia region, Chapada dos Veadeiros) probably remained stable and formed a single large Cerrado refugium during the late Pleistocene (AB'SÁBER, 1983).

During the Pleistocene, climatic conditions oscillated between dry and wet phases. This suggests that Pleistocene glacial oscillations caused the distribution of species to change considerably. This caused extinctions of many species. Species that

survived the glaciations responded by contracting into remnant habitat (refugia) during glacial maxima, and expanding during the interglacial periods. (AVISE, 2000).

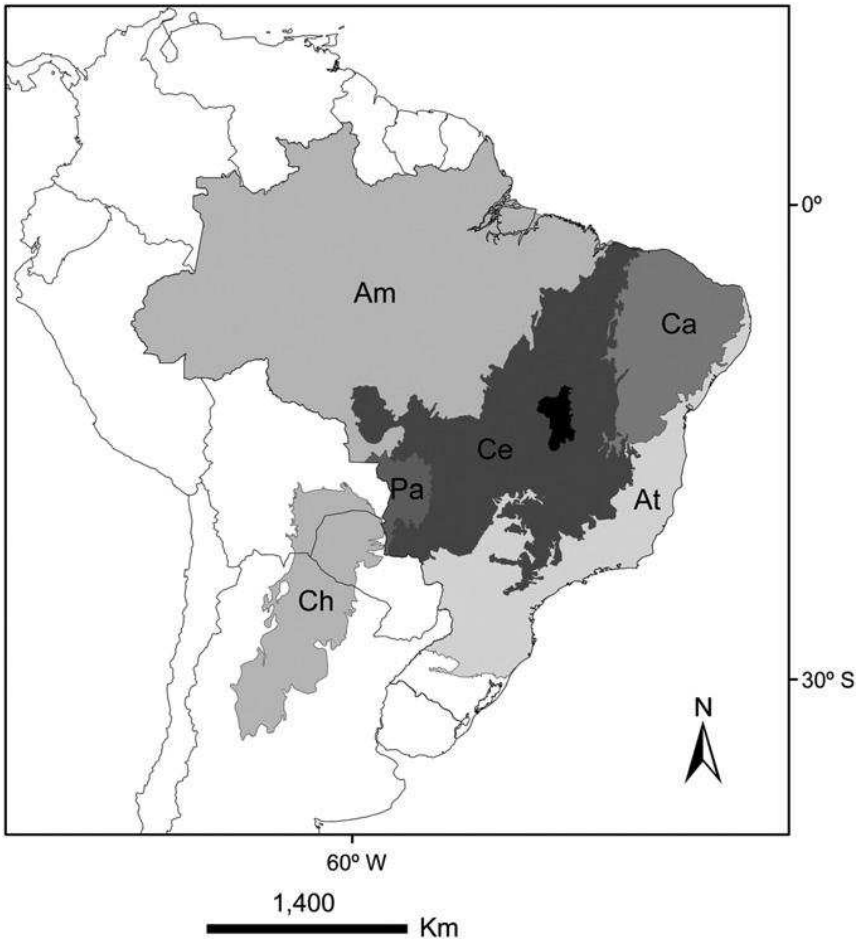

Figure 2. Georeferenced location of Cerrado (Ce), Chaco (Ch), and Caatinga SDTF nucleus (Ca) relative to other biomes (Amazon forest [Am], Atlantic forest [At], and Pantanal [Pa]). In black the Paranã River Valley, the major SDTF enclave region within the Cerrado biome in central Brazil. (Werneck, 2011)

Seasonal forests are characterized by a dual climatic seasonality, with a period of rainy season followed by a long dry period (up to five consecutive months), or in areas where precipitation shows little fluctuation, winter temperatures (< 15º C) cause physiological drought (OLIVEIRA-FILHO & FONTES, 2000; VELOSO et al., 1991). These forests represent a widely threatened ecosystem in Brazil (FUNDAÇÃO SOS Mata Atlântica; INPE, 2010); about 60% have been destroyed in South America (MILES et al., 2006). The main causes range from climate change, habitat fragmentation, fire to high density of human population, who are using the highly fertile soils, where occurs seasonal forests for extensive agriculture (MILES et al., 2006; OLIVEIRA-FILHO et al., 1994).

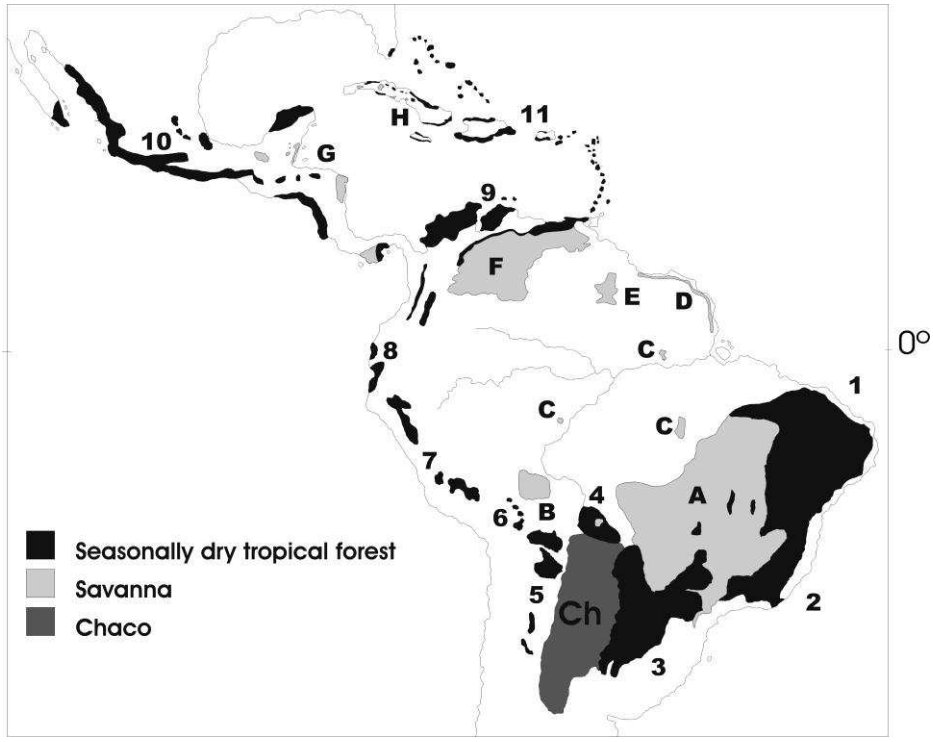

Figure 3. General distribution of rainforests, Seasonally Dry Tropical Forests (SDTFs) and other South American dry vegetation formations. SDTFs: 1. northeast Brazil (Caatinga); 2. southeast Brazilian seasonal forests; 3. Misiones Nucleus; 4. Bolivian Chiquitano region; 5. Piedmont Nucleus; 6. Bolivian inter Andean valleys; 7. Peruvian and Ecuadorian inter-Andean valleys; 8. Pacific coastal Peru and Ecuador; 9. Caribbean coast of Colombia and Venezuela; 10. Mexico and Central America; 11. Caribbean Islands (small islands colored black are not necessarily covered by SDTFs); 12. Florida. Savannas: (A) Cerrado; (B) Bolivian savannas; (C) Amazonian savannas (smaller areas not represented); (D) coastal (Amapá, Brazil to Guyana); (E) Rio Branco-Rupununi; (F) Llanos; (G) Mexico and Central America; (H) Cuba. Ce: Cerrado, Ch: Chaco (Pennington, et al, 2006b)

Knowledge about the status of the world´s rainforests, and particularly those in the Americas, is highly concentrating on wetlands, such as rain forests of the Amazon and Atlantic Forest biomes. Although the seasonal forests cover great part of the tropics, only 14% of the biodiversity studies were performed in these forest formations (SÁNCHEZ-AZOFEIFA et al. 2005). Recently, interest in seasonal Neotropical forests has increased since they have been diagnosed as areas of endemism (PRADO 2000; PENNINGTON et al. 2004) and are threatened by high levels of destruction due to urban expansion, conversion to agricultural use and wildfires (Fundação SOS Mata Atlântica and INPE 2009).

The study of the evolutionary history of tropical seasonal forests is of great importance for understanding the historical connections between the existing forest

fragments, the origins of the disjunction, and for understanding the processes of gene dispersal that may have occurred among the disjunction forest fragments. Molecular data can provide important information of historical process because demographic change will be reflected in the distribution and abundance of genetic diversity. Identifying and conserving the locations of high intraspecific genetic diversity is one a strategy needed to ensure that the evolutionary potential of a species is preserved for the future (SCOBLE, LOWE; 2010)

The knowledge of how genetic variation is partitioned among populations has important implications not only in evolutionary biology and ecology, but also in conservation biology (BALLOUX & LUGON-MOULIN, 2002). Genetic diversity is an important factor for the survival of populations in variable environments, and is recognized as a fundamental component of biodiversity (MACE et al., 1996). Studying genetic diversity in tree species is critical, because of the importance they have in structuring ecosystems and the fact of species with reduced genetic diversity, exhibiting risk of survival in the face biotic or abiotic stress.

Microsatellite markers are important tools which have been widely used to study genetic diversity of populations and demographics questions (ESTOUP et al., 1998). Microsatellite markers (LITT & LUTY, 1989) are short sequences of one to six base pairs, tandemly repeated, and are also called SSR (Simple Sequence Repeats, JACOB et al., 1991) or STR (Short Tandem Repeats, EDWARDS et al., 1991). Microsatellite presents randomization and scattered distribution (10^4 – 10^5) in the genome (TAUTZ, 1993). They are very abundant in the genomes of plants (DELSENY et al., 1983; TAUTZ & RENZ, 1984), are multi-allelic, have high degree of length polymorphism (ZANE et al., 2002), are codominant, are selectively neutral and have a high mutation rate. Thus, these markers are highly informative and recognized as being among the most efficient markers commonly used nowadays (ESTOUP et al., 1998; KALIA et al., 2011).

Our main hypothesis is that during past climate conditions that were drier and cooler than the present-day environment, Cedrela and co-distributed species found refuge in gallery forests that may have persisted along rivers in central Brazil. If so, exploring the Cedrela genetic diversity of present-day in areas nearby main existing rivers may reveal a genetic signature of these areas were likely refugia in the past. So to assess this hypothesis we address the following main objective. To characterize the genetic diversity within and among populations of Cedrela along upper Parana River, Brazil, as

well as to investigate phylogeographic patterns and relationships with the lineages, Chiquitano and Atlantic ranges. The specific objectives responded the following questions: (1) How is genetic diversity partitioned within and among populations? (2) What are the lineages obtained with microsatellite data, it is rare or unique genetic material? (3) How the obtained lineages are related with those previously established (Chiquitano and Atlantic ranges)? (4) Is there a refuge on the upper Paraná River?

MATERIALS AND METHODS

1. Plant sampling

We considered the ranges where the main lineages of Cedrela fissilis complex are established (Chiquitano, Central and Atlantic ranges) (Table 1).

We evaluated 192 specimens of Cedrela that were assigned to either C. fissilis or Cedrela spp. in the upper Parana River, Brazil. Theses specimens were sampled in 17 populations and the mean size was 11,3 individuals per population. 04 populations were collected in the Atlantic range (PEU, PRD, PSB, and CAP), east of the Cerrado; 13 populations were collected along the upper Paraná River in the Central range; 02 of these populations (ANA and FR1) were considered as a group genetically distinct unit (Cedrela sp.) because these populations showed morphological and phenotypical features distinct from common Cedrela. Individuals up to 50 km distant were grouped in the same population (Figure 4, Table 1). Leaf samples were stored in silica gel for subsequent DNA extraction.

2. DNA extraction and microsatellite markers analyzes

DNA was extracted according to the protocol of Cota-Sánchez et al. (2006) with modifications of Riahi et al. (2010).

We genotyped the Cedrela samples using 11 microsatellite loci. Eight markers (Ced2, Ced18, Ced41, Ced44, Ced54, Ced65, Ced95 and Ced131) were obtained from Cedrela odorata species (HERNÁNDEZ et al., 2008) and two markers (CF26, CF34 and CF66) were obtained from Cedrela fissilis (GANDARA, 2009). The primer pair CF66 amplified two loci (CF66A and CF66B) (Table 2), the primer pair CF34 revealed a tetraploid profile. For the 11 loci, the average percentage of lost data was 12.21% (ranging from 0.18%, for P66A, to 4.85%, for Ced95).

The polymerase chain reactions were conducted in multiplexing system (Mutiplex Ced54-Ced41-Ced95; Ced2-Ced65-Ced131 and CF26-Ced18-CF34) and Pseudomutiplex (diplex CF66; Ced44) with a final volume of 12 µL containing 10 ng of DNA, 1X buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl, 1% Triton X-100) 0, 2 mM of each primer (forward and reverse), 2.5mM MgCl2, 0.25 mg/ml BSA (Bovine Serum Albumin Invitrogen), 0.2 mM dNTPs and 0.5 U Taq polymerase (Phoneutria

Biotechnology). We used the following PCR program: 96°C for 2 minutes, 30 cycles of 94° C for 1 minute, annealing temperature 53° - 55 °C for 1 minute and 72 °C for 1 minute; and 72 °C for 20 minutes.

The forward primers were labeled with the fluorescence 6-FAM, HEX (MWG-Biotech) and NED (Applied Biosystems).The fragments were separated on a 96 capillary sequencer ABI PRISM 3130x1 DNA Analyzer (Applied Biosystems). The fragments were measured using Rox 500 size standard (Applied Biosystems) and analyzed in GeneMapper software v. 4.0 (Applied Biosystems).

Additionally, SSR dataset of 12 populations were added to our analyses from Mangaravite (2012). These 29 populations were used for running analyses of Population Structure (Figure 5 and 7, Table 1)

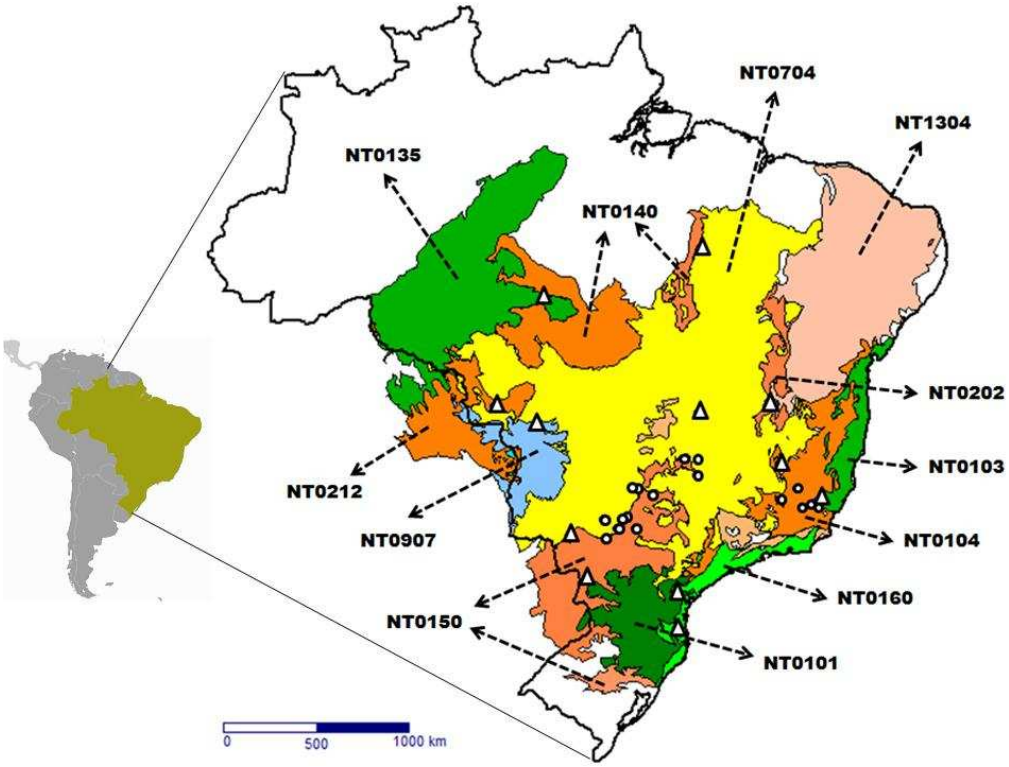

Figure 4. Geographic distribution of 29 populations of Cedrela and associated vegetal formations Terrestrial Ecoregions (OLSON et al., 2001): NT0101, Araucaria moist forest; NT0103, Bahia coastal forest; NT0104, Bahia interior forests; NT0135, Madeira-Tapajós moist forest; NT0150, Paraná-Paraíba interior forests; NT0160, Serra do Mar coastal forests; NT0202, Atlantic dry forests; NT0212, Chiquitano dry forests; NT0704, Cerrado; NT1304, Caatinga; NT0140, Mato Grosso seasonal forest e NT0907, Pantanal. Color Codes: green: Moist Forest; Orange: Seasonal Forest; Yellow: Savanna; Pink: Caatinga and blue: Pantanal.White triangles, 12 populations were taken from Mangaravite (2012); white circles, 17 populations from the present study.

Table 1. Populations of Cedrela used in this work, 12 populations were taken from Mangaravite (2012) and 17 populations were collected for this work, every population has information about their respective terrestrial ecoregions, codes, geographic coordinates and sample size. Color code: green: Moist Forest; Orange: Seasonal Forest.

Ecoregions	Population (code)	Latitude	Longitude	Sample size
Chiquitano Distribution Range				
NT0135	Alta Floresta/MT (ALT)*	-09 53’ 56,97”	-56 09’ 16,12”	21
NT0212	Figueirópolis D’Oeste/MT (FIG)*	-15 26’ 43,80”	-58 44’ 25,73”	22
NT0705	Tocantins (TOC)*	-07 12’ 26,92”	-47 45’ 49,04”	9
NT0907	Poconé/MT (POC)*	-16 19’ 08,50”	-56 31’ 52,50”	22
Central Distribution Range				
NT0150	Anaurilândia/MG(ANA)**	-22 12' 51.10"	-52 50' 37.20"	24
NT0150	Hidroeletrica1/ (HD1)	-21 45' 52.40"	-51 15' 47.70"	13
NT0150	Hidroeletrica2/ (HD2)	-21 44' 52.10"	-52 15' 41.80"	7
NT0150	Cecalpina/ (CEC)	-21 15' 26.80"	-51 58' 24.20"	7
NT0704	Hidroeletrica3/ (HD3)	-21 11' 07.70"	-51 51' 51.40"	7
NT0704	Cecalpina-Hidroeletrica/ (C-H)			2
NT0150	Foz Rio Aguapei1/ (FR1)**	-21 08' 10.00"	-51 47' 27.60"	11
NT0150	Foz Rio Aguapei2/ (FR2)	-21 07' 25.10"	-51 44' 29.70"	5
NT0150	Iturama/ (ITU)	-20 00' 40.90"	-50 21' 45.60"	18
NT0150	Paraiba/(PAR)	-19 43' 00.70"	-51 05' 37.10"	15
NT0150	Uberlândia1/ (UB1)	-18 06' 43.00"	-48 37' 00.00"	11
NT0704	Uberlândia2/ (UB2)	-18 11' 45.00"	-47 57' 21.00"	16
NT0704	Uberlândia3/ (UB3)	-18 58' 45.00"	-48 02' 12.00"	19
NT0704	Brasília/DF (BRA)*	-15 46’ 47,00”	-47 55’ 47,00”	2
NT0150	Itaporã/MS (ITA)*	-22 00’ 09,68”	-54 42’ 52,59”	9
NT0202	Januária/MG (JAN)*	-15 11’ 22,56”	-44 12’ 24,48”	9

Legend (*) Populations collected and SSR dataset obtained by Mangaravite (2012). (**) Populations belong to a genetically distinct unit.

(Continued on next page)

Table 01 (continued)

Ecoregions	Population (code)	Latitude	Longitude	Sample size
	Atlantic Distribution Range			
NT0160	Blumenau/SC (BLU)*	-26 55’ 07,51”	-49 03’ 57,69”	18
NT0150	Campinhos/PR (CAM)*	-25 2’ 17,00”	-49 5’ 26,00”	7
NT0104	Caparaó1/MG (CAP1)*	-20 31’ 45,60”	-41 55’ 14,10”	35
NT0150	Palotina/PR (PAL)*	-24 18’ 34,29”	-53 54’ 32,15”	26
NT0704	Diamantina/MG (DIA)*	-18 24’ 33,60”	-43 29’ 24,70”	8
NT0104	Parque Estadual Rio Doce/MG (PRD)	-19 42' 48.05"	-42 43' 55.30"	3
NT0104	Parque Estadual Uaimií/MG (PEU)	-20 14' 57.41"	-43 34' 16.76"	17
NT0104	Parque Serra Do Brigadeiro/MG (PSB)	-20 41' 01.00"	-42 26' 41.00"	10
NT0104	Caparaó/MG (CAP)	-20 27' 38.86"	-41 50' 14.70"	7
			TOTAL	378

Legend (*) Populations collected and SSR dataset obtained by Mangaravite (2012). (**) Populations belong to a genetically distinct unit.

Table 2. Information of the eleven microsatellite loci used in this work: Reference, locus, primers sequences, Annealing Temperature (T° C), microsatellite repeats, fluorescently labeled primers, total number of alleles per locus (N_A) and allelic range in base pairs (bp).

Reference	Locus	Primer Sequences (5'-3')	T(°C)	Repetition	Fluorescence	N_A	Allelic range (pb)
NCBI, 2011	**Ced2**	F: TTTGCTTTGAGAAACCTTGT R: AACTTTCGAATTGGTTAAGG	55	$(GA)_{20}$	6-FAM	30	131-241
	Ced18	F: CAAAGACCAAGATTTGATGC R: ACTATGGGTGGCACAACTAC	55	$(GA)_{23}$	HEX	22	113-161
	Ced41	F: TCATTCTTGGATCCTGCTAT R: GTGGGAAAGATTGTGAAGAA	55,5	$(TC)_{18}$	HEX	22	110-158
	Ced44	F: ACTCCATTAACTGCCATGAA R: ATTTTCATTCCCTTTTAGCC	55,5	$(TG)_{14}(AG)_{17}$	6-FAM	30	162-224
	Ced54	F: GATCTCACCCACTTGAAAAA R: GCTCATATTTGAGAGGCATT	55	$(GA)_{15}(AG)_6G(GA)_5$	6-FAM	20	172-218
	Ced65	F: GAGTGAGAAGAAGAATCGTGATAGC R: GAGGTTCGATCAGGTCTTGG	55,5	$(GA)_7(CA)_{14}$	HEX	24	157-193
	Ced95	F:ATTTTCATTCCCTTTTAGCC R:TTATCATCTCCCTCACTCCA	55	$(CT)_{17}(AC)_{13}$		27	80-120
	Ced131	F: CTCGTAATAATCCCATTCCA R: GGAGATATTTTTGGGGTTTT	55	$(CT)_{16}$	NED	19	66-128
Gandara, 2009	**CF26**	F: CCAAATTCCAGAGGAGAG R: GTTCTGCTTCATCGAAGG	56	$(AG/TC)_{13}$	6-FAM	0	139-187
	CF66A	F: CAGCAGTTCTGAAACAGTAA R: ATTCAGCAACTTGAGAGC	56	$(AG/TC)_{13}$	6-FAM	28	113-175
	CF66B			$(AG/TC)_{13}$		27	199-253

3. Data analyses

3.1. Analyses within Populations

The frequency of null alleles and scoring errors were estimated for our 17 populations using the software MICROCHEKER v. 2.2.3 (OOSTERHOUT et al. 2004). The statistical significance of deviation from Hardy–Weinberg equilibrium (HWE); the linkage disequilibrium loci, beyond the zero offset (proportions of genotypes in HWE), was assessed by allelic permutations within populations according to the Fisher exact test described by Weir (1996) were calculated according to the software FSTAT version 2.9.3.2 (GOUDET, 1995). For descriptive analyzes of genetic diversity was used the GDA program version 1.0 (d16c) (LEWIS; ZAYKIN, 2001). We computed for each population the average number of alleles per locus (*A*/loco), the number of private alleles (A_{PRIV}.); the expected heterozygosity (H_E); the observed heterozygosity (H_O); and the inbreeding coefficient (F_{IS}) (WRIGHT, 1951).

3.2. Analyses among Populations

Parameters of fixation indexes within populations (F_{IS}); total fixation index (F_{IT}) and genetic divergence among populations (F_{ST}) described by Weir and Cockerham (1984), G_{ST} index (NEI, 1973) and the estimated R_{ST} (SLATKIN, 1995) were estimated using the FSTAT version 2.9.3.2 software (GOUDET, 1995). These analyses were calculated for 17 populations and also separately for the different ranges along the upper Parana River (Table 1). For these analyses, the bootstrap resampling procedure was used with 1000 permutations and 95% confidence interval. The G_{ST} rate is analogous to F_{ST}, which assumes a infinite allele model (IAM). Whereas R_{ST} is based on the size of the allele (stepwise mutation model, SMM). R_{ST} is expected to be greater than the F_{ST} index whether the SMM contribute more to differentiation.

The differentiation of current 17 populations by analysis of molecular variance (AMOVA) for within and among population differences was obtained using the software Arlequin version 3.1 (EXCOFFIER et al., 2006). The AMOVA analysis was also performed with the partitioned data, grouping the populations in five groups corresponding to K=5 in structure analyses (Table 5). Significance was tested with 1,000 permutations.

We calculated the pairwise F_{ST} (WEIR; HILL, 2002) among the 17 populations using the software Arlequin version 3.1 (EXCOFFIER et al., 2006). The average gene flow was estimated assuming $Nm = [1/(F_{ST} -1)]/4$ (WHITLOCK; MCCAULEY, 1999), the absolute number of migrants ($M = 2Nm$) between populations was also estimated on the basis of F_{ST}, using the program Arlequin version 3.1 (EXCOFFIER et al., 2006).

The genetic relationships among populations were assessed through a Neighbor-Joining tree generated based on the D_A genetic distance (NEI et al., 1983) using the software Populations 1.2.30 (LANGELLA 1999). The significance of best topology was estimated with 1000 permutations.

To infer the genetic clusters of the populations of the Cedrela we used a Bayesian approach with Markov Chain Monte Carlo (MCMC), as implemented in the software STRUCTURE v. 2.2 (PRITCHARD et al, 2000; FALUSH et al, 2007). We used the default values for most parameters, the Admixture model and correlation of gene frequencies among populations, according to Falush et al. (2003). Twelve independent running were performed as suggested by Evanno et al (2005). We executed it with a burn-in period of 250,000 steps followed by 750,000 MCMC steps. The number of groups ranged from K = 1 to K = 20. To obtain the best K, the criterion used was the ΔK (EVANNO et al., 2005).

RESULTS

1. Genetic diversity of microsatellite loci

The 11 loci exhibited small to moderate frequency of null alleles, from -0.0082 (P66A) to 0.1424 (P66B) for all 17 populations. The primers CF26 and CF34 were removed from subsequent analyses, the first one because showed a frequency of null alleles of 0.1886 in all populations and the second one because showed tetrapliod characteristics and the software do not support tetraploid data. The linkage disequilibrium probability in all populations showed no associations between loci. We identified a total of 249 alleles for the 10 loci assessed; the number of alleles for each locus ranged from 19 (CED131) to 30 (CED44 and CED2), with an average of 25 alleles per locus (Table 2). Eleven populations exhibited a total of 54 private alleles. A population located in Central Brazil (UB1) exhibited the highest number of private allele ($A_{PRIV.}$= 13) and populations HD2, CEC, C-H, FR1, PRD, and CAP exhibited no private allele. The expected heterozygosity (H_E) varied from 0.53 (HD3) to 0.88 (UB1), with an average of 0.78 (± DP) (± 0.079); H_E was higher than the observed heterozygosity (H_O), which varied from 0.41 (FR1) to 0.83 (PRD), with an average of 0.65 ± 0.073. Overall, the inbreeding coefficient (F_{IS}) reached positive values, except for the populations HD3 (F_{IS}=-0.06) and PRD (F_{IS}=-0.01). It varied from -0.06 (HD3) to 0.43 (ITU), with 0.18 as the average; indicating an excess of homozygotes (Table 3).

2. Differentiation between populations

Indexes of diversity showed small to moderate differences between the 17 populations assessed (G_{ST}=0,094; F_{ST}=0,111; R_{ST}=0,1344); a certain level of endogamy is also present (F_{IS} = 0.171). Overall, the R_{ST} (0.1344) was higher than the F_{ST} (0.111), which suggests a high contribution of stepwise mutation model to the current differentiation (Table 4).

Analyses of molecular variance (AMOVA) with two hierarchical levels showed the majority variation within populations (84,93%, Φ_{ST}=0,151, P<0,001).

Likewise, the AMOVA considering three hierarchical levels, populations were grouped in C. fissilis and Cedrela sp., also exhibited a high genetic variation within populations (79,57%, Φ_{ST}=0,204, P<0,001). Posteriorly, the AMOVA was calculated for every group and all cases showed a high genetic variation within populations. The group Cedrela fissilis exhibited (88,95%, Φ_{ST}=0,111, P<0,001) and the group Cedrela sp. showed (81,22%, Φ_{ST}=0,188, P<0,001) (Table 5).

Between the 136 comparisons of pairs of F_{ST} (WEIR; HILL, 2002) among populations, 32,35% of data were no significant (P<0,05). The significance pairs ranged from 0,038 (PEU x UB2) to 1 (FR1 x C-H). In this analyses the populations HD3 (pairwise F_{ST} ranging from 0,1968 to 0,7269), FR1 (pairwise F_{ST} ranging from 0,1729 to 1,1031) and ANA (pairwise F_{ST} ranging from 0,1739 to 0,7675) were more divergent than the others populations. (Lower part of the Table 6).

The upper half of the table 6 shows the analyses of absolute number of migrants among the populations. The absolute number of migrants ranged to proximately 1 (C-H x FR1) to 13 (UB2 x PEU) and a value of 3. Comparing the two groups, we could see that within C. fissilis the mean absolute number of migrants was 3.2, while between the two populations from Cedrela sp. The mean absolute number of migrants was 1.6, this value is smaller than compare with C. fissilis. These results showed a greater genetic flow among populations of C. fissilis than populations of Cedrela sp. (Upper half of the Table 6), yet this result showed low levels of migrants.

We assessed the genetic relationships among 29 populations (12 taken from Mangaravite, 2012) through a Neighbor-Joining tree generated based on the D_A genetic distance, this analyses generated a best distance tree where showed 5 groups, 3 of them formed a well-supported bootstrap. The first one showed a bootstrap of 97 and grouped populations of ANA an FR1, the second one exhibited a bootstrap of 100 and grouped populations of HD (1, 2 and 3) and the last one showed a bootstrap of 67 and grouped populations of ITU and PRN, probably this value in comparison with the other is low because of these populations had individuals with different genetic information and was corroborated by the structure analyses. The others 2 groups corresponded to Chiquitano and Atlantic ranges. We found that populations of PEU, PRD, PSB and CAP were grouped in the Atlantic range, corresponding with their geographic distribution (Figures 5 and 8).

3. Population Genetic Structure

Population structure was assessed through the software STRUCTURE version 2.2 (PRITCHARD et al., 2000; FALUSH et al., 2007). The convergence of the cluster analyses was done according to the method suggested by Evanno et al. (2005) through ΔK, and it generated a K=5 (mean ln[Pr(X|K)]=-18153.145 ± 4,34) (Figure 6). These results also showed a high genetic diversity within the populations. The group denominated as Cedrela sp. lineage 1 group between of the five groups obtained herein, was characterized into the less admixtured specimens (represented in red) with the majority assigned into two populations (ANA and FRA1). The Cedrela sp. lineage 2 group (represented in yellow) was compounded also by not very admixtured specimens, assigned into tree populations (HD1, HD2, and HD3). The Cedrela sp. lineage 3 group (represented in orange) was assigned into two populations (ITU and PRN), with only some non-admixtured specimens. Both ITU and PRN populations were also assigned into all other groups. These two last groups were very admixtured and they occur into the last ten populations (CEC, C-H, FRA2, UB1, UB2, UB3, PEU, PRD, PSB and CAP). These two groups were the same of the lineages already described: the Chiquitano genealogical lineage (assigned in green) and the Atlantic genealogical lineage (assigned in blue) (GARCIA et al., 2011, MANGARAVITE, 2012). Considering the five groups, these two groups from the Chiquitano range and the Atlantic range were from west and east of the Cerrado, respectively. On the other hand, the three new groups arose from the Central range (Figure 7). Note that as the values of K increase, the three groups are held constant, and a group of individuals within the population CAP1 (Figure 9).

Table 3. Genetic diversity for the 17 populations using microsatellite polymorphic loci: mean sample size over all loci (N); average number of alleles per locus (A/locus); number of private alleles ($A_{PRIV.}$); expected heterozygosis (H_E); observed heterozygosis (H_O); and inbreeding coefficient (F_{IS}) (WRIGHT, 1951).

Population	**N**	**A/loco**	**$A_{PRIV.}$**	**H_E**	**H_O**	**F_{IS}**
Cedrela sp. lineage 1 group						
ANA	24	7,7	4	0,71	0,68	0,05
FR1	10,3	4,56	0	0,59	0,41	0,31
Mean	**17,15**	**6,13**	**(4)***	**0,65**	**0,55**	**0,18**
Cedrela sp. lineage 2 group						
HD1	12,8	7,22	1	0,75	0,61	0,19
HD2	6,9	4,89	0	0,74	0,65	0,13
HD3	6,9	4,25	2	0,53	0,56	-0,06
Mean	**8,87**	**5,45**	**(3)***	**0.45**	**0,61**	**0,09**
Cedrela sp. lineage 3 group						
ITU	17,3	8,67	4	0,81	0,47	0,43
PAR	14	8,11	2	0,76	0,53	0,31
Mean	**15,65**	**8,39**	**(6)***	**0,79**	**0,50**	**0,37**
Cedrela fissilis lineage 1 group						
C-H	1,9	3,25	0	0,76	0,61	0,27
UB1	10,9	10,8	13	0,88	0,78	0,12
UB2	15,2	12,9	9	0,87	0,77	0,12
Mean	**9,33**	**8,98**	**(22)***	**0,84**	**0,72**	**0,17**
Cedrela fissilis lineage 2 group						
CEC	6,8	6,44	0	0,83	0,61	0,29
FR2	4,8	6,11	2	0,88	0,65	0,29
UB3	17,8	11,6	9	0,86	0,76	0,12
PEU	16,2	11,4	2	0,84	0,79	0,06
PRD	3	3,9	0	0,82	0,83	-0,01
PSB	9,7	8,9	6	0,84	0,67	0,21
CAP	5,8	6,1	0	0,81	0,64	0,24
Mean	**9,16**	**7,78**	**(19)***	**0,84**	**0,71**	**0,17**
MEAN	**10,84**	**7,45**	**(54)****	**0,78**	**0,65**	**0,18**

Legend: (*) Refers to the sum of private alleles for each region;
(**) Refers to the total sum of private alleles

Table 4. Genetic Diversity Indexes G_{ST} (NEI, 1973); F_{IT}, F_{ST} and F_{IS} (WEIR; COCKERHAM, 1984) and R_{ST} (SLATKIN, 1995) for all data (17 populations) and different data grouped in five groups.

Group (size)	G_{ST}	F_{IT}	F_{ST}	F_{IS}	R_{ST}
All (17)	0,094	0,263**	0,111**	0,171**	0,1344
Cedrela lineage 1 (02)	0,046	0,191**	0,087**	0,114**	0,2277
Cedrela lineage 2 (03)	0,065	0,184**	0,063**	0,129**	0,0647
Cedrela lineage 3 (02)	0,016	0,396**	0,031**	0,376**	0,0523
Cedrela fissilis lineage 1 (03)	0,032	0,134**	0,025**	1,112**	0,0416
Cedrela fissilis lineage 2 (07)	0,020	0,177**	0,028**	0,153**	0,0527

Legend: Statistically significant (*) P<0,05; (**) P<0,01.

Table 5. AMOVA analyses for genetic structure of population with two and three hierarchical levels. The populations was grouped into Cedrela sp. lineages and Cedrela fissilis lineages

Source of variation	d.f.	Sum of squares	Variance components	Fixation index	Percentage Variation	P-Value
Two hierarchical levels						
Among population	16	67282.97	151.11705 Va	Φ_{ST}=0,151	15,07	<0,001
Within populations	367	312496.467	851.48901 Vb		84,93	<0,001
Total	383	379779.438	1002.60606			
Three hierarchical levels						
Among two Groups	1	16700.706	99.56826 Va	Φ_{CT}=0,093	9,30	<0,001
Among populations within groups	15	50582.264	119.01616 Vb	Φ_{SC}=0,123	11,12	<0,001
Within populations	367	312496.467	851.48901 Vc	Φ_{ST}=0,204	79,57	<0,001
Total	383	379779.438	1007.07343			
Analyses within Cedrela sp. lineages						
Among population	1	3333.817	96.64137 Va	Φ_{ST}=0,188	18,78	<0,001
Within populations	68	28424.583	418.00858 Vb		81,22	<0,001
Total	69	31758.400	514.64994			
Analyses within Cedrela fissilis lineages						
Among population	14	47248.447	188.07145 Va	Φ_{ST}=-0,111	11,05	<0,001
Within populations	299	284071.884	950.07319 Vb		88,95	<0,001
Total	313	331320.33	1068.14464			

Table 6. Matrix of pairs of F_{ST} (WEIR; HILL, 2002) among populations (lower half) and absolute migrants number (M=2Nm) among populations (upper half) generated based on F_{ST} values.

	ANA	HD1	HD2	CEC	HD3	C-HD	FR1	FR2	ITU	PRN	UB1	UB2	UB3	PEU	PRD	PDB	CAP
ANA		0.81841	0.886	1.483	0.726	0.651	2.162	1.707	2.353	0.982	0.922	2.874	2.212	1.878	1.087	2.570	2.613
HD1	0.6109*		222.535	4.007	2.541	-	0.884	1.684	2.902	6.927	6.437	3.884	3.317	3.430	1.632	1.613	2.917
HD2	0.5638*	0.0023		2.168	53.366	14.106	0.834	1.604	2.250	3.028	3.929	3.669	2.929	2.194	1.203	1.679	2.200
CEC	0.3370*	0.1247*	0.2305*		1.087	7.425	1.666	7.714	12.986	20.165	23.449	34.827	14.678	111.836	11.297	3.040	34.897
HD3	0.6880*	0.1967*	0.0093	0.4596*		1.541	0.687	1.407	1.086	1.117	1.696	1.823	1.807	1.040	0.711	1.206	1.228
C-HD	0.7675*	0	0.0354	0.0673	0.3244		0.453	1.556	2.835	144.906	-	25.228	8.428	7.171	1.462	2.173	1.807
FR1	0.2311*	0.5651*	0.5992*	0.2999*	0.7269*	1.000*		2.295	2.003	1.172	1.784	2.996	2.410	1.417	0.505	2.892	1.313
FR2	0.2928*	0.2968*	0.3116*	0.0648	0.3551*	0.3212	0.2177*		3.397	2.418	7.711	47.999	-	3.651	2.420	4.850	14.711
ITU	0.2124*	0.1722*	0.2221*	0.0385	0.4603*	0.1763	0.2496*	0.1471		7.318	4.505	9.821	5.806	6.589	1.717	3.455	4.023
PRN	0.5087*	0.0721*	0.1650*	0.0247	0.4475*	0.0034	0.4265*	0.2067*	0.0683		-	6.755	6.648	5.662	1.639	1.966	2.561
UB1	0.5423*	0.0776*	0.1272*	0.0213	0.2947*	0	0.2802*	0.0648	0.1109*	0		7.917	11.564	5.060	2.894	2.746	5.407
UB2	0.1739*	0.1287*	0.1362*	0.0143	0.2742*	0.0198	0.1668*	0.0104	0.0509*	0.0740*	0.0631*		-	13.277	3.389	4.377	25.099
UB3	0.2259*	0.1507*	0.1706*	0.0340	0.2766*	0.0593	0.2074*	0	0.0861*	0.0752*	0.0432	0		14.754	5.153	4.109	22.203
PEU	0.2662*	0.1457*	0.2278*	0.0044	0.4804*	0.0697	0.3528*	0.1369*	0.0758*	0.0883*	0.0988*	0.0376*	0.0338		88.435	3.264	19.870
PRD	0.4598*	0.3062*	0.4153	0.0442	0.7030*	0.3419	0.9887*	0.2065	0.2910*	0.3048*	0.1727*	0.1475*	0.0970	0.0056		2.856	16.243
PSB	0.1945*	0.3098*	0.2977*	0.1644*	0.4143*	0.2300*	0.1728*	0.1030*	0.1447*	0.2542*	0.1820*	0.1142*	0.1216*	0.1531*	0.1750*		7.820
CAP	0.1913*	0.1713*	0.2272*	0.0143	0.4071*	0.2765	0.3806*	0.0339	0.1242*	0.1952*	0.0924	0.0199	0.0225	0.0251	0.0307	0.0639	

Legend: (*) Significant (P < 0,05); (-) Estimates not calculated.

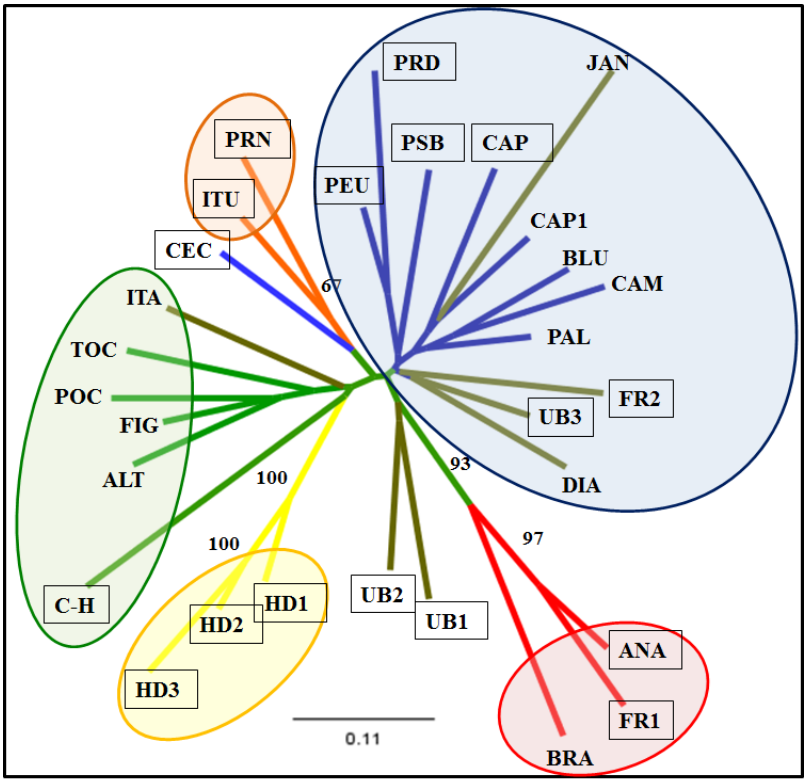

Figure 5. Unrooted tree of Neighbor-Joining generated based on the D_A genetic distance (NEI et al., 1983). In green ellipsoid, populations of the Chuquitano range; in light blue, populations of the Atlantic range; in red, orange and yellow, the new lineages exhibited in this work for the Central range, In dark green lines are shown admixtured individuals; names in square, the 17 populations from the present study; the values greater than 60 bootstrapping are shown.

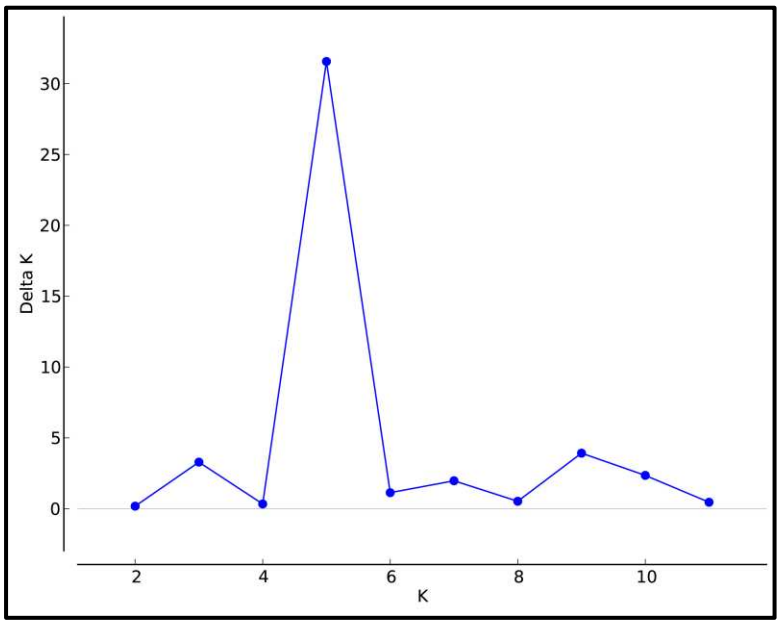

Figure 6. Graph obtained with ΔK values for display the best K=5.

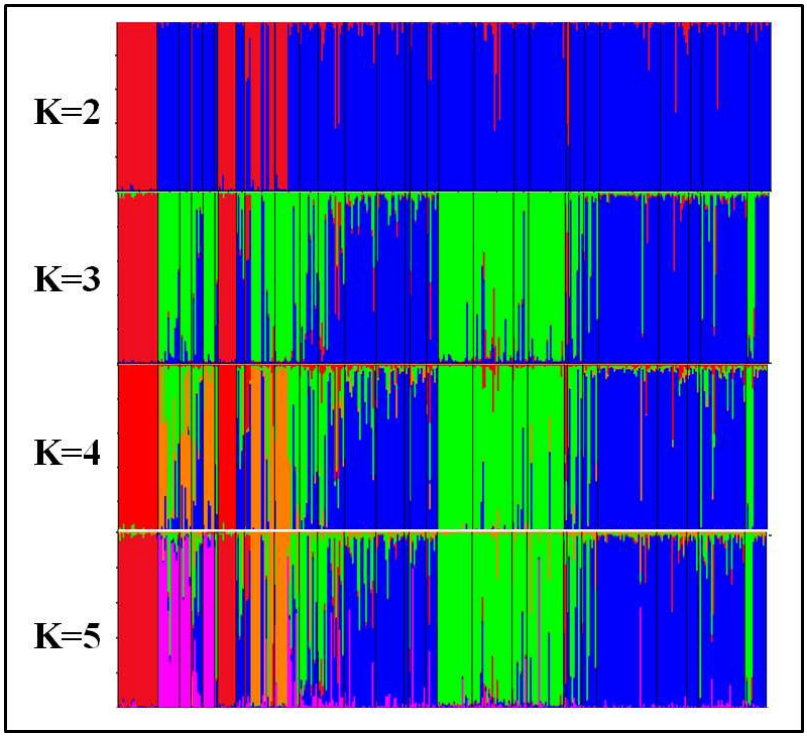

Figure 7. Figure obtained by the analysis of STRUCTURE with K ranging from 2 to 5, each group is represented by a color; each individual is represented by a vertical bar.

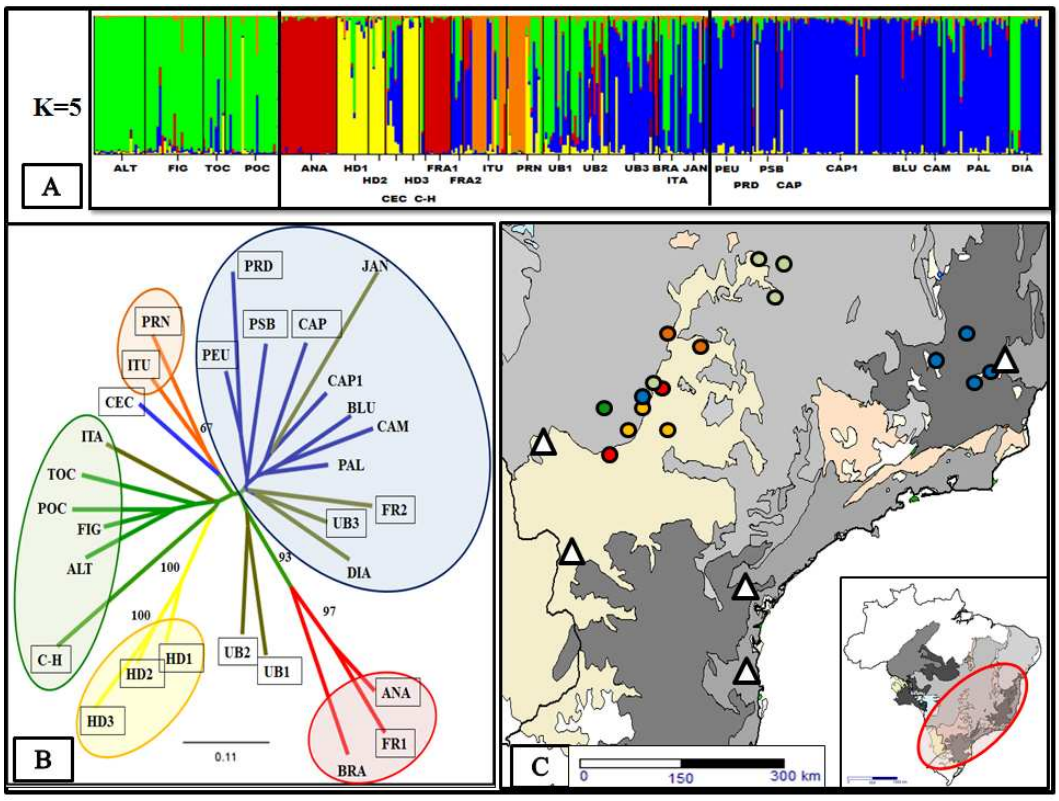

Figure 8. Graph of the correspondence between (A) Highlight the graph with K=5 (Cedrela sp. lineage 1, 2 and 3, and C. fissilis lineage.1 and 2), showing the distribution of individuals from Chiquitano (green), Atlantic (blue) and the Central regions, (B) the unrooted Neighbor-Joining tree and (C) Map of the distribution of populations, in colorful dots (17 populations used in this study) and in white triangles (12 populations from Mangaravite (2012) SSR dataset).

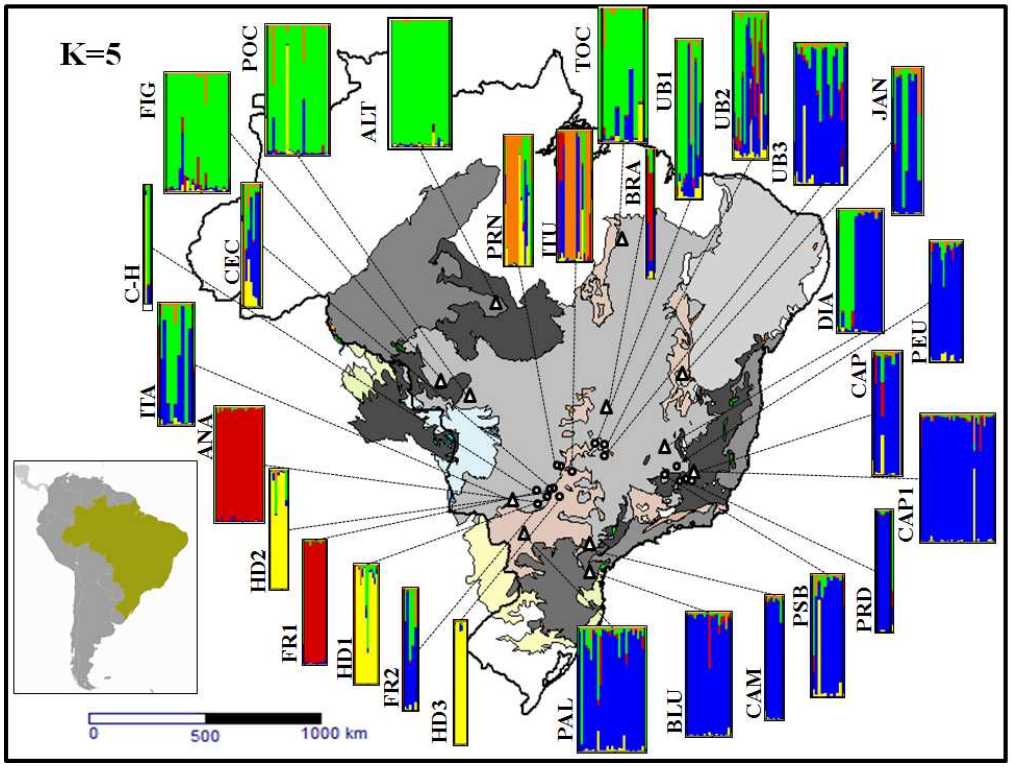

Figure 9. Distribution of 29 populations with their respective graph obtained by the analysis in STRUCTURE for K=5 (Cedrela sp. lineage 1 in red, Cedrela sp. lineage 2 in yellow, Cedrela sp. lineage 3 in orange, Cedrela fissilis lineage 1 in green, Chiquitano, Cedrela fissilis lineage 2 in blue, Atlantico), each group is represented by a color, each individual is represented by a vertical bar.

DISCUSSION

1. Genetic diversity among and within populations

Similar results found in our study was observed by Mangaravite (2012) (H_E=0,81) using SSR markers for the Cedrela fissilis complex and where her study bears out the hypothesis of Chiquitano and Atlantic ranges. Values of high heterozygosis using microsatellite was found by Sánchez (2008), in Cedrela odorata in central America (H_E=0,85), and by García et al. (2004), em Entandrophragma cylindricum (H_E=0,85). In others species of Meliaceae family, using microsatellite markers: Swietenia humilis (H_E=0,528, WHITE; POWELL, 1997; H_E=0,548, WHITE et al., 1999); Carapa guianensis (H_E=0,644, DAYANANDAN et al., 1999; H_E=0,69, VINSON et al., 2005; H_E=0,71, CLOUTIER et al., 2007); Swietenia macrophylla (H_E=0,781, LEMES et al., 2003; H_E=0,657, NOVICK et al., 2003; H_E=0,518, CÉSPEDES et al., 2003; H_E=0,799, LEMES et al., 2007; H_E=0,639 e H_E=0,176, LEMES et al., 2010); and Khaya senegalensis (H_E=0,636, SEXTON et al., 2010; H_E=0,739, KARAN et al., 2012). According to Hamrick et al. (1979), there is a relationship between high levels of genetic variability and species of wide distribution with long time of generation which could be attributed to Cedrela fissilis,

Despite the high genetic diversity observed in these 17 populations of Cedrela fissilis collected along to the Paraná River basin in Brazil, we observed positives levels of inbreeding and positives values of F_{IS} (mean F_{IS} = 0.18), indicating an excess of homozygotes, these values are higher than observed by Mangaravite (2012) (mean F_{IS} = 0,11). In addition, was observed the highest values of F_{IS} in this work (F_{IS} = 0,43 and F_{IS} =0,31) corresponding for the populations ITU, PAR respectively. According Frankham et al. (2002), deficiency of heterozygotes indicates that populations are not randomly crossing, and this can be expected for these data due to the large distances between populations.

The genetic structure among populations was slightly higher in populations of Cedrela sp. (F_{ST}=0,087; G_{ST}=0,046) than in populations of Cedrela fissilis (F_{ST}=0,071; G_{ST}=0,065), this result also was observed in the AMOVA analyses (Table 5). This scenario suggests closer proximity in populations of Cedrela fissilis group than populations of Cedrela sp. group. In general, the genetic structure among populations was higher (F_{ST}=0,111; G_{ST}=0,094) than the results observed for the C. fissilis complex

(Chiquitano and Atlantic range) study by Mangaravite (2012) (F_{ST}=0,075; G_{ST}=0,085). Our results showed interpopulation differences, these results were similar and slightly higher than those observed for Cedrela odorata from Central America (F_{ST}=0,08, SÁNCHEZ, 2008); Swietenia macrophylla (F_{ST}=0,097, LEMES et al., 2003; F_{ST}=0,176, LEMES et al., 2010; F_{ST}=0,117, NOVICK et al., 2003; F_{ST}=0,063, CÉSPEDES et al., 2003); Swietenia humilis (F_{ST}=0,036, WHITE; POWELL, 1997; F_{ST}=0,032, WHITE et al., 1999); Carapa guianensis (R_{ST}=0,017, DAYANANDAN et al., 1999). In constrats with our results species of Meliaceae showed higher levels of proximity. This moderate to slightly high differentiation observed in our results with the populations spread in a small geographical scale covered in the central range (HAMRICK et al., 1979) is in contrast with observed by Mangaravite (2012) that the populations were collected in large geographical scale covered; moreover, it could be observed highest levels of interpopulational diversity due to the presence of two distinct genetically units (Cedrela fissilis and Cedrela sp.), with the presence of low levels of historic gene flow among populations (FUCHS; HAMRICK, 2010), observed in the low migrations in the central range among populations (Upper half . Table 6).

2. Genetic diversity and structure among and within Bayesian groups

The obtaining of a K=5, unlike the Mangaravite (2012) presviously work, K=2 for the Cedrela fissilis complex, could be to explain by the presence of material more diverse but structured, besides the existence of materials from different genetically units, observed in the individuals in these groups remained as K values were increased; despite having used of a robust marker with higher mutational rate than rate of base substitution (SCHLÖTTERER, 2000). Other factors may have influenced the high K, such as hybridization, which has importance in variability (ROBERTSON et al., 2010). For our work done in the central range and some areas in the Atlantic range, apart from the heterogeneity of the age of the collected trees, this would add to the moderate to great morphological and phenological diversity of individuals in populations were grouped by geographic extent (50 km.), generated the value of K observed, with groups well-supported, this add a structured diversity (WHITE et al., 2007).

In this study was observed that in the central range the populations UB1, UB2, UB3, BRA, ITA and JAN showed genetic information from the five groups formed in the structure analyses, being constituted largely by genetic information from the Atlantic

and Chiquitano ranges, as described by Garcia et al. (2011) and bear out for C. fissilis complex studied by Mangaravite (2012). This could be explained by addition of that is spread out in areas of gathering and migration between the two main lineages (Chiquitano and Atlantic ranges). These have been a remnant given by the migration attempt between the two regions during drier periods (PRADO; GIBBS, 1993). The formation of last groups could be explained by the presence of different groups in addition to the two main lineages (Chiquitano and Atlantic ranges). As suggested by Mangaravite (2012) our work with major sampling along to the Paraná River basin served to confirm facts.

The analyses by groups of taxa showed differences between them. Populations of C. fissilis exhibited higher structure and more heterogeneous than populations of Cedrela sp., populations of C. fissilis showed higher A_{PRIV}., H_E, H_O, e lower F_{IS} as for descriptive analyses as AMOVA and fixation indexes (G_{ST}, F_{ST}, R_{ST} & F_{IT}). In contrast with the results of Mangaravite (2012) the values of A_{PRIV}. were higher only for the populations that correspondence to Central range, and in total values, 54, higher than Mangaravite (2012) results, moreover the area where correspondence populations of UB1, UB2 and UB3 (Uberlândia) exhibited 30 of A_{PRIV}., higher than the population of CAP (Caparao), 8 reported by Mangaravite (2012) and also showed higher values of H_E, H_O, and lower F_{IS}. This could be a new paradigm about refugia areas for this taxon, our work suggests to increase the analyses about relax molecular clock to aim to date evolutionary process about recent colonization.

3. Refugia and conservation implications

High levels of genetic diversity ($H_{E,}$ $A_{PRIV.}$) in the populations of Cedrela fissilis group in the central range and mainly in populations upper Parana River Basin (UB1, UB2 and UB3) which according to the structure analyses being shared genetic information from two lineages (Chiquitano and Atlactic ranges) suggests presences of refugia as observed through analyses of climatic stability in the Brazilian Cerrado (WERNECK et.al., 2012). Moreover beside the presence of refugia in the Atlantic range, where some lineages colonized others regions in the central range, observed in the structure results, the presence of historical forest refugia in the Atlantic range suggests spatial variation, predicting patterns of biodiversity in several taxa like C. fissilis (CARNAVAL; MORITZ, 2008; CARNAVAL et al., 2009), our study also bear

out the presence of genetic material from the Chiquitano range in the same populations which will be sharing genetic material from Atlantic range, this event suggest that this area is considered a refugia (GARCIA et. al., 2011; MANGARAVITE, 2012).

The presence of genetic material from Chiquitano and Atlatic ranges could explain the high diversity and private alleles in populations of UB1, UB2, and UB3 found in our study, could also explain the connectivity and the presence of historical forest refugia in the Atlantic region in the Pleistocene. In addition the high genetic diversity with presence of great number of private alleles of the populations of the Atlantic range for this in previous studies suggests this region could be a re-colonization area, received migrations from contiguous refugia (TZEDAKIS et al., 2002). In contrast with philogeographical studies where stable refugia and recently colonized areas expected to have different genetic signatures (CARNAVAL; MORITZ, 2008). Thus, as occur in the populations of Atlantic range, which are occurred altitude areas where constituted refugia areas because of the genetic flux maintains around the hills, this condition being a true genetic isolation (SHI et al., 2011); show higher levels of diversity and a single genetic material, as informed by Cavers et al. (2003) for populations of C. odorata in Costa Rica.

With the loss of habitats and overexploitation of species, is vital to generate the necessary resources to develop programs to protect habitats and ecosystems in areas where genetic diversity studies constitute a 'hotspots' to preserve species for the future. In this case habitat loss added to the high economic value of the timber, C. fissilis is listed as being 'Endangered A1acd+2cd' in the IUCN Red List of species (International Union for Conservation of Nature) (IUCN, 2013). In general as observed by Frankham (2002) threatened species and populations have lower genetic diversity compared with non-threatened species with large population size. Not always applying for all species, in our taxa due to the huge economic importance it is 'endangered'.

Genetic diversity showed high conservation values, because of diversity is important for survival of the species, especially in adverse factors. So in our study we observed the presence of new areas can be highlighted for conservation due to the presence of unique genetic material, as the UB1, UB2 and UB3 populations for the two main Chiquitano and Atlantic lineages (GARCÍA et.al., 2010; MANGARAVITE, 2012) and for new lineages that appear in our analyses (ANA, FR1, ITU and PRN). Other populations of the Atlantic range, especially the CAP and PSB populations, also had relevance for conservation due to the characteristics of refuge, in these geographical

areas today exists efforts to preserve the ecosystems and habitats, further contributing with our results.

The climatic changes occurred in the Quaternary were the force to form standard spatial models of Meliaceae, especially in Pleistocene and Pliocene (MUELLNER et al., 2006). It is proven that the current climate change has a negative effect on species C. odorata from Costa Rica (ESMAIL; OELBERMANN, 2011) and C. montana from Bolívia (PACHECO et al., 2010). According Muellner et al. (2010), diversity in the genus Cedrela was apparently given during the Oligocene and early Miocene, with more intensified in the late Miocene and early Pliocene. However, these plants experienced a strong selection pressure for cold tolerance after surviving the last ice age millions of years ago, with the implication that the heat-tolerant genes, as well as many species, may have been eliminated (COLWELL; RANGEL, 2010). Apparently, expected for the next century temperatures are even higher than estimated for the periods with higher temperatures the Paleocene-Eocene (CUI et al., 2011).

CONCLUSIONS

- ✓ Populations of Cedrela exhibited high intrapopulational genetic diversity and low inter populational distance.

- ✓ The populational structure exhibited five groups in the best ΔK (K=5); three groups of these five constitute new lineages never before sampled which will contain rare or unique genetic material when compared with those already existing. The taxon Cedrela sp. grouped in this study by populations of ANA and FR1 constitute one of those three groups, thus, our morphological, phenological and together with our molecular evidence by microsatellite markers, suggests, the presence of a new taxon, which will be botanically described. As well as non-admixture specimens of the last two lineages need to be botanically exanimated.

- ✓ The three new lineages found on the upper Parana River in Brazilian territory sharing genetic material information with the two main lineages (Chiquitano and Atlantic ranges). This information should be tested with the inclusion of major number of samples obtained from others areas that improved the data until now sampled.

- ✓ The Central range, where today are spread the upper Paraná River Basin according to our evidence suggest the presence of refugia in the past due to the presence of rare or unique genetic material and high genetic diversity found in populations of Cedrela established in this area, moreover this region constitute a place of migration between populations of Chiquitano and Atlantic ranges because of the relationship with populations in the Central range. This assumption will contrast with the Cerrado refugia hypothesis established around the Phytogeography distribution hypothesis for Tropical Seasonal Dry Forest in Brazil.

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