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Article

Timber tracking of *Jacaranda copaia* from the Amazon Forest using DNA fingerprinting

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Abstract: We investigated the utility of nuclear and cytoplasmic single nucleotide polymorphism (SNP) markers for timber tracking of the intensively logged and commercialized Amazonian tree *Jacaranda copaia*. Samples of 832 trees from 38 population samples in Bolivia, Brazil, French Guiana, and Peru were genotyped at 113 nuclear, 11 chloroplastic and 4 mitochondrial SNP markers. Bayesian cluster analyzes were carried out to group individuals into homogeneous genetic groups for self-assignment tests of groups of individuals or of individuals to their population of origin. Cluster analysis based on all SNP markers detected seven main genetic groups. Genetic differentiation was high among populations (0.484) and among genetic groups (0.415), and populations showed a strong isolation-by-distance pattern, favoring the group or individual tracking to the correct site of origin. Self-assignment test of groups of individual for all loci was able to determine the population origin of all samples with 100% accuracy. Self-assignment test of individuals was able to assign the origin of 94.5–100% of individuals (accuracy: 91.7–100%). Our results show that the use of 128 SNP makers is suitable to correctly determine the origin sampling site of *J. copaia* timber and they are a useful tool for customs and local and international police.

Keywords: illegal logging; forensics; SNP markers; timber tracking; tropical trees; *Jacaranda copaia*

1. Introduction

Timber logging, whether legal or illegal, contributes to biodiversity loss. Illegal logging is also an economic problem for the legal market, as extremely low-priced wood competes with legal logging, where costs are higher [1,2]. International laws have been established in countries around the world to avoid the importation of illegal timber, as for example, the timber regulation of the United States (Lacey Act), European Union (The EU Timber Regulation No. 995/2010), and Australia (Illegal Logging Prohibition Act), which declare illegal to import timber and its derived products (furniture, musical instruments, etc) originated from illegal timber extraction [2]. These regulations require that importing companies declare the country and specific place of origin, names of the exact species of all plants contained in their wood products, and guarantee that these wood products have a legal origin and have been extracted in a sustainable way in accordance with the laws of the country of origin. In addition to the legislation targeting illegal timber trade, the Convention on International Trade in Endangered Species- CITES [3,4] allows international trade of species listed in Appendix II only when sourced from plantations.

The main problems in the illegal timber trade are in the documentation of the origin of the wood, having falsified certificates and documentation, and with missing or false information [5]. According to the Brazilian Federal Police, in 2021, 90% of the wood extracted from the Amazon Forest had an illegal origin [5,6]. Timber tracking methods are required to fight against illegal timber trade, but methods based only on documentation are sensitive to manipulation and forgery. Therefore, timber export companies and institutions responsible for controlling the origin of imported timber, such as customs, federal police, and Interpol need reliable tools to prove and confirm the declared origin of wood and its derivative products, when traded internationally.

To be able to track the species, country, and specific site origin of imported timber, several methods exist, such as chemical analysis or mass spectrometry [7–9], stable isotopes [10], near-infrared spectroscopy [11], wood anatomy [12,13], and DNA fingerprint [14–21], all exploiting differences in the profiles among species and specific origins. These methods have been shown strong potential for species, country, and site origin determination, especially DNA fingerprinting. Some methods, such as wood anatomy, isotopic profiling, and spectrometric methods are limited in their capacity to identify the origin of timber due to variations among tissue types, individual sample age, individual and population genetic differences and/or

environmental influences on timber composition [20]. Thus, genetic methods that allow correct identification of tree species and tracking of timber origin are essential for controls on the legality of timber by public authorities, industry, and trading companies.

Here, we evaluate the use of a DNA fingerprinting method to track the intensively exploited and high value wood of the Neotropical pioneer tree *Jacaranda copaia* (Aubl.) D. Don. (Bignoniaceae). The genus *Jacaranda* sp. has ca. 53 species, all Neotropical, and most are found in the Cerrado around the Amazon Forest. *J. copaia* is the only species of *Jacaranda* widely distributed in the Amazon Forest [22]. The species occurs from Central America to western South America, from Belize to Bolivia, including Brazil, French Guiana, and Peru [22]. In Brazil, the species is found in the states of Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, and Roraima, and populations usually have more than one tree individuals per hectare [22]. *J. copaia* is a fast-growing species, with an average annual increase of 2.05 cm in diameter at breast height (DBH) and 1.98 m in height. Adult trees have straight stems, reaching 106 cm in DBH and 45 m in height [22,23]. Its wood is light and used for furniture [23,24]. The species is hermaphroditic and self-incompatible. About 40 species of bees, wasps, butterflies, and hummingbirds were detected as potential pollinators, although *Euglossa* spp. and *Centris* spp. bees was identified as the main pollinators in the Tapajós National Forest, Brazil [25]. **Reproduction occurs mainly by outcrossing (> 94%)** [26,27]. Each fruit can have up to 250 seeds and the winged seeds are dispersed by the wind [25]. Mating between related trees has been reported and attributed to the fact that populations have intrapopulation spatial genetic structure, ranging from 50 to 500 m, due to the short-distance seed dispersal (up to 100 m), but also due to short pollen dispersal distance, ranging from 34–90 m [27].

Here, samples of *J. copaia* collected in 43 sites in Bolivia, Brazil, French Guiana, and Peru were used to characterize genetic diversity and population structure, and to test the ability of chloroplast, mitochondrial, and nuclear single nucleotide polymorphism (SNPs) markers to determine the **geographic origin of wood** of the species harvested from the Amazon Forest.

2. Materials and methods

2.1. Sampling

There is considerable confusion in the literature regarding the naming of samples and populations, therefore, we specify here our naming convention: “individual sample” corresponds to the biological material sampled from a single individual tree; “sampling sites” are locations where individuals are collected; “population samples” are the collection of individuals sampled at a sampling site; “reporting groups” are the biological populations or genetic groups (i.e. groups of interbreeding individuals with a common ancestry) as estimated from population genetic analyses (i.e. STRUCTURE) and used to assign indi-

viduals; “country” are all population samples collected within the political borders of a country. Countries may include several reporting groups, and reporting groups may range across different countries.

Cambium or leaf samples were collected from 832 individual trees from 43 sampling sites (2–31 individuals) in the Amazon rainforest in four countries: Bolivia, Brazil, French Guiana, and Peru (Table 1, Figure 1). The minimum distance between individual trees was 50 m and the distance between sampling sites ranged from 23–2,648 km. All sampled trees were georeferenced using GPS and the plant material collected was stored in a plastic bag labeled with silica gel. In Bolivia, population samples were collected from 5 sampling sites on farms and forestry concessions. In Brazil, population samples were collected from 12 sampling sites in national forests, parks, extractive reserves, and ecological stations, with support from the Chico Mendes Institute of Biodiversity; In French Guiana, population samples were collected by the Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) together with the forest authorities (Office National des Forêts, ONF) in 4 sampling sites. Six additional individuals were collected in three sites; In Peru, population samples were collected from 19 sampling sites in national forests, parks, extractive reserves, ecological stations, farms, and forestry concession. However, due to the low sample size at 5 sampling sites (2 trees), for genetic analysis, these individuals were grouped with the closest sampling sites. We also divided the Brazilian samples into West (six population samples) and East (six population samples) origins and Peru into North (nine population samples) and South (eight population samples) origins. All individual samples were registered in a database at the Thünen Institute (SampleDataBase, Grosshansdorf, Germany).



Figure 1. Spatial distribution of sampling locations for *Jacaranda copaia* in South America.

Table 1. Information on the population sample size (*n*); sampling site latitude and longitude; population sample abbreviation (Abbrev); and sample size after combining population samples with only two individuals with the closest population sample (*n*₁).

Country	Sampling Site / population sample	<i>n</i>	Latitude	Longitude	Abbrev	<i>n</i> ₁
1-F. Guiana	Counami	30	5,41543	-53,175	1FG-Co	32
2-F. Guiana	Sinamary	2	5,2884	-52,916		
3-F. Guiana	Piste de Paul Isnard	27	5,33216	-53,957	2FG-Is	29
4-F. Guiana	Acapou	2	5,27343	-54,218		
5-F. Guiana	Route de Cacao	30	4,56779	-52,406	3FG-Ro	32
6-F. Guiana	Regina	2	4,13118	-52,088		
7-F. Guiana	Saut Maripa	28	3,87833	-51,857	4FG-Ma	28
8-Brazil	ESEC de Maraca-RR	31	3,37032	-61,444	5BW-Ma	31
9-Brazil	Flona de Anauá e arredores-Rorainópolis-RR	28	-0,9339	-60,451	6BW-An	28
10-Brazil	AMATA Flona do Jamari-RO	8	-9,4014	-62,911	7BW-Ja	8

11-Brazil	ESEC do Jarí	15	-0,4955	-52,829	8BW-Jr	15
12-Brazil	Resex Chico Mendes-Xapuri-AC (AMATA-Flona do Jamari)	16	-10,504	-68,595	9BW-Xa	16
13-Brazil	Resex Chico Mendes-Comunidade Cumaru-Assis-AC	15	-10,772	-69,647	10BW-Co	15
14-Brazil	FLONA Amapá-AP	20	0,52785	-51,128	11BE-Am	20
15-Brazil	PARNA da Ana Avilhanas-AM	11	-2,5345	-60,837	12BE-Av	11
16-Brazil	Flona de Tapajós-PA	27	-2,8687	-54,92	13BE-Ta	27
17-Brazil	Resex Tapajós-Arapins-PA	11	-3,0792	-55,278	14BE-Ar	11
18-Brazil	FLONA Tefé-AM	4	-3,5248	-64,972	15BE-Te	4
19-Brazil	FLONA do Carajás	23	-6,0628	-50,059	16BE-Ca	23
20-Peru	Dpto Loreto, Maynas, El Napo, Huiririma Native Community	26	-2,4761	-73,744	17PN-Hu	26
21-Peru	Huaman Urco	27	-3,3128	-73,198	18PN-Ur	27
22-Peru	Dpto Loreto, Maynas, Las Amazonas, Est. Biológica Madre-selva	28	-3,6312	-72,233	19PN-Ma	28
23-Peru	Dpto Loreto, Mayna, Iquitos, Comunidad Campesina Yarina	28	-3,827	-73,567	20PN-Ya	28
24-Peru	Allpahuayo	2	-3,9544	-73,422		
25-Peru	Dpto Loreto, Mar. Ramón Castilla, C. Poblado Unión Progre-sista	27	-3,9727	-70,841	21PN-Pr	29
26-Peru	Dpto Loreto, Requena, Jenaro Herrera Research Centre	11	-4,8966	-73,646	22PN-Re	11
27-Peru	Jenaro Herrera	25	-4,9158	-73,649	23PN-He	25
28-Peru	Dpto Loreto, Alto Amazonas, Jeberos, Centro Poblado Jeberos	26	-5,2598	-76,317	24PN-Je	26
29-Peru	Shucushuyacu	27	-6,0199	-75,854	25PN-Sh	27
30-Peru	Dpto Ucayali, Cor. Portillo, Con. Forestal-Oxigeno para el Mundo	29	-8,8869	-74,034	26PS-Po	29
31-Peru	Dpto Ucayali, Padre Abad, Macuya Forestry Research Station	30	-8,8766	-75,014	27PS-Pa	30
32-Peru	Dpto Ucayali, Atalaya, Tahuania, Concesión Forestal-Javier Díaz	29	-9,9803	-73,817	28PS-Di	29
33-Peru	Dpto Ucayali, Atalaya, Raymondi, Comunidad San Juan de Inuya	12	-10,582	-73,071	29PS-In	12
34-Peru	Dpto Madre de Dios, Tahuamanu, Concesión Forestal Madera-cre	31	-11,145	-69,758	30PS-Md	33
35-Peru	Ibérica	2	-11,299	-69,524		
36-Peru	Dpto Madre de Dios, P.N. Manu, Est. Biológica Cocha Cashu	15	-11,903	-71,403	31PS-Ca	15
37-Peru	Dpto Madre de Dios, Manu, Estación Biológica Los Amigos	30	-12,565	-70,088	32PS-Am	30
38-Peru	Dpto Madre de Dios, R. Nac. Tambopata, La Torre-Sandoval	24	-12,832	-69,284	33PS-Ta	24
39-Bolivia	Riberalta, MABET	15	-10,442	-65,55	34Bo-Ri	15
40-Bolivia	Riberalta, El Desvelo	11	-11,093	-65,746	35Bo-De	11
41-Bolivia	Cobija, Road - Bella Vista	13	-11,198	-68,287	36Bo-Vi	13
42-Bolivia	Riberalta, El Chorro	5	-11,514	-66,327	37Bo-Ch	5
43-Bolivia	Rurrenabaque, Área Protegida Madidi	29	-14,162	-67,905	38Bo-Ma	29

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2.2. DNA extraction and SNPs analysis

Cambium and leaf samples collected in Brazil were sent the Laboratory of Population Genetics and Forestry of São Paulo State University in Ilha Solteira, Brazil (UNESP-FEIS) for DNA isolation. Samples collected in French Guiana, Peru, and Bolivia were sent to Thünen Institute facilities in Großhansdorf, Germany, for DNA isolation. DNA

isolation from leaf and cambium was carried out according to Dumolin et al. [28]. The individual samples were screened for 128 SNP and insertion-deletion INDEL markers (nCpMtSNPs) according to Sebbenn et al. [29], using the MassARRAY® iPLEX™ (Assay Design Suite v2.0 [Agena Bioscience™, San Diego, EUA]) genotyping, including 113 nuclear SNPs (nSNPs), 11 chloroplast SNPs (CpSNPs), and 4 mitochondrial SNPs (MtSNPs). The combined nuclear, chloroplast, and mitochondrial SNP markers will hereafter be referred to as nCpMtSNP markers.

2.3. Genetic diversity

The genetic diversity for nSNPs was determined for each population sample and country by the total number of alleles (K), percent of polymorphic loci ($P\%$), observed (H_o), and expected (H_e) heterozygosity. The mean fixation index (F) was estimated to quantify the inbreeding coefficient within each population sample or country and the statistical significance of the F values was determined permuting alleles among individuals. For CpMtSNPs, the genetic diversity was determined for each site by K and percent of polymorphic loci ($P\%$). We also estimated the percent of polymorphic loci ($P\%_{128}$), observed for all nCpMtSNP markers. These analyses were carried out using the GDANT 1b software [30].

2.4. Bayesian clustering analysis

The Bayesian clustering method implemented in the software STRUCTURE v.2.3.4 [31] was used for the 128 nCpMtSNP markers to check for the number of reporting groups (*i.e.* genetic populations). We set the length of burn-in and Markov chain Monte Carlo to 10,000 and tested K values from 2–10 for 30 times. We used the admixture model with correlated allele frequencies. The optimal number of reporting groups was estimated with the ΔK method [32]. For each optimal K , data from the 30 STRUCTURE runs was permuted with CLUMPP v.1.1.2 [33] to obtain the final Q values for each individual. The optimal number of reporting groups was determined with the ΔK method described by Evanno et al. [31] using the software CLUMPAK [33].

2.5. Genetic differentiation among populations, countries and clusters

The overall and pairwise genetic differentiation (F_{st}) was estimated among all population sample (locals), clusters determined by STRUCTURE analysis, among countries, and population sample within country, and for all nCpMtSNP, nSNP, and CpMtSNPs markers. These analyses were carried out using the GDANT 1b software [30]. The pairwise F_{st} and spatial distance among population samples with sample size higher than 16 individuals and among clusters determined by STRUCTURE analysis, was used to investigate the isolation by distance (IBD) gene dispersal, using the Spearman correlation coefficient

(ρ). We also assessed the IBD by the estimate the spatial genetic structure (SGS) based on calculation of the coancestry coefficient (θ_{ij}), described in Loiselle et al. [34], between mean pairs of individuals within 10 distance-classes (0–198, 199–441, 442–655, 656–860, 861–1068, 1069–1409, 1410–1838, 1839–2770, 2771–2478, and 2479–2646 km) determined using the same number of pairs per classes, and using the SPAGEDI 1.5 software [35]. The statistical significance of the average θ_{ij} of each distance class was derived by comparing the limits of the confidence interval at 95% probability for the average θ_{ij} for each distance class, estimated permuting (1000 times) genotypes between distances classes using the SPAGEDI software.

2.6. Genetic assignment

No wood DNA samples were used here. The purpose of our study is to determine if the population structure and genetic differentiation among *J. copaia* across the species range is enough to allow correct determination of the origin of an individual sample. Provided that DNA can be extracted from the target material, the same analytical procedures can be used for DNA collected from wood stored by timber companies, sawn wood, instruments musicals, furniture, etc. Similar procedures have been used in other studies for the same purpose [16–19]. The Bayesian method [36] implemented in GeneClass 2.0 [37] was used to assign population samples (group of individuals collected in a sampling site) and individuals to its reporting group and country. The genetic grouping determined by the STRUCTURE analysis was used to group individuals into reporting groups. Both population sample and individual sample assignment were carried out for all nCpMtSNP markers, and the most likely reporting group determined by the highest score by the Bayesian criteria was used as an indicator of the power of the markers to compute the proportion of correctly assigned population sample or individuals in self-assignments tests [38]. Here, the individual samples from the population samples (reference data) were self-classified to the reporting groups and countries, using the leave-one-out approach [39]. We also estimated the overall, $\geq 80\%$ and $\geq 95\%$ score rate of population samples and individual samples assigned to correct origin reporting group.

3. Results

3.1. Genetic diversity

The rate of missing data ranged among loci from 0.1 to 47.1%, with average of 4.4% (Table S1) and among population samples ranged from 0.8 to 32.3%, average of 4.6% (Table 2). Across population samples, the total number of alleles (K) for 113 nSNPs ranged from 122–226 alleles (mean of 183) and for 15 chloroplast and mitochondrial loci (CpMtSNPs) from 14–19 alleles, with a mean of 15.9 alleles (Table 2).

The percent of polymorphic loci ($P\%$) for 113 nSNPs was higher (ranged among populations from 23–88.5%) than for 15 CpMtSNPs (0–26.7%) and 128 nCpMtSNP markers (20.3–78.1%, mean of 55.9%). The observed heterozygosity (H_o) ranged from 0.023–0.343 (mean of 0.178), the expected heterozygosity (H_e) for nSNPs ranged among populations from 0.028–0.348 (mean of 0.204). The values of H_e for nSNPs were high ($\geq 50\%$ of the maximum value for biallelic loci, 0.5) for 21 populations, where the highest values were observed for Brazil and Bolivia populations. The mean intrapopulation fixation index (F) ranged from -0.086–0.295 and was significantly ($P < 0.05$) higher than zero in six of the 38 populations, suggesting inbreeding. At country level, for nSNPs, K and $P\%$ were highest in Brazil for nSNPs (226, 100%, respectively) and CpMtSNPs (30, 100%, respectively), and lowest in French Guiana (nSNPs= 200, 76.1%, respectively; CpMtSNPs= 18; 20%, respectively). Bolivia presented the highest H_o (0.319) and H_e (0.359) values and lowest F_{IS} (0.113), where French Guiana presented the lowest H_o (0.095) and H_e (0.192) values and the highest F_{IS} (0.506).

Table 2. Genetic diversity of *J. Copaia* at different population samples and countries for 113 nuclear (nSNPs), 15 chloroplast and mitochondrial (CpMtSNPs), and 128 nuclear, chloroplast and mitochondrial (nCpMtSNPs) markers. * denotes $P < 0.05$; n is the sample size; $M\%$ is the percentage of missing data; $P\%$ is the percentage of polymorphic loci; $P\%_{128}$ is the percentage of polymorphic loci for all 128 nCpMtSNPs; K is the total number of alleles; H_o and H_e are the observed and expected heterozygosity, respectively; F is the fixation index.

Sample	n	nSNPs						CpMtSNPs		nCpMtSNPs
		$M(\%)$	K	$P\%$	H_o	H_e	F	K	$P\%$	$P\%_{128}$
1FG-Co	32	6.6	122	50.4	0.023	0.028	0.005	16	6.7	45.3
2FG-Is	29	4.9	190	69	0.109	0.197	0.25*	17	13.3	62.5
3FG-Ro	32	5.7	196	71.7	0.094	0.202	0.295*	16	6.7	64.1
4FG-Ma	28	3.5	195	72.6	0.148	0.254	0.286*	16	13.3	65.7
5BW-Ma	31	2.4	193	82.3	0.299	0.309	0.005	16	6.7	73.4
6BW-An	28	1.5	195	70.8	0.291	0.292	-0.022	15	0	72.7
7BW-Ja	8	32.3	207	80.5	0.194	0.298	0.198*	17	13.3	72.6
8BW-Jr	15	4.3	207	83.2	0.205	0.287	0.057	16	13.3	75
9BW-Xa	16	4.5	204	72.6	0.278	0.29	-0.021	16	6.7	75
10BW-Cu	15	1.8	147	83.2	0.261	0.275	0.016	15	0	73.5
11BE-Am	20	1.1	204	75.2	0.215	0.231	0.052	15	0	66.4
12BE-Av	11	7.5	207	61.9	0.278	0.29	-0.021	15	0	64.8
13BE-Ta	27	2.8	197	83.2	0.261	0.275	0.016	15	0	74.2
14BE-Ar	11	3.1	183	69	0.238	0.276	0.078	15	0	68
15BE-Te	4	1.4	207	83.2	0.141	0.257	0.057	15	0	73.5
16BE-Ca	23	5.3	190	81.4	0.275	0.282	-0.019	15	0	75
17PN-Hu	26	2.4	149	30.1	0.053	0.059	0.017	17	13.3	28.1
18PN-Ur	27	5.0	147	31.9	0.06	0.066	0.018	17	13.3	29.7
19PN-Ma	28	4.4	147	30.1	0.054	0.066	0.031	19	20	28.9
20PN-Ya	28	2.5	147	30.1	0.059	0.064	0.024	16	6.7	27.4
21PN-Pr	29	4.7	142	30.1	0.065	0.069	0.016	19	26.7	29.7

22PN-Re	11	1.8	146	25.7	0.043	0.065	0.072	15	0	22.7
23PN-He	25	3.8	145	29.2	0.05	0.056	0.018	16	20	28.1
24PN-Je	26	7.9	142	28.3	0.041	0.061	0.087*	19	26.7	28.1
25PN-Sh	27	3.2	147	26.5	0.047	0.053	0.028	14	0	23.4
26PS-Po	29	13.9	146	30.1	0.046	0.075	0.087*	16	6.7	27.4
27PS-Pa	30	3.1	140	30.1	0.063	0.064	0.02	14	0	26.6
28PS-Di	29	4.9	139	23.9	0.054	0.061	0.024	15	0	21.1
29PS-In	12	4.8	213	23	0.056	0.061	0.017	15	0	20.3
30PS-Ma	33	4.5	206	88.5	0.297	0.315	0.045	15	0	78.1
31PS-Ca	15	2.4	213	82.3	0.271	0.31	0.073	15	0	72.7
32PS-Am	30	4.5	209	88.5	0.312	0.317	-0.01	15	0	78.1
33PS-Ta	24	3.2	209	85	0.284	0.314	0.08	15	0	75
34Bo-Mb	15	2.8	207	84.1	0.321	0.324	-0.026	16	6.7	75
35Bo-De	11	1.3	206	84.1	0.297	0.312	-0.012	16	6.7	75
36Bo-Vi	13	0.8	205	82.3	0.313	0.313	-0.023	15	0	72.7
37Bo-Ch	5	1.4	212	82.3	0.34	0.348	-0.086	19	26.7	75.8
38Bo-Ma	29	1.3	226	87.6	0.322	0.313	-0.032	15	0	77.3
Overall	832	4.4	183	100	0.178	0.204	0.086*	15.9	6.7	55.9
F. Guiana	121	5.2	200	76.1	0.095	0.192	0.506*	18	20	69.5
Brazil	209	4.2	226	100	0.261	0.354	0.264*	30	100	100
Peru	429	4.7	217	92.9	0.111	0.222	0.498*	23	53.3	88.3
Bolivia	73	1.5	218	92.9	0.319	0.359	0.113*	20	33.3	85.9

3.2. Bayesian cluster analysis

The results of the STRUCTURE analysis showed that based on delta K, the best representation of the data is obtained with a K of 4 or 9 different reporting groups (i.e. genetic populations) (Figure 2). With 5 groups, there is less mixture of gene pools within individuals. With K= 5, 85% of all individuals had a gene pool membership coefficient above 0.9, while for K= 4, only 76% of all individuals had gene pool membership coefficients above 0.9. The STRUCTURE analysis based on CpMtSNPs showed a clear distinction between French Guiana and North Brazil sampling sites (red) from North Peru, South Brazil (6BW-An; 7B-Ja), East Bolivia (34Bo-Ri, 35Bo-De) (green and white), and from South Peru (29P-In; 30P-Md, 31P-Ca, 32P-Am, 33P-Ta), Southwest Brazil (9BW-Xa, 10B-Co) and Bolivia (36Bo-Vi, 37Bo-Ch; 38Bo-Ma) (blue) (Figure 2A). The STRUCTURE analysis based on nSNP markers, revealed that there are a few population samples with mixtures of individuals from different gene pools (e.g. Flona do Jamari (7BW-Ja), Resex Tapajos-Arapins (14BE-Ar), and ESEC Maraca (5BW-Ma) in Brazil and Piste de Paul Isnard (2FG-Is), Route de Cacao (3FG-Ro) and Saut Maripa (4FG-Ma) in French Guiana in Figure 3B). We found one genetic group (K3) which we only found in two Brazilian sampling sites (8BW-Jr, 11BE-Am) and another genetic group (K1) distributed more in the middle of the Amazon basin (Figure 2B).

312

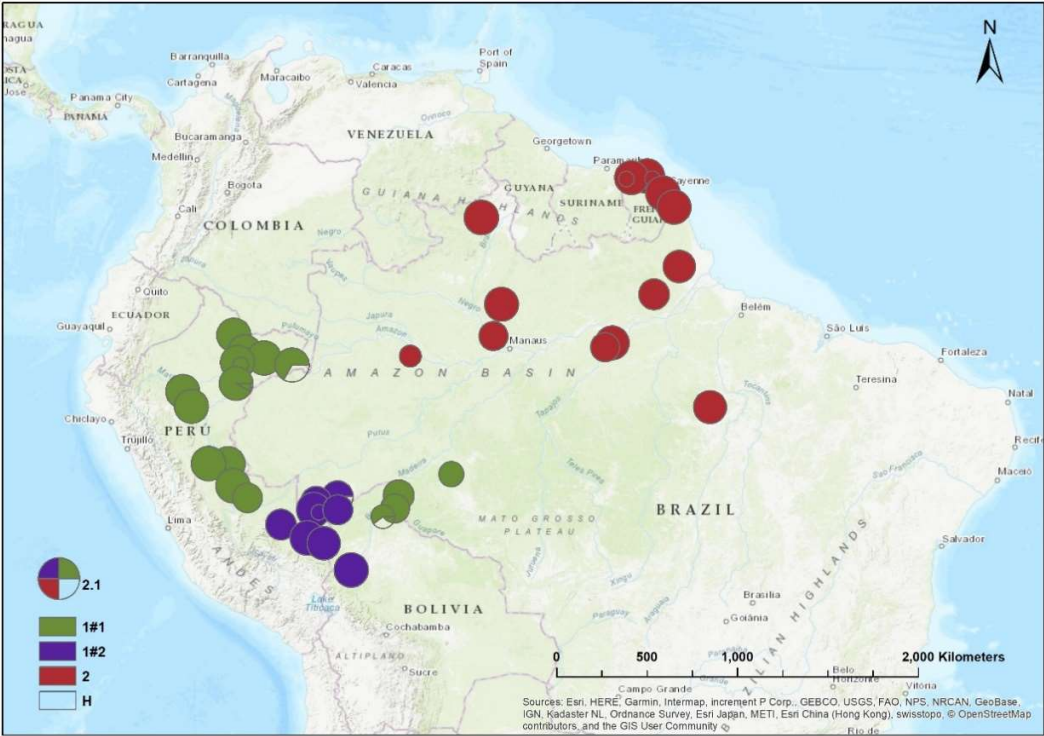


Figure 3A

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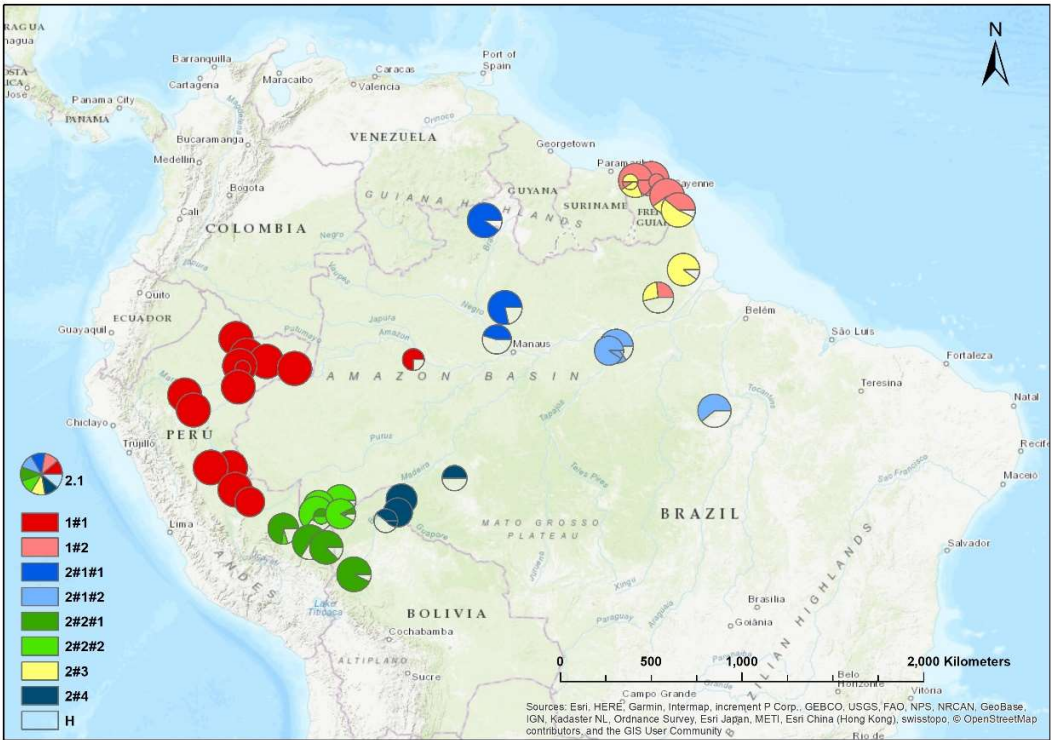


Figure 3B

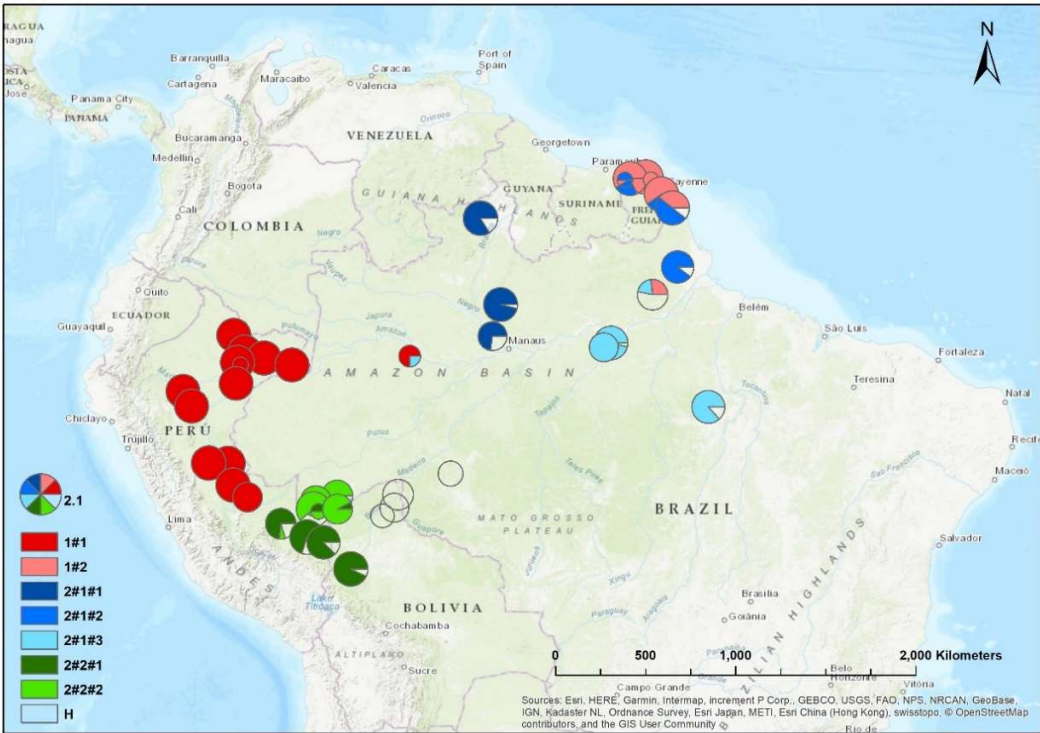


Figure 3C

Figure 2. Population structure of *Jacaranda copaia* in South America showing the Spatial distribution of reporting groups (i.e. genetic clusers) based on CpMtSNP (A, K=4), nSNP (B; K=9), and nCpMtSNP (C; K=8) markers estimated by STRUCTURE (K= 4 to 9).

3.3. Population differentiation

To determine differences between nuclear SNPs (nSNPs) and chloroplast and mitochondrial markers (CpMtSNPs) in estimating genetic differentiation (F_{st}) among population samples, we compared the results among all 128 nCpMtSNPs, 113 nSNPs, and 15 CpMtSNPs (Table 3). The results showed that the F_{st} among all population samples and countries was higher for CpMtSNPs (0.942 and 0.695, respectively) than for all nCpMtSNPs (0.484 and 0.295, respectively) and nSNPs (0.415 and 0.233, respectively). The F_{st} was also higher among clusters determined by STRUCTURE analysis for CpMtSNPs (0.896) than CpMtSNPs (0.401) and nSNPs (0.315). Among population samples within Brazil, Peru, and Bolivia, the F_{st} was also higher for CpMtSNPs (0.974, 0.741, and 0.735, respectively) than for all nCpMtSNPs (0.3, 0.466, and 0.142, respectively) and nSNPs (0.224, 0.456, and 0.107, respectively). In contrast, among population samples within French Guiana, the F_{st} was higher for nCpMtSNPs (0.12) and nSNPs (0.117) than CpMtSNPs (0.004). The results also showed that the estimates of F_{st} for nCpMtSNPs, nSNPs, and CpMtSNPs was generally highest within Peru and Brazil, where the number of sampling sites was highest (Peru= 17, Brazil= 12), indicating that the number of sampling sites is important to detect high population differentiation within countries. The highest F_{st} for CpMtSNPs and nCpMtSNPs than for nSNPs indicated that the combination of 113 nuclear and 15 cytoplasmatic SNP markers increases the capacity to detect genetic differences among populations.

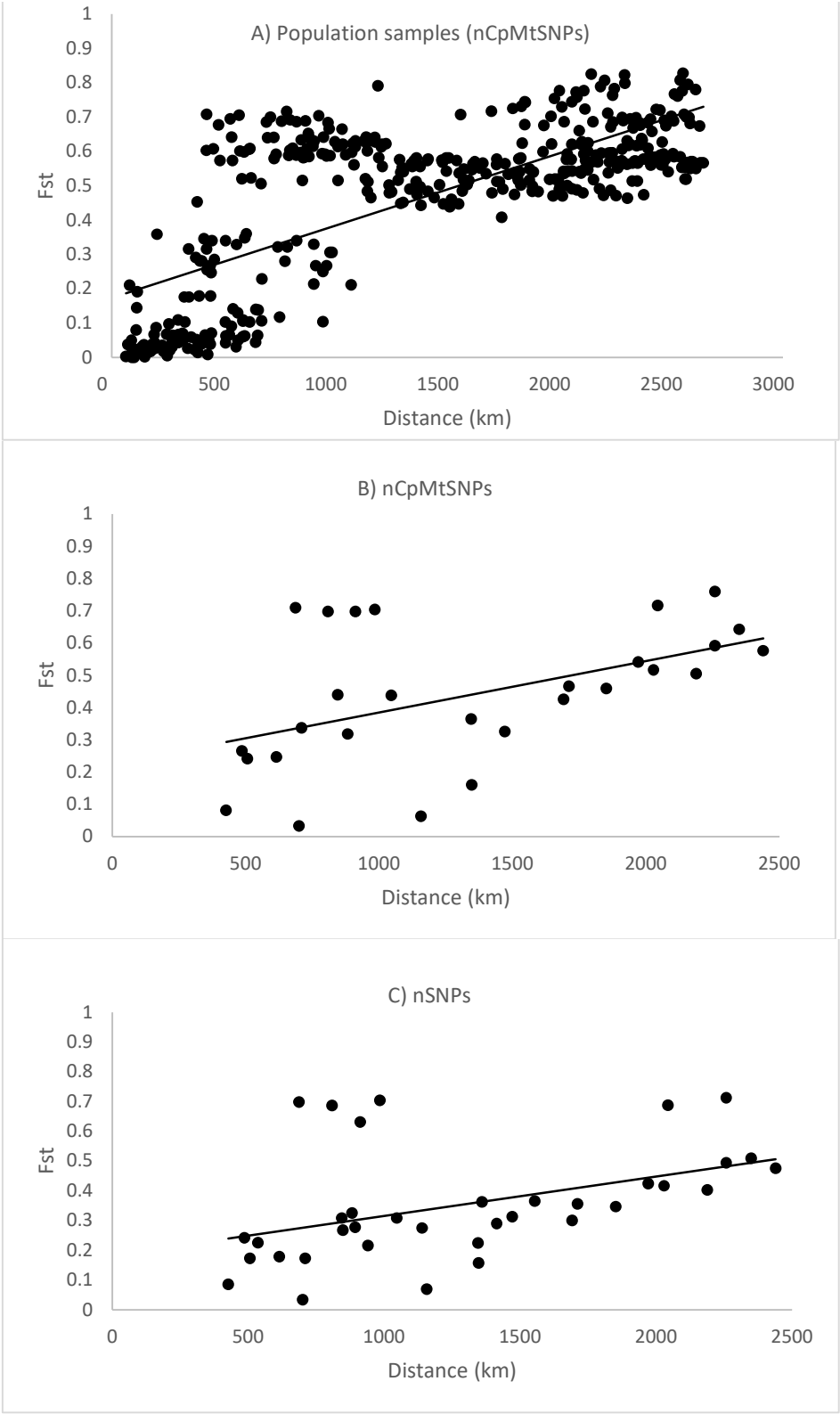
Table 3. Genetic differentiation (F_{st}) among all population samples, all countries, all population samples within countries for all nCpMtSNPs (128), nSNPs (113), and CpMtSNPs (15).

Sample	np	nCpMtSNPs (128)	nSNPs (113)	CpMtSNPs (15)
All populations	38	$0.484 \pm 0.043^*$	$0.415 \pm 0.032^*$	$0.942 \pm 0.042^*$
<u>Genetic groups</u>	8; 9; 4€	$0.401 \pm 0.044^*$	$0.315 \pm 0.03^*$	$0.896 \pm 0.094^*$
Countries	4	$0.295 \pm 0.036^*$	$0.233 \pm 0.022^*$	$0.695 \pm 0.144^*$
French Guiana	4	$0.120 \pm 0.017^*$	$0.117 \pm 0.017^*$	0.011 ± 0.002
Brazil	12	$0.299 \pm 0.049^*$	$0.224 \pm 0.03^*$	$0.925 \pm 0.103^*$
Peru	17	$0.466 \pm 0.056^*$	$0.456 \pm 0.056^*$	$0.741 \pm 0.267^*$
Bolivia	5	$0.142 \pm 0.034^*$	$0.107 \pm 0.024^*$	$0.735 \pm 0.383^*$

* $P < 0.05$; np is number of populations; $\pm$ is the 95% standard error, 1.96SE; € is the number the genetic groups determined for nCpMtSNPs, nSNPs, and CpMtSNPs, using Bayesian cluster analyzes.

3.4. Isolation by distance

The Spearman correlation coefficient (ρ) between pairwise F_{st} and spatial distance between population samples ($n > 16$ individuals) based on 128 nCpMtSNP **markers** was significantly ($P < 0.001$) higher than zero ($\rho = 0.626$), indicating an isolation by distance (IBD) genetic pattern (Figure 3A). The Spearman correlation coefficient (ρ) between pairwise F_{st} and spatial distance between clustered locals, as determined by STRUCTURE analysis was also significantly ($P < 0.001$) greater than zero for nCpMtSNPs ($\rho = 0.521$, **Figure 3B**) and nSNPs ($\rho = 0.552$, **Figures 3C**), reinforcing the occurrence of an isolation by distance genetic pattern. The estimate of pairwise coancestry for nSNP and nCpMtSNP **markers** between individuals within ten distance classes was significantly higher than zero up to the distance of 655 km, where for CpMtSNP **markers** was significant up to 1068 km, and non-significant or significantly lower than zero in the other distance classes (Figure S1).



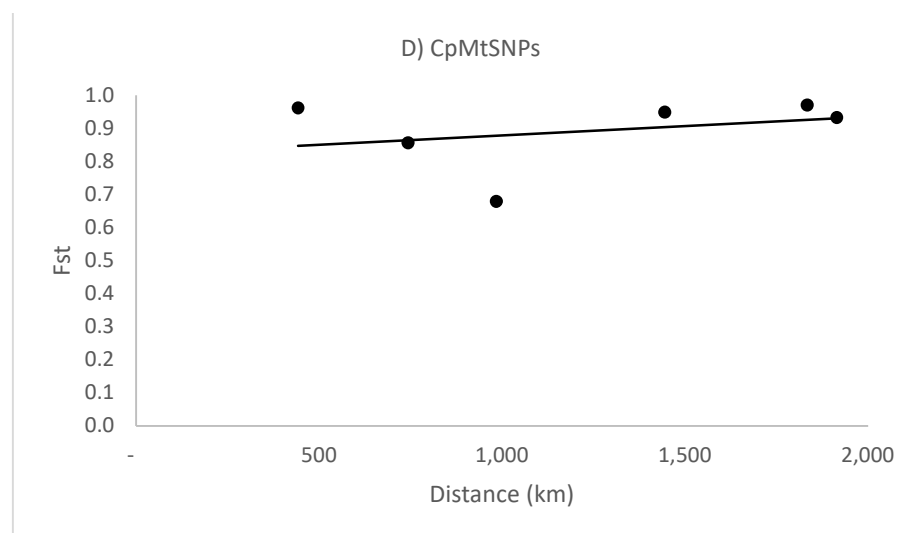


Figure 3. Pattern of isolation by distance in *Jacaranda copaia* population samples (A) and genetic populations determined by cluster analysis using STRUCTURE analysis (B, C, D). F_{ST} is the pairwise genetic differentiation between population samples (sample size higher than 16 individuals) for all 128 nCpMtSNP markers (A) and the pairwise genetic differentiations between clustered locals as determined by STRUCTURE analysis for all 128 nCpMtSNP (B), 113 nSNP (C), and 15 CpMtSNP (D), markers. The Spearman correlation coefficient (ρ) was significantly higher than zero ($P < 0.01$) between pairwise F_{ST} and spatial distance among populations (A) for all 128 nCpMtSNP markers (0.645), as well as between F_{ST} and spatial distance between clustered locals as determined by STRUCTURE analysis (B, C, and D) for nCpMtSNPs (0.521) and nSNPs (0.552), where non-significantly correlation was observed for CpMtSNPs (0.142).

3.5. Genetic assignment

We performed self-assignment tests based on seven main genetic clusters determined in STRUCTURE analysis (Figure 2C) using all 128 nCpMtSNP markers. Due to the genetic similarity and small sample size of the 15BE Te site (Figure 2A, B), it was grouped with the 12 population samples from Northern Peru for statistical analyses. Self-assignment tests were carried out to assign or exclude the seven clusters as the origin of individuals or groups of individuals (locations grouped by cluster) from our sample. The reporting groups assignment test was able to self-assign 100% of population samples (score of 100%) to the correct origin for seven reporting groups and four countries (Table 4). Self-assignment test of individuals was able to self-assign 94.5–100% (mean of 98.4%) of individuals to the correct origin reporting group (score ranging from 91.7–100%, mean of 97.3%). The rate of correct individual assignment for scores $\geq 80\%$ and $\geq 95\%$ were high (93.5 and 89.9%, respectively). The individual sample assignment test was able to self-assign from 87.6% of individual from French Guiana to 100% of individuals from Bolivia to the correct origin country (score ranging from 93.4–99.9%). The rate of correct individual assignment to the correct country source with scores $\geq 80\%$ and $\geq 95\%$ ranged from 87.8–100% and 72.8–100%, respectively.

Table 4. Corrected population sample and individual self-assignment rate and scores for reporting groups and countries (mean score) for all 128 nCpMtSNP markers. Rate means the percent of individuals assigned to the

correct source reporting group or country; Rates > 80 and > 95% are the rates of individuals assigned to the correct source reporting group or country, with a score values $\geq$ than 80 and 95%, respectively.

Reporting groups	Rate	Score	Rate	Score	Rate: Score> 80%	Rate: Score>95%
1	100	100	100	99.9	100	99.4
2	100	100	100	100	100	100
3	100	100	98.6	99.8	100	98.6
4	100	100	96.0	93.2	89.7	69.2
5	100	100	100	100	100	100
6	100	100	94.5	91.7	82.6	67.4
7	100	100	100	98.2	96.5	94.7
Overall	100	100	98.4	97.5	95.5	89.9
Country						
French Guiana	100	100	87.6	98.1	99.1	99.1
Brazil	100	100	98.1	99.9	100	98.5
Peru	100	100	98.1	93.4	87.8	72.8
Bolivia	100	100	100	99.9	100	100

Reporting groups:

1: 1FG-Co, 2FG-Is, 3FG-Ro, 4FG-Ma, 11BE-Am, 8BW-Jr;

2: 13BE-Ta, 14BEAr, 16BE-Ca;

3: 5BW-Ma, 12BE-Av, 6BE-An, 15BE-Te;

4: 17PN-Hu, 18PN-Ur, 19PN-Ma, 20PN-Ya, 21PN-Pr, 22PN-Re, 23PN-He, 24PN-Je, 25PN-Sh;

5: 7BW-Ja, 34Bo-Ri, 35Bo-De, 37Bo-Ch;

6: 26PS-Pa, 27PS-Pr, 28PS-Di, 29PS-I;

7: 9BW-Xa, 10BW-Co, 30PS-Md, 31PS-Ca, 32PS-Am, 33PS-Ta, 36Bo-Vi, 38Bo-Ma.

4. Discussion

4.1. Genetic diversity

The illegal timber trade, both species-specific and illegally sourced, has become a major problem for tropical forests. Control of the chain of custody for timber originating from the Amazonian Forest, as well as timber from other biomes and regions of the planet, is urgent to verify the species, countries, and place of origin declared in the transport and export documentation and restrict or even eradicate illegal logging. Our study shows the potential of DNA fingerprinting to track the country and origin of timber, as well as a method to follow and verify the chain of custody for *J. copaia* timber products. Our study shows only moderate levels of genetic diversity for nSNPs and low levels of genetic diversity for CpMtSNPs for *J. copaia*. However, the observed (H_o) and expected (H_e) heterozygosity were generally moderate to high, respectively, and are within the reported pattern identified for other Neotropical trees using nSNP markers. For example, in *Carapa* sp. H_o has been observed ranging among populations from 0.017–0.264 and H_e ranging from 0.02–0.251 [41], in *Dipteryx* sp. H_o has been observed ranging among populations from 0.03–0.39 and H_e ranging from 0.03–0.29 [19], and in *Handroanthus* sp. H_o has been observed

ranging among populations from 0.15–0.276 and H_e ranging from 0.071–0.371 [42]. The genetic diversity of *J. copaia* was especially low in some Peru population samples, due to the low percent of polymorphic loci ($P\%_{128}$) for all nCpMtSNP markers. The estimate of intrapopulation fixation index (F) indicated inbreeding in some populations. Inbreeding has also been observed for other Neotropical trees using SNP markers (Table S2). However, because *J. copaia* is self-incompatible [25], and our population samples were taken from geographically distant trees, the observed inbreeding is likely an artefact of the Wahlund effect [43] due to mixtures of samples from different subpopulations, as previously shown for *J. copaia* [44].

4.2. Population genetic differentiation

The presence of population genetic differentiation and intrapopulation spatial genetic structure (SGS), or the occurrence of isolation by distance patterns (IBD), is key for the assignment of timber to different origins [1,45], including country, population, and regions within countries. High genetic differentiation among the different reporting groups increases the success of genetic assignment [1,45]. Our results showed strong genetic differentiation among all reporting groups and between countries, as well as SGS and a pattern of IBD. The F_{st} for all nCpMtSNP, nSNPs, and CpMtSNPs was generally high, especially for CpMtSNPs. The F_{st} was higher among reporting groups than among countries, as well as the mean assignment test of individuals to correct origin reporting groups was higher than among correct country.

Within countries for all nCpMtSNP and CpMtSNP markers, F_{st} was lowest among French Guiana population samples. In agreement, the lowest self-assignment of individuals was found in French Guiana (87.6%). Brazil, Peru and Bolivia showed a high rate of self-assignment of individuals to the correct country of origin (> 98%). The results showed that the F_{st} among all population samples, reporting groups, and among countries was higher for CpMtSNPs than for nSNPs. This can be attributed to the fact that CpMtSNPs have lower variability due to uniparental inheritance, while nuclear SNPs have higher diversity due to biparental inheritance and recombination. Thus, CpMtSNPs can show low variation within or among closed reporting groups, where nSNPs can present high variation and share many alleles among reporting groups, as can be observed in Figure 2. Furthermore, the percentage of polymorphic loci was lower for CpMtSNPs than nSNPs, where the highest polymorphism was observed, and many population samples shared the same alleles, which reduces genetic differences between population samples. These results indicate the importance of

using both nSNP and CpMtSNP markers to detect greater population genetic structure and thus increase the potential for assigning wood origins to population samples, reporting groups, and countries.

4.3. Genetic assignment and practical application

The results indicate a high power to correctly assign reporting groups at both levels: among countries and reporting groups (100%). Our results confirm that this approach offers the possibility of high levels of success when grouping timber samples based on their origin sampling site. This success can be attributed to the general wide genetic differentiation among countries and reporting groups. Even when the differentiation was only moderate ($F_{st} < 0.2$) (French Guiana, Bolivia), differences in allele frequencies between analyzed groups were enough to produce high scores for the determination of the correct origin. However, it is important to emphasize that this is true so long as the reference data (collection of samples of individuals from sampling sites encompassing the species range) set contains genetic data of the timber's original population. Therefore, the success in determining the origin of *J. copaia* timber, as well as other species, greatly depends on the reference data set developed by the authorities involved in controlling the origin of timber (reference population samples). The reference data set should include genetic data from all sites where harvesting is legally practiced.

The results for assignment test of individuals for all nCpMtSNPs was lower than for assignment test of groups of individuals, but also showed a high power of correct assignment among countries and reporting groups. In general, the results for the assignment test of individuals indicate that this approach has the power to determine the reporting group of origin, and in some cases estimate the specific site of timber origin. When using CpMtSNP markers, it is necessary to develop a large number of loci to improve both population sample and individual assignment tests. Similar results of better self-assignment tests at the population sample level rather than at the individual was reported for *Hymenaea* sp. [18]. For *Swietenia macrophylla*, based on nuclear microsatellite loci (SSRs), the assignment test was higher at the country (82%) than population sample (71%) level [1]. For the Malaysian *Gonystylus bancanus* tree, the self-assignment rate using a set of 16 nSSRs was lower (55%) than that observed here at the population sample level [46]. For SNP markers of *Entandrophragma cylindricum*, the assignment at the country level ranged from 66–74%, depending on the assignment method [15]. Many other studies on tropical and temperate tree species have been developed using microsatellite and SNP markers to track timber showing that, thanks to spatial genetic structure and genetic differentiation, DNA fingerprinting is the most effective tool to track the country and population of origin of timber [45–51].

Finally, we recommend that the timber sector add such genetic controls as an independent audit beyond the paper-based proof of the chain of custody [1]. It is important to note that the power of the genetic reference data to detect false declarations reaches 100% if more than one sample with the same declaration is tested.

4.4. Conclusion

The genetic differentiation (F_{st}) among all population samples, reporting groups, and countries was generally high, especially based on CpMtSNP markers. Furthermore, there is a strong isolation by distance pattern among population samples, favoring the tracking of population samples or individual samples to the correct site. For self-assignment tests, we were able to obtain 100% accuracy to the country and reporting group for all samples using all 128 nCpMtSNPs and 113 nSNPs. Our results show that the use of all nCpMtSNP or nSNP markers are suitable to correctly determine the country and reporting group of origin for *J. copaia* timber, thus offering a very useful tool for customs and local and international police. The *J. copaia* reference database of our study represents a robust assignment tool available to timber companies or government agencies to test and validate origin declarations. We recommend the use of the method described herein for other native tropical species, since it is highly effective in identifying the origin of wood, thus helping the police and other relevant agencies in the definition of illegally deforested areas, as well as unsustainable extraction.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1. Spatial genetic structure (SGS) of *Jacaranda copaia* for all sample individuals in all populations for all 128 nCpMtSNP, 113 nSNP, and 15 CpMtSNP markers. Unfilled circles indicated mean θ_{ij} values significant different than zero ($P < 0.05$) of the hypothesis of absence of SGS ($H_0: \theta_{ij} = 0$); vertical bars show the standard error at 95% of probability; Table S1. Population sample size (n), missing data (%), genetic diversity, and differentiation among population samples (F_{st}) for each and mean nCpMtSNP markers: k= number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; Table S2. Population genetic diversity and population structure (F_{st}) for Neotropical tree species based on SNP markers.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to [prevent data from being used by private companies to make a profit](#)

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