ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392201902203

ORIGINAL ARTICLE

Genetic diversity and population structure of endangered rosewood from the Peruvian Amazon using ISSR markers

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ABSTRACT

Rosewood, *Aniba rosaeodora* is an endangered species in Amazon forests and its natural stands have been heavily depleted due to over-exploitation for the cosmetic industry. This study aimed to investigate the genetic diversity and population structure of 90 rosewood accessions from eight localities in the Peruvian Amazon through 11 Inter Simple Sequence Repeats (ISSR) primers. The ISSR primers produced a sum of 378 bands, of which 375 (99.2%) were polymorphic, with an average polymorphism information content (PIC) value of 0.774. The mean effective number of alleles (Ne), Shannon informative index (I), gene diversity (He) and total gene diversity (Ht) were 1.485, 0.294, 0.453 and 0.252, respectively. Analysis of molecular variance (AMOVA) showed the presence of maximum variability within populations (88%). The Structure algorithm, neighbor joining and principal coordinate analysis (PCoA) grouped the 90 rosewood accessions into three main populations (A, B and C). Diversity indices at the inter-population level revealed a greater genetic diversity in population A, due to higher gene flow. The neighbor-joining analysis grouped populations A and B, while population C was found to be divergent at the inter population level. We concluded that population A reflects higher genetic diversity and should be prioritized for future management and conservation plans.

KEYWORDS: *Aniba rosaeodora*, endangered species, gene flow, germplasm, molecular characterization

Diversidad genética y estructura poblacional de palo de rosa en peligro de extinción de la Amazonía Peruana utilizando marcadores ISSR

RESUMEN

Palo de rosa, *Aniba rosaeodora* es una especie en peligro de extinción en los bosques amazónicos. Sus rodales naturales se han agotado debido a la sobreexplotación para la industria cosmética. Este estudio tuvo como objetivo investigar la diversidad genética y estructura poblacional de 90 accesiones de palo de rosa de ocho localidades en la Amazonía Peruana utilizando 11 marcadores de Inter Secuencias Simples Repetidas (ISSR). Los marcadores ISSR produjeron una suma de 378 bandas, de las cuales 375 (99,2%) fueron polimórficas, con un valor promedio de contenido de información de polimorfismo (PIC) de 0,774. El promedio del número efectivo de alelos (Ne), índice informativo de Shannon (I), diversidad genética (He) y diversidad genética total (Ht) fueron 1,485; 0,294; 0,453 y 0,252; respectivamente. El análisis de varianza molecular (AMOVA) mostró la presencia de máxima variabilidad dentro de las poblaciones (88%). El algoritmo Structure, neighbor joining y análisis de coordenadas principales (PCoA) agruparon las 90 accesiones de palo de rosa en tres poblaciones principales (A, B y C). Los índices de diversidad a nivel interpoblacional revelaron una mayor diversidad genética en la población A, debido al mayor flujo de genes. El análisis de *neighbor joining* agrupó las poblaciones A y B, mientras la población C fué divergente a nivel interpoblacional. Concluimos que la población A refleja mayor diversidad genética y debería priorizarse para futuros planes de manejo y conservación.

PALABRAS-CLAVE: *Aniba rosaeodora*, especies en peligro de extinción, flujo de genes, germoplasma, caracterización molecular

CITE AS: Guizado, S.J.V.; Nadeem, M.A.; Ali, F.; Barut M.; Habyarimana, E.; Gómez, T.P.; Santillan, J.A.V.; Canales, E.T.; Gómez, J.C.C.; Chung, G.; Baloch, F.S. 2020. Genetic diversity and population structure of endangered rosewood from the Peruvian Amazon using ISSR markers. Acta Amazonica 50: 204-212.

INTRODUCTION

The first global assessment of plant extinction risk indicated that every fifth plant species in the world is threatened with extinction (Ibrahim *et al.* 2013). The Amazon region represents one of the richest reservoirs of biological diversity on the planet (Confalonieri *et al.* 2014; Gentry 1992), and is considered a biodiversity hotspot that can serve as a potential source of genetic variability for breeding perspectives of crops (Myers *et al.* 2000; Gentry 1992). Rosewood, *Aniba rosaeodora* Ducke (Lauraceae) has 2n = 24 chromosomes (Contim *et al.* 2005), and is distributed in the Amazon region of Brazil, Guyana, Suriname, Peru, Colombia, and Venezuela (Maia and Mourão 2016). The species is known for its essential oil, which is mainly characterized by a high content of linalool in the leaves and branches (74.4 - 81.8%) (Pimentel *et al.* 2018) and in the trunk wood (~ 100%) (Chantraine *et al.* 2009). Rosewood essential oil was extracted at a large scale from 1875 to 1975 in French Guiana, and trees were felled in such proportions that natural populations were significantly depleted (Bruleaux 1989). Export of rosewood essential oil has undergone a significant decline since 2001, and French Guiana banned the felling of this tree. Currently, Brazil is the only producer of rosewood essential oil (Amusant *et al.* 2015). Rosewood is now included as an endangered species in the database of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Salazar 2011).

Rosewood trade in Peru started in 1941, when Samuel Reggeroni, owner of the Pucabarranca farm on the Napo River, sent his first essential oil samples to Europe (MINAM 2015). During the 1950s, an increase in trade of rosewood essential oil was observed globally (MINAM 2015; Krainovic *et al.* 2017), reaching 300 tones year^{-1} in Brazil. In Peru, rosewood forests are located north of the Marañon - Amazonas river axis, along the rivers Tiger, Napo and Putumayo (MINAM 2015). It is believed that rosewood populations were greatly reduced by historical exploitation, fragmentation of habitats and deforestation resulting from the extraction of species of high timber value (Salazar 2011). Currently, rosewood is categorized as vulnerable in Peru (Salazar 2011), therefore the Peruvian government has taken strong actions to halt the decline of the species, and the export of rosewood wood and essential oil is forbidden since 1972. The Peruvian ministry of agriculture recommended the establishment of rosewood plantations in order to promote the conservation of the species in the wild and its commercial exploitation (Salazar 2011; MINAM 2015).

Studies on the genetic diversity and population structure of endangered species are necessary to design conservation and management strategies, including the selection of germplasm accessions for cultivation and improvement programs (Tabin *et al.* 2016; Ali et al. 2020a)commonly called Rhubarb, form an important component of the north western Himalayan flora and provide high value medicinal products to folks and pharmaceutical industries. Genetic diversity and structure of three Rheum species, namely, Rheum emodi, R. spiciforme and R. webbianum from Kashmir Himalaya was examined at the molecular level using Inter-Simple Sequence Repeat (ISSR. Characterization of wild germplasm is a necessary step to determine intraspecific variability for use in the design of breeding programs for plant species (Barut *et al.*2020; Nadeem *et al.* 2020). Molecular markers have been very helpful in investigating genetic diversity, and exploring the genetic relationship among the genotypes of various crops (Yaldiz *et al.* 2018; Yildiz *et al.* 2019; Karık *et al.* 2019). Various types of molecular markers have been developed according to their application efficiencies (Nadeem *et al.* 2018). Studies have confirmed that ISSR are abundant and widely distributed throughout plant nuclear genomes (González *et al.* 2007; Ekincialp *et al.* 2019; Nadeem *et al.* 2018) and have been successfully utilized for the assessment of population structure and genetic variation in various crop species (Cardoso *et al.* 2019; Ekincialp *et al.* 2019; Ali *et al.* 2020b).

In the case of rosewood, current efforts are being made globally to bring sustainability to the rosewood essential oil industry through *in-situ* and *ex-situ* germplasm collections (Amusant *et al.* 2016). Most studies on the species have been about the activity of its essential oil (Sarrazin *et al.* 2016; Amusant et al. 2016; Maia and Mourão 2016). Information about the genetic characterization of rosewood using molecular markers and its conservation management is available for populations in Brazil (Santos *et al.* 2004, Santos *et al.* 2008a, b; Angrizani *et al.* 2013), but is lacking for rosewood in Peru. There is no *ex situ* germplasm bank for this species in Peru, and the information available in Peruvian Amazonian herbaria on this species is limited. Therefore, the objective of this study was to investigate the genetic diversity and population structure of rosewood from remnants in the Peruvian Amazon using 11 ISSR primers.

MATERIAL AND METHODS

Plant material and DNA extraction

For this study, we collected leaves of 90 rosewood trees from eight different localities in the regions of Loreto and Ucayali, in the Peruvian Amazon (Figure 1; Supplemental Material, Table S1), which are considered main habitats for rosewood in Peru. Three localities are close to Iquitos, two of them accessible by road, and one on the margin of the Amazonas River (Figure 1). The rosewood population in the locality of Allpahuayo is adjacent to the Allpahuayo–Mishana National Reserve. The populations in Zungarococha, Mayriricay, Nanay, Tamshiyacu and Santa Marta are located within private estates, and those in Huajoya and Maria de Huajoya, within native community lands. The Instituto de Investigaciones de la Amazonía Peruana (IIAP) established a

pilot plantation of rosewood 25 years ago in the perimeter zone of the Allpahuayo National, Reserve. The populations in Zungarococha, Allpahuayo and Mairiricay are plantations from material originating from Tamshiyacu. The plantations are 25, 20 and 15 years old, respectively.

For the extraction of genomic DNA, leaves of each accession were packaged separately and kept on ice to avoid oxidation, and were transported to the laboratory of the specialized unit of biotechnology of the Centro de Investigación de Recursos Naturales de la Amazonía, in Iquitos, Peru. Species identification was based on the morphology of the collected material and was carried out at the Herbarium Amazonence of Universidad Nacional de la Amazonía Peruana (Iquitos, Peru). Leaves of each accession were kept in a freezer at -20 °C until isolation of the genomic DNA, which followed the protocol suggested by Castro *et al.* (2017). DNA was diluted in TE (Tris–EDTA) with a final volume of 35 µL per accession, and then stored at -20 °C. Genomic DNA quantification was performed by spectrophotometry using Nanodrop 2000c (Thermo Scientific, USA).

ISSR primer analysis

A total of 70 ISSR primers were screened using eight randomly selected rosewood accessions for PCR amplification. Out of the 70 ISSR primers, 11 most polymorphic primers were selected and used for the final PCR amplification, which resulted in high polymorphism with strong and clear band profiles, suitable for genotyping of all accessions (Table 1). A total reaction volume of 25 µL for PCR amplifications was comprised of 25 ng of template DNA, 4 µL dNTPs (0.2 mM) (Thermo Scientific), 0.2 µL U Taq DNA polymerase (Thermo Scientific), 1 mM primer , 2.5 µL 1_X PCR buffer (Thermo Scientific), 2 mM $MgCl_2$ and 11.3 µL distilled water. Reactions were performed in the sequence of denaturation at 94 °C for 3 min, followed by 30 denaturation cycles at 94 °C for 1 min, annealing temperature of 48-54 °C for one minute, depending upon the primer, and a final extension for 10 min at 72 °C. Agarose gel 1.8% (w/v) containing 0.5_X Tris-borate-EDTA (TBE) buffer was used for the electrophoreses of the amplified DNA fragments at a constant voltage of 120 V for 240 min. Ethidium bromide was used to perform the staining of the gel and Gel Doc XR+ system (Bio-Rad, USA) was used as gel imager to visualize the gel and to take photographs. Two µl of the 100 bp+ molecular weight marker was used for the measurement of the fragment patterns (Promega, Madison, South Dakota, USA).

Statistical analysis

ISSR bands (strong, clear, and unambiguous) were manually scored using the binary system as present (1) versus absent (0). The following genetic diversity indices were calculated by using PopGene ver. 1.32: effective allele number (Ne), Shannon's Information Index (I), gene diversity (He) and the overall gene diversity (Ht). Polymorphism information content (PIC) was calculated as suggested by Baloch *et al.* (2015) genetic studies in lentil are still in their infancy. Genetic diversity and relationships among wild Lens species from Turkey has seldom been investigated. Additionally, a limited number of simple sequence repeat (SSR. To evaluate the genetic relationship among the 90 rosewood accessions, the pairwise genetic distance (GDj) (Jaccard 1908) was calculated using the jaccard package in the R statistical software. Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed using GenAlEx v6.5 software (Peakall and Smouse 2012). A neighbor joining analysis was performed using the ape package in the R software. The

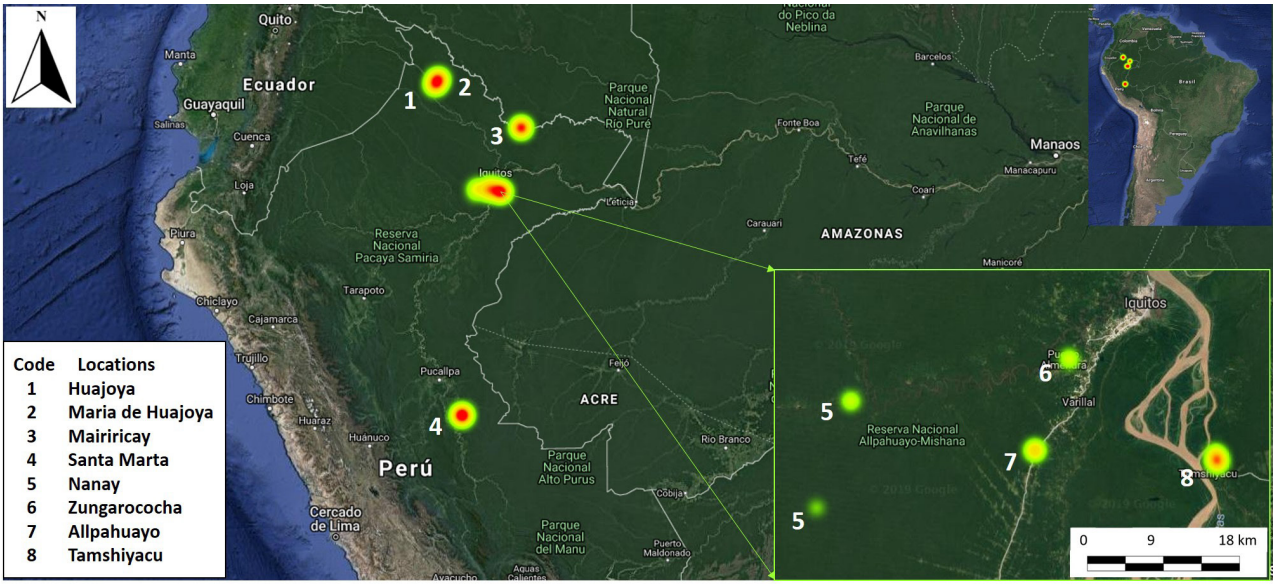

Figure 1. Collection localities of rosewood, *Aniba rosaeodora* accessions in the Peruvian Amazon. This figure is in color in the electronic version.

Table 1. List of the 11 most polymorphic primers (sequences and annealing temperature) used for the assessment of the genetic diversity and population structure of rosewood, *Aniba rosaeodora* germplasm from the Peruvian Amazon.

Primer	Sequence	Annealing temperature (°C)
807	AGAGAGAGAGAGAGAGT	50
810	GAGAGAGAGAGAGAGAT	50
812	GAGAGAGAGAGAGAGAA	50
814	CTCTCTCTCTCTCTCTA	50
815	CTCTCTCTCTCTCTCTG	52
817	CACACACACACACACAA	50
818	CACACACACACACACAG	52
819	GTGTGTGTGTGTGTGTA	50
826	ACACACACACACACACAG	52
834	AGAGAGAGAGAGAGAGYT	52
840	GAGAGAGAGAGAGAGAYT	52

population structure was calculated using the STRUCTURE software (Evanno *et al.* 2005). The initial burn-in period was set to 5000 with 100,000 MCMC (Markov chain Monte Carlo) iterations with no prior information on the origin of individuals. For each K and each run, 10 independent runs were set as parameters to estimate the population structure. We plotted the cluster number (K) against logarithm probability relative to standard deviation (ΔK) and the criteria by Evanno *et al.* (2005) were used to estimate the optimum number of clusters (K subpopulations). Each accession was assigned to its respective population on the basis of its membership coefficient being greater than or equal to 50% as suggested by Habyarimana (2016)assessment of GS strategies for grain yield improvement in this crop is still limited. This work aimed to evaluating the cross-validation accuracy (rcv. We calculated the same genetic diversity indices as above for the STRUCTURE populations using PopGene version 1.32. Gene flow among the STRUCTURE populations was also estimated following the methodology of Mallet (1999) and by performing the neighbor joining analysis using the ape package in the R software.

RESULTS

The eleven most polymorphic ISSR primers yielded a sum of 378 bands and 34.4 average bands per primer in the 90 accessions. Among the 378 scored bands, 375 (99.2%) were polymorphic, with an average of 34.1 bands per primer (Table 2). Maximum (51) and minimum (10) number of bands resulted with primers ISSR826 and ISSR819, respectively. PIC averaged 0.774, with minimum and maximum values of 0.592 (ISSR819) and 0.867 (ISSR834), respectively (Table 2). The average number of alleles was 1.485, with maximum and minimum values of 1.608 (ISSR812) and 1.427 (ISSR840), respectively. The average value for the Shannon information index was 0.294, with highest (0.356) and lowest (0.261) values resulting with ISSR812 and ISSR840, respectively (Table 2). The highest (0.532) level of gene diversity was recorded for ISSR812, and the lowest (0.406) for ISSR840, with an average of 0.453.

The overall mean genetic distance among accessions was 0.554, with a maximum distance of 0.83 between the Mairiricay-11 and Santamarta-4 accessions, and a minimum of 0.09 between Nanay-4 and Nanay-5. The STRUCTURE analysis divided the accessions into three populations (K = 3), with 29 (32.2%) accessions in population A, 41 (45.6%) in population B, and 20 (22.2%) in population C (Figure 2). Population A was the genetically most diverse, comprising accessions from Nanay, Mariadehuajoya, Mairiricay and Huajoya (Figure 2). Population B, the largest, clustered accessions from Allpahuayo, Zunagarococha, Tamshiyacu and Mairiricay. Population C was the least diverse and clustered all 20 accessions from Santa Marta (Figure 2), the location farthest away from the other localities (Figure 1).

Population A had higher genetic diversity compared to the other two populations, as indicated by its high number of effective alleles (1.44), gene diversity (0.27), Shannon information index (0.41) and gene flow (2.875) (Table 3). Mean genetic distance within populations was 0.36 for population A, 0.323 for population B, and 0.314 for population C. AMOVA indicated that 88% of variance in the

Table 2. Genetic diversity parameters calculated for 90 accessions of rosewood, *Aniba rosaeodora*, from eight localities in the Peruvian Amazon, using 11 polymorphic ISSR primers.

Primer	TB	PB	Polymorphism (%)	PIC	Ne	I	He	Ht
807	31	31	100	0.765	1.538	0.319	0.484	0.310
810	42	39	92.9	0.726	1.466	0.278	0.427	0.258
812	38	38	100	0.819	1.608	0.356	0.532	0.254
814	33	33	100	0.844	1.477	0.302	0.470	0.270
815	37	37	100	0.727	1.469	0.284	0.435	0.274
817	29	29	100	0.727	1.435	0.274	0.433	0.274
818	29	29	100	0.837	1.435	0.269	0.421	0.218
819	10	10	100	0.592	1.605	0.351	0.524	0.233
826	51	51	100	0.746	1.439	0.271	0.423	0.218
834	37	37	100	0.867	1.431	0.271	0.427	0.258
840	41	41	100	0.863	1.427	0.261	0.406	0.204
Average	34.4	34.1	99.4	0.774	1.485	0.294	0.453	0.252
Total	378	375						

TB: total bands, PB: polymorphic bands, PIC: polymorphism information content, Ne: effective allele number, I: Shannon information index, He: gene diversity, Ht: overall gene diversity

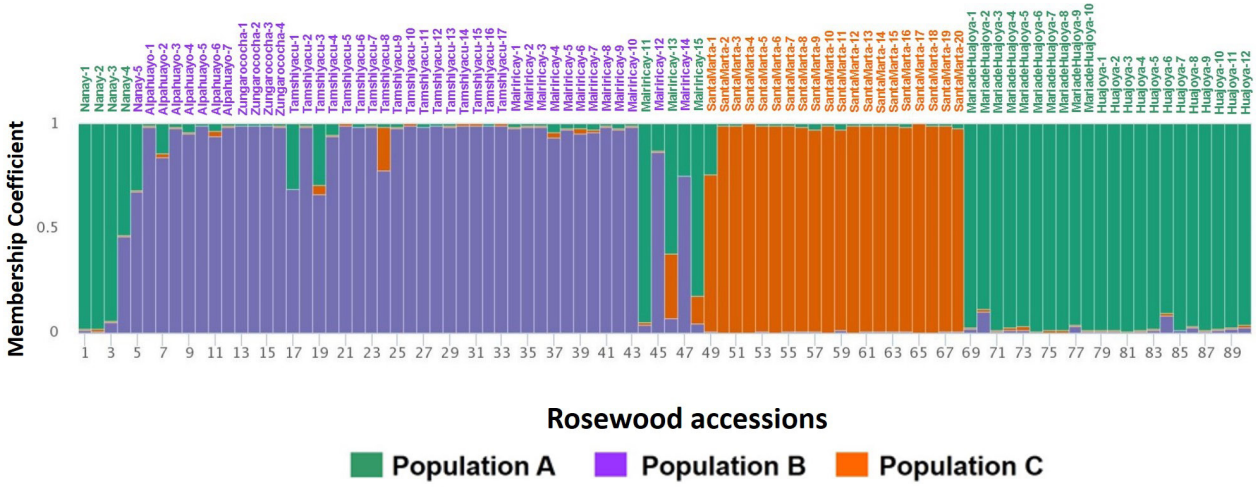

Figure 2. Population structure of 90 accessions of rosewood, *Aniba rosaeodora* from the Peruvian Amazon revealed by 11 ISSR primers. This figure is in color in the electronic version.

Peruvian rosewood accessions occurred within populations, and 12% among populations (Table 4).

The neighbor-joining-based clustering also grouped the 90 accessions into three populations (Figure 3). Neighbor joining based on the STRUCTURE populations grouped population A and B, while population C was found genetically distinct from the other two (Figure 4). PCoA clearly supported the results obtained through neighbor joining and grouped the 90 accessions into three main clusters (Figure 5).

DISCUSSION

To our best knowledge, this is the first study to assess the genetic diversity and population structure of Peruvian rosewood accessions using ISSR primers. We found a higher mean number of bands per primer than that found by Angrizani *et al.* (2013) using 11 SSR primers in 68 rosewood accessions from two localities in the central Amazon in Brazil. We also obtained higher mean polymorphism than that reported by Santos *et al.* (2008a) for one locality in the central Brazilian Amazon using RAPD in 94 rosewood accessions. We found a higher PIC value than that reported by Ebrahimi *et al.* (2016) in Persian walnut, *Juglans regia* L. (Juglandaceae) germplasm and by Zhu *et al.* (2016)African, and Asian countries using SSR markers","container-title":"Tree Genetics & Genomes","page":"114","volume":"12","issue":"6","source":"Springer Link","abstract":"Persian walnut (Juglans regia L. in *Lindera glauca* (Siebold & Zucc.) Blume (Lauraceae) using SSR markers. We also found a higher number of effective alleles then that reported for three other Lauraceae species using ISSR markers (Zhang *et al.* 2012). The mean number of effective alleles in our samples was higher than in *Nectandra megapotamica* (Spreng.) Mezz (Lauraceae) from southern Brazil using RAPD markers (1.22 to 1.39) (Costa *et al.* 2015). A high number of effective alleles is always desirable, as this stands for high genetic diversity in a population (Ali *et al.* 2019), thus indicating a potential of high genetic variability in Peruvian rosewood accessions. We obtained a higher Shannon index then that reported for *Neolitsea sericea* (Blume) Koidz. (Lauraceae) using RAPD markers (Wang *et al.* 2005). As ISSR markers are more informative than RAPD markers (Verma *et al.* 2017), this suggests that our rosewood accessions were more diverse than the species of Lauraceae listed above, with a genetic variability more evenly distributed throughout the analyzed accessions. The gene diversity in our samples was also much higher than that reported for the Lauraceae, *N. sericea*

Table 3. Diversity indices among the rosewood, *Aniba rosaeodora* populations from the Peruvian Amazon, as grouped by the STRUCTURE algorithm.

Population	Ne	He	I	Ht	Fst	Nm	GD
A	1.447	0.271	0.416	0.219	0.08	2.875	0.362
B	1.420	0.256	0.393	0.228	0.16	1.313	0.323
C	1.367	0.219	0.335	0.173	0.29	0.612	0.314

Ne: effective allele number, He: gene diversity, I: Shannon information index, Ht: overall gene diversity, Fst: measure of genetic structure, Nm: gene flow, GD: genetic distance

Table 4. Results for AMOVA among and within three rosewood, *Aniba rosaeodora* populations from the Peruvian Amazon as grouped by the cluster algorithm for 90 samples from eight localities.

Source of variance	df	SS	MS	Estimated variance	% variations
Among populations	2	594.539	297.270	8.220	12
Within populations	87	5290.461	60.810	60.810	88
Total	89	5885.000		69.030	100

df: degrees of freedom, SS: Sum of squares, MS: Mean square

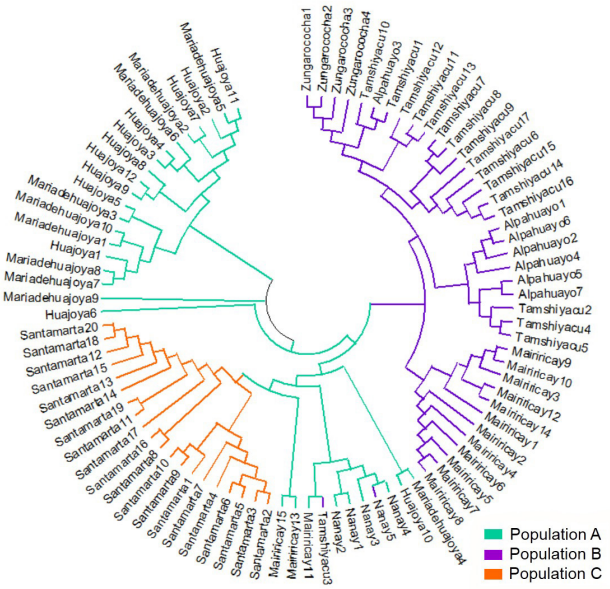

Figure 3. Neighbor-joining based clustering of 90 accessions of rosewood, *Aniba rosaeodora* from the Peruvian Amazon using 11 ISSR primers. This figure is in color in the electronic version.

Figure 4. Neighbor-joining based clustering among rosewood, *Aniba rosaeodora* populations from the Peruvian Amazon using 11 ISSR primers. This figure is in color in the electronic version.

(Wang *et al.* 2005) and *Lindera melissifolia* (Walter) Blume (Godt and Hamrick 1996).

Endangered species usually have low genetic diversity, mainly due to genetic drift and inbreeding in small remnant populations (Spielman *et al.* 2004). Our results indicate a higher genetic diversity in rosewood in the Peruvian Amazon than the reported values for rosewood in the central Brazilian Amazon (Santos *et al.* 2008a; Angrizani *et al.* 2013). Santos *et al.* (2008b) studied the genetic variability of four rosewood populations in central Amazonia using RAPD markers, and found higher genetic variations in Ducke Reserve, the only population under long-term protection. The higher genetic diversity in Peru may be owed to higher gene flow among the populations, and/or to the environmental heterogeneity and complex topography in the Peruvian Amazon, compared to the central region of the Amazonas River floodplain. The conditions in the sub-Andean Amazon may have provided optimal refuge habitat for rosewood during past events of climate change, enabling the conservation of a higher level of genetic diversity (Morelli *et al.* 2016).

Our AMOVA results confirmed higher variability within (98.1%) than among (1.9%) rosewood populations. In the central Brazilian Amazon, Santos *et al.* (2008b) also found higher (76.6%) genetic variations within (76.6%) than among (23.4%) populations in four populations of rosewood. The same pattern has also been reported in another tropical tree species in Belize (Pither *et al.* 2003). Similarly, Dong *et al.* (2016) studied the genetic diversity of five impacted and

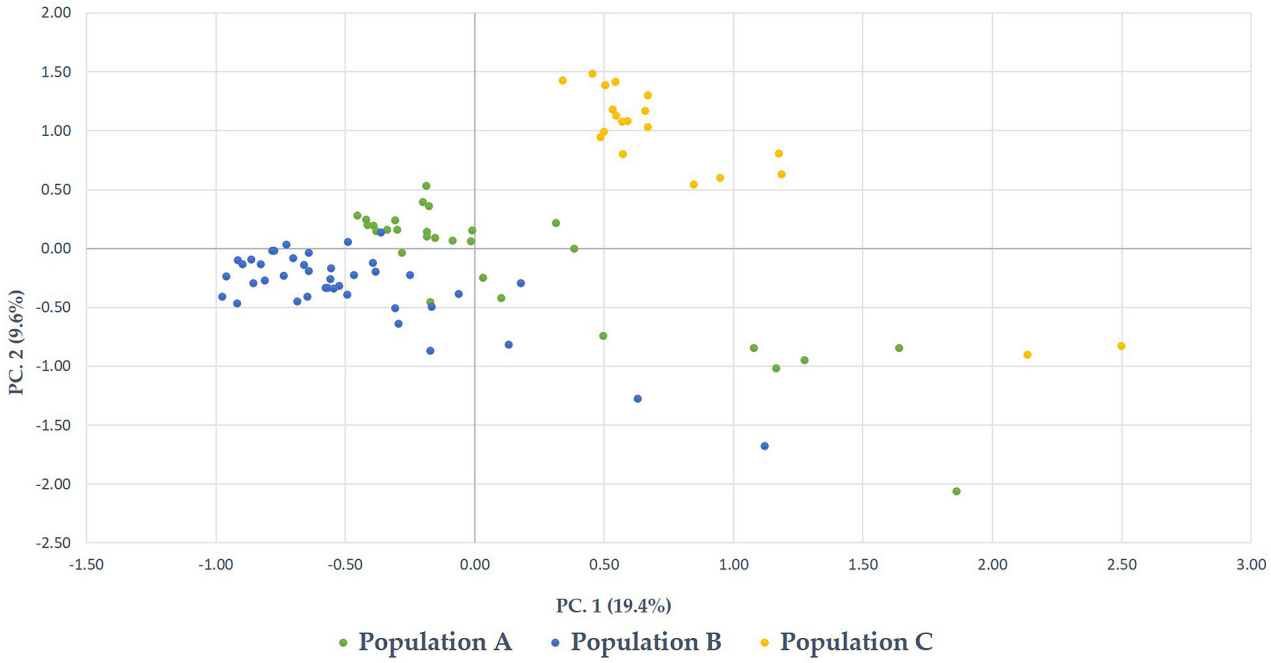

Figure 5. Principal coordinate analysis (PCoA) of rosewood, *Aniba rosaeodora* germplasm from the Peruvian Amazon revealed by 11 ISSR primers. This figure is in color in the electronic version.

fragmented populations of the narrowly distributed and rare *Cinnamomum chago* B.S. Sun & H.L. Zhao (Lauraceae) in a mountainous region in China using ISSR markers and found 17% of genetic variation among populations, and 83% within populations.

The STRUCTURE analysis separated more clearly the three populations A, B and C, as this software has better clustering power compared to other clustering algorithms. The differences with the neighbor joining clustering and PCoA might be due to their lower resolution power (Newell *et al.* 2013, Ali *et al.* 2019). In any case, accessions from the location of Santa Marta formed a distinct population, with lower genetic diversity, highest genetic distance and low gene flow respective to the other locations, which is likely related to the greater geographical distance and isolation of this stand from the other localities. Likewise, Santos *et al.* (2008b) found higher gene flow among rosewood populations closer to each other and also observed that increasing geographic distance resulted in decreased gene flow. Our population A, which had highest genetic diversity, grouped the wild populations of Nanay, Mariadehuajoya and Huajoya, indicating that frequent natural gene flow is maintained among these locations. Gene flow among populations conserves genetic diversity (Slatkin 1994), and high gene flow results in increased genetic diversity (Fu *et al.* 2016). The higher genetic similarity among the rosewood accessions grouped in population B of both clustering algorithms was expected, as those of Zunagarococha, Allpahuayo and Mairiricay were planted from material originating from the wild population of Tamshiyacu. Mairiircay was an interesting case, being a plantation, it was also grouped in population A, presumably because wild trees already existed in the area where the plantation was established, and/or part of the planted material was brought from population-A locations. Accessions Mairiricay-11 and Santamarta-4, which had maximum values of genetic distance are likely the more interesting for Peruvian rosewood breeding and conservation programs, as the evaluation of plants with variability in traits of interest is the main focus of breeders (Arystanbekkyzy *et al.* 2018).

CONCLUSIONS

Our genetic analysis of rosewood accessions from eight important remnant stands in the Peruvian Amazon revealed one distinct and more isolated population in the Ucayali region (Santa Marta). The populations at Zunagarococha, Allpahuayo and Mairiricay showed genetic similarity because they were planted from material originating from the wild population of Tamshiyacu. Genetic diversity in Peruvian rosewood germplasm was higher than that reported for rosewood populations in the central Brazilian Amazon, and higher within-population diversity is consistent with a pattern of fragmentation resulting from overexploitation. The Peruvian accessions show promising potential for use in germplasm enhancement and parental selection in breeding and genetic improvement programs. Accessions Mairiricay-11 and Santamarta-4 were found genetically distinct and can be suggested as candidate parents for rosewood breeding activities. This was the first attempt to investigate genetic diversity and population structure of Peruvian rosewood germplasm.

ACKNOWLEDGMENTS

The authors express their gratitude to Servicio Nacional Forestal y de Fauna Silvestre (SERFOR), Peru for providing the financial support for the collection of germplasm (1360-2018-MINAGRI-SERFOR-CAF). The authors also express their gratitude to Programa Nacional de Innovación Agraria (PNIA), Peru for providing a scientific internship to Stalin Juan Vasquez Guizado (156-2018-INIA-PNIA), at the Bolu Abant Izzet Baysal University, Bolu, Turkey.

REFERENCES

Ali, F.; Yılmaz, A.; Nadeem, M.A.; Habyarimana, E.; Subaşı, I.; Nawaz, M.A.; *et al.* 2019. Mobile genomic element diversity in world collection of safflower (*Carthamus tinctorius* L.) panel using iPBS-retrotransposon markers. *PLOS ONE*, 14: e0211985.

Ali, F.; Nadeem, M.A.; Barut, M.; Habyarimana, E.; Chaudhary, H.J.; Khalil, *et al*. 2020a. Genetic Diversity, Population Structure and Marker-Trait Association for 100-Seed Weight in International Safflower Panel Using SilicoDArT Marker Information. *Plants*, 9: doi.org/10.3390/plants9050652.

Ali, F.; Nadeem, M.A.; Habyarimana, E.; Yılmaz, A.; Nawaz, M.A.; Khalil, I.H.; *et al.* 2020b. Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers. *Brazilian Journal of Botany*, 43: 109–121.

Amusant, N.; Beauchène, J.; Digeon, A.; Chaix, G. 2016. Essential oil yield in rosewood (*Aniba Rosaeodora* Ducke): initial application of rapid prediction by near infrared spectroscopy based on wood spectra. *Journal of Near Infrared Spectroscopy*, 24: 507-515.

Amusant, N.; Digeon, A.; Descroix, L.; Bruneau, O.; Bezard, V.; Beauchene, J. 2015. Planting rosewood for sustainable essential oil production: Influence of surrounding forest and seed provenance on tree growth and essential oil yields. *Bois et Forest des Tropiques*, 326: 57–65.

Angrizani, R.C.; Contim, L.A.S.; Lemes, M.R. 2013. Development and characterization of microsatellite markers for the endangered Amazonian tree *Aniba rosaeodora* (*Lauraceae*). *Applications in Plant Sciences*, 1: 1200516.

Arystanbekkyzy, M.; Nadeem, M.A.; Aktas, H.; Yeken, M.Z.; Zencirci, N.; Nawaz, M.A.; *et al.* 2018. Phylogenetic and taxonomic relationship of turkish wild and cultivated emmer (*Triticum turgidum* ssp. dicoccoides) revealed by iPBSretrotransposons markers. *International Journal of Agriculture and Biology*, 10: 1-9.

Baloch, F.S.; Derya, M.; Andeden, E.E.; Alsaleh, A.; Cömertpay, G.; Kilian, B.; Özkan, H. 2015. Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild Lens species. *Biochemical Systematics and Ecology*, 58: 162-168.

Barut, M.; Nadeem, M.A.; Karaköy, T.; Baloch, F.S. 2020. DNA fingerprinting and genetic diversity analysis of world quinoa germplasm using iPBS-retrotransposon marker system. *Turkish Journal of Agriculture and Forestry*, 44: doi:10.3906/tar-2001-10

Bruleaux, A.M. 1989. Deux productions passées de la forêt guyanaise. L'essence de bois de rose et la gomme de balata. *Bois & Forets des Tropiques*, 219: 99-113.

Cardoso, M.N.; Nascimento, A.L.S.; de Oliveira, L.A.R.; de Assunção, D.A.; Machado, C.A.; de Oliveira, A.C.A.; Muniz, A.D.S. 2019. Genetic diversity in native *Genipa americana* (*Rubiaceae*) populations in Sergipe, Brazil. *Genetics and Molecular Research,* 18: 1-19.

Castro, J.C.; Rodríguez, H.N.; Maddox, J.D.; Jiu, B.; Petterman, J.B.; Marapara, J.L.; Cobos, M. 2017. A simple and efficient method for high-quality total Rna isolation from oleaginous microalgae. *Plant Cell Biotechnology and Molecular Biology*, 18: 15-21.

Confalonieri, U.E.; Margonari, C.; Quintão, A.F. 2014. Environmental change and the dynamics of parasitic diseases in the Amazon. *Acta Tropica*, 129: 33-41.

Contim, L.A.S.; Carvalho, C.R.; de Martins, F.A.; Freitas, D.V. 2005. Nuclear DNA content and karyotype of Rosewood (*Aniba rosaeodora*). *Genetics and Molecular Biology*, 28: 754–757.

Costa, L.S.; Reiniger, L.R.S.; Heinzmann, B.M.; Amaral, L.P.; Serrote, C.M. 2015. Study of the genetic diversity and structure of a natural population of *Nectandra megapotamica* (Spreng.) Mez. using RAPD markers. *Genetics and Molecular Research*, 14: 18407-18413.

Chantraine, J.M.; Dhénin, J.M.; Moretti, C. 2009. Chemical variability of rosewood (*Aniba rosaeodora* Ducke) essential oil in French Guiana. *Journal of Essential Oil Research*, 21: 486-495.

Dong, W.; Zhang, X.; Guansong, Y.; Yang, L.; Wang, Y.; Shen, S. 2016. Biological characteristics and conservation genetics of the narrowly distributed rare plant *Cinnamomum chago* (Lauraceae). *Plant Diversity*, 38: 247-252.

Ebrahimi, A.; Zarei, A.; Lawson, S.; Woeste, K.E.; Smulders, M.J.M. 2016. Genetic diversity and genetic structure of Persian walnut (*Juglans regia*) accessions from 14 European, African, and Asian countries using SSR markers. *Tree Genetics & Genomes*, 12: 95-114.

Ekincialp, A.; Erdinc, C.; Turan, S.; Cakmakci, O.; Nadeem, M.A.; Shehzad, F.S. 2019. Genetic characterization of *Rheum ribes* L. (Wild Rhubarb) genotypes in Lake Van basin of Turkey through ISSR and SSR markers. *International Journal of Agriculture and Biology*. 21: 795-802.

Evanno, G.; Regnaut, S.; Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, 14: 2611-2620.

Fu, P.C.; Gao, Q.B.; Zhang, F.Q.; Xing, R.; Wang, J.L.; Liu, H.R.; Chen, S.L. 2016. Gene flow results in high genetic similarity between Sibiraea (*Rosaceae*) species in the Qinghai-Tibetan Plateau. *Frontiers in Plant Science*, 7: 1596-1600.

Gentry, A.H. 1992. Tropical forest biodiversity: distributional patterns and their conservational significance. *Oikos*, 63: 19–28.

Godt, M.J.W.; Hamrick, J.L. 1996. Allozyme diversity in the endangered shrub *Lindera melissifolia* (Lauraceae) and its widespread congener *Lindera benzoin*. *Canadian Journal of Forest Research*, 26: 2080-2087.

González, A.; Souza, V.; Aguirre, X. 2007. Inter Simple Sequence Repeats (ISSRs). *ASM Science Journal*, 9: 567-571.

Habyarimana, E. 2016. Genomic prediction for yield improvement and safeguarding of genetic diversity in CIMMYT spring wheat (*Triticum aestivum* L.). *Australian Journal of Crop Science*, 10: 127-131.

Ibrahim, M.A.; Na, M.; Oh, J.; Schinazi, R.F.; McBrayer, T.R.; Whitaker, T.; *et al.* 2013. Significance of endangered and threatened plant natural products in the control of human disease. *Proceedings of the National Academy of Sciences of the United States of America*, 110: 16832-16837.

Jaccard, P. 1908. *Nouvelles recherches sur la distribution florale*. *Bulletin de la Société Vaudoise des Sciences Naturelles*, 44: 223–270.

Karik, U.; Nadeem, M.A.; Habyarimana, E.; Ercişli, S.; Yildiz, M.; Yılmaz, A.; Yang, S.H.; Chung, G.; Baloch, F.S. 2019. Exploring the genetic diversity and population structure of Turkish laurel germplasm by the iPBS-retrotransposon marker system. *Agronomy*. 9: 647, doi:10.3390/agronomy9100647.

Krainovic, P.; Almeida, D.; Sampaio, P. 2017. New allometric equations to support sustainable plantation management of rosewood (*Aniba rosaeodora* Ducke) in the Central Amazon. *Forests*, 8: 327, doi.org/10.3390/f8090327.

Maia, J.G.S.; Mourão, R.H.V. 2016. Amazon rosewood (*Aniba rosaeodora* Ducke) oils. In: Preedy, V.R. (Ed.). *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, New York, p.193-201.

Mallet, J. 1999. Gene flow. In: Woiwod, I.P.; Reynolds, D.R.; Thomas, C.D. (Ed.). *Insect Movement: Mechanisms and Consequences*. Symposium-Royal Entomological Society of London, CAB International, Wallingford, p.337–360.

MINAM. 2015. Dictamen de extracción no prejudicial de *Aniba rosaeodora* Ducke "palo de rosa" procedente del medio natural. Viceministerio de Desarrollo Estratégico de Recursos Naturales - Ministerio del Ambiente, Peru. 26p

Morelli, T.L.; Daly, C.; Dobrowski, S.Z.; Dulen, D.M.; Ebersole, J.L.; Jackson, S.T.; *et al.* 2016. Managing climate change refugia for climate adaptation. *PLoS ONE*, 11: e0169725.

Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Da Fonseca, G.A.; Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403: 845-853.

Nadeem, M.A.; Nawaz, M.A.; Shahid, M.Q.; Do ˘gan, Y.; Comertpay, G.; Yıldız, M.; *et al.*. 2018. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, 32: 261–285

Nadeem, M.A.; Gündoğdu, M.; Ercişli, S.; Karaköy, T.; Saracoğlu, O.; Habyarimana, E.; *et al.* 2020. Uncovering phenotypic

diversity and DArTseq marker loci associated with antioxidant activity in common bean. *Genes*, 11: 36. doi.org/10.3390/genes11010036

Newell, M.A.; Cook, D.; Hofmann, H.; Jannink, J.L. 2013. An algorithm for deciding the number of clusters and validation using simulated data with application to exploring crop population structure. *The Annals of Applied Statistics*, 7: 1898-1916.

Peakall, R.; Smouse, P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28: 2537-2539.

Pimentel, R.B.; Souza, D.P.; Albuquerque, P.M.; Fernandes, A.V.; Santos, A.S.; Duvoisin Jr,S.; Gonçalves, J.F. 2018. Variability and antifungal activity of volatile compounds from *Aniba rosaeodor*a Ducke, harvested from Central Amazonia in two different seasons. *Industrial Crops and Products*, 123: 1-9.

Pither, R.; Shore, J.S.; Kellman, M. 2003. Genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in naturally fragmented populations. *Heredity*, 91: 307-313.

Salazar, B.M. 2011. *List of Peruvian CITES Species Wild Flora*. Ministerio del Ambiente, Lima, 130p.

Santos, A.S.; Antunes, A.M.S.; D´Avila, L.A. 2004. New natural linalol sources: research and industrial application. *Perfumer & Flavorist*, 29: 38-43.

Santos, R.P.; Spironello, W.R.; Sampaio, P.T.B. 2008a. Genetic diversity in rosewood saplings (*Aniba rosaeodora* Ducke, Lauraceae): an ecological approach. *Acta Amazonica*, 38: 707-714.

Santos, R.P.; Ângelo, P.C.D.S.; Sampaio, P.D.T.B.; Quisen, R.C.; Leite, Â.M.C.; Oliveira, C.L.D. 2008b. Geographic pattern of genetic diversity in natural populations of Rosewood (*Aniba rosaeodora*), in the Central Amazonia. *Acta Amazonica*, 38: 459-466.

Sarrazin, S.L.F.; Oliveira, R.B.; Maia, J.G.S.M.; Mourão, R.H.V. 2016. Antibacterial activity of the rosewood (*Aniba rosaeodora* and *A. parviflora*) linalool-rich oils from the Amazon. *European Journal of Medicinal Plants*. 12: 1-9.

Slatkin, M. 1994. Gene flow and population structure. *Ecological Genetics*, 1: 3-17.

Spielman, D.; Brook, B.W.; Briscoe, D.A.; Frankham, R. 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics*, 5: 439-448.

Tabin, S.; Kamili, A.N.; Ganie, S.A.; Zargar, O.; Sharma, V.; Gupta, R.C. 2016. Genetic diversity and population structure of *Rheum* species in Kashmir Himalaya based on ISSR markers. *Flora*, 223: 121-128.

Verma, K.S.; ul Haq, S.; Kachhwaha, S.; Kothari, S.L. 2017. RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. *3 Biotech*, 7: 288. doi: 10.1007/s13205-017-0918-z.

Wang, Z.S.; An, S.Q.; Liu, H.; Leng, X.; Zheng, J.W.; Liu, Y.H. 2005. Genetic structure of the endangered plant *Neolitsea sericea* (Lauraceae) from the Zhoushan Archipelago using RAPD markers. *Annals of Botany*. 95: 305-313.

Yaldiz, G.; Çamlica, M.; Nadeem, M.A.; Nawaz, M.A.; Baloch, F.S. 2018. Genetic diversity assessment in *Nicotiana tabacum* L. with iPBS-retrotransposons. *Turkish Journal of Agriculture and Forestry*, 42: 154-164.

Yildiz, M.; Koçak, M.; Nadeem, M.A.; Cavagnaro, P.; Barboza, K.; Baloch, F.S.; Argün, D.; Keleş, D. 2019. Genetic diversity analysis in the Turkish pepper germplasm using iPBS retrotransposon-based markers. *Turkish Journal of Agriculture and Forestry*, 44: 1-14.

Zhang, R.; Zhou, Z.; Jin, G.; Wang, S.; Wang, X. 2012. Genetic diversity and differentiation within three species of the family Lauraceae in southeast China. *Biochemical Systematics and Ecology*, 44: 317-324.

Zhu, S.; Ding, Y.; Yap, Z.; Qiu, Y. 2016. De novo assembly and characterization of the floral transcriptome of an economically important tree species, *Lindera glauca* (*Lauraceae*), including the development of EST-SSR markers for population genetics. *Molecular Biology Reports*, 43: 1243-1250.

RECEIVED: 27/06/2019
ACCEPTED: 22/06/2020
ASSOCIATE EDITOR: Alexandre Pio Viana

SUPPLEMENTARY MATERIAL (only available in the electronic version)

Guizado *et al.* Genetic diversity and population structure of endangered rosewood from the Peruvian Amazon using ISSR markers

Table S1. Passport data of 90 rosewood, *Aniba rosaeodora* accessions collected from eight different localities in the Peruvian Amazon (see Figure 1).

Serial. Nr.	Accession name	Region	Province	District	Village	Latitude	Longitude	Altitude (m)
1	Nanay-1	Loreto	Alto Nanay	Santa maria del Nanay	Quebrada Curaca	9551691	638610	152
2	Nanay-2	Loreto	Alto Nanay	Santa maria del Nanay	Santa maria del nanay	9569683	644419	106
3	Nanay-3	Loreto	Alto Nanay	Santa maria del Nanay	Santa maria del nanay	9569689	644389	109
4	Nanay-4	Loreto	Alto Nanay	Santa maria del Nanay	Santa maria del nanay	9569727	644387	106
5	Nanay-5	Loreto	Alto Nanay	Santa maria del Nanay	Santa maria del nanay	9569721	644391	99
6	Allpahuayo-1	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561154	675470	158
7	Allpahuayo-2	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561182	675477	148
8	Allpahuayo-3	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561208	675492	144
9	Allpahuayo-4	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561236	675505	148
10	Allpahuayo-5	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561247	675500	142
11	Allpahuayo-6	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561262	675512	141
12	Allpahuayo-7	Loreto	Maynas	San Juan Bautista	Allpahuayo	9561300	675527	138
13	Zungarococha-1	Loreto	Maynas	San Juan Bautista	Zungarococha	9576628	681106	113
14	Zungarococha-2	Loreto	Maynas	San Juan Bautista	Zungarococha	9576631	681105	115
15	Zungarococha-3	Loreto	Maynas	San Juan Bautista	Zungarococha	9576625	681115	116
16	Zungarococha-4	Loreto	Maynas	San Juan Bautista	Zungarococha	9576650	681100	114
17	Tamshiyacu-1	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559735	706059	112
18	Tamshiyacu-2	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559801	706144	110
19	Tamshiyacu-3	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559783	706148	120
20	Tamshiyacu-4	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559741	706087	123
21	Tamshiyacu-5	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559669	706071	111
22	Tamshiyacu-6	Loreto	Maynas	Fernando Lores	Tamshiyacu	9560651	705900	125
23	Tamshiyacu-7	Loreto	Maynas	Fernando Lores	Tamshiyacu	9560660	705877	105
24	Tamshiyacu-8	Loreto	Maynas	Fernando Lores	Tamshiyacu	9560676	705862	116
25	Tamshiyacu-9	Loreto	Maynas	Fernando Lores	Tamshiyacu	9560681	705840	121
26	Tamshiyacu-10	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559356	706026	119
27	Tamshiyacu-11	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559220	706283	129
28	Tamshiyacu-12	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559223	706274	112
29	Tamshiyacu-13	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559205	706296	115
30	Tamshiyacu-14	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559076	706243	108
31	Tamshiyacu-15	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559096	706281	119
32	Tamshiyacu-16	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559092	706266	115
33	Tamshiyacu-17	Loreto	Maynas	Fernando Lores	Tamshiyacu	9559076	706269	110
34	Mairiricay-1	Loreto	Putumayo	Putumayo	Mairiricay	9726985	760695	136
35	Mairiricay-2	Loreto	Putumayo	Putumayo	Mairiricay	9726991	760701	132
36	Mairiricay-3	Loreto	Putumayo	Putumayo	Mairiricay	9726988	760714	134
37	Mairiricay-4	Loreto	Putumayo	Putumayo	Mairiricay	9727009	760707	132
38	Mairiricay-5	Loreto	Putumayo	Putumayo	Mairiricay	9727008	760702	131
39	Mairiricay-6	Loreto	Putumayo	Putumayo	Mairiricay	9726999	760690	130
40	Mairiricay-7	Loreto	Putumayo	Putumayo	Mairiricay	9726978	760714	125
41	Mairiricay-8	Loreto	Putumayo	Putumayo	Mairiricay	9726981	760726	126
42	Mairiricay-9	Loreto	Putumayo	Putumayo	Mairiricay	9726972	760715	125
43	Mairiricay-10	Loreto	Putumayo	Putumayo	Mairiricay	9726971	760716	127

Table S1. Continued.

Serial. Nr.	Accession name	Region	Province	District	Village	Latitude	Longitude	Altitude (m)
44	Mairiricay-11	Loreto	Putumayo	Putumayo	Mairiricay	9726971	760713	123
45	Mairiricay-12	Loreto	Putumayo	Putumayo	Mairiricay	9726982	760719	128
46	Mairiricay-13	Loreto	Putumayo	Putumayo	Mairiricay	9727003	760729	124
47	Mairiricay-14	Loreto	Putumayo	Putumayo	Mairiricay	9726994	760726	126
48	Mairiricay-15	Loreto	Putumayo	Putumayo	Mairiricay	9727007	760725	124
49	Santamarta-1	Ucayali	Atalaya	Masisea	Santa Marta	8980940	604385	171
50	Santamarta-2	Ucayali	Atalaya	Masisea	Santa Marta	8980933	604388	169
51	Santamarta-3	Ucayali	Atalaya	Masisea	Santa Marta	8980925	604386	170
52	Santamarta-4	Ucayali	Atalaya	Masisea	Santa Marta	8980934	604388	169
53	Santamarta-5	Ucayali	Atalaya	Masisea	Santa Marta	8980923	604387	172
54	Santamarta-6	Ucayali	Atalaya	Masisea	Santa Marta	8980943	604348	171
55	Santamarta-7	Ucayali	Atalaya	Masisea	Santa Marta	8981608	604180	171
56	Santamarta-8	Ucayali	Atalaya	Masisea	Santa Marta	8981590	604184	171
57	Santamarta-9	Ucayali	Atalaya	Masisea	Santa Marta	8981587	604200	173
58	Santamarta-10	Ucayali	Atalaya	Masisea	Santa Marta	8981586	604182	171
59	Santamarta-11	Ucayali	Atalaya	Masisea	Santa Marta	8981588	604231	174
60	Santamarta-12	Ucayali	Atalaya	Masisea	Santa Marta	8981574	604258	176
61	Santamarta-13	Ucayali	Atalaya	Masisea	Santa Marta	8981667	604622	174
62	Santamarta-14	Ucayali	Atalaya	Masisea	Santa Marta	8981668	604623	174
63	Santamarta-15	Ucayali	Atalaya	Masisea	Santa Marta	8981674	604632	175
64	Santamarta-16	Ucayali	Atalaya	Masisea	Santa Marta	8981978	604874	177
65	Santamarta-17	Ucayali	Atalaya	Masisea	Santa Marta	8981965	604878	175
66	Santamarta-18	Ucayali	Atalaya	Masisea	Santa Marta	8981959	604892	175
67	Santamarta-19	Ucayali	Atalaya	Masisea	Santa Marta	8981528	604688	172
68	Santamarta-20	Ucayali	Atalaya	Masisea	Santa Marta	8980586	604483	164
69	Mariadehuajoya-1	Loreto	Maynas	Napo	Maria de Huajoya	9838429	536797	120
70	Mariadehuajoya-2	Loreto	Maynas	Napo	Maria de Huajoya	9835376	537866	125
71	Mariadehuajoya-3	Loreto	Maynas	Napo	Maria de Huajoya	9833880	535209	116
72	Mariadehuajoya-4	Loreto	Maynas	Napo	Maria de Huajoya	9835834	531637	121
73	Mariadehuajoya-5	Loreto	Maynas	Napo	Maria de Huajoya	9838277	528614	118
74	Mariadehuajoya-6	Loreto	Maynas	Napo	Maria de Huajoya	9841544	530843	118
75	Mariadehuajoya-7	Loreto	Maynas	Napo	Maria de Huajoya	9839223	533377	123
76	Mariadehuajoya-8	Loreto	Maynas	Napo	Maria de Huajoya	9838429	535515	140
77	Mariadehuajoya-9	Loreto	Maynas	Napo	Maria de Huajoya	9841788	535393	135
78	Mariadehuajoya-10	Loreto	Maynas	Napo	Maria de Huajoya	9840811	537164	129
79	Huajoya-1	Loreto	Maynas	Napo	Huajoya	9852750	540889	146
80	Huajoya-2	Loreto	Maynas	Napo	Huajoya	9851987	543454	152
81	Huajoya-3	Loreto	Maynas	Napo	Huajoya	9852140	545255	134
82	Huajoya-4	Loreto	Maynas	Napo	Huajoya	9854918	544828	142
83	Huajoya-5	Loreto	Maynas	Napo	Huajoya	9855834	543179	127
84	Huajoya-6	Loreto	Maynas	Napo	Huajoya	9855010	539087	131
85	Huajoya-7	Loreto	Maynas	Napo	Huajoya	9854949	537744	135
86	Huajoya-8	Loreto	Maynas	Napo	Huajoya	9856109	539912	145
87	Huajoya-9	Loreto	Maynas	Napo	Huajoya	9855651	543576	155
88	Huajoya-10	Loreto	Maynas	Napo	Huajoya	9854430	544858	149
89	Huajoya-11	Loreto	Maynas	Napo	Huajoya	9852873	547362	138
90	Huajoya-12	Loreto	Maynas	Napo	Huajoya	9851040	546660	151