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 inter-poblacional 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 fue 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

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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 Regegorni, 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; Krainovc et al. 2017), reaching 300 tones year⁻¹ in Brazil. In Peru, rosewood forests are located north of the Maraçon - 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; Karik 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; Ekinciap 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; Ekinciap 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. 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, Mayirircay, 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, Alpahuayo and Mairirircay 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 Amazonense 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 × PCR buffer (Thermo Scientific), 2 mM MgCl₂ 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 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    | AGAGAGAGAGAGAGAT | 50 |
| 810    | GAGAGAGAGAGAGAT | 50 |
| 812    | GAGAGAGAGAGAGAA | 50 |
| 814    | CTCTCTCTCTCTCTCA | 50 |
| 815    | CTCTCTCTCTCTCTCG | 52 |
| 817    | CACACACACACACACAA | 50 |
| 818    | CACACACACACACACAG | 52 |
| 819    | GTGTGTGTGTGTGTGA | 50 |
| 826    | ACACACACACACACAC | 52 |
| 834    | AGAGAGAGAGAGAGAYT | 52 |
| 840    | AGAGAGAGAGAGAGAYT | 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 evaluate 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, Mariadehuayoa, 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 (% of total bands) | PB (% of polymorphic bands) | PIC | Ne | I | He | Ht |
|--------|-----------------------|-----------------------------|-----|----|---|----|----|
| 807    | 31                    | 31                          | 100 | 0.765 | 1.338 | 0.319 | 0.484 | 0.310 |
| 810    | 42                    | 39                          | 100 | 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 | 1.451 | 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 |

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
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) in 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.
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

(Wang et al. 2005) and Lindera melissifolia (Walter) Blume (Godt and Hamrick 1996).
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, Mairihehuajoya 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 Mairircay were planted from material originating from the wild population of Tamshiyacu. Mairircay 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 Mairircay-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 Mairircay 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 Mairircay-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.

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**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 | 9566683 | 644419  | 106         |
| 3          | Nanay-3        | Loreto | Alto Nanay | Santa Maria del Nanay | Santa Maria del nanay | 9566689 | 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  | 604982    | 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    | Mana de Huajoya | 9838429 | 536797    | 120          |
| 70         | Mariadehuajoya-2 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9835376 | 537866    | 125          |
| 71         | Mariadehuajoya-3 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9833880 | 535209    | 116          |
| 72         | Mariadehuajoya-4 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9835834 | 531637    | 121          |
| 73         | Mariadehuajoya-5 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9838277 | 528614    | 118          |
| 74         | Mariadehuajoya-6 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9841544 | 530843    | 118          |
| 75         | Mariadehuajoya-7 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9839223 | 533777    | 123          |
| 76         | Mariadehuajoya-8 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9838429 | 535515    | 140          |
| 77         | Mariadehuajoya-9 | Loreto   | Maynas   | Napo    | Mana de Huajoya | 9841788 | 533939    | 135          |
| 78         | Mariadehuajoya-10 | Loreto    | Maynas   | Napo    | Mana 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     | 9854094 | 537744    | 135          |
| 86         | Huajoya-8       | Loreto   | Maynas   | Napo    | Huajoya     | 9856109 | 539912    | 145          |
| 87         | Huajoya-9       | Loreto   | Maynas   | Napo    | Huajoya     | 9856651 | 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          |