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Genetic diversity of *aguaje* (*Mauritia flexuosa*) in Peruvian and Ecuadorian Amazon

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Declaration

I hereby declare that the master thesis entitled "Genetic diversity of *aguaje* (*Mauritia flexuosa*) in Peruvian and Ecuadorian Amazon" is all my own work and information sources I have used have been properly listed in references.

In Prague, May 2020

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Bc. Dita Mervartová

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Abstract

Mauritia flexuosa (*aguaje*) is a dioecious indigenous species from the family *Arecaceae* important for local people and also for Amazon forest as a whole. The specific environments along the banks of large rivers or inside the lowland jungle forest, where water is retained and where nutrients are slowly decomposed, are called *aguajales*. Particularly these swampy areas, where *M. flexuosa* occurs in abundance, provide a refuge for different kinds of fauna and flora. People from the local communities living in the Amazon depend on its nutritive fruits. The fact that it is a dioecious species presents a number of difficulties in cultivation. Therefore, a large part of the production is still obtained from wild populations, usually by cutting the female trees. Unfortunately, this bad management of the fruit collection leads to degradation of *aguajales* and to biodiversity loss.

The main objective of this research was to assess the intra-population and inter-population genetic diversity of *M. flexuosa* in Peruvian and Ecuadorian Amazon by SSR markers. Totally, 145 trees from 15 populations were sampled and successfully analysed using seven polymorphic microsatellite loci. The populations were characterized by high values of genetic diversity and very low levels of inbreeding. The high molecular variance was determined especially among individuals. According to STRUCTURE analysis, no different genetic composition was indicated in the populations. The UPGMA diagram divided populations into two main groups corresponding to the region's isolation; (1) Ecuador and (2) Peru. A very low correlation between geographic and genetic distances was determined using a Mantel test.

The results can be explained by the high level of gene flow along the Amazonian rain forest, where the rivers are used as bio corridors. Also, the human activity through the history of the Amazonian probably has had a significant impact on the *M. flexuosa* distribution. Based on the high genetic diversity, the selection of superior individuals for further breeding can be explored.

Key words: population genetics, genetic variability, microsatellites, biodiversity, *Arecaceae*

Abstrakt

Mauritia flexuosa (*aguaje*) je dvojdomý, hojně rozšířený druh z čeledi *Arecaceae,* jenž má nepostradatelný význam pro Amazonii i tamní obyvatele. Četnější uskupení jedinců této palmy vytváří specifické biocenózy, tzv. *aguajales,* které lemují především břehy mohutných řek. Nacházejí se ale také v depresích nížinných pralesů, kde se zadržuje voda a kde dochází k pomalejšímu rozkladu živin. Mnoho druhů rostlin a živočichů právě v takovém prostředí vyhledává útočiště. Místní lidé zase s oblibou konzumují výživné plody *aguaje,* ovšem dvoudomý charakter rostliny značně komplikuje její plošnější pěstování. Z tohoto důvodu stále převážná část produkce pochází z divokých populací *M. flexuosa*. Bohužel, nešetrný a nadměrný sběr plodů významně přispívá k degradaci *aguajales* a následně i k postupnému snižování biodiverzity.

Hlavním cílem této práce bylo porovnat s pomocí SSR markerů genetickou diverzitu mezi a uvnitř populacemi *M. flexuosa* na území peruánské a ekvádorské Amazonie. S využitím sedmi polymorfních mikrosatelitních lokusů bylo dohromady úspěšně analyzováno 145 vzorků z 15 populací.

Všechny populace se vyznačují vysokou úrovní genetické rozmanitosti a nízkým koeficientem inbreedingu. F statistiky odhalily minimální molekulární variabilitu mezi populacemi a naopak velmi vysokou uvnitř jednotlivců. Dle STRUCTURE analýzy nebyla indikována variabilní struktura populací. Diagram UPGMA rozdělil populace na dvě hlavní skupiny v závislosti na izolaci regionů; (1) Ekvádor a (2) Peru. Mantelovým testem byla stanovena nízká pozitivní korelace mezi geografickou a genetickou vzdáleností.

Výsledky poukazují na nepostradatelnou roli vodních toků na území Amazonského deštného pralesa, kde řeky ve velké míře fungují jako biokoridory a přispívají tak k nepřetržité fluktuaci genů. Také antropogenní činnost v historii Amazonie měla pravděpodobně významný vliv právě na rozšíření druhu *M. flexuosa*. V souvislosti s vysokými hodnotami genetické diverzity je možné vybrat adekvátní jedince k případnému šlechtění.

Klíčová slova: populační genetika, genetická variabilita, mikrosatelity, biodiverzita, *Arecaceae*

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List of abbreviations

AMOVA	Analysis of Molecular Variance
bp	Base Pair
CTAB	Cetyl trimethylammonium bromide
DNA	Deoxyribonucleic Acid
EDTA	Ethylendiaminetetraacetic acid
GPS	Global Positioning System
IIAP	Instituto de Investigaciones de la Amazonía Peruana
ISSR	Inter Simple Sequence Repeats
IUCN	The International Union for Conservation of Nature
LD	Linkage disequilibrium
LnP(D)	Mean Log-likelihood of K
L.f.	*Linnaeus filius*
NaCl	Sodium chloride
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
PVP	Polyvinylpyrrolidone
RAE	Retinol activity equivalents
RPM	Rotation per minute
SERNANP	National Service of Natural Areas Protected by Steate, Peru
SSR	Simple Sequence Repeats
TBE	Tris base, boric acid and EDTA buffer
TRIS-HLC	(Hydroxymethyl)aminomethane hydrochloride
UNU	Universidad Nacional de Ucayali, Peru
UPGMA	Unweighted Pair Group Method with Arithmetic mean
UV	Ultraviolet

1 Introduction

Due to demographic upsurge the natural habitats are gradually being replaced by artificial ones. Spreading out of the human being into highly biodiverse areas is one of the major threats we are facing nowadays. Above all, the challenge in the future will be to adapt crop production to the growing demographic curve, without destroying forested land (FAO, 2018). The deforestation of the Amazon forest and exploitation of natural resources causes large scale environmental and also economic problems (Padoch, 2019). A huge area is transformed into the agricultural crops production and agro-industrial plantations (Kahn et al., 1993). Insomuch as the local farmers are forced due to the population pressure to shorten fallow periods or move on (Mukul et al., 2016). SOFO (2012) notes that in the case of the Amazon the deforestation is generated mainly by commercial agriculture, but also the traditional shifting cultivation contributes to large scale deforestation and to soil degradation afterwards.

Habitat loss is also a serious problem for one of the most commonly found palms in the Peruvian Amazon; *Mauritia flexuosa*. Its fruits generate income and provide employment to many communities. Nevertheless, the high popularity of the species, the excessive pulp consumption and its unsustainable harvesting, consequently lead to threats for the whole biocenosis of *M. flexuosa.* (Pacheco Santos, 2005).

Generally, palms constitute one of the most important ecological components of tropical rainforests worldwide and represent a model group for research of the evolution of those ecosystems (Lasso et al., 2013). An interaction of palms with wildlife also represents a very useful resource for maintenance of indigenous ethnicities throughout its history. Particularly in America, where the interdependence between palms and humans is still in force (Lasso et al., 2013). The *Arecaceae* family has a great economic potential. It is utilized as edible fruit, oil, canned palm, fibres, starch, or constitute a resource that could be used for the genetic improvement of native cultivated species (Kahn et al., 1993).

The main objective of this study was to asses a genetic diversity of *M. flexuosa* in Peruvian and Ecuadorian Amazon and to find a correlation between geographical and genetic distances. The study could be also helpful for the IIAP – National Peruvian Research Institution, realizing many investigations related to Amazonian fruit species.

2 Literature review

2.1 Mauritia flexuosa L.f.

Mauritia flexuosa L.f, is a palm tree native to riparian borders of the Amazonian rainforest. Since there are many Amazonian tribes that use their own language, many local names exist for *M. flexuosa*. For example, in Peru people use the term *aguaje*, but in neighbouring country Ecuador it is already *morete* or *acho*, Colombian name is *cananguchazil*, Venezuelan *moriche* and the Bolivian one *caranday-guazú* or *palma real*. These are only official names of Spanish speaking countries. Other local names include: *buriti* (Brazil), *maurise* (Suriname), *aeta* (Guyana) and for instance *palmier bâche* (French Guyana) (Lasso et al., 2013).

2.1.1 Origin and distribution

Precisely, it is distributed to the North in the Orinoco basin, the Guayanas, Venezuela, Trinidad and Tobago, Brazil and in the Western valleys of the Andean foothills in Bolivia, Colombia, Ecuador and Peru (Rosati et al., 2012). The highest density of *aguaje* is localized into the Peruvian part of Amazon (Figure 1).

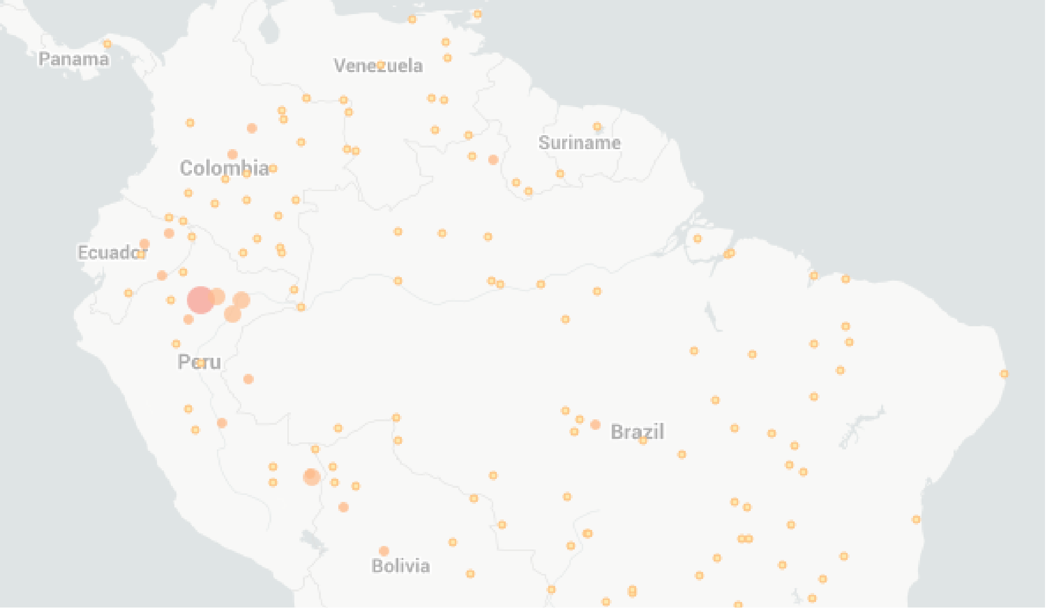


Figure 1. Map of distribution of *Mauritia flexuosa*.

(Source: https://www.gbif.org)

Apparently, the palm diversification started in the half of the Cretaceous period, approximately 100 million years ago. The ancestral biome and the reconstruction area for the whole family *Arecaceae*, confirms the hypothesis that palms diversified in a very similar environment to the current tropical rainforests, although at latitudes further north. The fact stands out that the highest concentration of species of palms is mainly associated with tropical rainforests. Couvreur et al. (2011) assume, that this type of forest has been maintained over time as of its origin in the mid-Cretaceous in the continent called Laurasia. Possibly there, the seeds and fruits of the original species migrated so much, via terrestrial as marine ways, and settled in the current tropical regions of South America, Africa and Southeast Asia, where they formed the same type of forest (Couvreur et al., 2011).

Nowadays, palm trees, including 200 genera and 2,450 species, are distributed in the tropical region, with some species that extend into subtropical areas of both hemispheres (Moraes et al., 2006). However, a few species have been found at latitudes reaching extremal locations in the northern hemisphere at 44 ° N (*Chamaerops humilis*). The southern boundary of palms distribution is over 46 ° S on the Chatham Islands near New Zealand, where only one species *Rhopalostylis sapida* occurs (Lasso et al., 2013).

2.1.2 Taxonomy and morphological description

According to IUCN, the taxonomical classification of Latin names are summarized as follow: Kingdom *Plantae*; Phylum *Tracheophyta*; Class *Liliopsida;* Order: *Arecales*; Family: *Arecaceae*; Genus: *Mauritia*; Species: *Mauritia flexuosa L.f.*

The subfamily *Calamoideae* is characterized by its four genera, *Lepidocaryum, Mauritîa, Mauritiela* and *Raphia* (Uhl and Dransfield, 1987). However, according to Dransfield et al. (2005), the genus *Raphia* belongs to the subtribe *Raphiinae*. The genus *Mauritia* includes only two species*: M. carana* and *M. flexuosa* (Lasso et al. 2013).

The synonyms of *Mauritia flexuosa* L.f. are: *Mauritia flexuosa var. venezuelana Steyerm. Mauritia minor Burret, Mauritia sagus Schult. & Schult.f., Mauritia setigera Griseb. & H.Wendl***,** *Mauritia sphaerocarpa Burret, Mauritia vinifera Mart.* and *Saguerus americanus H.Wendl.*

Palms are monocotyledonous plants with a specific shape. Although they have the same organs as dicotyledons do – roots, stem, leaves, flowers and fruits – the structure can be very different as well as the appearance itself (Lasso et al., 2013).

In the case of *M. flexuosa* (Figure 2), the plant can reach a height of 35 meters in its natural state (Brokamp, 2013). The stem is straight, smooth and cylindrical, with a diameter up to 60 centimetres (Gonzalez et al., 2011).

Figure 2. *Mauritia flexuosa* L.f.

Source: https://www.behance.net/gallery/34657057/Mauritia-flexuosa-Palma-Moriche

Aguaje is a dioecious palm, having separate inflorescences in female (Figure 3) and male (Figure 4) individuals. Each one is from 2 to 3 m long. In natural populations, it can reach 50 cm in diameter (Gonzalez et al., 2011).

The fruit is a drupe with an ellipsoid oval shape, covered by reddish cornea scales (Figure 5). It measures up to 7 cm long and the diameter reaches about 5 cm (Delgado et al., 2007). The fruit is composed of approximately 20% skin, 10–20% mesocarp and 15–20% endocarp and it contains from one to three seeds inside 40–45% (Almeida and Silva, 1994).

An inter-foliar space is always superior to 10 cm (Delgado et al., 2007). The juvenile leaf is rolled inside the foliar crown for 105 days. At this stage, the juvenile leaf of *M. flexuosa* is tougher and stiffer than whatever leaf of the dicotyledonous. That is caused by the higher fibre content per area. Both attributes are considered as an adaptation against insects. It is important for palms to be able to keep a reduced number of leaves at the top of the stem for a longer time. *Mauritia flexuosa* keeps only up to 16 of them (Gonzalez et al., 2011). The 8 to 25 leaves per plant measure up to 6 m long. Moreover, each leaf is divided from 120 to 236 segments, and the petiole measures up to 4 m long (Delgado et al., 2007).

A very interesting part of the plant are the special roots, adapted to life in the aquatic environment. Aerial roots are typical in the case of mangroves. Except *Mauritia flexuosa* and many other palms, according to Kahn et al. (1993), the species *Euterpe precatoria* (Figure 6) is another example of the pneumatophores development. The root system allows assimilating nutrients in the anaerobic conditions. It is characterized by the development of pneumatophores, respiratory roots of a vertical ageotropic growth (Figure 7). Part of the system is submerged in water and produces many tiny roots. The other part develops a special rings made of the aerenchyma, which are called "pneumatozones". The aerenchyma captures oxygen necessary for function of the submerged roots (Kahn et al., 1993).

Figure 3. Female adult plant of *M. flexuosa* and its inflorescence.

Figure 4. Male adult plant of *M. flexuosa* and its inflorescence.

Figure 5. *Shambo* fruit of *aguaje* with significant reddish cornea scales.

Figure 6. Aerial roots of *Euterpe precatoria* (*huasaí*).

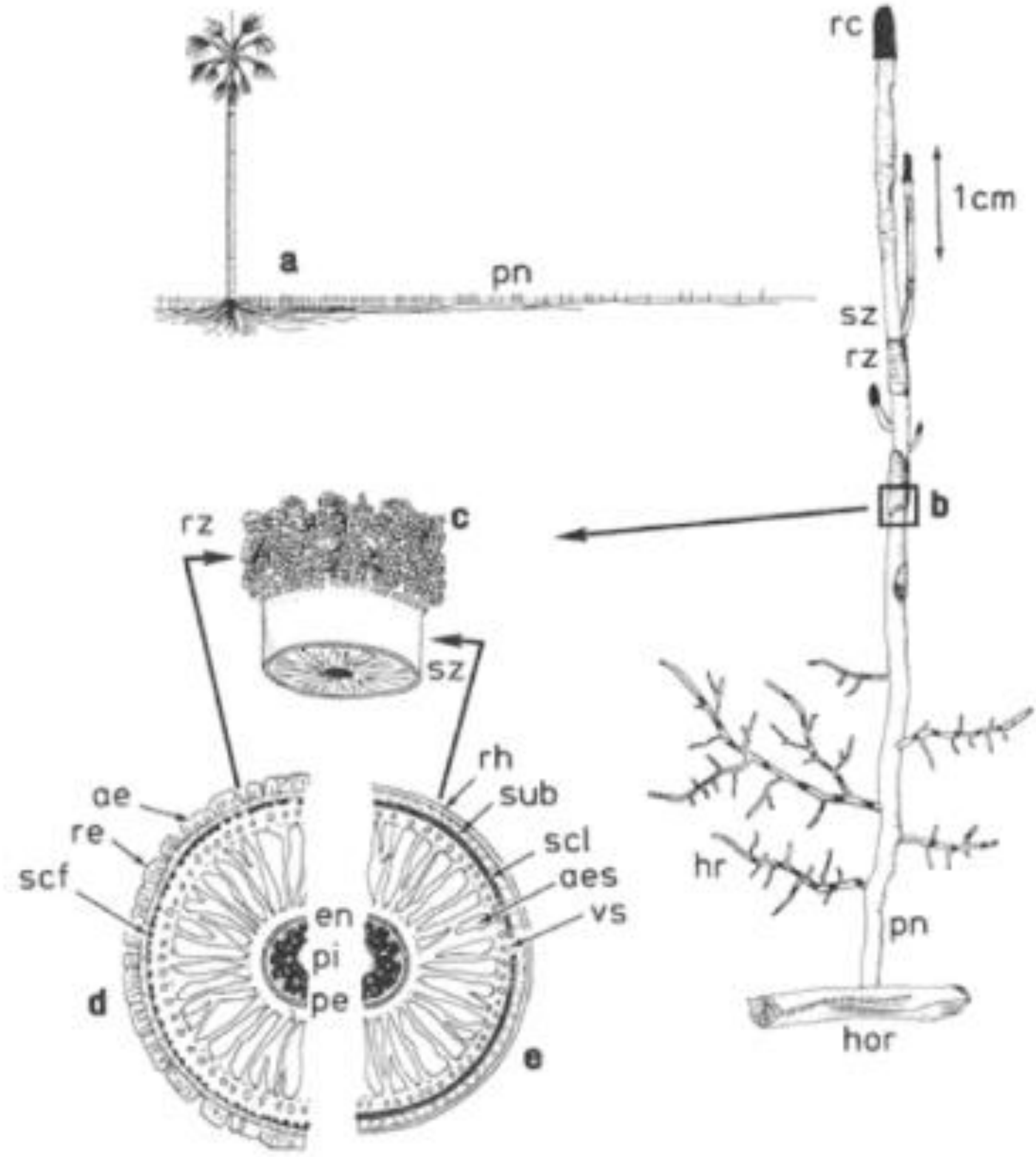


Figure 7. Respiratory roots of *Mauritia flexuosa*.

a) Root system of the palm tree; b) Pneumophore (pn) of ageotropic vertical growth with one part in the water, which produces many roots, and an aerial part with pneumatozones (oz); c): detail of a pneumatozone d) Rough zone and e) Smooth zone.

Ae aerenchyma; aes aeriferous sapce; en endodermis; hr hair roots; hor horizontal roots; pe pericycle; pi pith; pn pneumatophores; rc root carp; re remnant of rhizodermis; rh rhizodermis; rz rough zone; scf sclerifield fibers; scl sclerenchyma; sub suberous sclerified fibers; sz smooth zone; vs vessels. According to Kahn et al. (1993).

2.1.3 Ecological/Environmental requirements

Naturally *M. flexuosa* occurs in *aguajales.*, which are permanently or seasonally flooded swamps and depressions along great rivers throughout the Amazon, where the palms live in pure formations or mixed with other plants and diverse trees. In Brazil, the *aguaje* palm is considered as an indicator of a water source nearby. They play several important ecological roles, especially in terms of providing habitat and food resources for wildlife (Brokamp, 2013). Habitat characteristics (geomorphology, water regime) are results of an evolution of the geological origin of the landscape. They are completely different in the Orinoco plains, the Guyana and Brazilian shields, and within the Amazon. In the Amazon there are even differences that are determined by the hydrological regime, as occurs in the *aguajales.* of the Orinoco delta and in the uplands (Lasso et al., 2013). However, it also grows in a well-drained soils and in general, the species is suitable for cultivation in various soil conditions in the Amazonian basin (Alvarado & Bardales, 2019). These differences, given by habitat conditions, allow classification of *M. flexuosa* according to the landscape composition and structure. *Aguajales* of the Ecuadorian an Peruvian Amazon could be divided into three groups (Lasso et al., 2013):

I. On the riverbanks with very little sediment layer, on the planes and alluvial terraces, the latter with lateral contribution of groundwater. It is an environment as the seasonally or permanently flooded by rivers or streams with the typical white and black colours. This ecosystem is called *várzea* forests or *igapo* (Figure 8).

II. Sites far away from the great rivers, dependent only on the rainwater (greater than 2,000 mm per year). They are usually situated in a concave shape of the relief that is capable to retain water. Humid and warm weather, organic and peat soils, are significant for this ecosystem (Figure 9 d).

III. Extensive plains with a negligible layer of the peat soils, with no influence of the tides of the rivers and streams nearby. The small depressions, where the water is accumulated, alternates with a relief (Figure 9 b). These *aguajales* can be dense and mixed with primary forest (Lasso et al., 2013).

Figure 8. *Igapo* is a special kind of the Ecuadorian ecosystem.

The environmental conditions for development of *aguajales* are following: a maximum annual average temperature of 25.1 °C and minimum annual average of 17.2 °C; maximum annual precipitation per year of 3,419 mm and minimum annual average of 936 mm. The altitude varies from 50 to 850 m above sea level (Delgado et al., 2007).

This habitat supports permanent flooding of its root system, where, on unorganized soils, the palms grow from organic matter little decomposed in water (Kahn et al., 1993). The stem is able to remain immersed in water, without causing damage to the plant for long periods. The explanation is the presence of pneumatophores permitting growth in anaerobic conditions (Delgado et al., 2007). Moreover, a significant amount of carbon is stored in the waterlogged soils of *aguajales* (Vegas-Vilarúbia et al., 2010). The soils of the mentioned ecosystem contain approximately 532 - 632 t of carbon per hectare. Five million hectares of Peruvian tropical forests are constituted by swampy forests out of which approximately 700,000 hectares are pure *aguaje* forests distributed along the Amazon (Vegas-Vilarúbia et al., 2010).

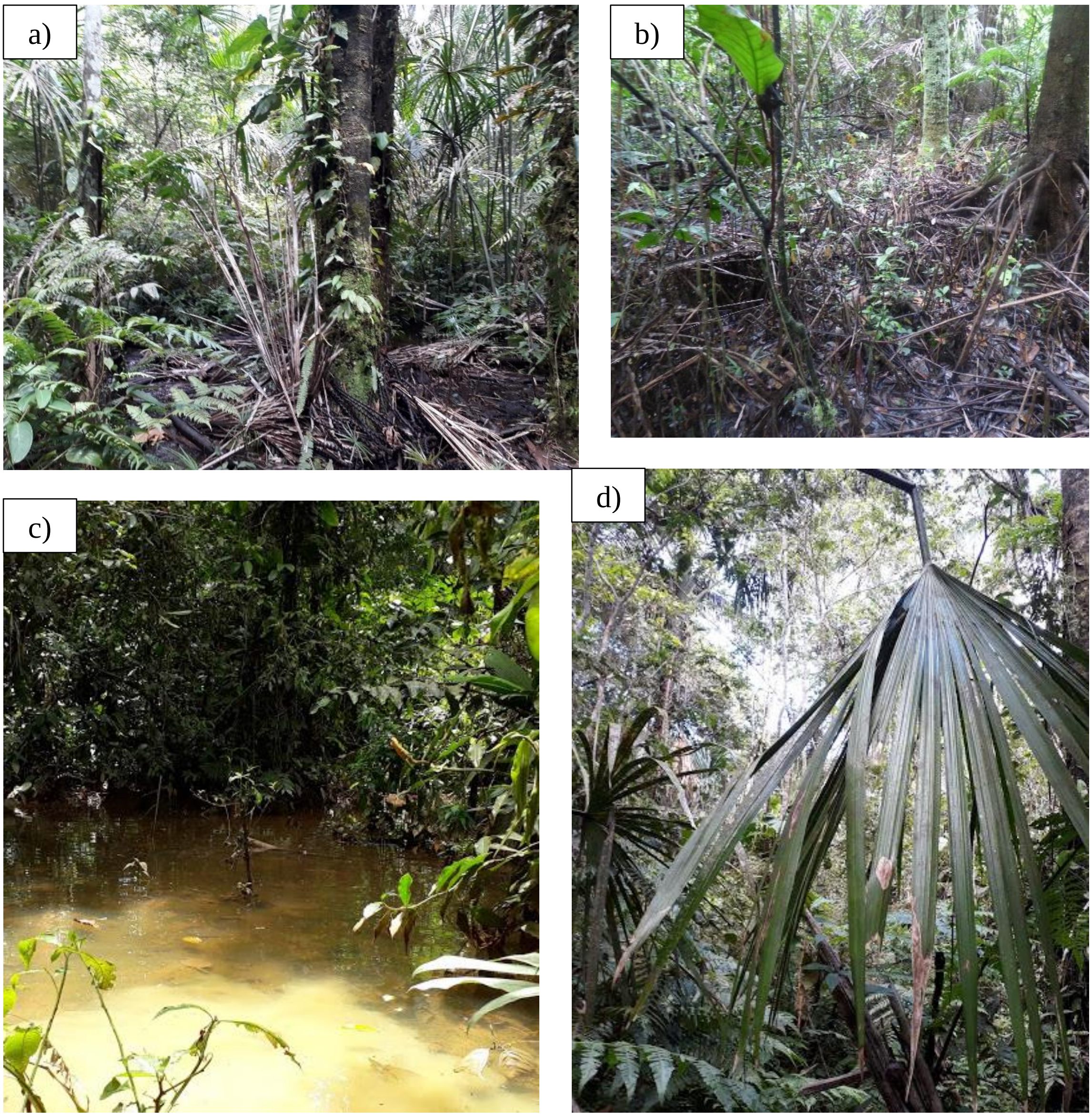


Figure 9. Examples of several natural *aguajales*.

a) and c) *Moretes* in the Cuyabeno Wildlife Reserve b) *Aguajal* close to the Cumaceba river d) *Aguajal* along the Laguna Venecia in Tarapoto.

Aguaje forms almost monospecific dense populations associated mainly with two other species*: Euterpe precetoria* and *Jesenia batua* (Kahn et al., 1993). On the other hand, *M. flexuosa* can form also a relatively continuous canopy, from a dense swamp palms, swampy grasslands with isolated individuals of *M. flexuosa* in small groups, constituting in of the various stages of life of the palm: juveniles (Figure 10), sub adults and already reproductive adults (Lasso et al., 2013).

Figure 10. A few weeks old juvenile plant of *aguaje*.

2.1.4 Ethnobotany and uses

Many different ethnic groups, with their habits, art and knowledge, transform the Amazon into a natural pharmacy known for its incredible variety of medicinal and magical plants (Jauregui et al., 2011). Traditional medicine is one of the most important ancestral memory of the Amazonian people. Various ethnic groups use a large number of plant species to cure their diseases and syndromes. Peruvian Amazonian flora is one of the largest collections of phytotherapeutic resources. The healing properties of Peruvian medicinal plants have attracted the attention of foreigners since the first years of the encounter of native people with Europeans (Mejia and Rengifo, 2000).

Over 60 plant species found in . are used for construction material, food, medicine, cultural ceremonies and they seem to form an important habitat for more than 20 species of mammals and birds (Gilmore et al., 2013). Several species of edible beetle larvae are also harvested from the trunks of the palms *M. flexuosa* and *Oenocarpus bataua* (Endress et al., 2013). For instance *suri*, the larva of the beetle *Rynchophorus palmarum,* which is an important food in rural areas (Gilmore et al., 2013).

Fruit pulp is obtained from the mature *aguaje* from which the *aguajina* (drink called "amazon milk") is prepared (Ruiz et al., 2001). In Iquitos (Peru), the inhabitants recognize according to the colour, flavour, size and thickness of the pulp, several varieties of *M. flexuosa*. *Carnoso* is typical thanks to the reddish superficial part of the thick and tasty pulp (Gonzalez et al., 2011). *Shambo* has reddish whole pulp and it is very preferred for its pleasant taste. That one has the highest price in the local market and it is the preferred variety for direct consumption. On the other hand, *shambo* rusts rapidly and the pulp becomes blackish, so making processed products lose its quality. *Ponguete* (yellow mesocarp) can be divided into yellow-yellow (does not change the pulp colour several minutes after the scales are taken down and it is used for *aguajina* preparation), pale yellow (*posheco*) and dark yellow groups (Delgado et al., 2007).

The leaves and stipes are used to build houses, to make ropes, hats, mats, brooms, purses, key holders etc. (Kahn et al., 1993). From seeds of *M. flexuosa* people get the material, used for the preparation of household utensils, buttons and different handcrafts (Ruiz et al., 2001). An unfermented liquid extracted from the pith is used by indigenous community Kalunga as "wine" against diabetes. They use the seeds from immature fruits to make a powder, helping women during childbirth. Roots are a medicine against rheumatism and squeezed petiole juice is used to cure snake bites. Oil extracted from the mesocarp is used against pneumonia, respiratory problems, asthma, coughs, influenza, fever and heart problems (Martins et al., 2012). Probably in the future *aguaje* could have significance as a source of lignocellulosic material, raw material in the pulp and paper industry from the stubs and petioles (Rivadeneyra1 et al., 1974).

Aguaje is considered as one of the most mythical plants for Amazonian tribes. The symbology, legends, stories and spiritual value have another important aspect for them. For example, they believe that *aguaje* palms were originated by creator beings from the estuary of the Great Amazon River. Another legend is about *yakumama*, a huge snake living in *aguajales* and rivers. The shamans are responsible for the welfare of the palms from the point of view of their knowledge and ceremonies (Lasso et al., 2013).

2.1.5 Nutritional value

The fruit is consumed directly or processed and according to some studies, it is considered as one of the richest natural sources of beta carotene (36 mg/100g of pulp) (Pacheco Santos, 2005). In comparison, the most popular source of β-carotene, orange carrot, contains 10 mg/100g (Bogacz-Radomska and Harasym, 2018). The oil of *M. flexuosa* also contains high levels of monounsaturated fatty acids, carotenoids and vitamin E (tocopherols). Moreover, a high antioxidant capacity is present (Aquino et al., 2012). The pulp contains 75.7% of oleic acid, 18.9% of palmitic acid, 2.1% of linoleic acid, 1.7% of arachidonic acid, 0.3% of palmitoleic and 1.3% of stearic acid (Darnet et al., 2011). According to Aquino et al. (2012) presence of myristic acid was reported by gas chromatography.

There are various benefits attributed to β-carotene, such as reducing of the risk of Alzheimer's disease, prevention of stomach cancer. A low concentration of β-carotene is associated with a risk of heart attack, and oral administration of this carotenoid provides a photo protective effect. It is claimed that its concomitant use with a topical sunscreen provides greater protection (Rosati et al., 2012). Animal studies showed an extremely high bioavailability, probably due to its oily composition (Pacheco Santos, 2005). Moreover, it contains various secondary metabolites serving to the pharmaceutical industry (Kahn et al., 1993). Sometimes it is believed that *aguaje* pulp contains a large amount of estrogen, and as such is not recommended for males (Rainforest Conservation Fund, 2003). The fruit is the main source of nutrients for Amazonian terrestrial animals, such as tapir (*Tapirus terrestris*) and white-lipped peccary (*Tayassu pecarie*).

2.1.6 Cultivation and destructive harvesting

M. flexuosa is a palm in the domestication process (Delgado et al., 2007). It is a dioecious plant species and until the first blooming there are no morphological differences between the male and female plant (Gonzalez et al., 2011). The germination period depends on the seed condition and its physiological development, the nursery humidity, the substratum and the temperature. In the nursery, the registered maximum germination of 88% was in 60 days and a minimum germination of 9% was in 61 days (Delgado et al., 2007). Flowering and fructification can happen during the whole year. Usually, the fruit production of *aguaje* in the Peruvian Amazon starts at the age about 8 years, but there are various reports noting about the starting of the fruit production at the age of 4 to 11 years (Gonzalez et al., 2011). Until then it is impossible to discern the sex of the plant, when the plant reaches a height of approximately 7 m. This fact complicates the cultivation of *aguaje* (Delgado et al., 2007). Although only the female plant produces fruits, several mature male palms should be kept in the plantation for pollination and maintenance of a good fruit production (Rainforest Conservation Fund, 2003).

The process of pollination, in the case of *aguaje*, is still not clear. Opened flowers emit a strong and pleasant fragrance that attracts an insect. Nevertheless, studies focused on *Mauritia flexuosa* and its pollination claimed that more opinions exist. In summary, due to the mentioned fragrance, the insect pollination was considered as the primary one. However, based on the recent research made by Khorsand Rosa and Koptur (2013), except the opened areas, the wind plays the most important role in the case of pollination of *M. flexuosa*. Short female plants are able to capture pollen more efficiently than tall ones. On the other hand, large male plants should disperse pollen more effectively than short ones do, according to the interaction between wind aerodynamics and height (Levin and Kerster 1974). In the study of Khorsand Rosa and Koptur (2013) focused on *M. flexuosa,* the relationship of height and sexual dimorphism were not as significant. The higher individuals are typical for forested habitats, probably due to the light competition and also to the wind pollen dispersal. The seed dispersal is provided by various kinds of mammals (Acevedo- Quintero and Zamora- Abrego, 2016). Also, an important fact is that the fruit is able to float in water (Moegenburg, 2002).

Staminate and pistillate flowers produce a sweet scent, stronger in male plants. There is no difference in the scent intensity between the day and night. According to Knudsen et al. (2001), in flowers of *M. flexuosa,* were found 44 volatile compounds, specifically fatty acid derivatives. Both, pistillate and staminate flowers, open continuously with an individual flower lasting of five days and a single inflorescence less than only one week (Rosa and Koptur, 2013). Thus, ambophily could be the appropriate term describing the process of *aguaje* pollination and could be a transitional state from entomophily to anemophily (Culley et al., 2002). Each female palm produces around eight inflorescences per year and each inflorescence produces approximately 900 fruits

(Delgado et al., 2007), but it can reach up to 2,000 fruits (Almeida and Silva, 1994). On well managed plantations, the production could be up to 25 tonnes of fruit per hectare (Shanley et al., 2011).

However, the over popularity of *aguaje* on the local markets attract people to harvest the fruit from wild populations. In Iquitos, for example, consumption is estimated to be 20 tonnes of fruits per day. This is an equivalent to the harvest of about two hundred palm trees (Alvarado and Bardales, 2019). A production of an *aguaje* plant, reaching up 30 meters in its natural environment, it is approximately 152 kg of fruit per plant (FAO, 2020). Recently there has been an increasing shift to growing *M. flexuosa* in small home gardens (Figure 11), however, most fruit of *aguaje* has been harvested from wild stands (Holm et al., 2008). People cut down especially the huge female plants to obtain the fruit more easily. Therefore, this species is endangered from ecological, but also genetic point of view. Simultaneously, there exists a correlation between the local communities and *M. flexuosa*, because many of them are economically dependent on the *aguaje* (Aspajo et al., 2008).

Figure 11. *M. flexuosa* cultivated in a home garden.

Therefore, to supply the demand of the local market in Iquitos, more than 6,000 single female palms of *M. flexuosa* must be harvested (usually cut down) monthly, and as much as 73,000 annually. During the dry season, the harvesting is very difficult. The workers have to carry sacks of the weight of 40 kilograms. Otherwise, during the rainy season, when the forest is covered by water, the natives get to the plants by small crafts, allowing them to harvest and transport major amount of fruits (Delgado et al., 2007).

Especially in rural communities, people still harvest the fruits by destructive practices. Therefore, as adult female trees are felled, the fruit collection causes also the degradation of *aguajales*. The number of female trees has been reduced. Certainly, cutting down the plant for the harvest of the fruit considerably reduces the livelihood of some animals, which are forced to migrate as a result of food shortages (Penn, 2008). This leads to severe degradation of the *aguajales* as an important natural ecosystem, but also to genetic deterioration of the whole species as the most valuable palms are disappearing.

Reported by Srinivas et al. (2016), the oil palm monoculture leads to biodiversity loss along the river Ucayali. During an experiment using mist nets in the previous habitats, it refers, that the mentioned crop provides territory to less than 5% of avifauna species than the forest does. Specifically, in the forest there were captured 159 birds of 44 different species and on the palm oil plantation there were captured only 99 birds of 19 species. According to the continuous expansion of the palm oil plantations in general, the loss of the bird species diversity connected to the biodiversity in the area could be expected. Extensive and largescale monoculture of *M. flexuosa* cultivation could also have consequently a similar negative effect on the biodiversity like the oil palm. That is the reason why it is necessary to think about the ecological way of yield increasing of *aguaje*.

2.1.7 Sustainable management of *M. flexuosa*

One of the possibilities to reduce the pressure on the natural populations of *aguaje* is the establishment of plantations with selection of commercial characteristics specific to fruit quality. It should be focused on solving of various problems in the production, environmental as well as social. Fruit plantations are considered a way to increase supply of raw material for the industry, and to recover deforested areas with new plant cover.

Another consequence is a greater uptake of water and improvement of environmental storage services and carbon sequestration. In addition, plantations generate income for producers, who manage areas close to their homes, minimizing distances for the harvest of the fruits, and thus promoting the development of regional agronomy (Alvarado and Bardales, 2019).

But there is another option. In communities in the basin of the river Marañón, people cultivate *aguaje* in pure plantations or associated with other fruit trees or with different animals (Bejerano et al., 2002). In the Loreto department, there exists the dwarfish form of *aguaje* called *enano*. But it has not been found in natural conditions. This palm has a thick stem and the internodes are less than 6 cm long. At the age of 5 years the fructification starts. Probably it is an adaptation to periodical seasonal floods and to opened swampy areas. Given these facts, the *enano* is recommended for monospecific cultivation or for cultivation in agroforestry systems (Delgado et al., 2007). Promotion to grow *aguaje* on farms or the promotion of agroforestry systems (Figure 12) would also improve the current situation (Delgado et al., 2007). *M. flexuosa* should be kept free of weeds and planted at least eight meters apart in full sun. It tolerates some flooding and can be mixed with other species (Rainforest Conservation Fund, 2003).

Figure 12. Silvopastoral system applied at the plantation of *aguaje*.

Protected areas and sustainable . bring also more income to people. Consideration of these various aspects lead researchers to focus on the genetics, phenology and the information clarifying the origin of *M. flexuosa* (Endress et al., 2013).

2.1.8 Pest and diseases

The main pest is *Eupalamides cyparissias Fab.* (*Lepidoptera: Castniidae).* It is a robust moth with measures up to 180 mm of wing expansion. The white larva damages the peduncle, rachis, rachillae and also petiole by building tunnels of 3.5 cm in diameter × 2.5 m long which interrupt the flow of water and nutrients and in general cause debility of the inflorescence, consequently leading to loss of flowers and fruits. If the infestation occurs in the advanced age stages, the fruit loss is partial. If it appears at the early stage, all fruits and flowers fall (Delgado et al., 2007).

2.1.9 Genetic diversity of *M. flexuosa*

It is more and more evident that people living along rivers nowadays, but also in pre-Colombian times, have impacted significantly the genetic forest patterns (Piperno et al., 2015) and probably subsequently also the genetic structure of individual species. The importance of the river flows, its direction and other hydrological factors to the genetic structure of species population, is still unknown (Sander et al., 2018). There is lack of information about *M. flexuosa* domestication. Nevertheless, the wide usage of the species by indigenous communities along the river basins could reflect the simple domestication process (Sander et al., 2018).

High genetic diversity is beneficial for a species' propagation, conservation and also domestication. According to Aspajo (2008), the area of origin of *aguaje* could be situated in the Peruvian Amazon. More detailed determination is reported by Villachicha (1996), who estimated Ucamara depression as one of the centres of origin for *M. flexuosa*. The area is characterised by huge and extended *aguajales* with high morphological diversity. The overconsumption and excessive fruit harvesting can cause ecological damages, while the deforestation of the Peruvian Amazon and the exploitation of natural resources could lead to irreversible losses of genetic variability across the populations of *aguaje* (Gomes et al., 2011). The above mentioned problems in a combination with artisanal gold mining, occurring along the rivers in the Peruvian and Brazilian Amazon, could reduce the gene flow in naturally fragmented populations (Federman et al., 2012). Thus, plant species depending on animal-mediated seed dispersal, are forced due to different barriers to the nearest-neighbour mating. Everything leads consequently to decrease of genetic diversity (Gomes et al., 2011).

Especially the tropics are characterized by high rates of landscape fragmentation and various barriers to genetic exchanges. The prediction of population's ability of adaptation to changing environmental condition is very important for the *M. flexuosa (*Federman et al., 2014). There may be genetic variants capable of adapting to new adverse natural conditions, such as shortening of the rainy periods and higher temperature during the dry periods.

Related to the existing microsatellite loci for *M. flexuosa*, Federman et al. (2012) isolated 13 of them (Table 1) from a single population in Estrada do Amapa (Brazil). In summary, the loci were tested on 25 individuals and the size varied from 180 to 291 bp. All loci were in Hardy-Weinberg equilibrium (HWE), with the expected heterozygosity ranging between 0.582 and 0.918. Number of alleles ranged from 6 to 15.

According to Federman et al. (2014), no significant differences were observed between expected and observed heterozygosities in any of the sampled collection sites of *M. flexuosa*. Also the F_{IS} values were not significant. The previous genetic research of the palm species *M. flexuosa* using ISSR markers, indicated high values of genetic diversity in comparison to other aquatic species (Gomes et al., 2011).

Huge amount of compounds involving in the biosynthesis of vitamin A is also an interesting for biotechnological industry (Quintanilla et al., 2014). The genetic diversity and genetic distance allows to the foundations for the development of programs of genetic improvement of plants (Meire et al., 2008). Knowledge of the genetic diversity of *M. flexuosa* could help consequently in its conservation and sustainable management development (Quintanilla et al., 2014).

Table 1. 13 isolated microsatellite loci for *Mauritia flexuosa* by Federman (2012).

Locus	Primer sequence (5’-3’)	T_A (°C)	Size (bp)
*Mf*04	F: CCACGGGGCTGTCATATTTC	54	224-260
	R: AGGAAAACGCAAGAAGTGC		
Mf13	F: TTACAAGCGACCCCTCGTC	57	230-264
	R: CGTCGAATAGGGTTTCAGTGG		
Mf14	F: TAGGTCCTGCTTCTGTGCC	54	233-275
	R: TGGATCCGGTCCGTTGATAG		
Mf17	F: GACAGCTTGTCATCCTCGC	54	210-232
	R: TTCCATCCCAGTTCTCCCC		
Mf19	F: AGCCACGTGACACTCTACC	57	239-261
	R: CTATAGGAACCGGCCACCTG		
Mf22	F: GCATGGTGTAGCTCGTATCTG	57	226-276
	R: CGCACCATACTTGGCTTGC		
Mf25	F: CCCCATTTTCCAATTTGATGCG	54	199-225
	R: TGGATGTTCAGTTTGGATGCC		
Mf34	F: GGACAGTTGCCTGTCTTGC	57	180-222
	R: CAAAGCTAGCACAACCTGGG		
Mf24	F: TCACATTAGTAGTCAAGGGTAGC	54	189-215
	R: GGGTGTTAAGCATTCGGGC		
Mf28	F: TCCCACACTCTCTTGCCAC	57	184-200
	R: TGAGGGCTGCGTTATGGTC		
Mf30	F: GAGGGGAGCTTCCTTGCTG	57	231-245
	R: ATTGGCGAAGGTCCAGGG		
Mf31	F: GCFCTAGAAGCATGATCAACC	54	225-259
	R: TCTCAGCCATCATATTCAGTTATCTTC		
Mf33	F: TGCCGCATTTAGGCTTTGG	57	215-229
	R: GGCCGGCGATTTATAAACGG		

2.2 Characterization of the study regions

2.2.1 Ucayali

The region Ucayali is located in the central part of the Peruvian Amazon (Figure 13), along the floodplain of Ucayali river. It is the fifth largest region in Peru, covering 102,410 km^2 (7.9% of the Peruvian territory). Also, it is the second largest Amazonian region in the country, with a still increasing population of 432,159. In the year 2007, approximately 77% of the inhabitants were concentrated in the city of Pucallpa (GOREU, 2008). About 12% of the total population consist of indigenous people, distributed among 296 native communities and 11 ethnic groups. Those communities belong to two linguistic families: the Panos and Arawakas (Jauregui et al., 2011). Ucayali basin is called as low jungle (*selva baja*).

Pucallpa is a constantly developing city lying on the river Ucayali. The typical activities of its inhabitants are logging valuable timber from Amazon and small-scale shifting cultivation leading to deforestation of the area. The biodiversity decreases every year because of the unsustainable utilization of natural resources and establishing crops in the place of primary and secondary forests.

Figure 13. Peru´s regions.

Source: https://commons.wikimedia.org/

All of the sampled Peruvian regions have a tropical climate (Figure 14). During most months of the year, there is significant rainfall in Pucallpa. The driest month is July, with 49 mm of precipitation. The greatest amount of precipitation occurs in March, with an average of 217 mm. The average annual temperature is 26.4 °C and the precipitation is about 1 667 mm. The warmest month of the year is December with an average of 27.1 °C and the coldest one is June, with 25.3 °C (Climate-data, 2020).

2.2.2 Loreto

Loreto is the largest region in the country, nevertheless, sparsely populated. It is characterized by the dense vegetation of primary and secondary forests along the Amazonian basin, where the low jungle dominates. This kind of jungle is important because it belongs to the least altered forests on the planet. Usually, it is formed by mosaic ecosystems connected in its origin with the Andes mountain range. There, the streams and various rivulets consequently give rise to the spectacular Amazon river, which is constituted close to the city Iquitos by the confluence of two huge rivers, Marañón and Ucayali (Alvarez and Shany, 2012). Iquitos has a significant amount of rainfall during the year, with the annual precipitation of 2,857 mm. The driest month is August, with 178 mm of precipitation. With an average of 295 mm, the highest rainfall is in March. The average annual temperature is 26.4 °C. The highest one is in October (26.9 °C) and the lowest in July (25.4 °C) (Climate-data, 2020).

The impact on the ecosystem that Amazonian native people had was minimal compared to starring immigration (mainly European), which has been particularly intense in the last two centuries. The occupation of the Amazonian forest is developed in a consequence with the extractive interventions due to international trade demands and to national policies, which have guided the intervention on the basis of the extraction of natural resources and colonization for agricultural purposes. It has a social and ecological impacts, for example: i) forest skimming by selective logging, and deforestation focused on riverside forests and roads annexes; ii) local extinction or threat of extinction of species such as for example: *Hevea brasilensis, Swietenia macrophyllia, Cedrela odorata, Aniba rosaeodora, Mauritia fluexuosa, Podocnemis expansa, Trichechus inunguis* and *Arapaima gigas*; iii) water pollution caused by the extraction of oil, gold and coca production; iv) 80% of the population with underemployment or self-employment, a sector characterized by a high dependence on extractive activities in the rural area and a very low level of income in the urban area; v) low education level; vi) chronic malnutrition in 56% of children between 5 and 6 years; vi) recessed regional economy, when the current productive level reaches 70% of what was recorded 20 years ago, when the population has doubled in the same period (Fernández, 2004).

2.2.3 San Martín

The San Martin region is situated in the north-eastern Peru and compose a central part of the Tropical Andes Biodiversity hotspot, considered as one of the highest conservation priorities worldwide (Alvarez and Shany, 2012). The capital of the region is Tarapoto, with an annual temperature of 25 °C and the annual precipitation about 1,188 mm. The driest month is July, with 61 mm of rainfall. The precipitation reaches its peak in March, with an average of 146 mm. The highest temperatures are in January, around 25.7 °C. 24.1 °C in an average temperature in July, the coldest month of the year (Climate-data, 2020).

2.2.4 Huánuco

The characteristic mountain known as "Sleeping Beauty" adorns the horizon of a nice town Tingo María. Also, it is a part of a National park with an area of 4,777 hectares (SERNANP, 2020). The presence of 76 species of mammals, 62 species, 33 species of amphibians, 29 species of reptiles and 291 species of birds were observed (Tamashiro, 2015). Peru occupies the first places in the world in terms of the number of butterfly species occurring. According to the previous study, in Tingo María National Park, there are more than 320 species of them.

There is significant rainfall throughout the year in Tingo Maria. Even the driest month is rainy. The annual average temperature is about 24.4 °C with a precipitation of 3,042 mm. The least amount of rainfall occurs in August (112 mm per month), while in the January it is 418 mm. The warmest month is November, around 24.9 °C, while the coldest one of the year is July (24.1 °C) (Climate-data, 2020).

2.2.5 Cuyabeno Wildlife Reserve in Ecuador

The Cuyabeno Wildlife Reserve is located in the provinces Orellana and Sucumbíos, in the northeast of the Amazon region of Ecuador. With an area of 590,112 ha, it is one of the most important biodiversity hotspots on the planet along with the Yasuni National Park. The reserve was decreed in 1979 in the context of the creation of a system of protected natural areas in Ecuador, based on an FAO study in 1976, initially with a dimension of 155,000 ha (Putney et al., 1976). Due to its large area and geographical location, Cuyabeno is a strategic protected area conformed by the country's largest lake system. A dark colour of the water is caused by a presence of certain substances from the decomposition of plant material that falls down. An example is the Cuyabeno and Lagartococha rivers. The flooded areas, known as igapos, can remain flooded for several months of a year (SNAP, 2020). Also, it is considered as an important Pleistocene refuge. 184 species of fish, 81 species of amphibians, 54 species of reptiles, 500 species of birds, 165 species of mammals, 473 tree species and 1,400 plant species are recorded in the Cuyabeno Wildlife Reserve. This protected area also has a great cultural value, due to 11 indigenous communities living in, for example, the Siona, Secoya and Cofán. Kichwa and Shuar have inhabited this zone since ancient times (Rivadeneira, 2007).

According to the IUCN Protected area categories system, the Cuyabeno Wildlife Production Reserve belongs to the VI category (Protected area with sustainable use of natural resources). "*These are protected areas that conserve ecosystems and habitats, together with associated cultural values and traditional natural resource management systems. They are generally large, with most of the area in a natural condition, where a proportion is under sustainable natural resource management and where low-level non-industrial use of natural resources compatible with nature conservation is seen as one of the main aims of the area*" (IUCN, 2020).

The tropical climate in Cuyabeno is characterized by a lot of rain, even in the driest month (Figure 15). The average annual temperature is 25.6 °C in Cuyabeno. In a year, the precipitation is 2,943 mm. November is the warmest month of the year. The average temperature in November is 26.1 °C. The lowest temperatures of the year occur in July, when it is around 24.5 °C (Climate-Data, 2020).

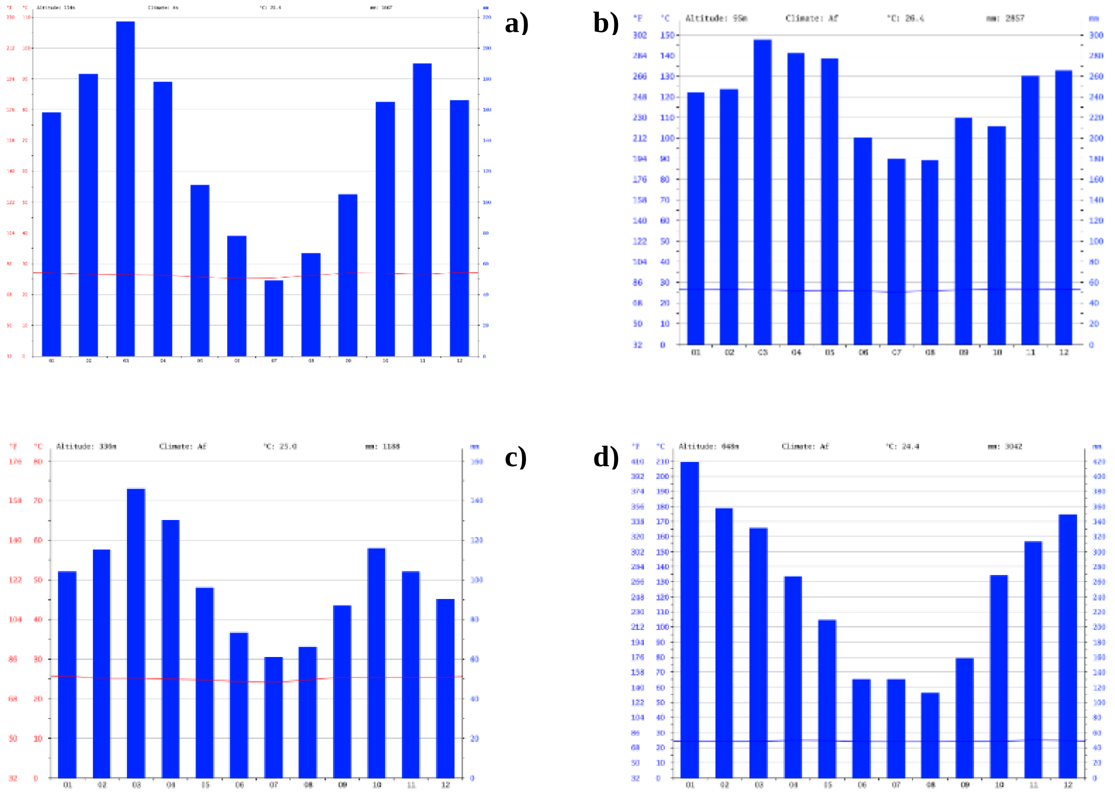


Figure 14. Climate diagrams of four distinct Peruvian regions. Pucallpa - Ucayali (A); Iquitos - Loreto (B); Tarapoto - San Martín (C); Tingo María - Huánuco (D).

(Source: https://de.climate-data.org).

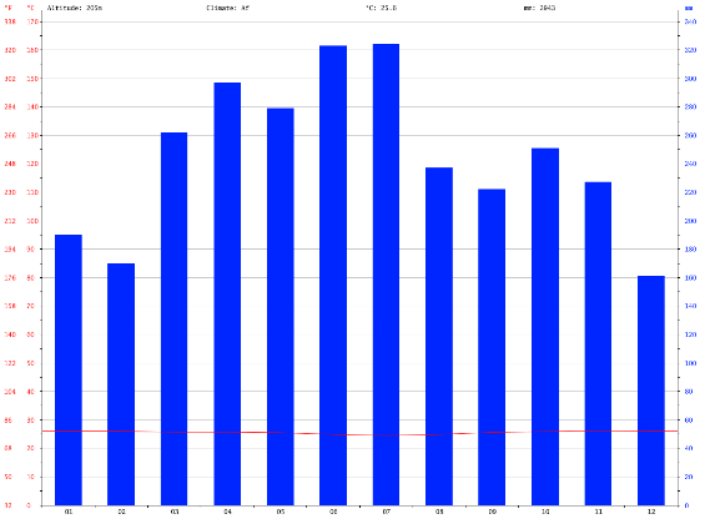


Figure 15. Climate diagram of Cuyabeno Wildlife Reserve.

(Source: https://de.climate-data.org).

Figure 16. Fauna of Cuyabeno Wildlife Reserve. The poisonous spider of the genus *Phoneutria* (a) and a typical snake *Eunectes murinus* (b).

Figure 17. One of the most common birds in the reserve - *Opisthocomus hoazin*. A picture by Jan Korba.

3 Objectives and hypothesis

The main objective of the present study was to assess the intra- and inter-population genetic diversity of *M. flexuosa* in Peruvian and Ecuadorian Amazon by SSR microsatellite markers.

The specific aims of this study were:

I. To determine the genetic structure of sampled populations.
II. To find a correlation between geographical distances and genetic diversity among selected provenances and individuals.
III. To compare genetic variability among wild, cultivated and IIAP (plantations managed by IIAP) populations.

We hypothesize that *Mauritia flexuosa* is cultivated mostly with the seeds of wild populations along the Amazonian rainforest. Due to the distances within the various isolated natural *aguajales* and cultivated plantation there could be the high genetic diversity determined.

4 Materials and methods

4.1 Study sites

In summary, 147 palm trees from 5 regions, 13 distinct geographical localities identified as provenances and divided into 15 populations (Figure 18), were sampled for evaluation of genetic diversity. However, in the case of two samples, the amplification was not successful, so the total number of counted samples is 145 (Table 2).

Samples were collected in the Peruvian regions of Ucayali, Huánuco, Loreto and San Martin and in Ecuadorian unique ecosystem called "igapo". Four populations IIAP 1, IIAP 1 R, IIAP 2 and IIAP 2 R come from two geographical localities (15 populations, 13 provenances), specifically from the experimental plantations managed by IIAP (Instituto de Investigaciones de la Amazonía Peruana). Those investigation plots are situated close to the district of Nueva Requena and they were established between the years 2001 and 2002. During this period, seeds from various localities along the Ucayali river (Appendix A) were collected from their original habitat and planted afterwards in the particular research plots. Since then, the plantations have been used for investigation purposes related to morphological observation and genetic analysis. Apart from the artificially cultivated individuals, the plantations also contain a naturally regenerated plants, which were inbred with *M. flexuosa*. For this reason, those samples are assigned in this paper to an especially divided populations marked as IIAP 1 - R and IIAP 2 – R. In other words, 55 individuals with the special focus were sampled related to the subsequent comparison of molecular variance and main genetic diversity parameters.

Apart from the samples coming from the IIAP plantations, other 49 samples are from the wild (natural) populations and 41 samples are from the cultivated (somehow domesticated or influenced by human activity) populations. (**Wild:** EC - Cuyabeno Natural Reserve Ecuador, IQP - Cumaceba river Iquitos, IQ - San Pedro de Huashalado, TAR - Laguna Venecia and Tarapoto town, LM - Laguna de los Milagros, LG – Limongema; **Cultivated**: PK19 - Km 19 Pucallpa Botanic garden, SF - San Francisco and San José villages, TM - Tingo María, P - Pucallpa, PUNU - Pucallpa plantation of the UNU; **IIAP:** IIAP 1 - Experimental plantation 1, IIAP 1 - R Experimental plantation 1 - regeneration, IIAP 2 – Experimental plantation 2, IIAP 2 - R Experimental plantation 2 – regeneration).

4.2 Data collection

Leaf samples were collected between June and September 2018 in Peruvian and Ecuadorian Amazon. The aim was to get the data based on the genetically diverse populations of the species *Mauritia flexuosa*, covering habitats with different human intervention.

Samples were individually collected and stored afterwards in plastic tubes with silica gel and the leaves were kept in dry conditions until further DNA extraction. GPS coordinates and pictures of each sampled plant were taken. In addition, *Mauritia flexuosa* is a common palm tree species in the Amazon and is not under any protection.

Table 2. 15 populations from Peruvian and Ecuadorian Amazon.

Code	Region	Number of samples	Coordinates		Altitude (m.a.s.l.)
			Latitude S	Longitude W	
Wild					
EC	Ecuador	7	0.00575	-76.179333	234-241
IQP	Loreto	7	-4.531806	-73.412056	102-108
IQ	Loreto	7	-3.624667	-73.130167	86-91
TAR	San Martin	9	-6.496944	-76.335	321-400
LM	Huánuco	8	-9.141667	-75.995833	680-713
JG	Ucayali	11	-8.524167	-74.398306	145-158
Cultivated					
PK19	Ucayali	5	-8.422667	-74.700306	145-156
SF	Ucayali	10	-8.273056	-74.637778	134-162
TM	Huánuco	5	-9.308333	-75.998056	663-720
P	Ucayali	10	-8.392833	-74.574611	149-167
PUNU	Ucayali	11	-8.406667	-74.575	153-157
IIAP					
IIAP 1	Ucayali	33	-8.379414	-74.881639	160-163
IIAP 1 - R	Ucayali	7	-8.379414	-74.881639	160-163
IIAP 2	Ucayali	8	-8.3475	-74.853333	169-178
IIAP 2 - R	Ucayali	7	-8.3475	-74.853333	169-178
Wild total		49			
Cultivated total		41			
IIAP total		55			
Overall		145			

The table is divided into three parts, according to the populations and its origin (natural populations, cultivated populations and the populations with special focus investigated by IIAP).

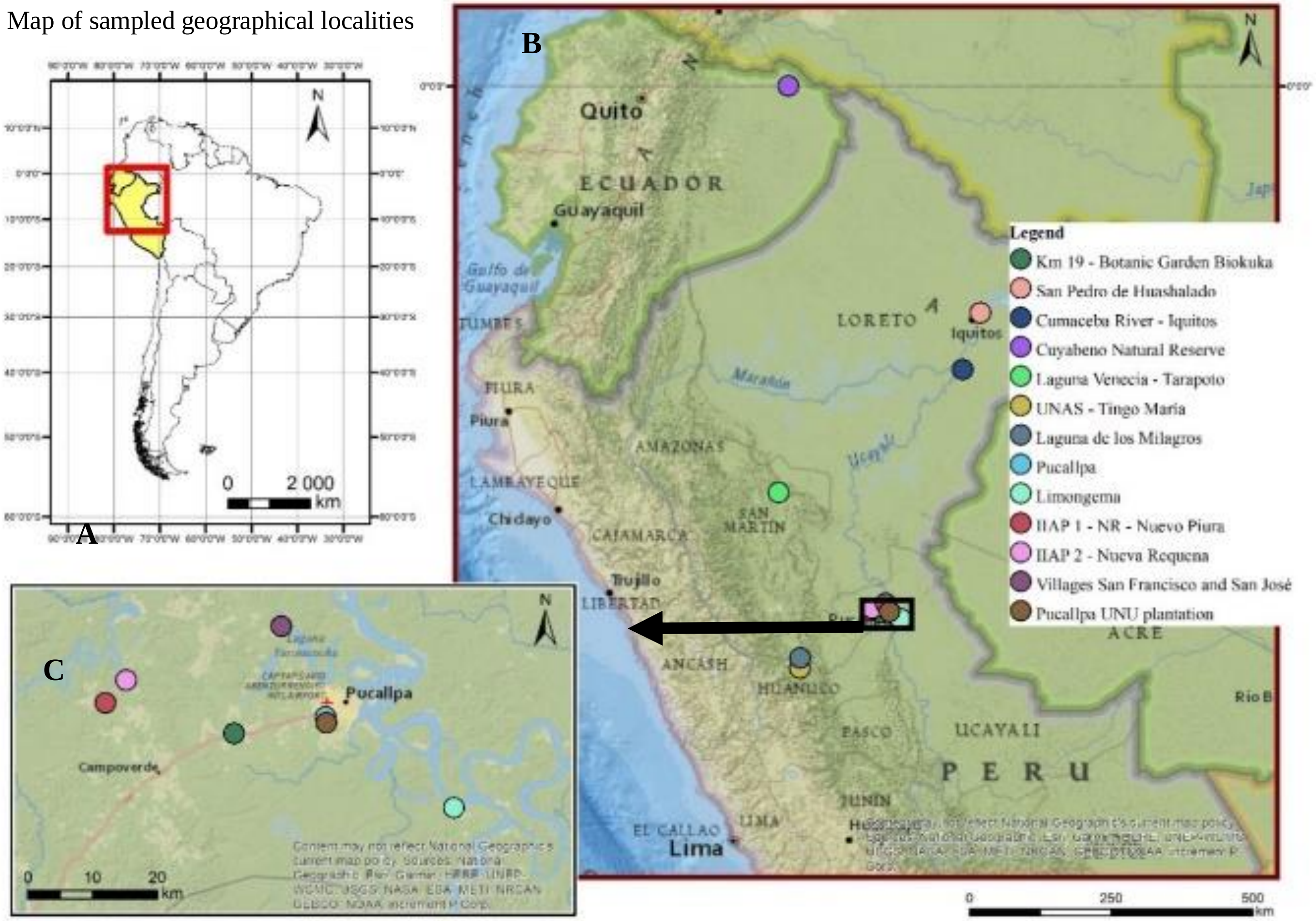


Figure 18. 13 provenances, where samples of *Mauritia flexuosa* were collected.

General distribution of the sampled populations (A). Detailed map of the Peruvian provenances (B). Detailed map the Pucallpa's one (C).

4.3 DNA analysis

4.3.1 DNA extraction

DNA was isolated from dried leaf tissue following the CTAB (cationic hexadecyl trimethyl ammonium bromide) method (Doyle et al., 1987). Using the laboratory sand, the sampled material was pulverized. Fot cell lysis, the powdered tissue was mixed with 800 μL of extraction buffer (CTAB 2.8%, NaCl 1.3 M, EDTA 20 mM, TRIS-HCl 100 mM, PVP 1%, mercaptoethanol 0.2%) and 5 μL of proteinase K, in 1.5 mL test-tubes.

After one hour of incubation at the default temperature of 65°C and mixing the content every 10 minutes, 700 μL of chloroform:isoamylalcohol (24:1) was added to the test-tubes to denature the contaminants. While mixing at the machine Silamat S6 and centrifuging afterwards for 14000 RPM and 4 °C, the mixture of phase materials was separated and the solid part was deposited at the bottom of the tube. The liquid phase was pipetted into new test tubes with 7% CTAB (55 μL) and then with 700 μL of chloroform:isoamylalcohol (24:1), mixed, centrifuged and transferred again into new micro tubes.

The supernatant was diluted with isopropanol and stored for an hour in the freezer. Next 10 minutes in the centrifuge, the dense and white nucleic acid was obtained and purified firstly with 400 μL of 96% ethanol, secondly with 400 μL of 70% ethanol. Finally, the DNA pellet was dried at a room temperature in the lab and then dissolved in TE buffer (100 μL) and RNase (1 μL).

The DNA concentration was adjusted to 20 ng/μL according to the measurement of DNA purity and content with NanoDrop UV Spectrophotometer (Thermo Scientific, USA). Diluted DNA was stored at -20 °C.

The DNA extraction was realized in the laboratory of molecular Biology of Faculty of Tropical AgriSciences at Czech University of Life Sciences (CZU), as well as the PCR optimization and analysis afterward.

4.3.2 Microsatellite analysis

To find the optimal temperature of annealing for a PCR analysis in the case of particular markers, the PCR optimization had to be done. The gel consisting of 1 g of agarose and 100 ml of TBE buffer was stained with ethidium bromide. As a size standard was used the GeneRuler 100 bp DNA Ladders (Thermo Scientific, USA). Using the electrophoresis set up for 120 V, 400 A and 30 minutes, the experimental markers were visualised by UV lamp and the pictures were taken by a camera.

Out of originally designed 13 microsatellite loci for *M. flexuosa* (Federman et al., 2012) (Table 1) identified in a gene bank database (Integrated DNA Technologies Inc., USA) 8 of them (Mf04, Mf22, Mf34, Mf24, Mf13, Mf19, Mf25, Mf31) were chosen due to previous research for the PCR optimization (realized also in the laboratory of molecular biology - CZU). However, Mf31 had to be excluded consequently due to the null allele exceeding 5% error. The PCR optimization resulted in a suitable annealing temperature 64°C for all primers. Forward primers were diluted with H_2O PCR Ultra in a proportion 1:9.

Primers were divided into two multiplexes (Table 3). The first multiplex M1 with a total volume 6 μL was composed of the primers Mf04 (0.1μL forward and 0.1 μL reverse primer), Mf22 (0.15 μL forward and 0.15 μL reverse primer), Mf34 (0.5 μL forward and 0.5 μL reverse primer), Mf24 (0.5 μL forward and 0.5 μL reverse primer), with 2.5 μL Quiagen Multiplex PCR Master Mix (Qiagen, Germany) and 1 μL DNA diluted to the concentration of 20 ng/μL.

The second multiplex M2 with a total volume 7 μL was composed of the primers Mf13 (0.2 μL forward and 0.2 μL reverse primer), Mf19 (0.15 μL reverse and 0.15 μL forward primer), Mf25 (0.15 μL forward and 0.15 μL reverse primer), Mf31 (1 μL forward and 1 μL reverse primer) with 2 μL Quiagen Multiplex PCR Master Mix and 1 μL DNA diluted to the concentration of 20 ng/μL.

Table 3. Seven microsatellite loci for *M. flexuosa* divided into two multiplexes.

Multiplex	Locus	Primer sequence (5’-3’)	Size (bp)	Volume (μL)
M1	*Mf*04	F: CCACGGGGCTGTCATATTTC	(194-264)	0.1
		R: AGGAAAACGCAAGAAGTGC		0.1
	Mf22	F: GCATGGTGTAGCTCGTATCTG	(219-259)	0.15
		R: CGCACCATACTTGGCTTGC		0.15
	Mf34	F: GGACAGTTGCCTGTCTTGC	(144-208)	0.5
		R: CAAAGCTAGCACAACCTGGG		0.5
	Mf24	F: TCACATTAGTAGTCAAGGGTAGC	(177-231)	0.5
		R: GGGTGTTAAGCATTCGGGC		0.5
M2	*Mf13*	F: TTACAAGCGACCCCTCGTC	(213-257)	0.2
		R: CGTCGAATAGGGTTTCAGTGG		0.2
	Mf19	F: AGCCACGTGACACTCTACC	(220-244)	0.15
		R: CTATAGGAACCGGCCACCTG		0.15
	Mf25	F: CCCCATTTTCCAATTTGATGCG	(174-216)	0.15
		R: TGGATGTTCAGTTTGGATGCC		0.15

To prevent any DNA contamination, evaporation and spilling of the diluted DNA, the 96-welled PCR microplates (8 rows and 12 columns) were closed properly with a plastic foil and stored in the freezer, before the PCR analysis had started. The PCR amplifications was performed in T100 Thermal Cycler (Bio-Rad, USA). The PCR profile was the same for both multiplexes: denaturation (95°C for 15 min, 94°C for 1 min), annealing (64°C for 1 min), extension (72°C for 90 s, GOTO step 2 repeated 34 times, 72°C for 15 min) and finally the PCR products were held at 4°C.

The capillary analysis was done consequently in a laboratory of molecular genetics at the University Centre for Environmental Sciences of CZU, where the products of PCR were separated by electrophoresis in an 3500 Genetic Analyzer (Applied Biosystems, USA). The laboratory data were analysed in the software GeneMarker version 2.6.0 (SoftGenetics, USA), using the standard size GeneScan 500 LIZ.

4.3.3 Data analysis

Number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) (Nei, 1987), fixation index (F_{IS}) (Weir and Cockerham, 1984), tests for Hardy-Weinberg equilibrium, analysis of molecular variance (AMOVA), Mantel test comparing matrices of the geographic and genetic distance, PCoA based on Nei's genetic distance and spatial structure analysis were calculated in Microsoft Excel 2016, extended by add in GenAlEx 6.5 (Peakall and Smouse 2012). The allelic richness (A_R) was evaluated in R software (R Core Team, 2018).

In the PAST software (Hammer et al., 2017), principal coordinates analysis (PCoA) based on the geographic coordinates (latitude and longitude) was performed. Consequently, the hierarchical clustering of populations and of individuals was done with the paired group (UPGMA) algorithm and the similarity Euclidean index.

The estimation of the number of genetic clusters (K) and fractional assignment of individuals into the inferred clusters was revealed by the STRUCTURE software (Department of Statistics University of Oxford, United Kingdom) (Pritchard et al., 2000). Each of ten runs comprised a burn-in period of 25,000 iterations and by 100,000 Markov chain Monte Carlo (MCMC) steps. The output Structure data was parsed using the Structure Harvester software (Earl et al., 2012). By ΔK distribution statistic of Evanno et al. (2005), the best single value was identified out of a range of K values through the detection of the second rate of change in LnP(D). The final results were performed in the Clumpp and Distruct software (Department of Biology of Stanford University, USA) (Jakobsson and Rosenberg, 2007).

Similar analyses were done simultaneously in Clumpak software (Kopelman et al., 2015), where the best alignment of the results across the range of K values was found and the structure was shown for various number of clusters.

In an accordance to Structure output data, several maps showing the genotype proportion of individual clusters were performed in the ArcMap 10.6.1. (ESRI, 2011). The projected geographical system WGS 84 World Mercator was used due to the UTM coordinates.

5 Results

5.1 Main parameters of genetic diversity

Seven polymorphic SSR primers and 145 individual samples were used for analysis. All microsatellite loci were polymorphic, with a total of 139 alleles identified. The length of amplified DNA fragments ranged from 144 to 264 bp (Table 3). All loci and populations were in Hardy-Weinberg equilibrium ($P > 0.05$).

Table 4. Main parameters of genetic diversity by locus.

Locus	N_a	N_e	A_R	H_o	H_e	F_{IS}	F_{ST}	N_m	S
Mf04	10.000	7.768	6.282	0.766	0.855	0.105	0.090	2.530	ns
Mf22	8.600	6.446	5.898	0.787	0.832	0.054	0.090	2.514	ns
Mf34	10.000	7.258	6.307	0.899	0.852	-0.055	0.064	3.653	ns
Mf24	7.467	5.715	5.350	0.737	0.801	0.081	0.086	2.660	ns
Mf13	7.800	5.320	5.300	0.766	0.801	0.043	0.068	3.400	ns
Mf19	5.800	4.261	4.504	0.699	0.751	0.069	0.076	3.028	ns
Mf25	8.400	5.974	5.662	0.834	0.816	-0.022	0.084	2.729	ns
Mean	8.295	6.106	5.615	0.784	0.815	0.039	0.080	2.931	
SE	0.287	0.209		0.018	0.007	0.022	0.004	0.169	

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001.

Number of alleles (N_a), effective number of alleles (N_e), allele richness (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), fixation index (F_{ST}), gene flow (Nm), significance of deviation from Hardy-Weinberg equilibrium (S), standard error (SE).

According to the Table 4, the average Na was 8.3 ranging from 5.8 to 10 different alleles per locus. The allelic richness was 5.615. The lowest value of N_e had a locus Mf19 (4.261) and the highest Mf04 (7.768). H_o and H_e was the lowest in the case of locus Mf19 (0.699 and 0.751), otherwise, the highest H_O had Mf34 (0.899) and H_e had Mf04 (0.855). F-Statistics and N_m (2.931) over all populations for each locus were 0.039 (F_{IS}), 0.116

(F_{IT}) and 0.080 (F_{ST}). Totally, the observed heterozygosity by population was lower than the expected one (Table 5). The coefficient of inbreeding is not significant, but in some populations is higher than 0.1. According to output data from G-Statistics, overall coefficient of genetic differentiation (G_{ST}) was 0.015, showing that 1.5% of the genetic variability was distributed among provenances (Appendix B).

The highest value of private alleles per population was found in IIAP1 (0.857), EC and PUNU (0.571). The Tarapoto population had no private alleles detected.

Table 5. Main parameters of genetic diversity by population

Population	N_a	N_e	A_R	H_o	H_e	F_{ST}	S
IQ	6.571	4.619	5.057	0.614	0.765	0.193	ns
IQP	7.857	5.886	5.699	0.714	0.821	0.132	ns
LG	9.000	6.511	5.801	0.842	0.839	-0.003	ns
PK19	6.714	5.655	5.567	0.829	0.800	-0.028	ns
EC	6.714	5.190	5.181	0.724	0.789	0.071	ns
SF	10.286	7.872	6.402	0.871	0.865	-0.004	ns
TAR	8.000	6.085	5.612	0.762	0.822	0.082	ns
IIAP 1 - R	7.000	5.550	5.385	0.918	0.808	-0.145	ns
IIAP 2 - R	7.429	5.551	5.503	0.714	0.806	0.113	ns
P	9.857	7.515	6.267	0.824	0.857	0.041	ns
PUNU	9.286	6.212	5.670	0.805	0.815	0.023	ns
LM	7.429	5.124	5.250	0.857	0.787	-0.084	ns
TM	5.429	4.461	4.724	0.714	0.763	0.069	ns
IIAP 2	8.143	6.168	5.671	0.768	0.820	0.059	ns
IIAP 1	14.714	9.190	6.431	0.804	0.877	0.080	ns
Mean	8.295	6.106	5.615	0.784	0.815	0.040	
SE	0.287	0.209		0.018	0.007	0.020	

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001.

Number of alleles (N_a), effective number of alleles (N_e), allele richness (A_R), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F_{ST}), significance of deviation from Hardy-Weinberg equilibrium (S), standard error (SE).

5.1.1 AMOVA

The most notable molecular variance was revealed within individuals (87%), whereas the variation among individuals was 11%, and among regions and populations it was only 1% (Table 6). The genetic variability within individuals and among regions was higher in the case of the cultivated populations, on the other hand among individuals and among populations the lower values of molecular variance were indicated in the wild populations.

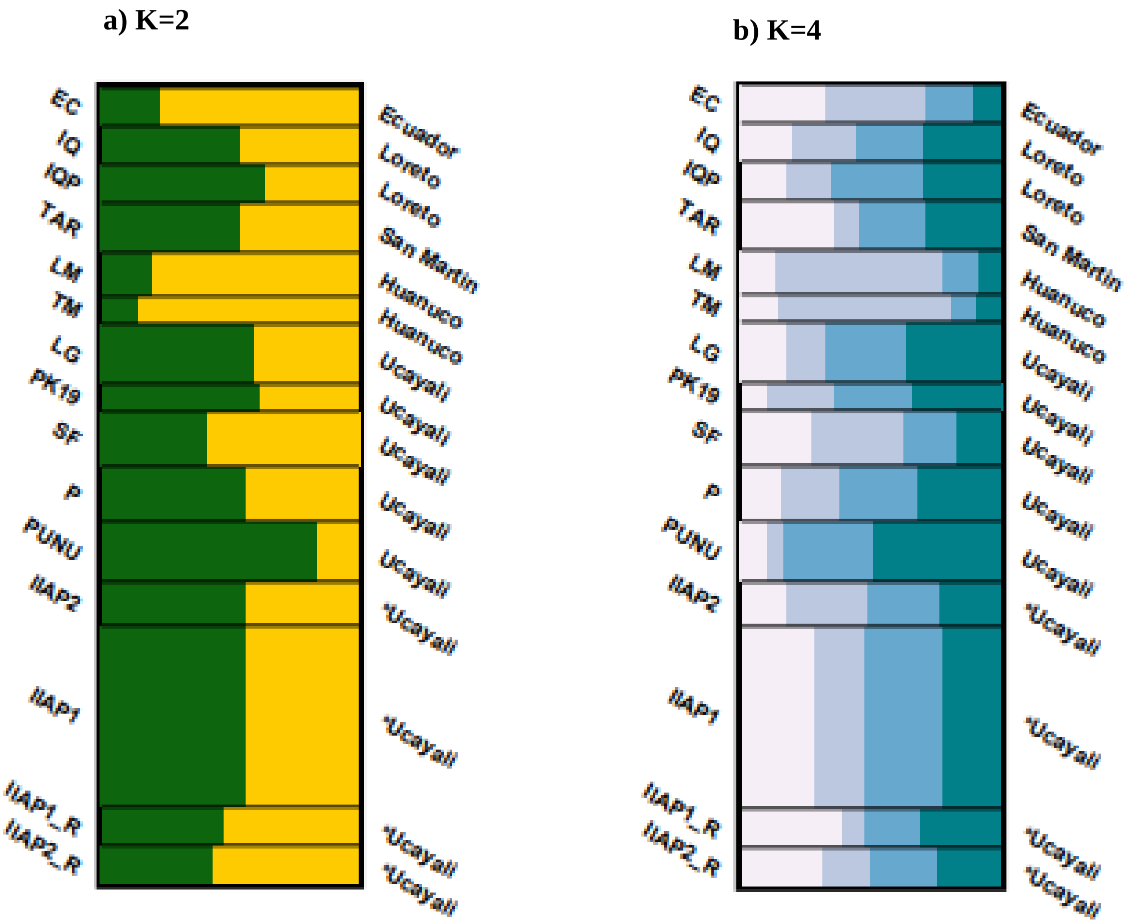
Table 6. Analysis of molecular variance (AMOVA) of the *M. flexuosa* populations.

Source	df	SS	MS	EV	%
All populations					
Among Regions	4	20.093	5.023	0.029	1%
Among Populations	10	41.295	4.129	0.035	1%
Among Individuals	130	446.105	3.432	0.343	11%
Within Individuals	145	398.000	2.745	2.745	87%
Total	289	905.493		3.153	100%
Wild populations					
Among Regions	4	19.103	4.776	0.010	0%
Among Populations	1	4.393	4.393	0.066	2%
Among Individuals	43	149.178	3.469	0.418	13%
Within Individuals	49	129.000	2.633	2.633	84%
Total	97	301.673		3.127	100%
Cultivated populations					
Among Regions	1	4.197	4.197	0.016	0%
Among Populations	3	12.720	4.240	0.049	2%
Among Individuals	36	121.327	3.370	0.258	8%
Within Individuals	41	117.000	2.854	2.854	90%
Total	81	255.244		3.177	100%
IIAP populations					
Among Populations	3	11.900	3.967	0.024	1%
Among Individuals	51	175.600	3.443	0.340	11%
Within Individuals	55	152.000	2.764	2.764	88%
Total	109	339.500		3.128	100%

degrees of freedom (df), sum of squares (SS), mean squares (MS), estimate of variance (EV), % - percentage of total variation.

5.2 Genetic structure

The mean Log-likelihood of K LnP(D) ± SD recommended several numbers of clusters, but ΔK indicated two clusters as the most informative (Appendix C). The cluster analysis was permuted in Clumpp and Distruct softwares (Figure 19), based on the output data from the Structure software (Appendix D). The Clumpak results are shown in Appendix E. The results of those analyses did not show any significant genotype classification related to the geographical parameters. To show more detailed segregation, the data of Structure were transmitted to a map in form of pie charts showing divisions of wild populations into two, three and four clusters (Figure 20). In an accordance with recommended 2K division, all wild and cultivated populations are shown on the map in more detail (Appendix F).


a) K=2

b) K=4

Figure 19. Population structure. a) Division into two clusters; b) Division into four clusters.

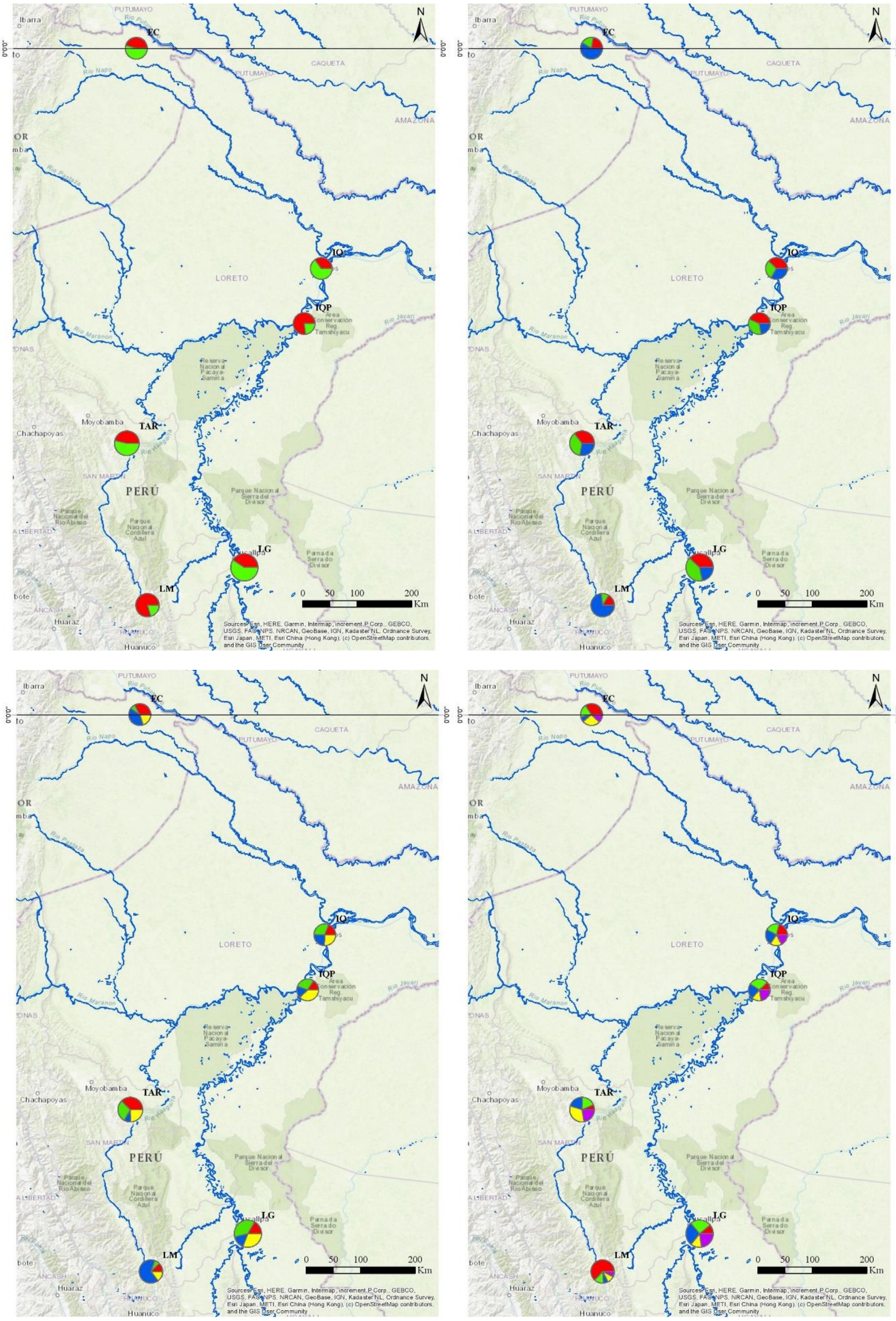


Figure 20. Division of a wild populations into two, three, four and five clusters.

5.3 Genetic and geographic distances

Nei's genetic distances among provenances varied from 0.219 to 0.973. The values of geographical distance were from 1.5 to 1,036 kilometres (Table 7). The lowest genetic distance was indicated between IIAP1 and TAR populations (264 km), which could reflect the genes exchanging caused by the human activity in the past. The map, where the Structure results are split into the pie charts due to the five clusters diversification, proves the genotype similarity of the San Martin population and Ucayali populations. Otherwise, the highest one was suggested between PUNU and TM populations. Although the geographical distance is only about 186 kilometres, the genotype differentiates extremely, probably in relation to the different river basins.

Despite the geographic isolation of Ecuadorian population, the Nei's genetic distance does not reach any high values related to Peruvian populations. The geographical distance of TM and EC populations is 1,036 km, however, the genetic distance is only 0.493. Populations from investigation plots (IIAP) indicated low numbers of genetic diversity with populations from Ecuador, Ucayali (except PUNU population) and San Martín regions. On the other hand, genetically the Huánuco and Loreto provenances vary significantly.

Stratigraphic Based on the Pairwise Population Matrix of Nei's Genetic Identity was performed a diagram (Figure 21). The unique group consists of Ecuadorian population, which was geographically the furthest and according to data collection the most isolated from the other provenances. The "Peruvian" main group is subdivided into three more parts. All populations belonging to the IIAP classification form the own group distinguished by two different provenances.

Based on Pairwise Population Matrix of Nei's Genetic Distance, also UPGMA hierarchy clustering diagram of individuals (Appendix G) was performed in Past software, using Euclidean distances similarity index.

The explanation of the results may be influenced by the distinct river basins. IQP population is situated along the Cumaceba river, which consequently flows into Ucayali. Otherwise IQ population comes from the Amazon river basin, same as the rest of the populations forming their own subgroup, except TAR, LM and TM.

Table 7. Pairwise population matrix of Nei's genetic distance and geographic tri matrix distance (km) among the provenances of *M. flexuosa*.

EC	IIAP1	IIAP1_R	IIAP2	IIAP2_R	IQ	IQP	LG	LM	P	PK19	PUNU	SF	TAR	TM	
***	943.4	943.4	940.4	940.4	527.0	590.8	968.8	1017.4	950.7	951.4	952.2	936.3	723.3	1035.9	EC
0.315	***	0.0	4.7	4.7	563.1	457.6	55.5	148.9	33.8	20.5	33.9	29.3	263.6	160.4	IIAP1
0.661	0.307	***	0.0	4.7	563.1	457.6	55.5	148.9	33.8	20.5	33.9	29.3	263.6	160.4	IIAP1_R
0.478	0.309	0.505	***	0.0	558.6	453.2	53.8	153.5	31.1	18.8	31.3	25.1	262.7	165.0	IIAP2
0.477	0.373	0.549	0.443	***	558.6	453.2	53.8	153.5	31.1	18.8	31.3	25.1	262.7	165.0	IIAP2_R
0.671	0.583	0.658	0.628	0.563	***	105.6	562.5	690.3	553.7	561.0	555.2	543.1	477.5	706.9	IQ
0.703	0.522	0.484	0.650	0.495	0.570	***	457.1	586.6	448.1	455.5	449.6	437.5	390.4	602.9	IQP
0.599	0.305	0.364	0.611	0.493	0.461	0.517	***	188.5	24.3	35.1	23.4	38.4	310.5	196.2	LG
0.459	0.389	0.573	0.335	0.539	0.630	0.701	0.609	***	177.0	163.3	176.2	177.8	296.4	18.5	LM
0.402	0.316	0.492	0.374	0.525	0.592	0.654	0.372	0.362	***	14.2	1.5	15.0	286.6	186.6	P
0.598	0.375	0.586	0.622	0.657	0.441	0.670	0.398	0.592	0.504	***	13.9	18.0	279.9	173.3	PK19
0.699	0.531	0.558	0.640	0.619	0.706	0.514	0.503	0.773	0.412	0.642	***	16.4	287.7	185.7	PUNU
0.418	0.219	0.404	0.439	0.456	0.687	0.664	0.458	0.387	0.419	0.571	0.764	***	272.1	188.7	SF
0.485	0.288	0.355	0.420	0.519	0.533	0.528	0.488	0.460	0.425	0.516	0.469	0.388	***	314.8	TAR
0.493	0.450	0.759	0.664	0.536	0.527	0.734	0.526	0.393	0.491	0.538	0.973	0.417	0.674	***	TM

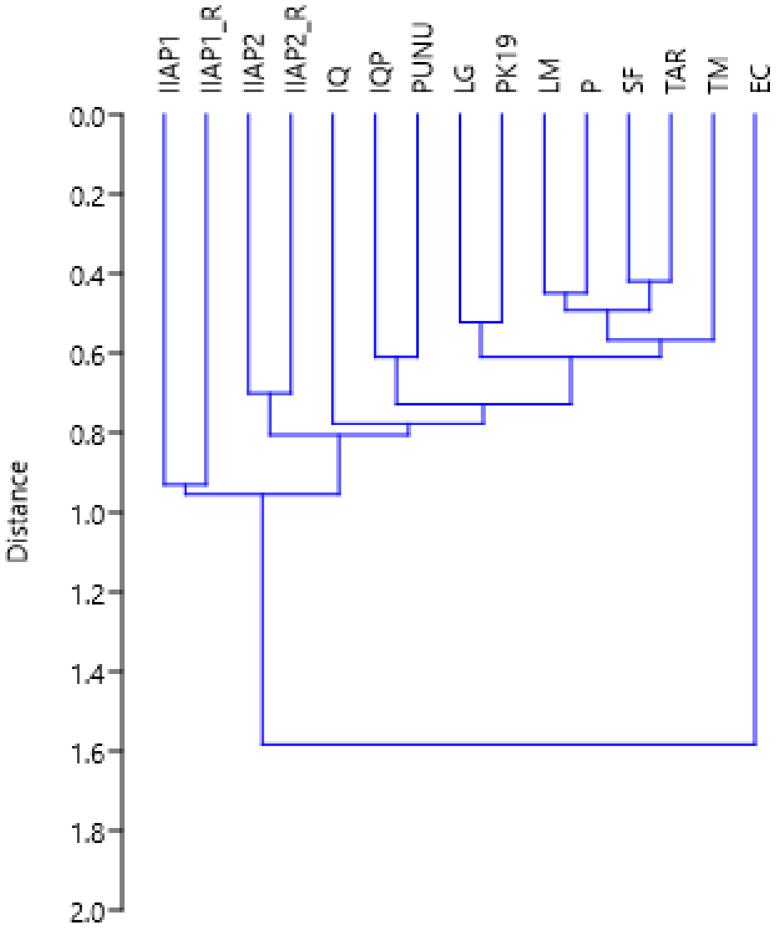


Figure 21. Stratigraphic UPGMA hierarchy clustering diagram of populations based on the Pairwise Population Matrix of Nei's Genetic Identity

The Mantel test revealed a weak correlation (r = 0.136) between geographic and genetic tri matrix distances among the populations of *M. flexuosa* (Appendix H). If the populations were divided into the regions according the river basin (Ucayali; Marańon; Huallaga), the positive relationships would be a bit greater (r = 0.162; 0.136 and 0.157). Generally, it means that geographic distance had a small effect on genetic differentiation among individuals and also among populations.

PCoA analysis based on the geographical coordinates was performed in the PAST software (Figure 22). *S*amples with the original coordinates from IIAP experimental plantations are shown individually, nevertheless, the data of other provenances figure out only as the populations. A principal coordinate analysis (PCoA) was performed using GenAlEx 6.5 (Peakall and Smouse, 2012), based on the pairwise Nei's genetic distance matrix. All individuals are shown on the Figure 23.

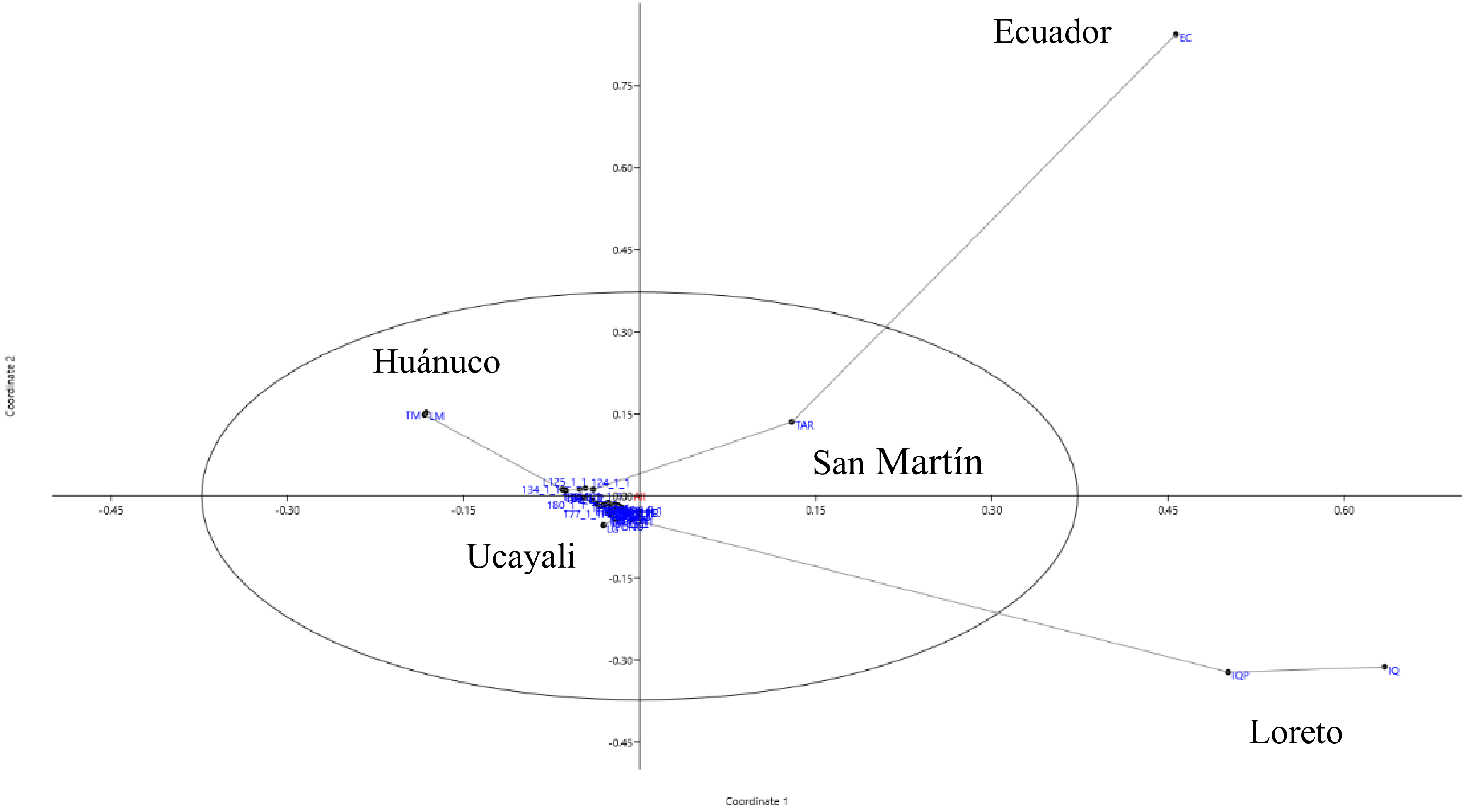


Figure 22. PCoA analysis performed in PAST software based on the geographic distance matrix (longitude and latitude).

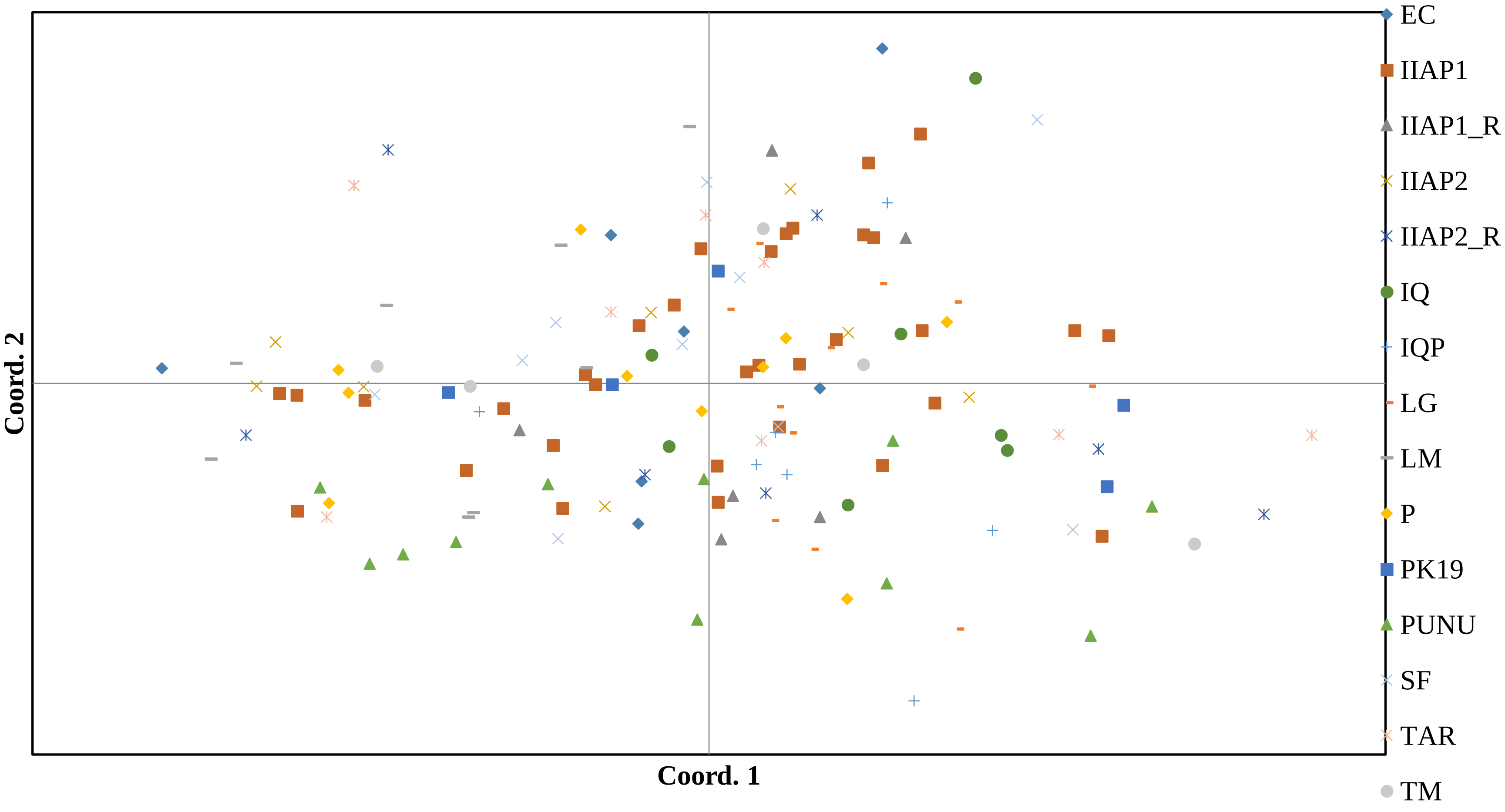


Figure 23. Principal Coordinates Analysis of all individuals divided by populations.

6 Discussion

6.1 Genetic diversity

The high genetic diversity within populations in the case of *Mauritia flexuosa* and other palms occurring in the riparian ecosystems, has been estimated in the study made by Gomes et al. (2011). On the other hand, in the case of the study made in Brazil by Rossi et al. (2014) using the ISSR markers, the AMOVA results showed 84.1% of the total genetic variation within populations and 15.9% among populations. There are comparable AMOVA results with the study made by Sander et al. (2018), indicating that 78% of the molecular variation was found within individuals and only 4.3% of the molecular variance among populations. Also values of observed and expected heterozygosity are similar, as well as the congruent with high levels of gene flow among populations. In general, the high heterozygosity could be due to the excessive adaptability to different water conditions, as well as the ability to compete in a difficult environment. With higher genetic variability, the chance of perpetuation of the species increases (Estopa et al., 2006). Within each studied population, high genetic variability was reported. The high genetic diversity of *Mauritia flexuosa* allows the adaptation to changing ecological conditions, whether it is an island, the Amazon lowlands or the relief areas. There are many widespread species along the Amazon having a high genetic diversity. For example *Myrciaria dubia* (Šmíd et al., 2017), *Inga edulis* (Rollo et al., 2016) and another very abundant palm, *Euterpe precatoria* (Santos et al., 2015).

Thus, genetic drift and fixation of different alleles in subpopulations does not play an important role. Significant values of fixation index in the case of the populations from the Loreto region indicate a slight influence of the gene flow between populations and point to the effect of genetic drift. However, in populations as large as those in Loreto, genetic drift is unlikely to have a significant effect. Otherwise, populations from the Ucayali region obviously imply the lowest degree of differentiation among populations. It could be due to the isolation of sampled wild populations in Loreto region. Moreover, IQ population was sampled in the basin of the Ucayali river while IQP population was sampled basin of the Amazon river. Although the collection of samples was sometimes carried out in relatively isolated and distant locations, *M. flexuosa* has been always in abundance there.

All populations and loci are characterized by high values of heterozygosity. Mostly the observed heterozygosity was significantly lower than the expected one, which could reflect a slight inbreeding effect, especially in the less numerous and remote populations. This situation occurred in all wild populations, except one from Laguna Milagros (Huánuco region). It has a more noticeable negative F_{ST} value, probably the mixing of two previously isolated populations could have occurred in the sampled locality. Based on the special and conservation status of the provenances managed by the national Peruvian research institution, as expected, the IIAP populations have the highest values for H_e (0.828) and H_o (0.801). In the case of IIAP_R1, results show significantly higher values of observed heterozygosity than expected was. Since the establishment of the research plantations, probably the original populations of *M. flexuosa* have been merged with the populations intended for research purposes, which might imply an isolate-breaking effect. Nowadays, the juvenile plants also come from the original populations around the investigated plot, which is shown on the UPGMA diagrams. Regenerated juvenile individuals on the investigated plots are characterized, as it was supposed, by high level of genetic diversity and of significantly higher values of the fixation index

6.2 Genetic structure and genetic and geographic distance

The overall results of a genetic structure of sampled populations do not reflect existence of any clear stratification. Little genetic differentiation indicates low population structuring and variability between populations, except populations from Huánuco region and population from the plantation belonging to UNU (Universidad Nacional de Ucayali). The results could be the recommendation for IIAP, to add individuals out of the mentioned plantation to the investigated plots of *M. flexuosa*.

Maybe it is necessary to look for various genetic structures in very remote and isolated populations, where the gene flow is low. For instance, according to Gomes et al. (2011), the research focus on the genetic diversity of *aguaje* showed a low values of allelic flow among populations and great genetic diversity within populations. This could be due to the isolation of sampled populations, which confirms the previous statement. As well Sander et al. (2018) reported, that populations from the same river basin are highly connected from genotypic point of view. Obtaining an important result from small-scale spatial genetic structure studies provides consequently the predictions and responds to changing natural conditions (Federman et al., 2013). Sampled population out of the Huánuco region are more structured, probably due to the isolation in a consequence of higher altitude. Tingo María is already designated as *selva central* and certainly, there are other hydrological and ecological conditions comparing to lowland areas along a large river basins. The genotype has to differ after a certain period of time due to the adaptation to new ecological conditions.

To get a different kind of data of genetic structure between *aguaje* populations, in my opinion, the geographic distance is not such a significant point of view. Even in the research made by Gomes et al. (2011), no correlation was found between the genetic and the geographic distances in the case of *M. flexuosa* populations in Brazil. Based on the study by Aspajo et al. (2008), related to the interpretation of the morphological data, only greater geographical distances played a role in an accordance to genetic diversity and gene flow influence. The rest of observations was probably just phenotypic variations. Actually, those facts correspond to the results of this study, where only the Ecuadorian and Peruvian populations were differentiated at the level of genetics in the UPGMA diagram (Figure 21). However, to distinguish the Peruvians populations from each other was not significant and low values of F_{ST} indicate the presence of a weak structure.

The positively determined correlation between geographical distances and genetic diversity, among selected provenances and individuals of *M. flexuosa*, was confirmed by Quintanilla et al. (2014), who explained the genetic similarity dependence on the geographical distance between the populations of *aguaje* in the Loreto region. Also, the relatively low genetic diversity among populations was indicated in their research.

6.2.1 Importance of rivers in shaping genetic structure

Obviously, a significant geographic distance could not be considered as a barrier to genetic migration leading to gene flow in the case of *M. flexuosa*. High degree of gene flow, providing by effective seeds and pollen dispersal usually maintain the high degrees of genetic diversity (Dick et al., 2008). During the rainy seasons, the water level rises and the flow accelerates, so the *aguaje* seeds can reach much longer distance in the limited time. Moreover, water is poured from the riverbed into the surrounding environment, increasing the chances of settling the new stand. The hard fruit protects the seed inside, against mechanical damage. *M. flexuosa* is very adaptive, the seeds spread over long distances by the water, but also the nutritive value of the fruits attracts different kinds of animals. The animals can distribute the seeds at a time when the drought is prolonged or the site dries up, the same as people do.

The results absolutely correspond to the study made by Sander et al. (2018), focused, inter alia, on the mapping of the *M. flexuosa* populations along the basin of Orinoco. The importance of the rivers and their water flows correlates to the gene flow within and among the population of the aquatic plant species. Particularly, the seed dispersal fully depends on the rivers. Sander et al. (2018) also suggest, that the gene flow patterns revealed that the migrants are primarily sourced from the same river basin.

The importance of rivers related to species propagation is probably much greater than is seems. If there are two populations situated many kilometres apart from each other, but close to the bank of the same river, the genetic structure would be probably almost the same. All the genes from different sites are mixed along the river basins and finally the huge rivers make the gene collection itself. That is the explanation, why in Iquitos, Ucayali all genotypes were found out.

6.2.2 Gene flow and human activity

The theory about the people settlement (Sander et al., 2018) of the Amazonian forests supports the results of the thesis, dealing with not logically differentiated structure of individual populations sampled from sites remote from each other. Related to research made by Vašek et al. (2017), the genetic structure of *Plukenetia volubilis* in the Peruvian Amazon is caused also due to socio-economic connectivity among the various sites, because the transportation of the seeds is possible over huge distances. Except for gene flow, probably the human factor also plays a role in the case of *M. flexuosa* as well. The provenances along the upper reaches of rivers are characterised by not clearly differentiated genotypes.

Throughout the history of the Amazonian forest settlement by human, people have always lived nearby the water sources. Their lives were closely connected to the banks of large rivers, as same as the *M. flexuosa* occurrence. No genetic structure with any logical genotypes clustering may reflect the *aguaje* consumption and transportation by various tribes living in the past and also, nowadays. As it was mentioned in the literature review in an accordance to several studies (Gilmore et al., 2013; Endress et al., 2013; Ruiz et al., 2001; Kahn et al., 1993; Martins et al., 2012), numbers of ethnic groups have used *aguaje*, in a large scale, for generations. Generations in the case of less contacted communities, having different languages, different traditions and different habits, could mean centuries or Millenia of isolation and the natural resources exploring and their using consequently.

Related to the results, it seems that people can also contribute to the higher genetic diversity of *M. flexuosa* through different kind of intervention. Perhaps the gene flow and the significant values of gene variability may be affected by long term human activity. Thus, the obvious differences between the genetic structure of individual populations from different river basins has been lost. However, a positive effect could also be a permanent influx of new genes to remote populations of *M. flexuosa* and increasing of the genetic diversity of the species afterwards. Even according to the structure analysis, it is evident that the cultivated populations come from the nearby wild populations. If there were not such a large scale deforestation and rainforest fragmentation, the cultivation of the individuals from the local wild population would be positive for the further existence of the native species. In this context, the value of inbreeding decreases as same as the chance of the emergence of a new subspecies.

On the other hand, the fluctuation of genes across unfragmented ecosystems keeps on and the new hybrids are able to adapt the unstable environment. But according to Federman et al. (2014), the artificial anthropogenic and destructive changes in the landscape have created barriers to gene flow on the island Trinidad, which lead to the nearest-neighbour mating afterwards, because the seed dispersal and pollination mediating by animals was disturbed. If the watercourse were changed and if the Amazon fragmentation carried on, the genetic diversity would be significantly influenced as same as the *Mauritia flexuosa* populations on the island.

7 Conclusion

According to this study, seven out of used eight SSR primers could be recommended for further genetic studies focused on *M. flexuosa*. The polymorphic microsatellite loci revealed a low molecular variance among the regions and among the populations, however, within individuals extremely higher values were shown. All populations are characterized by high levels of genetic diversity and low levels of inbreeding. Based on the results of this study, *M. flexuosa* is definitely not endangered from the point of view of genetics.

Although the research composed of the sampled populations from various watersheds, there was no fundamental genetic structure shown in-between them. Obviously, gene flow plays an important role in the Amazon basin. An open ecosystem without significant barriers within the river networks, such as the Amazon, leads to the constant and free spreading of genes. The propagation and existence of many species depend on the hydrology conditions. Probably also human activity contributes to the high values of genetic diversity of *aguaje*. However, any interference in the culmination of the rivers could irreversibly break long-lasting linkages and could easily result in an immediate decline in genetic diversity, not only in the case of the species *M. flexuosa*. That is the reason why the protection of Amazonian rainforest is so important in the global way of thinking. However, it will be necessary to gather the information and focus in the future, particularly, on less explored areas from a genetic diversity perspective. To prevent the destruction of *aguajales* due to the increasing demand and population growth, the efficient breeding based on the results of various studies has to be done.

The high genetic diversity will be very useful for hybridization. Based on the heterosis effect, genotypically specific individuals can be crossbred consequently. New hybrids could thus be more adaptable to climate changes in the tropics, where, especially the prolongation of drought periods causes an enormous problem. According to the data extensions of genetic diversity, the study could be also helpful for the IIAP – Instituto de Investigaciones de la Amazonía Peruana, realizing many investigations related to Amazonian indigenous species.

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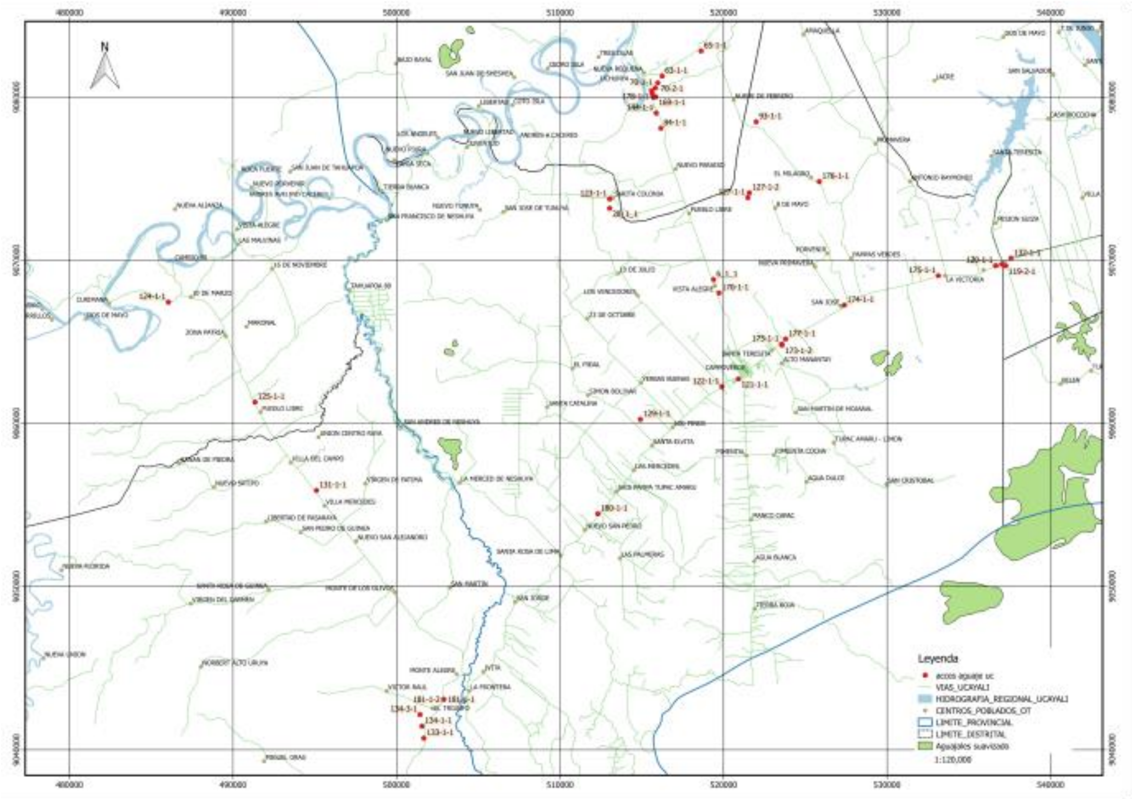
APPENDIX A.

Original provenances of collected seeds of *aguaje* by IIAP.



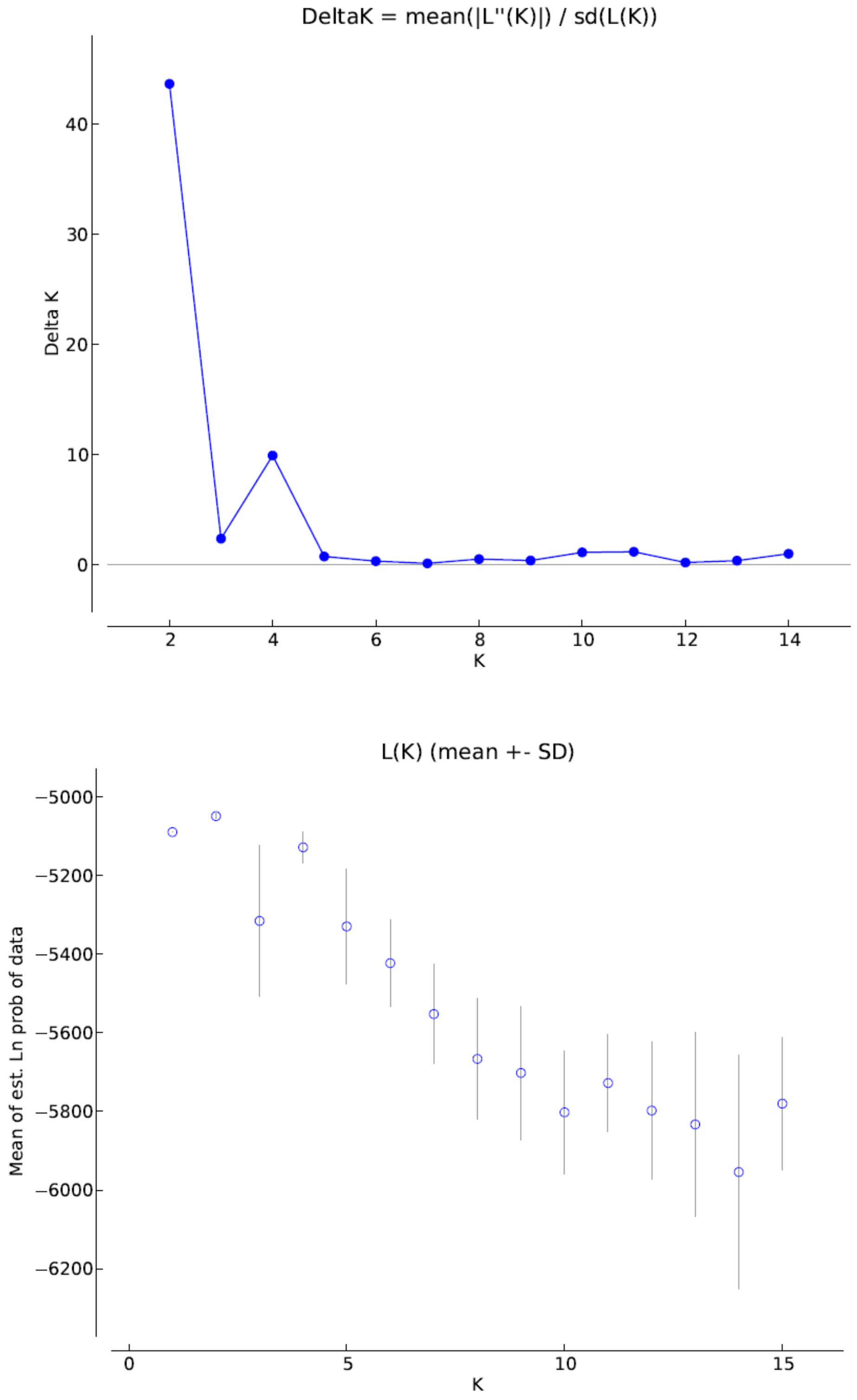
They were planted consequently on the experimental plantations owning by IIAP within the project Desarrollo de Tecnologías Sostenibles para el Manejo de Poblaciones Naturales de *aguaje* en Ucayali en los distritos de Campo Verde y Nueva Requena en los distritos de Campo Verde y Nueva Requena, elaborated by Jorge Manuel Revilla Chávez, Diego Gonzalo García Soria.

APPENDIX B.

Summary of G-Statistics.

Locus	Gst	G'stN	G'stH	G''st	Dest	P (Gst)
Mf04	0.022	0.024	0.310	0.311	0.294	0.003
Mf22	0.024	0.025	0.245	0.246	0.226	0.006
Mf34	0.004	0.005	0.052	0.053	0.048	0.237
Mf24	0.018	0.020	0.145	0.146	0.129	0.036
Mf13	0.003	0.003	0.025	0.025	0.021	0.355
Mf19	0.009	0.010	0.050	0.051	0.042	0.226
Mf25	0.023	0.024	0.192	0.194	0.173	0.009
Mean	0.015	0.016	0.130	0.131	0.117	0.001

APPENDIX C.

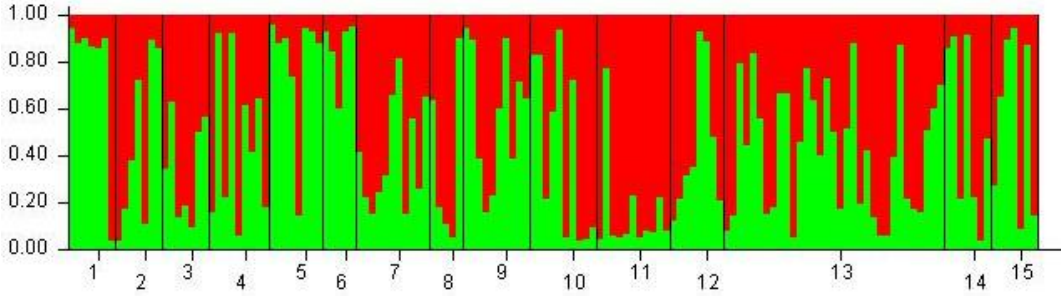


Delta K graph, showing the most appropriate number of genetic clusters (K=2) and mean estimated log-normal (Ln) probability of the data for K = 1–15. Related to the Structure output data, the results for 10 repeats were summerized by Structure Harvester software.

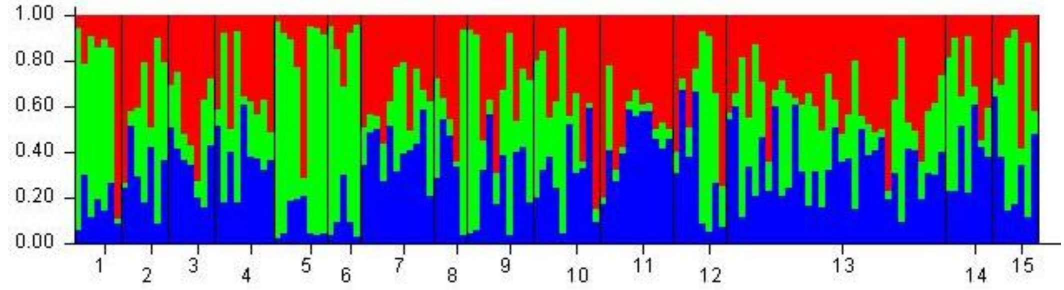
APPENDIX D.

Fractional assignment of individuals to the inferred clusters in Structure software.

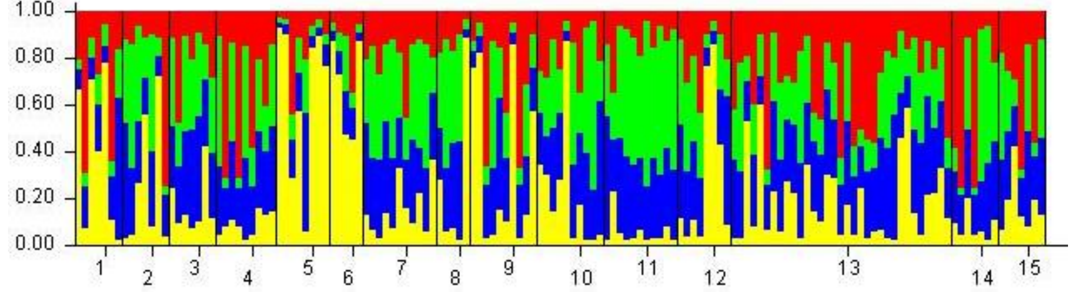
a) K = 2



b) K = 3



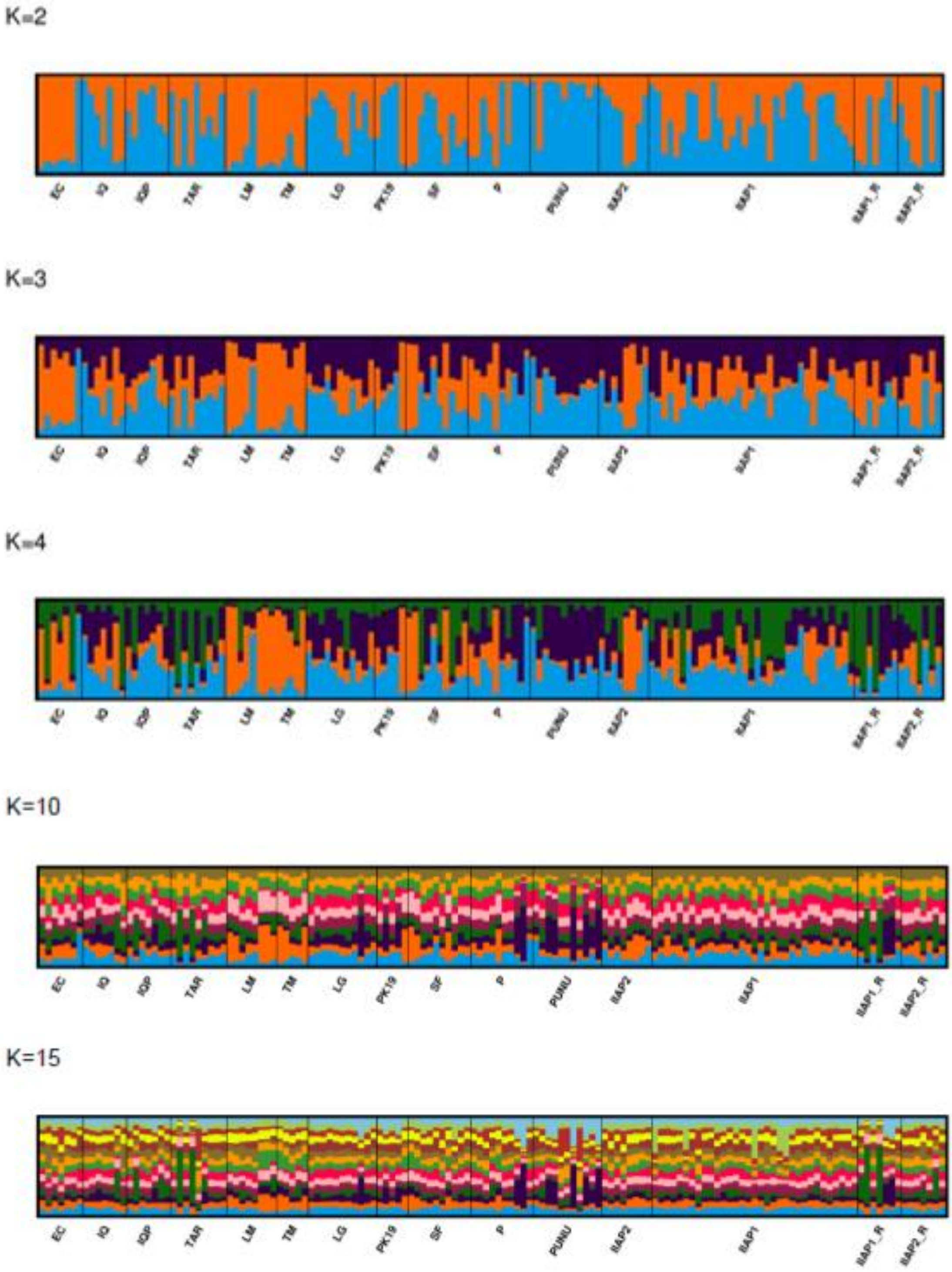
c) K = 4



Populations: 1 EC, 2 IQ, 3 IQP, 4 TAR, 5 LM, 6 TM, 7 LG, 8 PK19, 9 SF, 10 P, 11 PUNU, 12 IIAP2, 13 IIAP1, 14 IIAP1–R, 15 IIAP2-R

An example bar plots and tree plot of the first run. X-axis generates the number of the population divided by the grey lines between.. According to the genotype affinity to the particular cluster, the samples figured by the individual vertical lines are split with significant colours. Due to the estimation of Ln probability data they were distinguished several groups. 145 individual samples divided to a) Two clusters K=2; cluster 1 green, cluster 2 red. b) Three clusters K=3; cluster 1 blue, cluster 2 green, cluster 3 red. c) Four clusters K=4; cluster 1 yellow, cluster 2 blue, cluster 3 green, cluster 4 red.

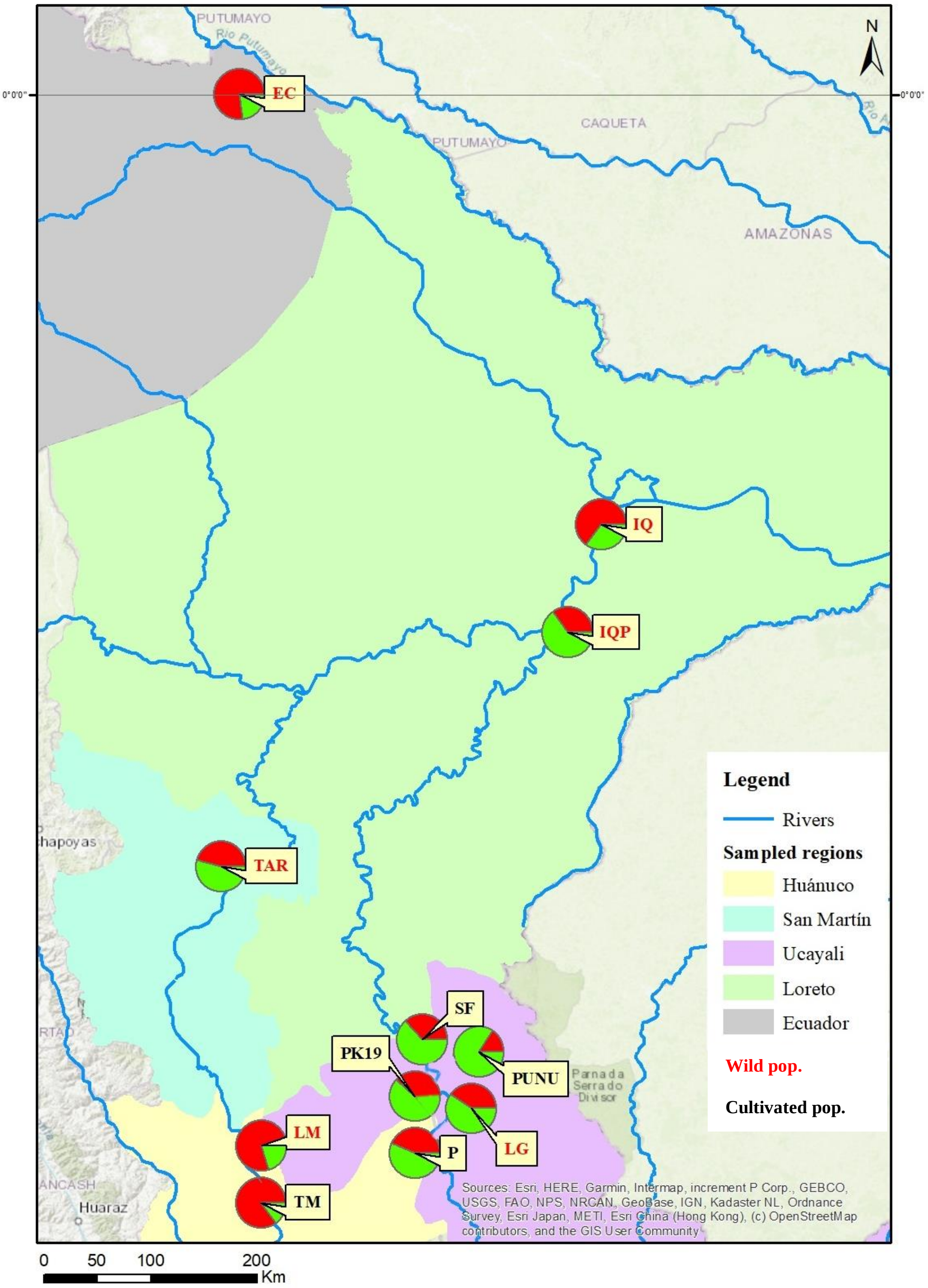
APPENDIX E.



Results generated in the Clumpak software according to various number of clusters. The structure is not differentiated even in the case of 10 or 15 genotypes groups.

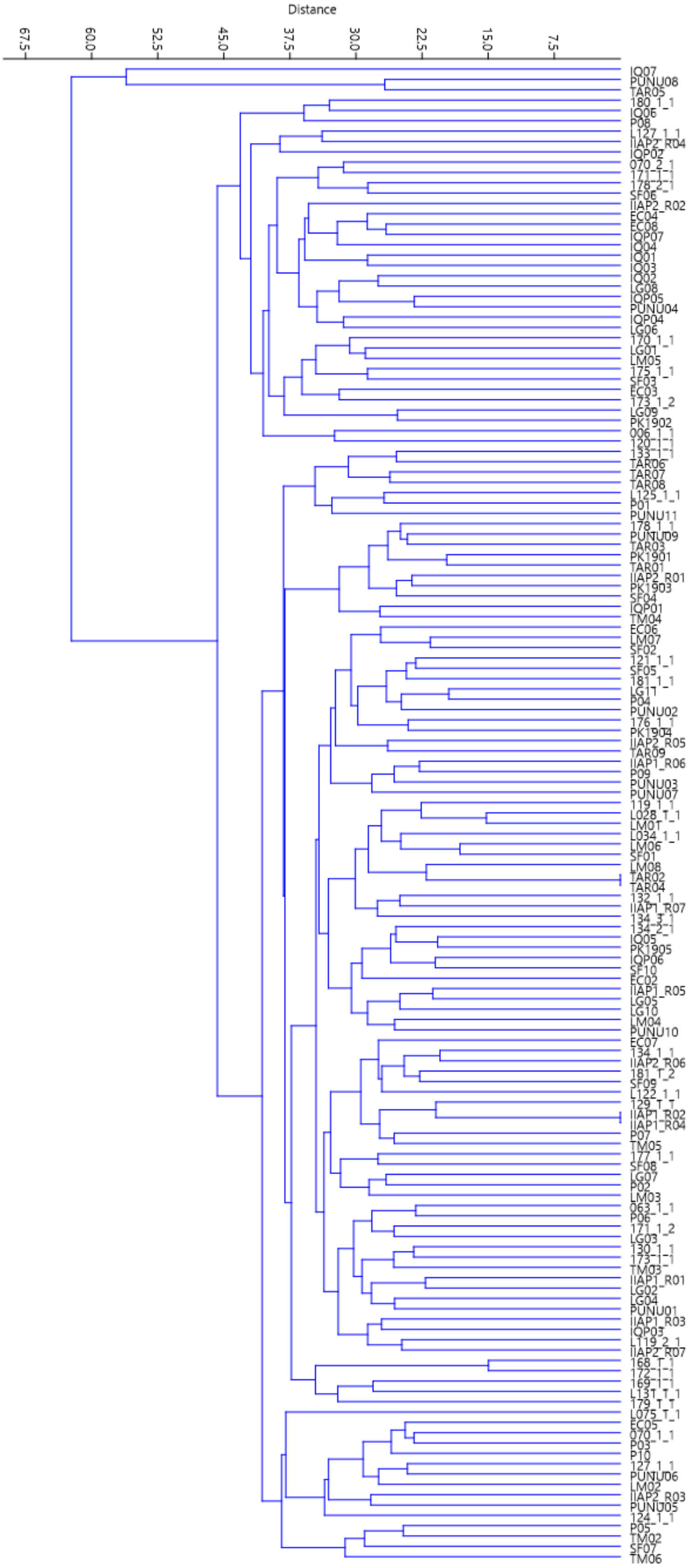
APPENDIX F.

Division of wild and cultivated populations into two genetic clusters.



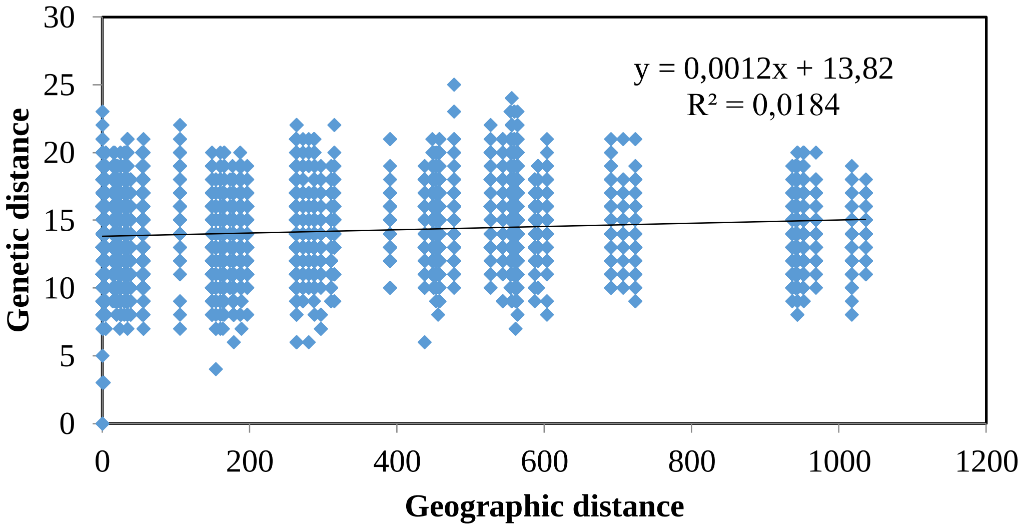
Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

APPENDIX G.



UPGMA hierarchy clustering diagram of all individuals.

APPENDIX H.



Mantel test showing weak correlation between geographic and genetic distances at the level of individuals.