Phylogeography and phylogenetic diversity of Amazon tree species and communities

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Abstract

The Amazon rain forest is the most diverse ecosystem on Earth, harbouring more than ten thousand tree species. In this project, I used ecological and molecular information to explore how ecological factors and historical events have determined the species distributions and population genetic structure of tree species and the phylogenetic diversity of tree communities in the Amazon rain forest.

Chapter 2 indicates that seasonally dry vegetation in northern South America represents a barrier to migration for *Ficus insipida* (Moraceae) and other wet-adapted Amazonian tree species as they have different plastid haplotypes restricted to Mesoamerica and Amazonia. Conversely, the ability of some pioneer species to survive seasonal drought may explain the weakly differentiated phylogeographic structure within these species, with some haplotypes occurring on both sides of this barrier. Chapter 3 explores whether patterns of population genetic structure in five widespread western Amazonian tree species are consistent with historical explanations. My results show that the genetic patterns among species are not entirely congruent suggesting that tropical rain forest species respond differently to long-term geological and climatic changes. Despite this, some tentative generalisations emerge, notably high genetic diversity and a strong geographic structure for plastid sequences suggesting long-term population stability across western Amazonia, and recent population expansions in the south-western Amazon. Chapter 4 uses 283 floristic inventories from the RAINFOR plot network to explore patterns of phylogenetic diversity across Amazonia. This study reveals that the species-rich communities of central Amazonia are dominated by phylogenetic close relatives compared to the equally species-rich communities of the north-west that tend to contain more distantly related species. Across Amazonia, an east-west gradient of the abundance of early divergent angiosperm clades was found, with the greatest percentage of tree species of Magnoliids and Monocots in the west. As these early diverging clades are also characteristic of pre-montane habitats, these results suggest that migration events from cooler environments at different geological times has played an important role in the assemblage of the most phylogenetically diverse communities in Amazonia.

The findings from these three chapters corroborate the notion that both ecological factors and historical events have been important in determining species distributions and the phylogenetic diversity of tropical tree communities in Amazonia. Regional differences in genetic structure among populations, and phylogenetic diversity among communities, should both be taken into account in forest conservation planning and management.

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Abbreviations

Al	Aluminium
AMOVA	Analysis of molecular variance
Ca	Calcium
CES	Combinatorial enhancer solution
CI	Confidence interval
cpDNA	Chloroplast DNA
DBH	Diameter at breast height
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate (A, C, G, T)
ECEC	Effective cation exchange capacity
ha	Hectare
indels	Insertions or deletions in DNA sequence
ITS	Internal transcribed spacer
IV	Inversion in DNA sequence
K	Potassium
Km	Kilometre
Ma	Million years before present
Mg	Magnesium
MPDt	Mean phylogenetic distance among unique taxa

n	sample size
Na	Sodium
nrDNA	Nuclear ribosomal DNA
NS	Nucleotide substitution in DNA sequence
PCR	Polymerase chain reaction
PD	Phylogenetic diversity
Φ_{CT}	Genetic differentiation among the groups
R	R Statistical Software
RAINFOR	Amazon Forest Inventory Network
SAMOVA	Spatial analysis of molecular variance
SB	Sum of bases
SD	Standard deviation
SDTF	Seasonally dry tropical forest
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
Tukey's HSD	Tukey's Honestly Significant Difference Test

Chapter 1. Introduction

1. 1. PROJECT RATIONALE

Ecological factors and historical events together determine the spatial patterns in the distribution of species and floristic communities. Ecological factors include biotic interactions and habitat filtering while historical events include specific geological and climatic changes, speciation, extinction, migration and so on. Although ecology and history may appear distinct, this is perhaps an artefact of our perspective from one point in time. Thus, it is clear that ecological factors may have wide geographic effects and can operate over million-year timescales (Crisp *et al.*, 2009; Pennington *et al.*, 2009), while on the other hand some historical events may also be responsible for local distributional patterns (Dexter *et al.*, 2012) and ecosystem properties (Hoorn *et al.*, 2010; Higgins *et al.*, 2011).

Early naturalist-explorers (e.g. Ruiz and Pavon in 1777-1786, von Humboldt and Bonplant in 1799-1805, and others) were the first people to start thinking about ecological and historical processes. For example, they were interested in the high diversity of tree species in Amazonia. However, this early work was descriptive, and quantitative data were produced only since the 1930s (e.g. Davis & Richards, 1934; Black *et al.*, 1950; Takeuchi, 1960). Now, modern floristic and molecular studies are used to unreveal the role of both ecological and historical processes. For example, floristic inventory plots have been used for testing the effect of the current environment on the spatial patterns in the distribution of tropical trees (e.g. Gentry, 1988a; Terborgh & Andresen, 1998; Ter Steege *et al.*, 2006). These studies have shown that tree diversity is unevenly distributed across Amazonia and that considerable species turnover occurs both locally and among different geographical regions. In addition, molecular biology has shown that historical events have played major roles in establishing present-day distribution patterns of species. The use of dated phylogenies has helped to reconstruct the origin and dispersal events of taxa across continents (Pennington & Dick, 2010) and at a finer taxonomic level, the effect of historical processes has been shown studying the genetic structure of populations of widespread species (e.g. Dick & Heuertz, 2008; Rymer *et al.*, 2013). However, for six million square

kilometres of Amazon rain forest, the practical challenges of accessibility and species identification have restricted the number of studies that can examine both historical and ecological processes at large geographical scales.

In this research project, I focus on the Amazon rain forest because of its high species diversity and its wide range of environmental and ecological conditions as well as its long geological history. Forests of north-western and central Amazonia are the most diverse on Earth, harbouring up to c. 300 species of trees with a diameter at breast height (DBH) $\geq$ 10 cm (Gentry, 1988b; Valencia *et al.*, 1994; De Oliveira & Mori, 1999). In spite of the high number of species represented by single individuals, and the great diversity of these forests, relatively few species may tend to be ecologically important because of their abundance, basal area and frequency. In some cases, locally abundant species can also dominate over large areas (oligarchies; Pitman *et al.*, 1999). One could expect that ecological adaptations may favour the abundance and widespread distribution of these species, but how they have responded over long-periods of time to historical events such as geological and climatic changes remains unknown. Therefore, this study focuses on comparing genetic patterns with historical events in widespread species to give insights into the history of Amazonia.

Over timescales of millions of years, historical events such as plate tectonics and climate change have promoted speciation, adaptation and extinction of floras, including in the Neotropics (Gentry, 1982; Pennington *et al.*, 2004; 2006b). For example, in South America, the uplift of the Andes developed new habitats for many species (e.g. *Inga*; Richardson *et al.*, 2001), and also promoted local extinctions and allopatric speciation in others (e.g. *Mauritia*; Rull, 1998). In addition, the closure of the Panama Isthmus that began in the Miocene and finished ca. 3 Ma facilated inter-continental migration (Coates & Obando, 1996) of Laurasian taxa into South America, and Gondwana taxa into North America. However, other past and current ecological factors are also likely to have had an important effect. For example, the effect of current environmental conditions such as rainfall and temperature (Gentry, 1988a; Ter Steege *et al.*, 2006), past and present edaphic differentiation (Gentry, 1981; Salo *et al.*, 1986; Tuomisto *et al.*, 1995; Fine *et al.*, 2005),

competition, herbivory (Fine *et al.*, 2004), past variation in climate (Zachos *et al.*, 2001), dispersal limitation (Condit *et al.*, 2002) and successful long-distance dispersal (Pennington & Dick, 2004; Pennington *et al.*, 2009) have all been proposed as potential factors to drive diversification and determine species distributions in Amazonia.

By using a series of large datasets of the phylogenetic relationship among populations, species and communities, this thesis tests whether ecological factors and historical events have determined the biogeography of neotropical tree species and the distribution of the phylogenetic diversity of tree communities across Amazonia. In particular, genetic patterns which are developed over long periods of time are compared to past climatic and geological changes and to current ecological preferences of the study taxa and communities. This thesis addresses the following questions: Are there ecological and historical barriers to gene flow between Mesoamerica and Amazonia for widespread wet-adapted species?; Have widespread species responded similarly to climatic and geological changes across western Amazonia?; and How is phylogenetic diversity of floristic communities distributed across the Amazon basin?

1. 2. ECOLOGY AND HISTORY OF THE AMAZON RAIN FOREST

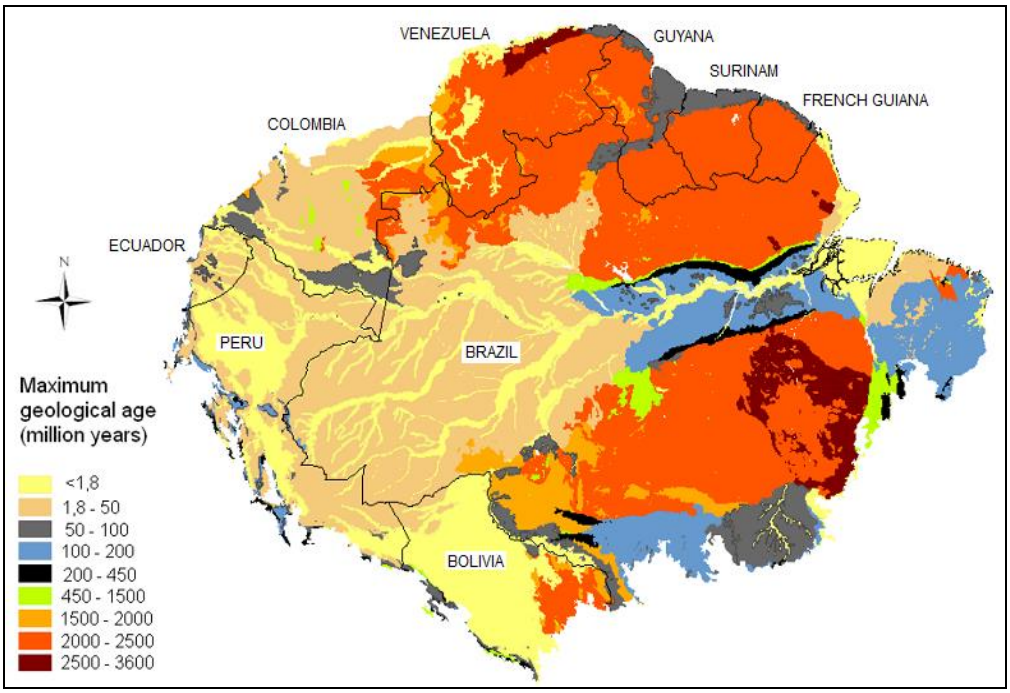
1.2.1. The Amazon rain forest

The Amazon rain forest is the most species-rich and the largest area of tropical rain forest in the world (Myers *et al.*, 2000), harbouring an estimated eleven thousand tree species with DBH $\geq$ 10 cm (Hubbell *et al.*, 2008) and covering ca. 6 million km^2 in nine countries - Colombia, Venezuela, Guyana, Suriname, French Guiana, Brazil, Bolivia, Peru and Ecuador (Figure 1.1). Amazonia is characterized by high alpha and beta diversity, with forests containing as much as 300 species per hectare of trees with DBH $\geq$ 10 cm and a high turnover of species at local (Phillips *et al.*, 2003; Tuomisto *et al.*, 2003b) and regional scales (Ter Steege *et al.*, 2006). Broad gradients in geology, soil fertility, and climate occur (Sombroek, 2001; Quesada *et al.*, 2011). For example, soils in the Guiana Shield and the Brazilian Shield are generally geologically older and less fertile than those in western

Amazonia (Figure 1.1a) where sediments from the Andes have been deposited more recently (Quesada *et al.*, 2011). Previous studies have suggested that spatial patterns in tree floristic composition (Ter Steege *et al.*, 2006; Honorio Coronado *et al.*, 2009), wood density (Baker *et al.*, 2004), above-ground biomass, productivity and mortality (Quesada *et al.*, 2012) are all influenced by differences in soil fertility and soil depth across Amazonia. Therefore, soil fertility is a key ecological factor to be considered when studying the Amazon flora. Dry season length also varies across the basin with more seasonal climates in the southeast, to almost no dry season in the northwest (Figure 1.1b). Mean annual rainfall can vary from 1250 mm in the southeast to more than 3000 mm in the northwest (Sombroek, 2001).

The Amazon rain forest is considered as a tropical moist forest in different classification systems available for the world because of its tropical location and characteristics of the vegetation and fauna. Within Amazonia, I will use a summarized classification of forest formations by Whitmore (1998) which is based on the general structure and physiognomy of the vegetation and on environmental and ecological factors such as climate, water availability, soil properties and altitude. In this classification, at least eight of the 14 formations of tropical rain forests of the world occur in Amazonia (Table 1.1). In terms of climate in Amazonia, seasonal regions can have up to 4-5 consecutive months with less than 100 mm rainfall, while rainfall in ever wet regions never falls below 100 mm per month (Sombroek, 2001). Flood frequency is determined by topography and river level fluctuations (Salo *et al.*, 1986) leading to areas subject to inundation (flood plain) and areas that never flood (terra firme). Rivers in Amazonia are of two main types that differ in their pH, nutrient content and humic matter (Prance, 1979). Black water rivers contain slowly degradable phenolic substances (organic matter), and have low level of nutrients and pH. In contrast, white water rivers have high pH, large amounts of heavy suspended sediments and a high level of nutrients (Kvist & Nebel, 2001). The most important forest formations are described below including characteristics of the geomorphology, soil types (based on the world reference base for soil resources; IUSS Working Group WRB, 2006) and location within Amazonia.

a)



b)

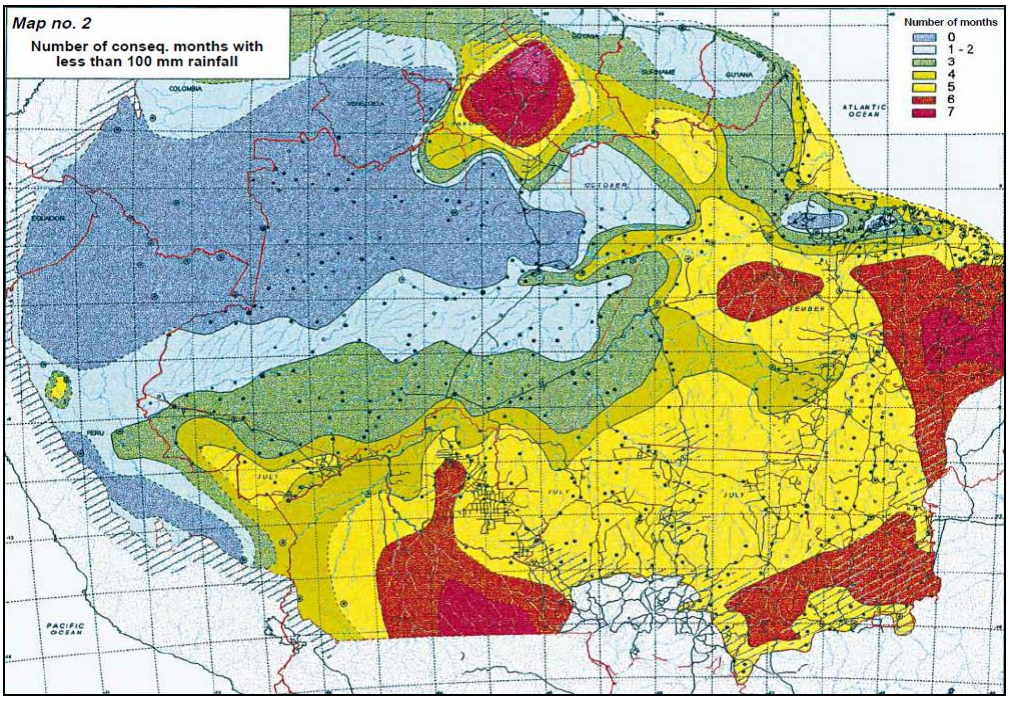


Figure 1.1. (a) Ages of geological sediments (from Quesada *et al.*, 2011) and (b) dry season length (from Sombroek, 2001) across the Amazon rain forest.

Table 1.1. The formations in tropical rain forests (Whitmore, 1998). The most important forests occurring in Amazonia are highlighted with an asterisk.

Climate	Soil water		Soils	Elevation	Forest formation
Seasonally dry	Strong annual shortage Slight annual shortage				Monsoon forests (various formations) Rain forests: *Semi-evergreen rain forest
Ever wet (perhumid)	Dry land		Zonal (mainly oxisols, ultisols)	Lowlands	*Lowland evergreen rain forest
				(750) 1200-1500 m	*Lower montane rain forest
				Mountains (600) 1500-3000 m	*Upper montane rain forest
				Mountains 3000 (3350) m to tree line	Subalpine forest
			Podzolized sands	Mostly lowlands	*Heath forest
			Limestone	Mostly lowlands	Forest over limestone
			Ultrabasic rocks	Mostly lowlands	Forest over ultrabasics
	Water table high (at least periodi-cally)	Coastal salt-water			Beach vegetation Mangrove forest Brackish water forest
		Island fresh-water	Oligotrophic peats		*Peat swamp forest
			Eutrophic (muck and mineral) soils	± Permanently wet Periodically wet	*Freshwater swamp forest *Freshwater periodic swamp forest

(1) *Lowland evergreen rain forest* dominates most of Amazonia. This formation has a stratified structure with emergent, canopy and understory trees. Emergent trees often have buttresses and woody lianas are frequently abundant. Topography is determined by terraces and low hills (Encarnación, 1985). Soils are characterised by the deep reddish to yellowish colour of the clay, and are typically well-drained. These vary across the basin with a dominance of Ferrasols in the Guiana and Brazilian Shields, Plinthosols in central Amazonia, and Acrisols and Cambisols in the west (Quesada *et al.*, 2011).

(2) *Semi-evergreen rain forest* is characteristic of the seasonal regions of Brazil, Bolivia and some parts of Peru. This formation has deciduous and evergreen species but deciduous species are common in the canopy. Trees have buttresses and climbing lianas and bamboos are common. Soils are shallow with high fertility (Quesada *et al.*, 2011).

(3) *Heath forest* or white sand forest comprises a dense vegetation of thin-stemmed trees. This forest is known as “varillal” in Peru and Colombia, and “campinarana” in Brazil. The most extensive areas are located in the upper Rio Negro in Brazil and on the Rio Orinoco in Venezuela; scattered patches of white sand forest also occur in Peru and Colombia. Woody climbers are rare. In Amazonia, this formation develops on deposits of quartzitic sands. Streams draining this formation contain black water. Soils are Podzols, highly acidic with low fertility, and also Arenosols (Quesada *et al.*, 2011).

(4) *Freshwater swamp forest* is defined by the permanent flooding by river water and (5) *freshwater periodic swamp forest* occurs when the flooding is episodic (daily, monthly) or seasonal. The terms *igapó* and *várzea* are commonly used to describe different types of flooded forest based on the type of water that these forests receive. *Igapó* refers to forests inundated by black water and *várzea* to those inundated by white water (Prance, 1979). In Amazonia, large areas of freshwater swamps are located near Iquitos in Peru and near Manaus in Brazil (Prance, 1979). Nutrients in these minerotrophic swamps are obtained from the periodical or permanent floodwater, rather than rainwater, and in some cases peat accumulation has been reported in *igapó* forest (Lahteenoja *et al.*, 2009). Soils are classified as Gleysols, Fluvisols and Histosols (Quesada *et al.*, 2011).

(6) *Peat swamp forest* is an unusual habitat that accumulates partly decomposed organic matter under anoxic conditions (Lahteenoja *et al.*, 2009). These permanently waterlogged forests are not common in Amazonia, but large areas are located in northern Peru and other scattered patches are found in central Brazil. This forest type also includes forests growing around ox-bow lakes across the basin. These nutrient-poor ombrotrophic swamps receive nutrients from atmospheric deposition and sometimes from reworked alluvial deposits. Soils are also classified as Gleysols (Prance, 1979).

(7) *Montane rain forest* occurs at 750-3000 metres above sea level along the Andes of Venezuela, Colombia, Ecuador, Peru and Bolivia. These forests are often covered by clouds that generate an ever wet environment rich in epiphytes, ferns, and lichens. They contain a high level of species diversity and endemism. While many lowland rain forest species reach lower montane areas (< 1500 m), the upper areas which are lower in temperature are

dominated by a different set of species that rarely reach the lowlands. Soils tend to be superficial with high organic matter content.

Two other main forested biomes occur in tropical South America, which are distinct from the Amazon rain forest – the seasonally dry tropical forest (SDTF) and the woody savannas. SDTF occurs on highly fertile soils and where the annual rainfall is less than 1600 mm, with at least 5-6 months receiving less than 100 mm (Gentry et al., 1995b). Savannas are found on poor soils similar to rain forest, and with similar or slightly higher annual rainfall than SDTF (Sarmiento, 1992). Large areas of SDTF (Caatingas and Misiones Nucleus) and savannas (Cerrado) are located mainly in Brazil and Bolivia (Pennington *et al.*, 2000). SDTF patches occur along the Caribbean coast, and in inter-Andean valleys of Colombia, Ecuador, Peru and Bolivia, while other savannas also occur in Venezuela (Los Llanos) and the Guianas (Rupununi; Pennington et al., 2000). The flora of SDTF is adapted to seasonal water stress with the vegetation mostly deciduous during dry season, while the savanna flora is adapted to fire with a grass dominated ground layer and sclerophyllous trees with evergreen leaves (Ratter *et al.*, 1997).

1.2.2. Plate tectonics in South America

The current Amazon rain forest lies on the Amazonian Craton, a large tectonic plate which formed in the Proterozoic (3000 - 1000 million years ago, Ma; Cordani *et al.*, 2009). In the middle of the Jurassic period, the supercontinent of Pangaea started to break up, forming Gondwanaland (South America, Africa, Australia, Antarctica, Indian subcontinent, and the Arabian peninsula) and Laurasia (the remaining continents), and at the end of the Jurassic, ca. 150 Ma, South America began to separate from Africa (Figure 1.2) (Dietz & Holden, 1970). The pressure among the geological plates during continental drift caused some structural faults in South America (Carauarí, Purus, Monte Aleg, and Gurupa arches) that divided the drainage basins into western and eastern portions (Roddaz *et al.*, 2005; Haffer, 2008). During the Early Cretaceous, the formation of the Southern Andes of Bolivia, Chile and Argentina began (120 Ma; Graham, 2009). During the mid-Cretaceous, South America started its separation from Antarctica. This split finished in the early Oligocene, initiating

the circum-Antarctic current and the formation of the Antarctic ice sheet in the middle Eocene (38 Ma; Ehrmann & Mackensen, 1992).

Geological changes are closely related to global changes in climate, and both have promoted the diversification of animals and plants, as well as migration and extinction of organisms around the world. While warm global conditions characterized the period of the dinosaurs (Mesozoic) and promoted the expansion of tropical climates after the Late Cretaceous (Figure 1.2), a gradual global cooling characterized the Tertiary (65 Ma to 2.6 Ma described below) that included the diversification and proliferation of mammals and angiosperms. At the boundary between the Palaeocene and the Eocene occurred one of the most extreme temperature increases in Earth history, known as the Palaeocene-Eocene Thermal Maximum (PETM; Zachos *et al.*, 2001). By the start of the Palaeocene (65 Ma), South America was considerably distant from Africa. During the Eocene the Central Andes uplift (40 Ma; Graham, 2009) started. During this same period, two marine incursions are thought to have occurred in South America caused by high sea levels, one from the Pacific separating the Northern and Central Andes (Western Andean Portal) and a second from the Caribbean (~35 Ma; Antonelli *et al.*, 2009). During the Miocene (23-6 Ma), it has been suggested that western Amazonia was dominated by a wetland system (Pebas Lake; Hoorn, 1996; Hoorn & Vonhof, 2006) and a fluvial system in the east that subsequently formed the Amazon River (Figueiredo *et al.*, 2009). The separation of these regions was probably caused by the presence of extended uplands or arches (Roddaz *et al.*, 2005; Haffer, 2008) that were probably used as corridors for animals and plants between the Guiana and Brazilian Shields until their isolation caused by the formation of the Amazon river (Haffer, 2008).

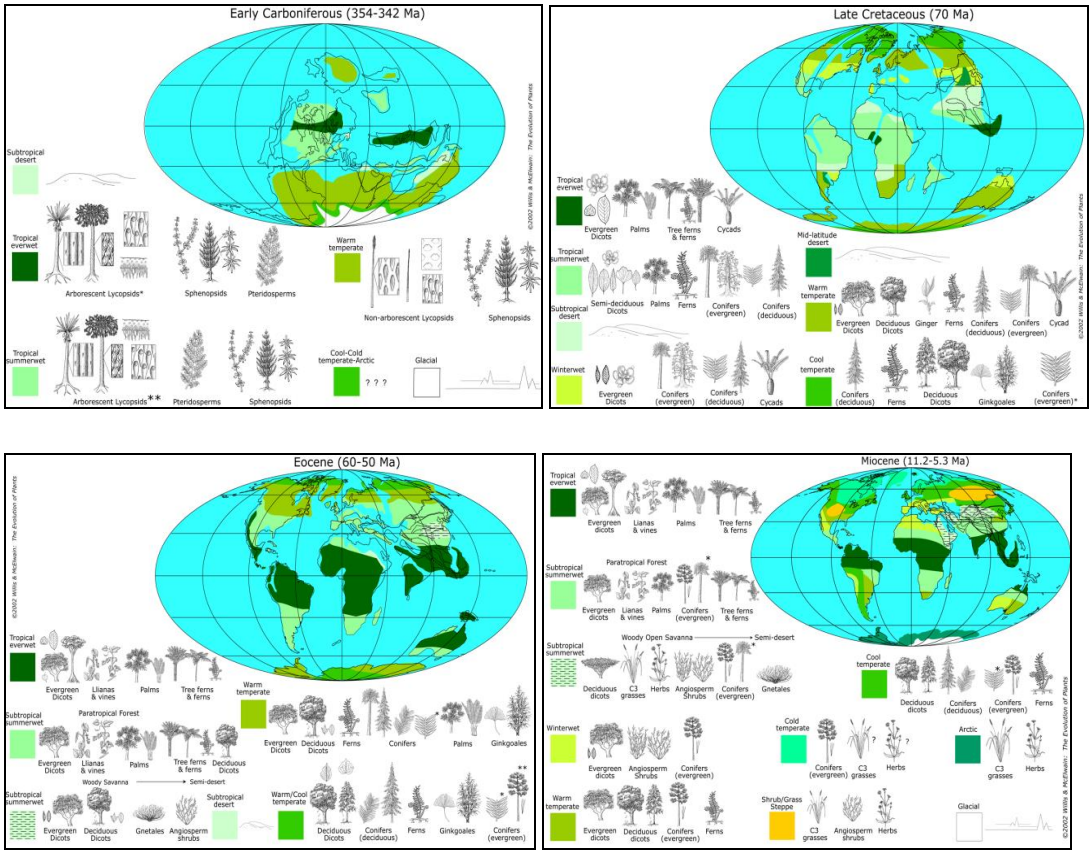


Figure 1.2. Plate tectonics and the evolution of plants (from Willis & Mc Elwain, 2002). © Oxford University Press

In the Late Miocene (11-7 Ma), a rapid uplift of the northeast Andes caused by the collision of South America and the Nazca plates connected the paleo-Amazon in the east with Lake Pebas in the west forming the Amazon river (Hoorn *et al.*, 1995; Figueiredo *et al.*, 2009). Evidence of this connection was found in the Atlantic in the form of Andean sediments dating from this time (Figueiredo *et al.*, 2009). The drainage patterns of other major rivers such as the Magdalena and the Orinoco were also changed. During the Late Miocene and the Pliocene, Andean uplift was still active causing the uplift of new features in western Amazonia, the Iquitos (Roddaz *et al.*, 2005) and the Fitzcarrald arches (Regard *et al.*, 2009), that together with the formation of the Amazon River caused aquatic habitats of the wetland system to become fragmented and dissected (Hoorn & Wesselingh, 2010). Then,

North America and South America joined with the closure of Panama Isthmus around 3.5 Ma (Coates & Obando, 1996), though there is now speculation that this event may have happened during the early Oligocene to early Miocene (see Bacon *et al.*, 2013).

The Quaternary (< 2 Ma) is characterized by the incidence of more than 20 cycles of cold and warm periods, defining the glacial and interglacial cycles (Zachos *et al.*, 2001). Within the northern hemisphere, pollen fossil and molecular studies have confirmed the refugia hypothesis, suggesting large-scale colonization events of newly deglaciated land and the restriction of tree species to refugial areas during glacial periods (Hewitt, 1999). For Amazonia, it has been proposed that cycles of dry periods led to the reduction and fragmentation of the Amazon rain forest (refugia) and expansion of dry habitats (Haffer, 1969; Prance, 1982). However, the effect of the oscillation in Pleistocene climatic conditions in Amazonia remains in debate. Studies of pollen fossils propose cooler, wet conditions across Amazonia and potentially drier areas at the edges of this biome (Van Der Hammen, 1974; Bush & Colinvaux, 1990; Colinvaux *et al.*, 1996). Other studies also suggest that cool periods during the Quaternary were related to a reduction in atmospheric CO_2 that affected the distribution of some montane taxa (Cárdenas *et al.*, 2011). Moreover, evidence of rain forest expansion after the last glacial maximum based on pollen fossils is suggested for eastern Bolivia, on the margin of Amazon rain forest, potentially related to changes in the position of the Intertropical Convergence Zone (ITCZ; Mayle *et al.*, 2000). This study indicates that the ITCZ, which determines a seasonally dry climate in south-western Amazonia, is expected to have changes in latitudinal position explained by Milankovitch astronomic forces and changing the distribution of the southernmost Amazon rain forest.

Although the first hominids appeared in the African fossil record at 4-7 Ma; it is not until the Holocene that people colonized South America. The Holocene, the last 11,500 years, has been relatively stable in climate (Zachos et al 2001). Settlement, hunting and the development of agriculture in South America have likely had substantial influence on the current landscape of Amazonia. For example, the arrival of humans in South America coincides with the extinction of the local megafauna (Martin, 1973). Other examples include the American oil palm, *Elaeis oleifera*, whose current distribution pattern is

probably related to human settlements (Henderson *et al.*, 1995). A summary of major historical events impacting the tropical South American flora is presented in Table 1.2.

Table 1.2. Potential historical events that might have influenced the distribution of tropical flora in South America

Period	Age (Ma)	Geological and climatological events
Jurassic	175	Pangea starts break up forming Gondwanaland (South America, Africa, Asia, Antarctica) and Laurasia (rest of the continents)
	150	South America starts its separation from Africa
Cretaceous	120	Formation of the Southern Andes in South America Development of suitable climate for tropical flora
Palaeocene	65	Cretaceous-Tertiary mass extinction
Eocene	55	Thermal maximum (expansion of tropical rain forest)
	44	Gradual global cooling
	40	Formation of Central Andes
	35	Marine incursions
Miocene	23	Wetland system in western Amazonia
	11	Rapid uplift of the northeast Andes and consequently formation of Amazon river
Pliocene	3.5	Closure of Panama Isthmus by the joining of South and North America
Pleistocene	2.5	Cold and warm periods
Holocene	0.01	Human modification of the landscape

1.2.3. The origin and diversification of tropical rain forest flora in South America

Abundant evidence from macrofossils and fossil pollen of arborescent lycopods and pteridophytes suggests that a climate suitable for rain forest developed across the continents of the northern hemisphere during the Early Carboniferous (Figure 1.2) (Willis & Mc Elwain, 2002). In the southern hemisphere, similar evidence suggests that a tropical flora developed during the Late Cretaceous (Morley, 2000; Maslin *et al.*, 2005), a period that coincides with the diversification of the angiosperms in tropical environments (Judd *et al.*, 2002). For the Neotropics, Late Cretaceous fossil records probably reflect the existence of tropical lineages in South America but not the presence of any ecosystem corresponding to the modern Amazon rain forest (Burnham & Johnson, 2004). The earliest evidence from fossil pollen indicating families characteristic of modern rain forests and leaves with drip-tips indicating rain forest vegetation is reported from northern Colombia from the Late

Paleocene (Wing *et al.*, 2009). Although this record contains moderately high pollen diversity, it is not until the Eocene that fossil records reached the diversity of current neotropical floras (Burnham & Johnson, 2004), including a flora more diverse during the Eocene compared to the Holocene (Jaramillo *et al.*, 2006). Although we know that tropical rain forest in the Neotropics may have been resilient to different geological and climatic changes (Jaramillo *et al.*, 2010), the detailed history of the Amazon rain forest remains uncertain because of the extreme rarity of well-preserved macrofossils in tropical environments where rapid decomposition rates and often extremely dynamic landscapes mitigate against preservation.

For South America, an important general question is whether the current high species richness of the Amazon rain forest is a result of *in situ* diversification since the split of South America from Africa (the break-up of west Gondwana), or if it is a result of dispersal from other land masses (e.g. from Laurasia). After the acceptance of the theory of plate tectonics, most authors supported the idea that the diversification of plants in South America developed in relative isolation from other land masses (west Gondwana origin) during the Cenozoic (Paleocene to Miocene; Simpson, 1980). For example, based on a preliminary study that assigned an area of origin to families to predict if they originated in west Gondwana or Laurasia (Raven & Axelrod, 1974), Gentry (1982) concluded that the Laurasian contribution to the South American rain forest flora was small.

Recent studies based on fossil records and molecular techniques have shown that the South American flora has received numerous taxa that originated outside the continent (immigrants) even when it was physically isolated as a continental island (Morley, 2003; Pennington & Dick, 2004). Based on fossil pollen, different routes for the dispersal of megathermal (frost-intolerant) species to South America have been proposed (Morley, 2003): 1) from North America via an island arc between the Yucatan and Colombia in the Late Cretaceous – Middle Eocene and via the Caribbean “Gaarlandia” landbridge or Caribbean Islands in Eocene – Oligocene, 2) from Africa via putative island chains - the Wallis Ridge in the late Cretaceous – Eocene – and the Rio Grande Rise and Sierra Leone Ridge in the Oligocene, 3) from Antarctica through New Zealand and Australia via the South Sandwich Islands and South Georgia in the late Cretaceous and Palaeocene. In

addition, dated molecular phylogenies indicate that in many taxonomic groups, long-distance transoceanic dispersal has been frequent (e.g. Fabaceae, Lavin *et al.*, 2004; *Renealmia*, Särkinen *et al.*, 2007; *Symphonia globulifera*, Dick & Heuertz, 2008) throughout the Cenozoic. These dispersal routes suggest that the Cenozoic is the most relevant era for the dispersal of megathermal species, coinciding with the extension of tropical climates in the northern hemisphere ("boreotropics") and subtropical temperatures in Antarctica (Figure 1.2) (Morley, 2000).

Good examples of immigrant lineages are found in the ecologically dominant South American rain forest families Fabaceae (Schrire *et al.*, 2005), Annonaceae (Richardson *et al.*, 2004), Lauraceae (Chanderbali *et al.*, 2001) and Melastomataceae (Renner *et al.*, 2001). Based on these and other examples, the percent of species belonging to "immigrant" clades was estimated in a 25-hectare Amazonian rain forest in Yasuni, Ecuador. Of the 1104 total species, 21% belong to families and genera classified as immigrants (Pennington & Dick, 2010), percentage that will almost certainly increase as more dated phylogenies are published. These studies show that tropical rain forest in Amazonia is formed by a complex mix of species of west Gondwanan origin that evolved locally in South America and also immigrant lineages that initially evolved elsewhere.

1. 3. NEOTROPICAL RAIN FOREST BIOGEOGRAPHY

Biogeography is the study of the geographical distribution of organisms in space and time (Morrone, 2008) and can be divided into ecological and historical biogeography. Ecological biogeography attempts to explain species distributions in terms of interactions with present-day physical and biotic environments while historical biogeography tries to reconstruct the origin, dispersal and extinction of taxa and biota (Cox & Moore, 2005). Whilst these approaches appear distinct, it is clear that ecological factors may have wide geographic effects and can operate over million-year timescales (Crisp *et al.*, 2009; Pennington *et al.*, 2009), and some historical events may also be responsible for local distributional patterns (Dexter *et al.*, 2012). This leads to the conclusion that ecology and

history are not independent processes acting on different spatial and temporal scales, but act together over all time scales (e.g. Webb *et al.*, 2002).

Different mechanisms have been proposed to explain the biogeographic patterns of tropical plants, but the most important is undoubtedly plate tectonics. The discovery of continental drift (Wegener, 1912) revolutionized the study of plant biogeography particularly following its acceptance in the 1960s when the mechanism that drove this process (plate tectonics) was elucidated. Plate tectonics is one of the most important mechanisms used to explain the current wide distribution of higher taxa (e.g. families, genera) in association with subsequent independent evolution of species in each tropical region from ancestral plant populations (Renner, 2004). Before plate tectonics, the broad distribution of tropical families and genera was explained by other mechanisms such as long distance dispersal (e.g. Darwin, 1859) or the existence of land bridges that had connected static continents in the past (e.g. Van Steenis, 1962).

A second important mechanism is the effect of past fluctuations of climate, both for generating suitable habitats for tropical rain forest and also for influencing species distributions. For example, pollen fossils and macrofossils suggest that neotropical rain forest was established during the Paleocene (Wing *et al.*, 2009) and was maintained during the Tertiary even in the face of a worldwide long-term cooling trend (Rull, 2008). During the Quaternary, as mentioned above, a reduction in temperatures promoted the migration of montane taxa to the lowland rain forest (Cárdenas *et al.*, 2011).

A third important mechanism is the effect of mountain building, both for creating new habitats and for dividing populations. For example, the development of the Andes in South America is suggested to have caused large scale rearrangement of the landscape through sediment deposition and changes of river drainage. Although the Andean uplift created a new corridor for the migration of cold adapted species at high altitudes, this physical barrier may have had a strong effect on cold intolerant plants leaving a potential signature in the genetic structure of previously continuous populations of widespread neotropical plants. Another potential barrier for wet adapted neotropical species may be the extended areas of dry vegetation in northern South America, restricting the migration of species between rain forests of Central and South America (see chapter 2).

A fourth important mechanism driving species distribution patterns is the effect of edaphic specialization (Salo *et al.*, 1986; Tuomisto *et al.*, 1995; Fine *et al.*, 2005). Soil types in Amazonia originated by several events and from different parent materials (Sombroek, 2001). For example, the Guiana and Brazilian Shields are formations of Pre-Cambrian origin, with highly weathered soils of low fertility. By contrast, young and relative fertile sediments were deposited more recently in western Amazonia during the Pliocene uplift of the Andes (Hoorn *et al.*, 2010; Quesada *et al.*, 2011). These spatial differences in soil fertility determine tree species turnover in Amazonia, both locally (Tuomisto *et al.*, 1995; Phillips *et al.*, 2003; Fine *et al.*, 2005; Honorio Coronado *et al.*, 2009; Higgins *et al.*, 2011) and among different geographical regions (Gentry, 1988a; Terborgh & Andresen, 1998; Ter Steege *et al.*, 2006). Another factor causing floristic differentiation is the influence of the rivers which can fluctuate during the year, creating new habitats and maintaining secondary succession across Amazonia (Salo *et al.*, 1986). Current climate also influences floristic composition in Amazonia, as species distributions are often related to variation in the seasonality of rainfall across the basin (Ter Steege *et al.*, 2006; Butt *et al.*, 2008).

The availability of DNA sequence data has offered a new opportunity to examine the role of historical processes over the past two decades. Such data have been used to examine biogeographic processes of populations, species, genera, families and whole communities (Dick & Kress, 2009). To understand how biogeographic hypotheses can be tested using molecular data, the techniques and examples of their application to phylogenetics, population genetics, and phylogeography are detailed below.

1. 4. TESTING BIOGEOGRAPHIC PROCESSES USING MOLECULAR DATA

1.4.1. Molecular phylogenetics

Molecular phylogenetics studies the evolutionary relationships of species and higher taxa using DNA sequence data (Avise, 2009). These relationships are presented in phylogenetic trees where the branches represent lineages that have diverged from a common ancestor (Simpson, 2006). Plastid DNA has been particularly useful in plants because it is easily

amplified via PCR and its genes evolve at a slow enough rate to resolve relationships at higher taxonomic levels (Avise *et al.*, 1987). However, genealogies are obtained using information from several regions of both plastid and nuclear DNA which can then be combined to try and estimate the phylogeny.

A most common use of molecular phylogenies is in phylogenetic systematics (e.g. APG; Bremer *et al.*, 1998; Bremer *et al.*, 2003; Bremer *et al.*, 2009). Molecular phylogenetics is also widely applied to detect the imprint of historical events by using molecular clocks and fossil records (e.g. Särkinen *et al.*, 2007; Antonelli *et al.*, 2009). When fossil records are available, they can be applied to the phylogeny at the position of specific nodes that contain all extant species with a similar morphological character to the fossils (synapomorphy; Figure 1.3). Using this method, the age of one or more branching nodes can be constrained to a given age of divergence (Lavin, 2006). Even though this method has tested time scales and patterns in the diversification of tropical flora, it is important to consider the limitations and possible alternatives. For example, fossils can give ambiguous results if they are not accurately dated or if they have been applied to a wrong node in the phylogeny (Pennington & Dick, 2010). The use of multiple fossils (Renner, 2005) and a good knowledge of the morphology of the extant species can improve the calibration (Figure 1.3). In addition, nucleotide substitution rates are not constant across all species and can change over time. Therefore, an appropriate algorithm must be chosen to correct the different rates of substitution, the dating process should be performed using different fossils for multiple nodes (Rutschmann, 2006), and the analysis should include the uncertainty in evolutionary rates and calibration times (Drummond *et al.*, 2006). When no fossils are available, nucleotide substitution rates inferred from other taxa can be used to estimate the relative age of divergence of lineages, though this approach is less preferable.

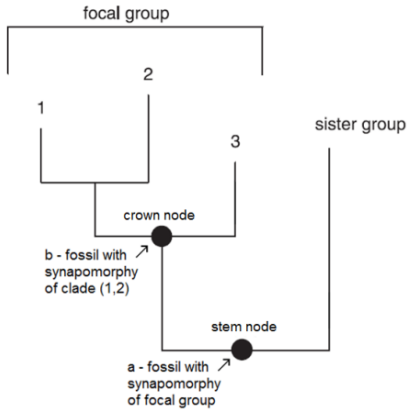


Figure 1.3. Diagram of a molecular phylogenetic tree representing the evolutionary relationships of a focal group formed by taxa 1, 2 and 3. The sister group is the closest relative of the focal group. Branch lengths represent the number of nucleotide substitutions, while rates of substitution can vary among lineages. Two fossils (a) and (b) are allocated to the nodes based upon sharing a common character or synapomorphy. In many cases fossils are taken to represent a minimum age of divergence, but recent software allows a probability distribution of ages to be applied (Drummond & Rambaut, 2007). The age of origin of the focal group is given by the stem node and the age of diversification is given by the crown node. Modified from Pennington *et al.* (2006b).

Dated molecular phylogenies of tropical clades have helped to understand the origin of different taxa and the role of vicariance and dispersal as mechanisms for species distribution and speciation. Therefore, this technique allows one to test whether the current high species richness of the Amazon rain forest is a result of *in situ* diversification since the break-up of west Gondwana, or if is a result of dispersal from other land masses. It was mentioned above that some families and genera present in South America originated and evolved outside the continent even when it was physically isolated as a continental island. For example, the arrivals in South America of the herbaceous genus *Renealmia* (Zingiberaceae) (Särkinen *et al.*, 2007) and the tree species *Symphonia globulifera* (Clusiaceae) (Dick & Heuertz, 2008) have been estimated by phylogenetic dating to the Pliocene and Miocene, much more recent than the split of west Gondwana,

suggesting transoceanic long-distance dispersal from Africa. Other geological processes involved in the creation of new habitats can be assessed. For example, in the study of *Renealmia* a recent diversification of the genus was also observed in the phylogeny related to the uplift of the Andes. A similar pattern was suggested in the genus *Inga* (Fabaceae) where the uplift of the Andes coincides with the rapid diversification of the genus (Richardson *et al.*, 2001). Furthermore, the drainage of Lake Pebas has also been suggested to have promoted the recent colonization and speciation of plants in western Amazonia. For example, this hypothesis has been recently suggested for the palm genus *Astrocaryum*: the crown node for the species restricted to western Amazonia post-dated the Lake Pebas (c. 6 Ma; Roncal *et al.*, 2013), suggesting the importance of the drainage of the lake in the diversification and colonization of western Amazonia.

At regional scale, phylogenies have also been used to investigate how soil types have influenced species diversification within Amazonia. In this case, the hypothesis of environmental heterogeneity leading to habitat specialization is tested (e.g. independent speciation events to different soils) against an alternative hypothesis of lineages exhibiting phylogenetic niche conservatism (e.g. species specialized to each soil are clustered in monophyletic groups). In a phylogenetic study of the genus *Protium* (Burseraceae) in north-western Amazonia, the species occurring on each soil type (forests growing on brown soils, clay soils, and white-sand soils) were not grouped in the phylogeny (Fine *et al.*, 2005). The scattered phylogenetic distribution suggests the occurrence of independent speciation on to white-sand and clay soil types, with the progenitors associated with forests on brown soils. This result supports previous ecological studies that provided evidence for the importance of edaphic niches in shaping patterns of diversity in western Amazonia (Gentry, 1992; Tuomisto *et al.*, 1995; Phillips *et al.*, 2003; Tuomisto *et al.*, 2003c; Fine *et al.*, 2004; Vormisto *et al.*, 2004; Ruokolainen *et al.*, 2007).

1.4.2. Population genetics and phylogeography

Population genetics studies the amount and distribution of genetic variation (allele frequency) present in populations by describing changes that occur as a result of mutation, genetic drift, gene flow, natural selection and/or sexual selection (Templeton, 2006).

Highly variable genetic regions are required such as simple sequence repeats (SSR, microsatellites). The development of these variable markers can have a relatively high cost, they are often specific to a species, and the sampling requires large numbers of individuals per population (Dick & Kress, 2009). Because of these reasons, few studies have used population genetics to study the biogeography of tropical plant species across Amazonia (e.g. Dick & Heuertz, 2008). Despite these challenges, the population genetic approach offers powerful tools for elucidating the history of particular species, and therefore gives an opportunity for understanding the demographic history of tropical vegetation (Naciri-Graven *et al.*, 2006).

Phylogeography is a similar approach that studies population genetic data in a spatial context. It can be defined as "the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species" (Avise, 2000). The term phylogeography was first used in 1987 (Avise *et al.*, 1987) in an attempt to link the two existing disciplines of population genetics and phylogenetic biology (Avise, 2009). Similar to phylogenetics, phylogeography uses DNA sequences as markers providing information of mutational events and uses statistical approaches for testing hypotheses. Phylogeography can also use population genetic methods to complement more phylogenetic approaches. Like phylogenetics and population genetics, phylogeographic studies consider both plastid and nuclear genomes, specifically in phylogeography to reduce the effects of stochasticity of population processes (e.g. genetic drift). Since plastid DNA markers have become easier and cheaper to sequence and have shown to be variable within individual plant species (Shaw *et al.*, 2005; Shaw *et al.*, 2007), phylogeographic studies using these markers have rapidly increased in quantity.

Molecular studies at the population level or within and among closely related species typically examine a more recent time scale than molecular phylogenetics and are perhaps more powerful for uncovering the influence of ecological processes on genetic patterns. For example, seed dispersal has been traditionally explored by comparing the floristic similarity between communities at different geographical scales (beta diversity) under a neutral expectation that predicts a decrease in similarity with distance (e.g. Condit *et al.*, 2002; Duque *et al.*, 2009). Patterns of seed dispersal and pollen flow can be studied using

sequence data from different genomes. Chloroplast DNA, which is maternally inherited, reflects patterns of seed dispersal (Petit *et al.*, 2003), so its use allows the quantification of gene flow from mothers to offspring through seeds. Nuclear DNA is biparentally inherited and therefore reflects both pollen and seed flow (Dick & Kress, 2009). Similarly, using markers form plastid and nuclear markers, the impact of major dispersal barriers on species evolution can be assessed, such as the uplift of the Andes separating widespread populations located at either side of the cordillera.

Phylogeography has also been applied to assessing the effect of past climate changes on the distribution of species. For example, in the northern hemisphere, a comparative approach of assessing concordance of phylogeographic patterns across several tree species has confirmed the location of Pleistocene refugia, post-glacial colonization routes, and secondary contact zones (e.g. Taberlet *et al.*, 1998; Brunsfeld *et al.*, 2001; Petit *et al.*, 2003; Soltis *et al.*, 2006). These comparative phylogeographic studies are based on the assumption that different extant organisms may accumulate similar genetic signatures as a response to a common environmental history (Avise *et al.*, 1987).

Phylogeography has also been used to detect the geographic origin of species, migration routes, and the vicariance of ancient populations (Ouborg *et al.*, 1999; Petit *et al.*, 2003). For example, a study of the population genetics of the sub-canopy tree *Symphonia globulifera* (Clusiaceae) shows that this species originated in Africa and arrived in South America by at least three trans-oceanic dispersal events, followed by early establishment of populations in Central America and north-western South America, and a more recent expansion in the Amazonian region (Dick *et al.*, 2003; Dick & Heuertz, 2008). A recent expansion is supported by lack of genetic variation among populations from the Amazon basin and Guiana, which contrast with the high differentiation among populations from Central America and West Ecuador. Another large geographic-scale population genetic study of the wind-dispersed, emergent tree *Ceiba pentandra* (Malvaceae) has shown the importance of successful long-distance dispersals from South America to Africa and vice versa (Dick *et al.*, 2007). No genetic variation was found across the entire distribution of *C. pentandra*, with the exception of one population located in western Ecuador. A contrasting pattern has been found among populations of *Hevea brasiliensis* (Euphorbiaceae) in south-

western Amazonia, where differentiated genetic clusters correspond to hydrological basins of tributaries of the Amazon River (Le Guen *et al.*, 2009). In the case of *Swietenia macrophylla* (Meliaceae), long distance seed dispersal was inferred in Central America while strong genetic structure was reported among populations in southern Amazonia (Lemes *et al.*, 2010). Lemes *et al*. suggested that the absence of cyclones in Amazonia compared to Central America may limit the dispersal of large wind-dispersed seeds, such as those of *S. macrophylla*, in the basin. However, this may also simply indicate that the single factor of dispersal syndrome is not sufficient to satisfactorily explain patterns of genetic structure. Other species traits, ecological factors and historical events may be responsible for these genetic patterns, but the extremely limited number of species covered by previous studies makes it difficult to draw general conclusions.

At a smaller geographic scale, using plastid DNA markers, gene flow of communities of eight widespread *Inga* species (Fabaceae) was estimated across a 250-km transect in south-western Amazonia (Dexter *et al.*, 2012). Low floristic community similarity and low seed flow was found between two geographically adjacent localities. This area in southern Peru was suggested as a transition zone where two historically separate populations are coming together, one extending from northern Peru to the Manu region and the second from Central Bolivia to the Los Amigos-Tambopata region. Using an approach based upon coalescent theory in population genetics, migration by seed dispersal between these two basins was estimated. The authors suggested that historical processes may be responsible for this pattern, as opposed to any difference in modern soil or climatic conditions, and date the separation of ancient populations as estimated as 42,000-612,000 years ago (Dexter *et al.*, 2012).

Phylogenetic, phylogeographic and population genetic studies have been applied to test different hypotheses of diversification of tropical fauna (e.g. refugia, riverine and gradient models; Moritz *et al.*, 2000) or to determine areas of high genetic differentiation among populations. In the Neotropics, comparative phylogeographic studies have been developed in mammals (Da Silva & Patton, 1998), bees (Dick *et al.*, 2004), ants (Solomon *et al.*, 2008), and birds (Burney & Brumfield, 2009). These studies showed genetic differentiation among populations in different parts of the tropical rain forest biome related to physical barriers

such as the Andes for understory birds, and geological arches for small mammals. In contrast, bees that pollinate orchids and canopy birds show high gene flow across the Andes, while ants show more ancient subdivision of populations related to Pliocene-Pleistocene events. Recent studies have also been able to detect species-specific faunal refugia in the Brazilian Atlantic forest (Carnaval *et al.*, 2009; Porto *et al.*, 2013).

1.4.3. Phylogenetic diversity and community phylogenetics

In contrast to phylogenetic studies that focus on single clades (e.g. a genus or family) or on particular species, the phylogenetic approach can also be applied at the community level in order to evaluate the evolutionary diversity of forests (phylogenetic diversity and its application to conservation; Faith, 1992) and to test hypotheses of processes determining community assembly (community phylogenetics; Webb, 2000).

The use of phylogenetic diversity in biodiversity was proposed in order to develop metrics that not only accounts for species richness but also for the evolutionary diversity of lineages (Vane-Wright *et al.*, 1991; Faith, 1992). For example, a study of the Cape flora in South Africa found that some areas have more, or less, phylogenetic diversity than would be expected from their taxon richness (Forest *et al.*, 2007). In this case, the Western Cape region with high species richness has closely related genera with multiple recent radiations, whereas the more species-poor eastern region was dominated by more distantly related taxa. Therefore, this study showed that phylogenetic diversity cannot necessarily be predicted from patterns of species diversity. The only study to date measuring phylogenetic diversity in South America (Chave *et al.*, 2007) used floristic inventories of trees carried out by Alwyn Gentry (Gentry, 1988a). In this study, a wide range of phylogenetic diversity values were reported but because this dataset is largely Andean and pre-Andean, it was not possible to explore the Amazon basin extensively. Therefore, phylogenetic diversity patterns across this vast, biodiverse ecosystem remain largely unknown.

Community phylogenetics have been applied to testing hypotheses of processes determining community assembly at local scales of a few square metres to a few hectares (e.g. Webb *et al.*, 2002; Kembel & Hubbell, 2006; Swenson *et al.*, 2006; Hardy & Senterre,

2007; Emerson & Gillespie, 2008; Cavender-Bares *et al.*, 2009; Kembel, 2009; Baraloto *et al.*, 2012). Using a phylogenetic tree of the species pool, metrics of phylogenetic community structure for each plot are calculated and compared to values for randomly generated null communities or phylogenies (Webb, 2000). Clustered phylogenetic structure signifies when more closely related species occur in the community than expected by chance, which indicates environmental filtering due to, for example, habitat partitioning. Over-dispersion occurs when species are more dispersed across the phylogeny than would be expected by chance, indicating that competitive interactions due to interspecific competition or attack by herbivores and pathogens may be dictating community structure. A large deviation from the null expectation of phylogenetic randomness is likely for clustered and overdispersed phylogenetic community structure, whereas in examples where a small deviation from the null expectation is found, this may indicate that opposing mechanisms or other stochastic processes dictate community structure (e.g. Webb & Pitman, 2002).

Some studies have pointed out that the results of community structure analyses can differ significantly depending on the method used to construct the phylogeny of the species pool and the spatial scale of the species pool (e.g. Cavender-Bares *et al.*, 2006; Kembel & Hubbell, 2006; Swenson *et al.*, 2006). For example, the phylogenetic structure of tree communities within a 50-ha plot in Panama was compared using poorly resolved phylogenies produced in Phylomatic (Kembel & Hubbell, 2006) and more resolved phylogenies generated from barcoding DNA markers of all species that occur in the area (Kress *et al.*, 2009). The results of these studies show significant differences in evolutionary and ecological inferences. While the phylogenetic structure of tree communities was close to random in the Phylomatic study (Kembel & Hubbell, 2006), significant deviations from random were found using phylogenies produced with sequences from DNA barcodes (Kress *et al.*, 2009). In the latter study, habitat filtering was inferred to be important in structuring communities of low plateau and slope habitats (that were phylogenetically clustered), biotic interactions were suggested to act on the high plateau, and in mixed and young forests (all phylogenetically over-dispersed), and no phylogenetic structure was found in streams and swamp forests (random phylogenetic structure). In addition, studies

on tree communities of Peru (Fine & Kembel, 2011) and French Guiana (Gonzalez *et al.*, 2010; Baraloto *et al.*, 2012) have shown that patterns of phylogenetic community structure were similar irrespective of the resolution of the phylogeny used. Results using a poorly resolved phylogeny provided by Phylomatic were compared to a phylogeny that was resolved to the genus level by hand based on published phylogenetic trees in the first study and to a phylogeny produced with DNA barcode sequences in the second study.

Despite discrepancies and difficulties in using poorly resolved phylogenies, phylogenetic diversity and community phylogenetics can still be applied if floristic community data are available. For example, plot data now available for Amazonia could be used to determine the spatial variation of phylogenetic diversity across the basin, which would have implications for conservation planning, if conservation of evolutionary diversity is to be taken into account (cf. Forest *et al.*, 2007).

1. 5. SYNTHESIS

This review has covered the geological history of Amazonia and the timing of different key historical and climatic events that may have affected to the diversification of the tropical rain forest flora in South America. Moreover, I described the different molecular techniques used for testing biogeographic processes. These molecular biogeographic studies indicate that *vicariance* processes, as well as *dispersal* and *chance* are all important for explaining current patterns of species distributions of the Amazonian tropical flora. However, large geographic-scale datasets of species, allied to studies at the community level, are needed to understand the role of history and ecology in the biogeography of Amazonian plants.

Current phylogeographic studies lack information of ecological factors that could determine genetic patterns at the population and species levels. Therefore, research is required to ascertain whether ecological factors are important in the biogeographic history of Amazonian species and whether different sets of species show similar genetic patterns. For example, the phylogeographic study of *Ceiba pentandra* (Dick *et al.*, 2007), *Cordia*

alliodora (Rymer *et al.*, 2013) and *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b), could be used to infer that long-lived, drought-tolerant pioneer trees with wind-dispersed seeds can overcome one or both of the potential barriers to migration between Amazonia and Mesoamerica: the cold temperatures of the Andean Cordillera and the seasonal drought of biomes of northern South America. These studies need complementing with others of tree species confined to rain forest environments and with animal-dispersed seeds, to test which, if any, ecological aspects of widespread neotropical species are critical in determining their large-scale biogeographies.

In contrast to the large amount of data available for the northern hemisphere, only two comparative phylogeographic studies of plants exist for the Neotropics and these are restricted to the regions of southern Peru and southern Central America (Dexter *et al.*, 2012; Poelchau & Hamrick, 2013). Therefore, it is important to perform more comparative phylogeographic studies of widespread Amazonian species over much greater geographic scales in order to test whether concordant genetic patterns among species can be linked to historical climatic and geological changes. In addition, it will be also important to determine if their widespread distribution across Amazonia have occurred before or after the Quaternary period.

At the community level, the spatial pattern of angiosperm phylogenetic diversity in the Amazon remains to be assessed and further research is required to test if conventional diversity metrics are enough to predict phylogenetic diversity across Amazonia. For example, it is not known whether terra firme forests of western and central Amazonia, which harbour exceptional high tree species diversity, will show high or low phylogenetic diversity. It could be expected that communities of western Amazonia might show lower phylogenetic diversity than expected by their taxon diversity because of the recent evolutionary radiations in some species-rich genera driven by the Andean orogeny or other recent historical processes (e.g. Richardson *et al.*, 2001). In contrast, less species-rich communities of the Brazilian and the Guiana Shields that are geologically much older and which have had no direct impact from the Andean orogeny might contain higher phylogenetic diversity than expected by their taxon richness because they may have accumulated more lineages, and may have fewer recent species radiations.

1. 6. PROJECT AIM AND OBJECTIVES

1.6.1. Thesis aim

The overall aim of this PhD is to understand the mechanisms that determine tree species distributions, population genetic structure, and phylogenetic diversity in the Amazon rain forest.

1.6.2. Thesis objectives

Objective 1: Determine within-species genetic diversity and the effect of ecological and physical barriers to gene flow in a widespread neotropical tree species adapted to wet environments (Chapter 2).

a) Compare genetic diversity within populations and between Mesoamerica and Amazonia.
b) Determine population genetic structure and the spatial location of genetic breaks.
c) Estimate divergence time among differentiated lineages.

Objective 2: Identify areas of concordance of genetic differentiation among five widespread western Amazonian tree species (Chapter 3).

a) Determine edaphic preferences of each species and assess how population genetic structure and genetic diversity vary across western Amazonia.
b) Determine concordant genetic breaks represented by areas of high genetic differentiation among populations.
c) Explore whether these genetic patterns are consistent with historical explanations.

Objective 3: Investigate the spatial distribution of phylogenetic diversity of tree communities across Amazonia (Chapter 4).

a) Determine the spatial distribution of phylogenetic diversity of tree communities across Amazonia.
b) Assess how phylogenetic diversity metrics correlate with species richness and the percentage of major angiosperm clades.

c) Explore whether phylogenetic diversity patterns can be explain by the maximum geological age and the different forest types sampled in each region.

1.6.3. Thesis approach

The study uses three approaches to achieve these objectives: 1) I develop and utilise a large dataset of DNA sequences of a widespread neotropical species which includes populations collected from Central and South America, from both sides of the Andes and from either side of seasonally dry environments of northern South America (objective 1); 2) I develop and use a dataset of DNA sequences of five different species which includes populations collected over a broad geographical area of western Amazonia (objective 2); and 3) I compiled and analysed a large dataset of floristic inventories across Amazonia including plot community data of regions with different maximum geological age and different forest types (objective 3). A brief introduction to methods is given below and further details are provided in the extended versions of each chapter.

Neotropical dataset

This study uses a large dataset of DNA sequences including plastid and nuclear markers to investigate the phylogeography of *Ficus insipida* subsp. *insipida*. This species is a good exemplar taxon for widespread neotropical rain forest trees because it is distributed across the Andes and into Mesoamerica, is a long-lived pioneer tree, and like the majority of rain forest trees, it has animal-dispersed seeds and importantly, in contrast to previous studies of pioneer species (Dick *et al.*, 2007; Lemes *et al.*, 2010; Rymer *et al.*, 2013; Scotti-Saintagne *et al.*, 2013b), *Ficus insipida* is confined to rain forest environments.This study also aims to examine genetic patterns in western Amazonia, using an intensive geographic sampling scheme. A significant contribution of plastid DNA sequences comes from Monica Poelchau who studied this species in Mesoamerica (Poelchau & Hamrick, 2013). In total, 54 populations from Mexico, Belize, El Salvador, Nicaragua, Costa Rica, Panama, Ecuador, Peru, and Bolivia were visited, covering the breadth of the distribution of the study taxon across Mesoamerica (n = 34 sites) and Amazonia (n = 24 sites). The dataset comprises 410 sequences for the plastid marker and 85 sequences for the nuclear marker.

Large geographic-scale phylogeographic studies are subject to severe practical challenges in Amazonia (e.g. high diversity, accessibility, etc) which reduce the possibility of covering the biome using thorough sampling. International collaboration, as was used in this thesis, is therefore important to be able to cover gaps in the sampling. An emphasis on long field campaigns, as I carried out in South America, is important to collect samples from geographically scattered populations.

Western Amazonia dataset

In order to further test genetic patterns in western Amazonia, target species were prioritized depending on their abundance, their frequency, if they were easy to identify in the field, and if they were restricted to western Amazonia. Based on these criteria, I chose *Jacaratia digitata* (Caricaceae), *Clarisia biflora*, *Ficus insipida* subsp. *insipida*, *Poulsenia armata* (all Moraceae), and *Otoba parvifolia* (Myristicaceae). This study aims to identify areas of genetic differentiation in western Amazonia that are concordant among the five species and which could be related to geological or climatic changes. Geographically extensive field work was necessary to accomplish the sampled required, and for compiling ecological information for each species. Leaf samples were collected for the five species sampled in 34 populations across 2500 km of western Amazonia and soil samples were taken at each site to assess the edaphic environment where the species occur. Plastid and nuclear DNA markers were used and sequenced for 674 and 214 individuals, respectively. This dataset adds substantial information to the understanding of the biological history of western Amazonia which has been poorly sampled in previous phylogeographic studies. Moreover, a new set of species with variable ecological traits (e.g. degree of shade tolerance, breeding system) is now available, to test further whether ecological traits are important in the biogeographic history of neotropical tree species.

Community dataset

In the fourth chapter, a total of 283 floristic tree inventories of the RAINFOR forest plot network were compiled using the forestplots database (Lopez-Gonzalez *et al.*, 2011) in order to assess spatial variation in the distribution of phylogenetic diversity across the Amazon rain forest. I tested different hypotheses related to potential long-term stability of

old formations versus the incidence of recent historical events in younger geological formations. For example, I expected that communities of western Amazonia might have lower phylogenetic diversity because of the recent evolutionary radiation events in some species-rich genera driven by the Andean orogeny (Gentry, 1982) compared to the Brazilian and the Guiana Shields that are geologically much older and which have had no direct impact from the Andean orogeny (Stebbins, 1974).

The dataset used in the analysis includes 157,340 individuals, belonging to 3,868 species, 732 genera and 126 families of angiosperms sampled in nine countries of South America. This dataset was kindly provided by many collaborators of the network, representing the result of more than two decades of work by botanical teams from South America, Europe and the United States. In the analysis, two phylogenetic diversity metrics are calculated using a phylogenetic hypothesis resolved to the family level and provided by Phylomatic in PHYLOCOM version 4.2 (Webb *et al.*, 2008). Results were compared by using a classification of plots by maximum geological age and forest type. In addition, values of phylogenetic diversity of forest types with marked ecological constraints of nutrient and water supply within the Amazon rain forest were compared to more nutrient-rich and ever wet environments such as terra firme or montane forests.

1. 7. THESIS SYNOPSIS

Chapter 2 investigates the role of ecology in the phylogeography of a widespread neotropical rain forest tree species. In chapter 3, areas of high genetic differentiation concordant among five widespread western Amazonian species are identified and compared to climatic and geological changes. Chapter 4 reveals for the first time the uneven spatial distribution of phylogenetic diversity in tree communities across Amazonia. Chapter 5 synthesises the key results, considers the implications of findings, and identifies emerging research directions.

Chapter 2. *Ficus insipida* subsp. *insipida* (Moraceae) reveals the role of ecology in the phylogeography of widespread neotropical rain forest tree species

2. 1. ABSTRACT

This study aims to examine the phylogeography of *Ficus insipida* subsp. *insipida* in order to investigate evidence for large-scale spatial genetic structure in its widespread neotropical distribution across montane and xeric barriers and to reveal its evolutionary and demographic history. We collected 410 individuals in 54 populations from Mexico to Bolivia, representing the full extent of the species' distribution. All individuals were sequenced for the plastid *trn*H-*psb*A DNA marker, of which 85 individuals were also sequenced for the nuclear ribosomal internal transcribed spacer (ITS). Genetic diversity was assessed with indices of haplotype and nucleotide diversities and genetic structure was examined using spatial analysis of molecular variance (SAMOVA) and haplotype networks. Divergence of plastid lineages was dated using a Bayesian coalescent approach. The *trn*H-*psb*A sequences yielded 19 haplotypes restricted to either Mesoamerica or Amazonia and six haplotypes were found among ITS sequences. Haplotype diversity was higher in Amazonia while nucleotide diversity was similar in both regions. Seven genetically differentiated SAMOVA groups were described for *trn*H-*psb*A, of which two were also supported by the presence of unique ITS sequences. Diversification of the plastid DNA haplotypes probably began during the Miocene (c. 17.6 Ma), and diversification of Mesoamerican lineages in the Pliocene (c. 3.5 Ma). In contrast to other neotropical pioneer species tolerant to dry habitats and with weak genetic structure between Mesoamerica and Amazonia, marked structure in *F. insipida* implies that the Andes and seasonally dry biomes of northern South America are eco-climatic barriers to its migration. A long residence time of *F. insipida* in Amazonia may underlie the population differentiation found in some areas there. However, the presence of genetically uniform populations also suggests recent colonization events, especially in southern Amazonia, which is consistent

with independent palaoecological data that indicate rain forest expansion in this area since the last glacial maximum.

2. 2. INTRODUCTION

Phylogeographic studies can give insights into past changes in the species distributions that can be related to the history of the landscape and the ecology of the species (Avise, 2000). Although large geographic-scale studies have rapidly increased in the last decades, phylogeographic studies of plants in the Neotropics are still scarce (Beheregaray, 2008). Therefore, past vegetation dynamics of the most species-rich region of the world (Fine & Ree, 2006) that contains the largest continuous rain forest globally in the Amazon basin remains poorly known.

In recent years, a few pioneering phylogeographic studies of widespread neotropical species have given insights into the relationships of populations of the Amazon basin and those west of the Andes, including Central America. Results of these studies are not consistent. *Symphonia globulifera* (Dick & Heuertz, 2008), *Carapichea ipecacuanha* (Oliveira et al., 2010), *Swietenia macrophylla* (Lemes *et al.*, 2010), *Simarouba amara* (Hardesty *et al.*, 2010), and *Schizolobium parahyba* (Turchetto-Zolet *et al.*, 2012) show restricted historical gene flow between populations in Mesoamerica and Amazonia. In contrast, *Ceiba pentandra* (Dick *et al.*, 2007), *Cordia alliodora* (Rymer *et al.*, 2013), and *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b) show weak phylogeographic structure over the same area, suggesting recent dispersal between these regions. These last three studies have hypothesized that those long-lived pioneer trees with wind-dispersed seeds and tolerance to drought can overcome two potential barriers between Amazonia and Mesoamerica: the Andean Cordillera and seasonally dry biomes of northern South America. In this study, we explore further which species traits are associated with overcoming potential physical and ecological barriers to gene flow between Mesoamerica and Amazonia in rain forest trees using *Ficus insipida* Willd. subsp. *insipida* (Moraceae). This species is a good exemplar taxon for widespread neotropical rain forest trees because it is distributed across the Andes and into Mesoamerica, is a long-lived pioneer tree

confined to rain forest environments, and like the majority of rain forest trees, it has animal-dispersed seeds.

Ficus insipida subsp. *insipida* is also widespread in the western portion of the Amazon basin and a second aim of this study is to examine genetic patterns at regional scale, using an intensive geographic sampling scheme. Prior phylogeographic studies of Amazonian trees used detailed sampling in a particular restricted geographical location (250-km transect; Dexter *et al.*, 2012), a region representing part of the species' range (1500- to 2500-km transect; Lemes *et al.*, 2010; Turchetto-Zolet *et al.*, 2012), or sparse population sampling across a wide geographic range (Dick *et al.*, 2007; Dick & Heuertz, 2008). Only two studies have moderate, range-wide sample densities in Amazonia (Rymer *et al.*, 2013; Scotti-Saintagne *et al.*, 2013b). Collectively, all the studies mentioned above have demonstrated contrasting genetic patterns across the Amazon basin. For example, genetic differentiation among multiple species of *Inga* (Fabaceae) in south-eastern Peru suggest a zone of secondary contact between two historically isolated populations (Dexter *et al.*, 2012). High genetic differentiation was also reported among populations of *Swietenia macrophylla* in the Brazilian Amazon (Lemes *et al.*, 2010), and among subspecies of *Schizolobium parahyba* (Turchetto-Zolet *et al.*, 2012) and *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b). In contrast, *Ceiba pentandra* (Dick *et al.*, 2007) and *Symphonia globulifera* (Dick & Heuertz, 2008) have low genetic variation for both chloroplast and nuclear DNA across Amazonia.

Strong genetic structure in Amazonia may reflect the effects of historical events in generating isolation in some areas where no current physical barriers to gene flow apparently exist. These historical events could include large fluvial rearrangements during the Late Miocene (Hoorn *et al.*, 1995), high frequency of fluvial dynamics of lateral erosion and deposition during the Pliocene - Pleistocene (Salo *et al.*, 1986), or Quaternary climatic fluctuations (Haffer, 1969). A similar genetic pattern can be related to areas of secondary contact where lineages converge from differences sources. Low genetic variation across the vast Amazon basin in other species is consistent with recent population expansion (Dick & Heuertz, 2008). Nevertheless, comprehensive evaluation of population genetic structure across the range of widely distributed Amazon species is a demanding task. Here

we set out to use an intensive geographic sampling scheme in order to establish where populations of *F. insipida* subsp. *insipida* show genetic uniformity or distinctiveness in western Amazonia and to establish if the patterns we find might reflect Quaternary climate changes or the legacies of more ancient geological events. In comparing the patterns of genetic structure of *F. insipida* with other species, we also explore the effect of species traits such as dispersal mode and floral sexuality.

In summary, the main objective of this study is to infer which geological or climatic factors may underlie patterns of genetic variation in *F. insipida* subsp *insipida* across the Neotropics and within the western Amazon Basin. We worked with both maternally inherited plastid DNA that reflects patterns of seed dispersal, and biparentally inherited nuclear DNA that reflects both pollen and seed flow. Our specific objectives are (1) to compare genetic diversity within populations and between Mesoamerica and Amazonia, (2) to test population genetic structure and the spatial location of genetic breaks, and (3) to estimate divergence time among differentiated lineages.

2. 3. METHODS

2.3.1. The study species and population sampling

The pantropical genus *Ficus* L. comprises about 750 species. *Ficus insipida* is the most morphologically distinct and widespread neotropical species of the section Pharmacosycea, a relatively species-poor group of about 20 tree species (Berg, 2001). In this study, we focus on *F. insipida* subsp. *insipida*, which is distributed from Mexico through the Andean region to the lowland rain forest of western Amazonia. The morphologically distinct subspecies *F. insipida* subsp. *scabra* C.C. Berg from eastern Brazil, the Guianas, and north-eastern Venezuela was not included. Our study is based principally on fresh leaf samples collected from the field for 410 individual trees. A total of 54 populations from Mexico, Belize, El Salvador, Nicaragua, Costa Rica, Panama, Ecuador, Peru, and Bolivia were visited, covering the breadth of the distribution of *F. insipida* subsp. *insipida* across Mesoamerica (n = 34 sites) and Amazonia (n = 24 sites). At least 10

individuals were collected at each site, but fewer samples were sourced from sites where the species was rare, and in some cases our sampling was supplemented using herbarium specimens. Leaf samples were dried and stored in silica gel and the locations of individuals were recorded using a handheld GPS. At least one herbarium voucher was collected from each population to allow subsequent verification of identification (see Appendix S2.1 in Supporting Information). Hereafter, we refer to the study taxon as *F. insipida*.

2.3.2. DNA extraction and sequencing

Total genomic DNA was extracted using the CTAB method (Doyle & Doyle, 1987). Seven plastid markers were tested for amplification and sequencing: *rpl*32-*trn*L*, trn*Q-5'-*rps*16, 3'*trn*V-*nd*HC, *atp*I-*atp*H, *trn*D-*trn*T, *trn*H-*psb*A, and *trn*L-*trn*F (Shaw *et al.*, 2007). The non-coding marker *trn*H-*psb*A was chosen for the full-scale study because of its high amplification success and variability within and among populations of *F. insipida*. In addition, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA was amplified and sequenced for 77 samples from Amazonia using ITS1 and ITS4 primers (White *et al.*, 1990). Eight additional ITS sequences were obtained from other regions (1 Mexico, 1 Costa Rica, 5 Panama, and 1 Brazil). Reaction conditions varied slightly between Mesoamerican and Amazonian samples, because amplifications were performed in different laboratories (Mesoamerican ITS data were contributed by other collaborators; see acknowledgements). However, care was taken to match the conditions as closely as possible. PCR reaction and sequencing conditions for *trn*H-*psb*A for the Mesoamerican samples are described in Poelchau and Hamrick (2013). PCR for the Amazonian samples was performed in 20 µl solutions containing 2 µl of template DNA, 2 µl of PCR Buffer 10x, 2 µl of 10mM total dNTP, 1 µl of 50mM $MgCl_2$, 1 µl of each primer, 4 µl of combinatorial enhancer solution (CES), 0.2 µl of Taq polymerase (Bioline, UK), and 6.8 µl of distilled H_2O. The thermal cycle for *trn*H-*psb*A (and ITS) was 94^0C for 5 min (3 min), followed by 35 (30) cycles at 94^0C for 30 sec (1 min), 55^0C (56^0C) for 30 sec (1 min) and 72^0C for 1 min (90 sec), and a final extension at 72^0C for 10 min (5 min). PCR products were visualized via 1% agarose gel electrophoresis, and products were purified using ExoSAP-IT (USB Corporation). Cycle sequencing was conducted in 10 µl solutions containing 3 µl PCR product, 0.5 µl of BigDye (Applied

Biosystems), 2 µl sequencing reaction buffer 5x, 0.32 µl of primer, and 4.18 µl of distilled H_2O.

2.3.3. Haplotype definition and networks

All forward and reverse strands were edited in SEQUENCHER version 5.0 (Gene Codes Corp.) and nucleotide substitutions, indels (i.e. insertions or deletions) and inversions were visually checked against the original electropherograms. The alignment was created manually in MESQUITE version 2.74 (Maddison & Maddison, 2001). Poly-T and poly-A length polymorphisms were excluded from subsequent analyses, and indels and inversions were treated as single events. The genealogical relationship of haplotypes was estimated independently for *trn*H-*psb*A and ITS sequences using statistical parsimony in TCS version 1.21 (Clement *et al.*, 2000), with 95% parsimony connection limit.

2.3.4. Statistical analyses

Because of uneven sampling of ITS sequences between Mesoamerica and Amazonia, the following analyses were performed only for *trn*H-*psb*A sequences, and the ITS data were used for comparison of general patterns.

Genetic diversity and population structure

Haplotype and nucleotide diversities were calculated for each population in ARLEQUIN version 3.5 (Excoffier & Lischer, 2010). We compared genetic diversity between Mesoamerica and Amazonia using a rarefaction procedure set to 100 runs that standardized each region to the same number of sequences (n = 182 individuals) using packages 'ape' and 'pegas' in R Statistical Software version 2.15.2. A spatial analysis of molecular variance was performed to determine the position of genetic breaks among populations using SAMOVA version 1.0 (Dupanloup *et al.*, 2002). Several runs were performed using increasing numbers of groups (k = 1 - 20) and 100 annealing simulations for each k. In each run, populations were clustered into genetically and geographically homogenous groups (Dupanloup *et al.*, 2002). The number of groups was chosen so as to maximize genetic differentiation among the groups (Φ_{CT}). Genetic structure using pairwise nucleotide differences was further examined by analysis of molecular variance in ARLEQUIN

version 3.5 (Excoffier & Lischer, 2010), for all populations and for groups of populations defined by geographical region and by SAMOVA. Significance of genetic structure indices was tested using a non-parametric randomization procedure (Excoffier & Lischer, 2010).

Divergence of plastid lineages

Bayesian inference was used to estimate divergence time among plastid DNA haplotypes. The ingroup was defined by all plastid haplotypes of *F. insipida* and five sequences downloaded from GenBank of *Ficus* species of sections *Americana* (GQ982218, GQ982219, and GQ982224), *Sycomorus* (EU213825) and *Galoglychia* (EU213821), and the taxon *Poulsenia armata* (tribe Castilleae) were used as multiple outgroups. Phylogenetic reconstruction of the haplotypes was performed in BEAST version 1.5.4 (Drummond & Rambaut, 2007) using the uncorrelated lognormal relaxed molecular clock and the GTR+G nucleotide substitution model suggested by JMODELTEST version 0.0.1 (Posada, 2008). The tree prior model was set using the birth-death speciation process, and using a log-normal distribution for the prior on tree root age with a mean value of 47 Ma and a standard deviation including the fossil age estimates for *Ficus* (40-55; Zerega *et al.*, 2005). Three replicate runs were performed using a chain of 100,000,000 states sampling every 10,000 generations. All trees were combined after the exclusion of the first 1,000 trees of each run as burn-in to avoid including trees sampled before convergence of the Markov chains. Posterior probabilities of trees were averaged on 27,000 sampled trees.

2. 4. RESULTS

2.4.1. Plastid DNA

For the plastid *trn*H-*psb*A marker, a total of 410 individuals collected in 54 populations were successfully sequenced (Table 2.1), 228 samples from Mesoamerica (n = 31 sites) and 182 from Amazonia (n = 23 sites). After the exclusion of two variable poly-T and poly-A length polymorphisms in the alignment, 340 bp of aligned sequences remained. A total of 19 polymorphic sites were detected including 14 substitutions, four indels, and one inversion (see Appendix S2.2); these mutations defined a total of 19 haplotypes. The

haplotypes were geographically restricted; seven were confined to Mesoamerica and twelve confined to Amazonia (Figure 2.1). The analysis at the population level indicates high values of genetic diversity, both in Mesoamerican (codes 10, 19, and 30) and Amazonian populations (32, 33, 39, 41, 47, and 50; Table 2.1). Haplotype diversity was higher in Amazonia (95% CI: 0.694 - 0.703) than Mesoamerica (95% CI: 0.646 - 0.654), and nucleotide diversity was similar for both regions (95% CI: Mesoamerica 0.281% - 0.348%; Amazonia 0.328% - 0.404%).

2.4.2. Population structure

The SAMOVA analysis showed increasing values of differentiation among groups up to a k value of seven (Φ_{CT} = 0.8). Four groups were defined in Mesoamerica (Table 2.1), separating seven populations from Costa Rica and Nicaragua containing mainly haplotypes H8 (group I), H9 (group II), and H7 (group III) from the rest of the populations (group IV). Three groups were found in Amazonia: group V containing two populations from north-eastern Peru and mainly haplotypes H15 and H19; group VI containing two populations from Ecuador and one from central Bolivia with mainly haplotype H4; and group VII containing the rest of populations. SAMOVA groups IV and VII contain most of the populations and are dominated by a few widespread haplotypes; H1 and H5 in Amazonia and H2 in Mesoamerica. Mesoamerican haplotype H9 is more related to Amazonian haplotypes, while haplotype H4 shows a disjunct distribution, occurring in Ecuador and in one population in Bolivia (Figure 2.1). Genetic structure was mainly explained by differences among populations (81%), and grouping of populations by SAMOVA groups explained more of the genetic variation (75%) than grouping by two geographical regions (56%; Table 2.2).

2.4.3. Divergence time

Diversification of plastid DNA haplotypes in *F. insipida* appears to have began in the Miocene with a split into two main lineages at 17.6 Ma (95% HPD: 7.4 - 30.8 Ma; Figure 2.2). A Pliocene age was estimated for the main diversification of Mesoamerican haplotypes (3.5 Ma; 95% HPD: 0.5 - 7.6 Ma; Figure 2.2), but the analysis also shows a second Mesoamerican group with a Pleistocene crown age of 1.3 Ma (95% HPD: 0 - 3.2

Ma). A third Mesoamerican lineage corresponding to haplotype H9 was nested within the most diverse clade of Amazonian lineages (Figure 2.2).

2.4.4. Nuclear ribosomal DNA

A total of 85 samples were sequenced for ITS, covering the entire study area. Five polymorphic sites, three substitutions and two indels (see Appendix S2.2) were found in the 635 bp of aligned sequences. These mutations defined six haplotypes of which haplotype H1 is widespread across Amazonia and extends to Panama in Central America. Haplotype H3 occurs in three localities in Amazonia and the remaining four haplotypes each occur in single populations in Amazonia and Mesoamerica (Figure 2.3).

2. 5. DISCUSSION

2.5.1. Genetic structure between Mesoamerica and Amazonia

We found clear structure in the plastid DNA data, with differentiation between Mesoamerican and Amazonian haplotypes demonstrated by the haplotype network (Figure 2.1), AMOVA analysis (Table 2.2) and phylogenetic tree (Figure 2.2). This pattern contrasts with previous results from other fast growing and light demanding pioneer species (Table 2.3) that indicated weak genetic structure with similar plastid DNA haplotypes spanning Mesoamerica and Amazonia (e.g. *Ceiba pentandra*; Dick *et al.*, 2007; *Cordia alliodora*; Rymer *et al.*, 2013; *Jacaranda copaia*; Scotti-Saintagne *et al.*, 2013b). Our results instead resemble more the distinct sets of plastid DNA haplotypes found in other neotropical tree species with animal-dispersed seeds (Table 2.3) such as *Symphonia globulifera* (Dick & Heuertz, 2008), and in wind-dispersed taxa such as *Swietenia macrophylla* (Lemes *et al.*, 2010) and *Schizolobium parahyba* (Turchetto-Zolet *et al.*, 2012).

At least two potential present-day barriers can be invoked to explain limitation of seed dispersal between Mesoamerica and Amazonia. The first and most obvious is the Andes Mountains that stretch along the entire western edge of South America with altitudes (> 4000 m) greatly exceeding the current elevational limits of lowland rain forest trees. The

species mentioned above grow mainly in lowland rain forest and in pre-montane environments reaching altitudes of 1,500 m a.s.l. and with rare records at 1,800 - 2,000 m a.s.l. Therefore, the Andes are a significant potential dispersal barrier and must have been for at least 3 Ma when the most recent uplift of the Eastern Cordillera caused it to reach 2,500 m a.s.l. (Gregory-Wodzicki, 2000). A second barrier for wet-adapted species is seasonally dry vegetation: extensive savannas in Colombia and Venezuela (the Llanos) and seasonally dry forests along the Caribbean coast of northern South America (Pennington *et al.*, 2006a). Both of these seasonally dry biomes may prevent dispersal to and from Mesoamerica of rain forest species such as *Ficus insipida*, *Symphonia globulifera* and *Schizolobium parahyba* that cannot tolerate seasonal drought (Table 2.3). Species able to tolerate more seasonally dry habitats could utilise the northern seasonally dry tropical forest and savannah biomes as a migration route. In particular, *Cordia alliodora* and *Ceiba pentandra* are frequently recorded in inventories of seasonally dry tropical forests on the Caribbean coast of Colombia (see http://www.biovirtual.unal.edu.co/ICN) and seed flow via this route may explain their low genetic population differentiation between Mesoamerica and Amazonia. In contrast, dispersal mode and floral sexuality seem to be less explanatory of the degree of genetic differentiation between Mesoamerica and Amazonia. For example, *Ficus insipida* with animal-dispersed seeds and monoecious flowers, and *Schizolobium parahyba* with wind-dispersed seeds and hermaphrodite flowers, both show high genetic differentiation. Therefore, it appears that the ecology of widespread neotropical species is critical in determining their large-scale biogeographies and perhaps more so than dispersal mode and floral sexuality.

2.5.2. Genetic structure and demographic history within Amazonia

Our phylogenetic analysis of the plastid DNA data indicates an Amazonian origin for *F. insipida* and one to three subsequent migration events to Mesoamerica during the last 10 Ma (Figure 2.2). This is further supported by the greater haplotype diversity of the *trn*H-*psb*A marker in Amazonia than in Mesoamerica. In Amazonia, *F. insipida* shows high genetic differentiation in the northern part of the basin where three SAMOVA groups separate populations of Ecuador and north-eastern Peru from the rest of populations. Haplotypes are also unique to these SAMOVA groups and belong to phylogenetically

distant lineages (e.g. H3 and H11 of Ecuador, and H15 and H19 of NE Peru). Regional genetic differentiation may be caused by the long-residence time of *F. insipida* in this region (mean estimate of 17.6 Ma) which is greater than in other neotropical tree species (0 - 9.9 Ma; Dick *et al.*, 2012). High genetic differentiation for plastid DNA has also been reported for *Swietenia macrophylla* in southern Brazilian Amazon (Lemes *et al.*, 2010). Like patterns of genetic differentiation between Amazonia and Mesoamerica, dispersal mode does not explain regional genetic differentiation in these species (Table 2.3).

Despite the genetic differentiation of some *Ficus insipida* populations, there are widespread haplotypes in both plastid and ITS markers. In particular, the lesser variation found in ITS and the presence of a single widespread haplotype in Amazonia (Figure 2.3) are suggestive of effective nuclear gene flow via pollen. Pollen of *F. insipida* is dispersed by tiny aganoid wasps of the genus *Tetrapus* (Machado *et al.*, 2001). Wasps that pollinate monoecious fig species such as *F. insipida* have been reported to travel over distances of up to 14 km in the Neotropics (Nason *et al.*, 1998) and up to more than 150 km in riparian vegetation of the Namibian desert (Ahmed *et al.*, 2009), indicating that pollen dispersal may succeed even among geographically distant individuals.

The population differentiation for the plastid DNA data in some areas of Amazonia suggests that seed flow must in some cases have been more restricted. However, an interesting pattern of no genetic diversity is observed within the Bolivian populations. This uniformity, which is also present at the northernmost range in Mesoamerica, may reflect recent colonization events. This is consistent with palaeoecological data suggesting that the Amazon rain forest expanded south in the last 3,000 years, meaning that the current vegetation in the region (near population 54) may represent the southernmost distribution of rain forest over the last 50,000 years (Mayle *et al.*, 2000). This southern region of Amazonia has a marked dry season where monthly rainfall can be less than 100 mm for 4-6 months (Sombroek, 2001). If drier conditions occurred during the Pleistocene, *F. insipida* (and other rain forest tree species) may have experienced range contraction at the southern end of its range and a reduction in effective population size. It is possible that *F. insipida* may have persisted in some restricted “micro refugia” in wetter forest at the base of the Andes, which may be the explanation of the haplotype found at Sacta (population

53 in Bolivia). This pattern might also reflect a recent long distance dispersal event from Ecuador because this haplotype is shared with populations found 2000 km away in Ecuador, but more dense population sampling in the intervening areas would be necessary to confirm this.

The occurrence of widespread haplotypes in some areas of the range of *F. insipida* is consistent with episodes of long-distance seed dispersal. Fig fruits are important for frugivores in the Neotropics, and fish and bats are the most important seed dispersers of *F. insipida* (Banack *et al.*, 2002). The mobility of these dispersal agents could help explain long-distance seed dispersal events. In particular, fish contribute significantly to the upstream dispersal of riparian plants (Reys *et al.*, 2009) and fruit-eating bats are known for travelling long distances from fruiting trees (Janzen, 1978; Pennington & De Lima, 1995). Successful establishment after long-distance dispersal is also favoured by the ecology of *F. insipida*, which is a light-demanding species that grows in riparian and disturbed open areas (Gentry, 1993; Banack *et al.*, 2002).

2. 6. CONCLUSIONS

By intensely sampling populations across the entire range of *F. insipida* subsp. *insipida*, we found genetic differentiation between and within Amazon and Mesoamerican populations. This contrasts with previous studies of other neotropical trees - *Cordia alliodora*, *Ceiba pentandra*, *Jacaranda copaia* - that show little differentiation between these areas. Although all these species are pioneers, only *F. insipida* is confined to ever-wet habitats. Therefore, it appears that the tolerance of seasonally dry climates in the other species means that dry forest and savanna vegetations in northern South America has presented a lesser migration barrier. In contrast, seasonally dry climates may have acted as major barriers to *F. insipida*. Further phylogeographic studies of widespread species are required to determine the key species traits that allow successful dispersal of seeds across the physical and environmental barriers of the Neotropics.

Within Amazonia, *F. insipida* also shows genetic differentiation among populations of the northern basin. A long residence time of the species in this region may underlie the population differentiation found there. However, in southern Amazonia, the presence of genetically uniform populations may indicate recent colonization events, a scenario consistent with palaeoecological data from the region that indicates post-glacial rain forest expansion.

Table 2.1. Haplotype diversity and nucleotide diversity (Mean ± SD for both indices) for the plastid *trn*H-*psb*A marker in 54 *Ficus insipida* populations in Mesoamerica and Amazonia. The metrics were not applicable for populations with less than three individuals sampled. SAMOVA groups (I-VII) and the number of sequences are provided for each population. Regional genetic diversity was estimated using rarefaction procedure.

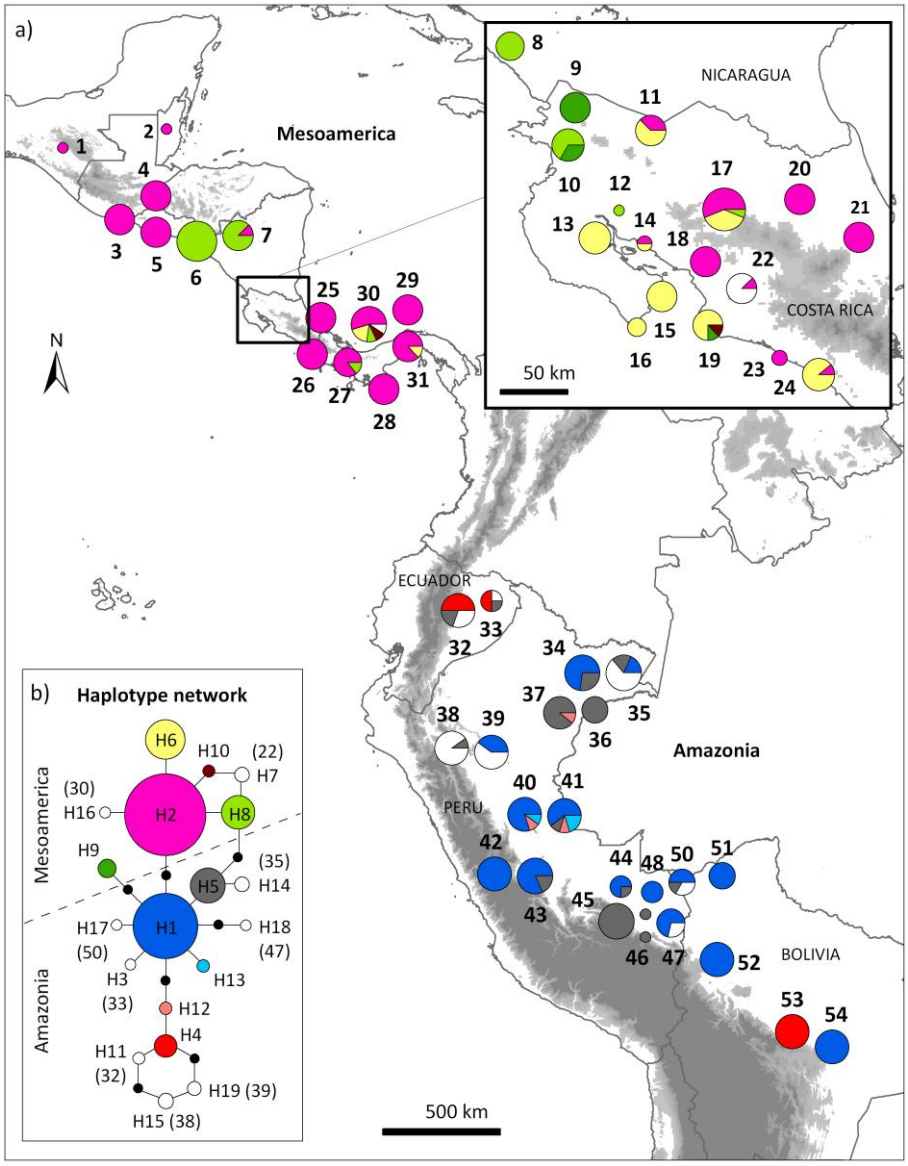
Nº	Code	Country	Group	Ind.	Hapl. div.	Nucl. div. (%)
1	MEX	Mexico	IV	1	n.a.	n.a.
2	BEL	Belize	IV	1	n.a.	n.a.
3	ElI	El Salvador	IV	8	0	0
4	Dei	El Salvador	IV	8	0	0
5	Nan	El Salvador	IV	8	0	0
6	VoC	Nicaragua	I	14	0	0
7	Mir	Nicaragua	I	8	0.25 ± 0.18	0.07 ± 0.11
8	ElO	Nicaragua	I	7	0	0
9	HLl	Costa Rica	II	8	0	0
10	RiT	Costa Rica	I	9	0.50 ± 0.13	0.74 ± 0.50
11	CaN	Costa Rica	IV	8	0.54 ± 0.12	0.16 ± 0.17
12	RiB	Costa Rica	I	1	n.a.	n.a.
13	RiN	Costa Rica	IV	9	0	0
14	LaE	Costa Rica	IV	2	n.a.	n.a.
15	Cur	Costa Rica	IV	8	0	0
16	CaB	Costa Rica	IV	3	0	0
17	RSC	Costa Rica	IV	16	0.58 ± 0.08	0.19 ± 0.17
18	Esp	Costa Rica	IV	8	0	0
19	Jac	Costa Rica	IV	8	0.46 ± 0.20	0.50 ± 0.37
20	LaS	Costa Rica	IV	8	0	0
21	EaU	Costa Rica	IV	8	0	0
22	Car	Costa Rica	III	8	0.25 ± 0.18	0.15 ± 0.16
23	MaA	Costa Rica	IV	2	n.a.	n.a.
24	HaB	Costa Rica	IV	9	0.22 ± 0.17	0.07 ± 0.10
25	Cah	Costa Rica	IV	8	0	0
26	PiB	Costa Rica	IV	8	0	0
27	CeB	Panama	IV	7	0.29 ± 0.20	0.09 ± 0.12
28	LaT	Panama	IV	8	0	0
29	FtS	Panama	IV	8	0	0
30	PLR	Panama	IV	11	0.71 ± 0.14	0.26 ± 0.22
31	PNM	Panama	IV	8	0.25 ± 0.18	0.07 ± 0.11
32	JaS	Ecuador	VI	10	0.69 ± 0.10	0.48 ± 0.35
33	Bog	Ecuador	VI	4	0.83 ± 0.22	0.89 ± 0.69
34	Yan	Peru	VII	11	0.44 ± 0.13	0.13 ± 0.14
35	Mad	Peru	VII	11	0.58 ± 0.14	0.25 ± 0.22
36	SaJ	Peru	VII	6	0	0
37	JeH	Peru	VII	9	0.22 ± 0.17	0.20 ± 0.19
38	Mar	Peru	V	10	0.20 ± 0.15	0.30 ± 0.25
39	Ura	Peru	V	10	0.53 ± 0.09	0.79 ± 0.52
40	vHu	Peru	VII	10	0.38 ± 0.18	0.18 ± 0.17
41	Mac	Peru	VII	10	0.64 ± 0.15	0.28 ± 0.24
42	LaG	Peru	VII	10	0	0
43	SaT	Peru	VII	11	0.33 ± 0.15	0.10 ± 0.12
44	CoC	Peru	VII	4	0.50 ± 0.27	0.15 ± 0.18
45	Qon	Peru	VII	11	0	0
46	SaG	Peru	VII	1	n.a.	n.a.
47	Tam	Peru	VII	7	0.48 ± 0.17	0.28 ± 0.25
48	LoA	Peru	VII	4	0	0
49	LaP	Peru	VII	1	n.a.	n.a.
50	Tah	Bolivia	VII	6	0.73 ± 0.16	0.26 ± 0.24
51	Aba	Bolivia	VII	6	0	0
52	Mai	Bolivia	VII	10	0	0
53	Sac	Bolivia	VI	10	0	0
54	LaEn	Bolivia	VII	10	0	0
MESOAMERICA			I-IV	228	0.65 ± 0.03	0.31 ± 0.23
AMAZONIA			V-VII	182	0.70 ± 0.03	0.37 ± 0.26

Table 2.2. Analysis of molecular variance (AMOVA) based on pairwise differences of the plastid *trn*H-*psb*A marker for *F. insipida*. The analysis was run independently using all populations, populations grouped by region (Mesoamerica and Amazonia) and populations grouped by SAMOVA.

Group level	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation indices ($p < 0.001$)
Populations	Among populations	53	421	1.02	81.15	ΦST = 0.81
	Within populations	356	84	0.24	18.85	
Regions	Among groups	1	203	0.98	56.21	ΦCT = 0.56
	Among pops within groups	52	218	0.53	30.18	ΦSC = 0.69
	Within populations	356	84	0.24	13.61	ΦST = 0.86
SAMOVA	Among groups	6	353	1.21	75.14	ΦCT = 0.75
groups	Among pops within groups	47	68	0.16	10.13	ΦSC = 0.41
	Within populations	356	84	0.24	14.73	ΦST = 0.85

Table 2.3. Summary of large-scale phylogeographic studies of widespread neotropical species. Genetic markers including nuclear ribosomal DNA (nrDNA), chloroplast DNA (cpDNA), and nuclear and chloroplast simple sequence repeats (nuSSR and cpSSR, respectively) are indicated. Pattern of genetic structure between Mesoamerica and Amazonia is also provided.

Species and family	Ecology	Habitat	Floral sexuality	Genetic marker	Pollen dispersal	Seed dispersal	Genetic structure		Reference
							Nuclear	Plastid	
Carapichea ipecacuanha RUBIACEAE	Shade tolerant perennial shrub	Wet to dry	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*T-*trn*L)	Insects (small bees)	Vertebrates (understory birds)	High	High	Oliveira *et al.* (2010)
Symphonia globulifera CLUSIACEAE	Shade tolerant tree	Wet	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Vertebrates (hummingbirds)	Vertebrates (bats, birds, monkeys)	High	High	Dick and Heuertz (2008)
Ceiba pentandra MALVACEAE	Fast growing, light demanding tree	Wet to dry	Hermaphrodite	nrDNA (ITS), cpDNA (*psb*B-*psb*F)	Vertebrates (bats) Insects (moths)	Wind and water	Low	Low	Dick *et al.* (2007)
Cordia alliodora BORAGINACEAE	Fast growing, light demanding tree	Wet to dry	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A), cpSSR	Insects (moths)	Wind	Low	Low	Rymer *et al.* (2013)
Jacaranda copaia BIGNONIACEAE	Fast growing, light demanding tree	Wet to dry	Hermaphrodite	cpDNA (*trn*H-*psb*A, *trn*C-*ycf6*), nuSSR, cpSSR	Insects (large bees)	Wind	Low	Low	Scotti-Saintagne *et al.* (2013b)
Swietenia macrophylla MELIACEAE	Fast growing, light demanding tree	Wet to dry	Monoecious	cpSSR	Insects	Wind		High	Lemes *et al.* (2010)
Simarouba amara SIMAROUBACEAE	Fast growing, light demanding tree	Wet	Dioecious	nuSSR	Insects (bees & moths)	Vertebrates (monkeys, birds)	High		Hardesty *et al.* (2010)
Schizolobium parahyba FABACEAE	Fast growing, light demanding tree	Wet	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A, *trn*L-*trn*F, *mat*K)	Insects (bees)	Wind	Low	High	Turchetto-Zolet *et al.* (2012)
Ficus insipida MORACEAE	Fast growing, light demanding tree	Wet	Monoecious	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Insects (wasps)	Vertebrates (fish, bats, others)	Low	High	This study



c)

SAMOVA groups	Population code
I	6-8,10,12
II	9
III	22
IV	Other Meso.
V	38,39
VI	32,33,53
VII	Other Amaz.

Figure 2.1. (a) Haplotype distribution, (b) haplotype network of *trn*H-*psb*A sequences, and (c) SAMOVA groups for *Ficus insipida* populations sampled in 54 sites in Mesoamerica and Amazonia. Haplotype distributions at the border between Costa Rica and Nicaragua are shown separated. Pie charts are labelled with population numbers as shown in Table 2.1. Colours represent the haplotypes (H1-H19). Haplotypes unique to single population are shown in white with population number given in brackets in the network. Circle is proportional to sample size for each population (n = 1 - 16 individuals) and for each haplotype (n = 1- 119 individuals). Missing haplotypes in the network are shown as black dots, and a dashed line separates haplotypes of each region. The Andean Cordillera and other mountains are shown in shaded grey.

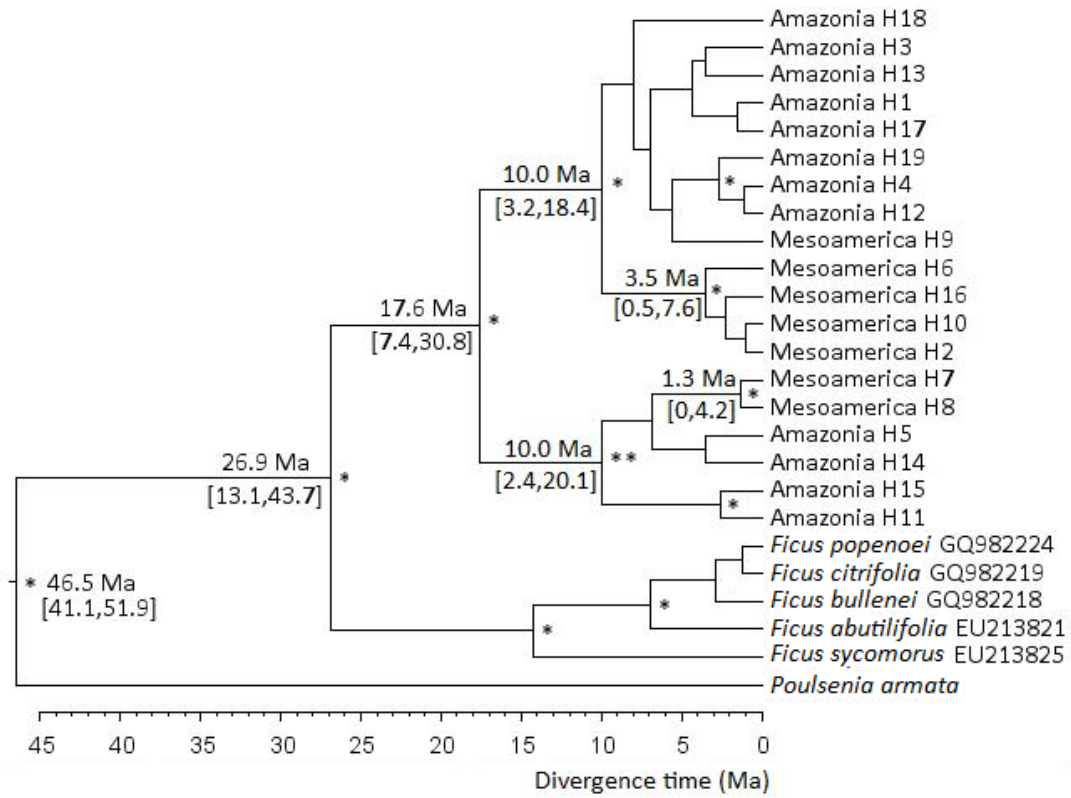


Figure 2.2. Lineage divergence dating for all plastid DNA haplotypes (H1-H19) of *Ficus insipida* occurring in Mesoamerica and Amazonia. Mean divergence dates are given with 95% highest posterior density values in brackets. Nodes with posterior probabilities ≥ 0.90 are indicated with an asterisk and two asterisks indicate that the value is equal to 0.88.

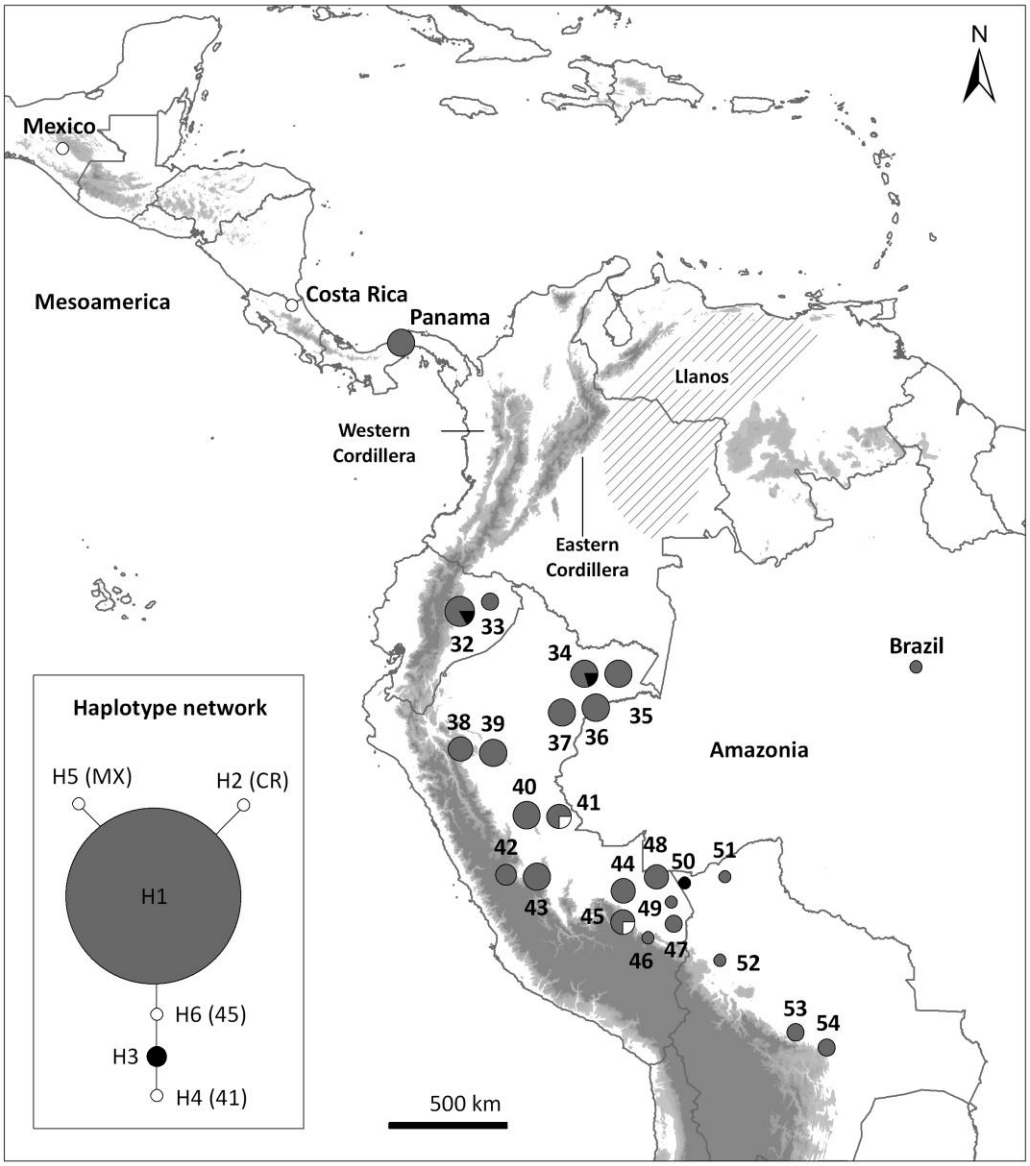


Figure 2.3. Haplotype distribution and haplotype network of ITS sequences for *Ficus insipida* populations sampled in 27 sites in Mesoamerica and Amazonia. Pie charts are labelled with population numbers as shown in Table 2.1. Colours represent the haplotypes (H1-H6). Haplotypes unique to single population are shown in white with population number given in brackets in the network. Circles is proportional to sample size for each population (n = 1 - 6 individuals) and for each haplotype (n = 1 - 76 individuals). The Andean Cordillera and other mountains are shown in shaded grey, and the dashed area represents the Llanos. Additional ITS sequences obtained from collaborators are indicated as Mexico (MX), Costa Rica (CR), Panama, and Brazil (see acknowledgements).

Chapter 3. Comparative phylogeography of widespread tree species of western Amazonia

3. 1. ABSTRACT

This study aims to identify concordant areas of genetic differentiation among five widespread western Amazonian tree species: *Jacaratia digitata* (Caricaceae), *Clarisia biflora*, *Ficus insipida* subsp. *insipida*, *Poulsenia armata* (all Moraceae), and *Otoba parvifolia* (Myristicaceae). Leaf samples were collected in 34 populations spanning most of the species' geographical ranges in western Amazonia. In addition, soil samples were collected for all populations to enable investigation of correlation of genetic variation with edaphic factors. Plastid (*trnH-psbA*) and nuclear (ITS) DNA markers were sequenced for 674 and 214 individuals, respectively, across all species. Genetic diversity was assessed with indices of haplotype and nucleotide diversities and genetic structure was examined using spatial analysis of molecular variance (SAMOVA) and haplotype networks. Divergence of plastid lineages was dated using a Bayesian coalescent approach. Our results show that genetic breaks among species are not entirely congruent suggesting that tropical rain forest species respond differently to long-term geological and climatic changes. In addition, some genetic patterns correlate with edaphic preferences as in the case of *Jacaratia digitata* and *Clarisia biflora*. The high amount of genetic variation and strong genetic structure found in the plastid DNA marker indicate a long residence time for all species in western Amazonia. However, the presence of recent lineages in the south of Amazonia is potentially related to Pleistocene climatic changes and expansion of rain forest in this area. Forest conservation in areas where genetic breaks are located may help to maintain the intraspecific genetic diversity of western Amazonia species.

3. 2. INTRODUCTION

Forests growing in the wet climates and varied soils of western Amazonia have the greatest tree diversity of any ecosystem on Earth (Gentry, 1988b; Clinebell *et al.*, 1995). Understanding how the extent, composition and great diversity of western Amazonian

forests has developed and changed over time scales of thousands to millions of years has long been an important research question (Haffer, 1969; Simpson & Haffer, 1978; Prance, 1982). Previous work addressing it has employed a range of techniques such as biostratigraphy (Hoorn & Wesselingh, 2010), palynology (Colinvaux *et al.*, 2000; Jaramillo *et al.*, 2006), biogeography (Haffer, 1969), and vegetation modelling (Cowling *et al.*, 2004). Molecular biology has the potential to examine in great detail the demographic history of species at large scales but has barely been used in the Amazonian plant context (e.g. Dick & Kress, 2009). Elsewhere, the determination of DNA nucleotide sequences has allowed assessment of whether concordant genetic patterns in co-occurring species can be linked to historical climatic and geological changes (Bermingham & Moritz, 1998). This area of study, called comparative phylogeography, is based on the assumption that different extant organisms may accumulate similar genetic signatures as a response to a common environmental history (Avise *et al.*, 1987).

In the Neotropics, comparative phylogeographic studies have been developed mainly in animals such as mammals (Da Silva & Patton, 1998), bees (Dick *et al.*, 2004), ants (Solomon *et al.*, 2008) and birds (Burney & Brumfield, 2009). These studies showed genetic differentiation among populations in different parts of the neotropical rain forests related to physical barriers such as the Andes for understory birds, and geological arches for small mammals. In contrast, bees that pollinate orchids and canopy birds show high gene flow across the Andes, while ants show more ancient subdivision of populations presumed to be related to Pliocene-Pleistocene events. Studies in plants have been limited by the lack of availability of sufficiently variable DNA markers (Ouborg *et al.*, 1999). However, several fast evolving plastid DNA regions have now been identified (Shaw *et al.*, 2005; Shaw *et al.*, 2007) and used in phylogeographic studies of plants for corroborating palynological observations about Pleistocene refugia and post-glacial colonization events in temperate vegetation in the northern hemisphere (Taberlet *et al.*, 1998; Brunsfeld *et al.*, 2001; Petit *et al.*, 2003; Soltis *et al.*, 2006).

In comparison with the large amount of data available for the northern hemisphere, only two comparative phylogeographic studies of plants exist for the Neotropics (Dexter *et al.*, 2012; Poelchau & Hamrick, 2013). In Central America, a study of one species of Burseraceae and two of Moraceae showed a concordant genetic break between Costa Rica

and Nicaragua, probably corresponding to the boundary of Pliocene islands (Poelchau & Hamrick, 2013). In South America, patterns of genetic differentiation among communities of several *Inga* species (Fabaceae) were reported across a 250-km transect in southern Peru (Dexter *et al.*, 2012). This study suggested a zone of secondary contact between two historically isolated populations attributed to either past climatic changes or the uplift of the Fitzcarrald arch (Dexter *et al.*, 2012). In contrast, most studies examining a single species across the Amazon basin have shown low genetic variation in the nuclear and plastid DNA, for example in *Ceiba pentandra* (Dick *et al.*, 2007), *Cordia alliodora* (Rymer *et al.*, 2013), and *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b).

Geographic genetic structure in western Amazonia may reflect the effects of historical events in generating isolation of populations in some areas where no current physical barriers to gene flow apparently exist. These historical events could include the presence of a wetland system during the Miocene (Pebas Lake; Hoorn, 1996; Hoorn & Vonhof, 2006), high frequency of fluvial dynamics of lateral erosion and deposition during the Pliocene - Pleistocene (Salo *et al.*, 1986), or Quaternary climatic fluctuations (Haffer, 1969). Low genetic variation across the Amazon basin in other species is consistent with recent population expansion (Dick & Heuertz, 2008). However, the extremely limited number of species, sampled in geographically scattered locations across Amazonia, makes it difficult to draw general conclusions. Here, we study genetic structure, based on more thorough geographic sampling of populations, of five widespread Amazonian tree species. The main objective is to test if there are concordant patterns of genetic differentiation among populations of these species and to establish if the patterns reflect Quaternary climate changes or more ancient geological events. In comparing the patterns of genetic structure, the effect of species traits such as floral sexuality and dispersal mode will be explored.

This project is the first comparative phylogeographic study of tree species across the hyper- diverse western-Amazon forest, which also includes analyses of edaphic preference for the studied species. Using plastid and nuclear markers of five tree species sampled in 34 populations across 2500 km, we address the following questions: 1) How do population genetic structure and genetic diversity vary across western Amazonia?, 2) Is there any signature of concordant genetic breaks?, and 3) Are these genetic patterns consistent with historical climatic or geological events?.

3. 3. METHODS

3.3.1. Sampling strategy

Collection effort focused on sampling as much as possible of each species' distribution within Amazonia. Five abundant and frequent species restricted to the western region were chosen: *Jacaratia digitata* (Poepp. & Endl.) Solms (Caricaceae), *Clarisia biflora* Ruiz & Pav., *Ficus insipida* Willd. subsp. *insipida*, *Poulsenia armata* (Miq.) Standl. (all Moraceae), and *Otoba parvifolia* (Markgr.) A.H. Gentry (Myristicaceae). These species were chosen among other species because of their ease of taxonomic recognition and their high genetic variation showed in a pilot study. Populations of these species were sampled in 34 localities in Ecuador, Peru and Bolivia (see Appendix S3.1).Individuals separated by at least 40 m were sampled to reduce the chance of collecting closely related individuals (e.g. parent trees and their progeny). Leaf samples were collected, dried and stored in silica gel and the locations of individuals were recorded using a handheld GPS. At least one herbarium voucher was collected from each population to allow subsequent verification of identification. Additional samples were also provided by local botanists; these are distinguishable by the collector name of the herbarium specimen (see Appendix S3.2). In each site, soil samples were collected next to the trees at 30 cm depth. These samples were combined for each species to represent soil properties of each population and analyzed at the School of Geography of the University of Leeds using a standard protocol (Quesada *et al.*, 2010). Soil properties included pH-H_2O, particule sizes, cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and Al^{3+}), sum of bases (SB = Ca^{2+}+ Mg^{2+} + K^{+} + Na^{+}), and aluminium saturation (AS = Al^{3+} / [SB + Al^{3+}]).

3.3.2. The study species

All of the study species are trees that are characteristic of primary forest, except *Ficus insipida* and *Jacaratia digitata* that also grow in secondary forest. They are readily distinguished from congeners, except for *Otoba parvifolia* and *O. glycycarpa* (Ducke) W. Rodrigues & T.S. Jaramillo. Therefore, to mitigate problems of confusing both species, *Otoba glycycarpa* is included in the analysis because it is not consistently distinguishable morphologically and is not genetically distinct from *O. parvifolia* for the *trn*H-*psb*A marker (Honorio, unpublished data). Both species are treated as *O. parvifolia* in the text. Pollen of

all the focal species is dispersed by insects (Bawa *et al.*, 1985a), and seeds are dispersed by a variety of animals. All species are outcrossing, although they show different floral sexuality; while *Ficus insipida* and *Poulsenia armata* are monoecious, the remaining species are dioecious. *Jacaratia digitata* is entirely restricted to western Amazonia, while the other four species also occur in Central America. Populations from Central America and the Andean Pacific slope were not sampled in this study, but this should not greatly affect our inferences because clear genetic differentiation between populations in Amazonia and these areas west and north of the Andean Cordillera have already been shown for numerous tree species (e.g. Dick & Heuertz, 2008; Hardesty *et al.*, 2010; Lemes *et al.*, 2010), and particularly for those species adapted to wet environments and indeed for one of the focal species here (see chapter 2).

3.3.3. DNA extraction and sequencing

Total genomic DNA was extracted using the CTAB method (Doyle & Doyle, 1987) for most species and the DNEasy Plant Mini Kit was used in *Otoba parvifolia*. Seven plastid markers were tested for amplification and sequencing: *rpl*32-*trn*L, *trn*Q-5'-*rps*16, 3'*trn*V-*nd*HC, *atp*I-*atp*H, *trn*D-*trn*T, *trn*H-*psb*A, and *trn*L-*trn*F (Shaw *et al.*, 2007). The non-coding marker *trn*H-*psb*A was chosen because of its high amplification success and variability among populations for all species. In addition, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA was amplified and sequenced using ITS1 and ITS4 primers (White *et al.*, 1990). PCR reactions were performed in 20 µl solutions containing 2 µl of template DNA, 2 µl of PCR Buffer 10x, 2 µl of 10mM total dNTP, 1 µl of 50mM $MgCl_2$, 1 µl of each primer, 4 µl of combinatorial enhancer solution (CES), 0.2 µl of Taq polymerase (Bioline, UK), and 6.8 µl of distilled H_2O. The thermal cycle for *trn*H-*psb*A (and ITS) was 94^0C for 5 min (3 min), followed by 35 (30) cycles at 94^0C for 30 sec (1 min), 55^0C (56^0C) for 30 sec (1 min) and 72^0C for 1 min (90 sec), and a final extension at 72^0C for 10 min (5 min). PCR products were visualized via 1% agarose gel electrophoresis, and products were purified using ExoSAP-IT (USB Corporation). Cycle sequencing was conducted in 10 µl solutions containing 3 µl PCR product, 0.5 µl of BigDye (Applied Biosystems), 2 µl sequencing reaction buffer 5x, 0.32 µl of primer, and 4.18 µl of distilled H_2O.

3.3.4. Statistical analysis

Haplotype definition and networks

All forward and reverse strands were edited in SEQUENCHER version 5.0 (Gene Codes Corp.) and nucleotide substitutions, indels (i.e. insertions or deletions) and inversions were visually checked against the original electropherograms. The alignment was created manually in MESQUITE version 2.74 (Maddison & Maddison, 2001). Poly-T and poly-A length polymorphisms for *trn*H-*psb*A and poly-G polymorphisms for ITS were excluded from subsequent analyses, and indels and inversions were treated as single mutation events (see Appendix S3.3). The genealogical relationship of haplotypes was independently estimated for *trn*H-*psb*A and ITS sequences using statistical parsimony in package 'pegas' of R Statistical Software version 2.15.2.

Genetic diversity and population structure

Haplotype and nucleotide diversities were calculated for each species and each genetic marker using packages 'ape' and 'pegas' in R version 2.15.2. Genetic diversity was compared among species using a rarefaction procedure set to 100 runs that standardized each species to the same number of individuals ($n_{trnH\text{-}psbA}$ = 61 individuals; n_{ITS} = 12 individuals). A spatial analysis of molecular variance was performed independently for each species and each marker to determine the position of genetic breaks among populations using SAMOVA version 1.0 (Dupanloup *et al.*, 2002). Several runs were performed using increasing numbers of groups (k = 1 - 20) and 100 annealing simulations for each k. In each run, populations were clustered into genetically and geographically homogenous groups (Dupanloup *et al.*, 2002). The number of groups was chosen so as to maximize genetic differentiation among the groups (Φ_{CT}). Genetic structure using pairwise nucleotide differences was further examined by analysis of molecular variance in ARLEQUIN version 3.5 (Excoffier & Lischer, 2010), for all populations and for groups of populations defined by SAMOVA and by geographical region. Populations were divided into north (population codes 1-19) and south (population codes 20-34) regional groups in order to test the presence of two genetic pools as suggested for *Inga* species by Dexter *et al.* (2012). The division line coincides with the midpoint between the two most distant populations in this present study and with the location of a geological feature called the Fitzcarrald Arch.

Significance of genetic structure indices was tested using a non-parametric randomization procedure.

Divergence of lineages

Bayesian inference was used to estimate divergence time among lineages for the plastid DNA sequences. We did not use ITS sequences for these dating analyses that aim to date the divergences within a single species because processes of concerted evolution that homogenise sequences within the genome are likely to affect overall sequence divergence across an interbreeding population (Elder & Turner, 1995). Divergence time was independently assessed for Caricaceae (*Jacaratia digitata*), Moraceae (*Clarisia biflora*, *Poulsenia armata*, and *Ficus insipida*), and Myristicaceae (*Otoba parvifolia*). The ingroups were defined by all plastid haplotypes for each species and different outgroup taxa were used for each family for which sequences were obtained from GenBank (Table 3.1). Phylogenetic reconstruction of the haplotypes was performed in BEAST version 1.6.2 (Drummond & Rambaut, 2007) using the uncorrelated lognormal relaxed molecular clock and the GTR+G nucleotide substitution model. The tree prior model was set using the birth-death speciation process, and using a lognormal distribution for the prior on tree root age with variable mean values for each family. Estimates for calibration dates for nodes were taken from previous phylogenetic studies and are based on the divergence time between the closest relative of the study species (e.g. 18.8 Ma between *Vasconcellea* and *Jacaratia*; Antunes Carvalho & Renner, 2012) or the crown node in the case of Moraceae (89.1 Ma; Zerega *et al.*, 2005) and Myristicaceae (18.0 Ma; Doyle *et al.*, 2004) (Table 3.1). Three replicate runs were performed using a chain of 100,000,000 states sampling every 10,000 generations. All trees were combined after the exclusion of the first 1,000 trees of each run as burn-in to avoid including trees sampled before convergence of the Markov chains. Posterior probabilities were averaged on 27,000 sampled trees.

Edaphic preferences

We compared physical and chemical properties for soil of the five species to values compiled from 49 sites sampled in western Amazonia by Quesada *et al.* (2010). Soil samples in the 49 sites were taken randomly within about one hectare of forest, which was representive of a given forest formation. We expect that this soil dataset represents an

unbiased sampled of different soil conditions in western Amazonia. Soil fertility was classified based on the sum of base cations and aluminium saturation. Low-fertility soils have low concentration of base cations (≤ 3 $cmol_c\ kg^{-1}$) and variable concentration of aluminium (0 - 95%), while high-fertility soils have high concentration of base cations (> 3 $cmol_c\ kg^{-1}$) and low concentration of aluminium (0 - 35%). Kruskal-Wallis tests were applied to test for significant difference in the different edaphic properties (pH-H_2O, base cations, Al, etc).

3. 4. RESULTS

3.4.1. Haplotype definition and genetic diversity

A total of 674 individuals were sequenced for the plastid *trn*H-*psb*A marker and 214 individuals for the nuclear ITS marker. For *trn*H-*psb*A, 16 haplotypes were defined in *Clarisia biflora* and *Otoba parvifolia*, 12 haplotypes in *Ficus insipida*, seven in *Poulsenia armata*, and six in *Jacaratia digitata*. For ITS, 27 haplotypes were defined in *Clarisia biflora*, seven in *Poulsenia armata*, four in *Ficus insipida*, and three haplotypes in *Jacaratia digitata* (Table 3.2). Haplotype and nucleotide diversities were consistently high in *Clarisia biflora* and *Poulsenia armata* for the plastid and nuclear DNA markers, and in *Otoba parvifolia* for the plastid marker (Table 3.2). *Ficus insipida* and *Jacaratia digitata* have the lowest genetic diversity for ITS and intermediate genetic diversity for *trn*H-*psb*A, except for a high value for nucleotide diversity in *Jacaratia digitata* for the plastid region (Table 3.2).

3.4.2. Population genetic structure

The spatial distribution of the plastid haplotypes shows geographic structure in all of the species, that is, haplotypes are mainly fixed in particular populations or regions (Figure 3.1a). The SAMOVA analysis defined four groups of populations for each species for the plastid DNA (Figure 3.2a) with various values of genetic differentiation: *Ficus insipida* (Φ_{CT} = 0.69), *Jacaratia digitata* (Φ_{CT} = 0.98), *Clarisia biflora* (Φ_{CT} = 0.49), *Poulsenia armata* (Φ_{CT} = 0.95), and *Otoba parvifolia* (Φ_{CT} = 0.61). While SAMOVA recognises more groups in the northern populations of *Ficus insipida* and *Jacaratia digitata*, two SAMOVA groups occur in each of both northern and southern populations in the rest of species. *Ficus insipida* has

the lowest genetic differentiation explained by nucleotide differences among populations (67 %), while the rest of species have more than 90 % of the genetic variation explained among populations (Table 3.3).

In the case of the nuclear DNA, *Ficus insipida* and *Jacaratia digitata* have widespread nuclear haplotypes (Figure 3.1b) and no geographic genetic structure, with populations forming one SAMOVA group (Figure 3.2b). In the case of *Clarisia biflora* only 55 % of genetic variation was explained by differentiation among populations, while 96 % was explained in *Poulsenia armata* (Table 3.3). SAMOVA analysis defined three groups for *Clarisia biflora* (Φ_{CT} = 0.61) and four groups for *Poulsenia armata* (Φ_{CT} = 0.92).

Regional genetic structure was present in the plastid DNA with distinct haplotypes dominating northern and southern regions (Figure 3.3). Genetic differentiation between these two regional groups was lower than among SAMOVA groups but still significant in *Jacaratia digitata* (44 %) and *Otoba parvifolia* (31 %), followed by *Poulsenia armata* (17 %) and to a lesser extent in *Clarisia biflora* (3 %). In the case of the nuclear DNA, north-south genetic structure only remained significant in *Clarisia biflora* (26 %) and *Poulsenia armata* (11 %; Table 3.3 and Figure 3.4).

3.4.3. Lineage divergence estimates

The crown nodes (date of divergence of extant intraspecific lineages) dated from the late Miocene for all species with mean values of 12.01 Ma for *Poulsenia armata* (95% HPD: 8.56 - 38.56 Ma), 10.89 Ma for *Ficus insipida* (95% HPD: 3.67 - 19.71 Ma), 9.80 Ma for *Jacaratia digitata* (95% HPD: 3.83 - 16.03 Ma), 9.16 Ma for *Clarisia biflora* (95% HPD: 3.29 - 16.61 Ma), and 7.28 Ma for *Otoba parvifolia* (95% HPD: 3.35 - 11.59 Ma). Haplotypes of the southern region are nested within lineages of the north for *Ficus insipida*, *Jacaratia digitata* and *Otoba parvifolia*. In the case of *Clarisia biflora* and *Poulsenia armata*, two main groups of haplotypes occur and both contain lineages of the north and the south (Figure 3.5).

3.4.4. Edaphic preferences

The five species occur on loamy soils with a pH of 5 to 6, with low aluminium saturation (< 20 %) and high concentration of base cations, including mean values per species of 3-7

$cmol_c\ Kg^{-1}$ (Table 3.4 and Appendix S3.6). Particle sizes were variable among species, with values varying from 10-30 % in clay, 30-50 % in silt, and 20-60 % in sand. Compared to the 49 RAINFOR sites in western Amazonia (see Appendix S3.7), values of pH and base cations, especially of Ca and Mg, were high for the five study species (Kruskal-Wallis test: X^2_{pH} = 34.5, X^2_{Ca} = 26.3, X^2_{Mg} = 22.3, X^2_{SB} = 25.1, df = 5, $p < 0.001$), and values of concentration of aluminium were low (X^2_{Al} = 25.8, df = 5, $p < 0.001$). In the case of particle sizes, no difference was observed in the percentage of sand, but greater values were obtained in the study species for silt and lower for clay than in RAINFOR sites (X^2_{Silt} = 13.6, X^2_{Clay} = 19.4, df = 5, $p < 0.05$; see Appendix S3.8).

3. 5. DISCUSSION

Our results show that genetic patterns among the five study species are not entirely congruent. All of the species are shown to have a preference to fertile soils suggesting that tropical rain forest tree species found on these soil types have complex histories and may have responded differently to geological and climatic changes. Despite this, some tentative generalisations emerge such as strong genetic structure for plastid *trn*H-*psb*A sequences, long-term population stability in western Amazonia, and recent population expansion in the south.

3.5.1. Genetic structure in the plastid DNA

This study reveals geographic genetic structure for all species in the plastid DNA marker *trn*H-*psb*A. The presence of platid haplotypes restricted to particular areas of Amazonia is consistent with the study of *Symphonia globulifera* (Dick & Heuertz, 2008). Contrary to this pattern, low geographic genetic structure has been shown for *Ceiba pentandra* (Dick *et al.*, 2007), *Cordia alliodora* (Rymer *et al.*, 2013), and *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b) in which plastid haplotypes are widespread across Amazonia. This low geographic structure might reflect wind seed dispersal, which occurs in all these species, whereas *Symphonia globulifera* and the species studied here are dispersed by animals (Table 3.5). Seeds dispersed by animals may travel shorter distances than wind-dispersed seeds promoting more genetic structure in the plastid genome (Loveless & Hamrick, 1984). An exception is *Swietenia macrophylla*, which is wind-dispersed and shows low geographic

structure in Central America, but strong genetic structure among populations in southern Amazonia (Lemes *et al.*, 2010). The authors suggested that the absence of hurricanes in Amazonia may restrict the distribution of large wind-dispersed seeds in the basin. The high genetic structure of *Swietenia macrophylla* and *Schizolobium parahyba* in Amazonia (Table 3.5) could also indicate that large seeds of these species (seed mass of 0.566 g and 0.954 g, respectively; Kew, 2008) travel shorter distances than other small wind-dispersed seeds (e.g. 0.059 g in *Ceiba pentandra*, 0.033 g in *Cordia alliodora*, and 0.005 g in *Jacaranda copaia*; Kew, 2008). Because c. 80 % of tree species in neotropical rain forests are dispersed by animals (Howe & Smallwood 1982), we predict that most tree species, very few of which have been sampled over large scales until now, will show high genetic structure in plastid DNA across the basin.

Other species traits (e.g. growth strategy and floral sexuality) may contribute to genetic structure and merit further attention. For example, while hermaphrodite species with pioneer growth (*Ceiba pentandra*, *Cordia alliodora*, *Jacaranda copaia*) show low genetic structure, monoecy (e.g. *Ficus insipida*, *Swietenia macrophylla*) and dioecy (*Jacaratia digitata*) in light demanding species may increase the occurrence of genetic structure. However, this is not the case of shade tolerant species, in which medium to high population genetic structure occurs irrespective of floral sexuality (Table 3.5). Floral sexuality can sometimes inform about breeding system which determine different patterns of population genetic structure. For example, inbreeding in some hermaphrodite species promotes high genetic differentiation among populations, while outcrossing in monoecious or dioecious taxa promotes low levels of differentiation and high gene flow among populations (Bawa, 1992). In the case of tropical rain forest species, most tree species are outcrossing irrespective of their floral sexuality (Bawa *et al.*, 1985b). However genetic differentiation can be high in some species and low in others (Bawa, 1992). These contradictory genetic patterns in the tropics may be an effect of other ecological factors such as the spatial isolation of reproductive individuals and flowering phenology (Sebbenn *et al.*, 2012). For example, low species density and dioecy (only female individuals reproducing) may increase the isolation of reproductive individuals and create more spatial genetic structure. These results indicate that the study of more species with different

combinations of species traits are needed to further explain genetic structure in neotropical rain forest species (Loveless & Hamrick, 1984).

While more SAMOVA groups are found among the northern populations in the two light demanding species *Ficus insipida* and *Jacaratia digitata*, the groups occur more evenly in both northern and southern regions in the shade tolerant species *Clarisia biflora*, *Poulsenia armata* and *Otoba parvifolia*. In addition, a remarkably high genetic differentiation for the plastid DNA among SAMOVA groups of *Jacaratia digitata* is reported in the north. A possible explanation for this pattern might be related to short distances that large birds and mammals may travel when dispersing its large fleshy fruits. However, in this northern area, populations of *Jacaratia digitata* are restricted to patches of forest of high soil fertility that are sparsely scattered geographically, whereas it grows on more extensive areas of terra firme forest with rich soils in the south, so these patterns may reflect variation in the geographic distribution of its preferred edaphic habitat. In north-western Amazonia, strong turnover of tree species has been reported between forest patches located on these areas of fertile soils (e.g. Jatun Sacha, Yanamono, Madreselva, Buenavista) and nearby sites of lower soil fertility (Honorio Coronado *et al.*, 2009). Some of these fertile soil patches coincide with the Miocene Pebas formation, which are sediments of higher soil fertility than the sediments of the Nauta formation which dominate much of this region (Higgins *et al.*, 2011). The presence of these two formations has been suggested to drive floristic discontinuities in north-western Amazonia (Higgins *et al.*, 2011).

In southern Peru, high genetic differentiation was recently reported between Cocha Cashu and Los Amigos (codes 20 and 25 respectively in Figure 3.2) for eight different species of *Inga*, an area that also coincided with high turnover in species composition of *Inga* communities (Dexter *et al.*, 2012). This study suggested the presence of two gene pools in western Amazonia for *Inga*, one extending from northern Peru to the Manu region and the second from Central Bolivia to the Los Amigos-Tambopata region. However my results across a much wider taxonomic range of species show a more complex picture with different genetic structures in western Amazonia. This is confirmed by AMOVA analysis which shows high values of genetic differentiation among SAMOVA groups and lower values between north and south regional groups (Table 3.3). The area of high genetic differentiation of *Inga* species is co-incident with two SAMOVA groups of *Poulsenia*

armata. However, the area of high differentiation is located further north for *Clarisia biflora* and *Otoba parvifolia* and not present in *Ficus insipida* and *Jacaratia digitata* (Figure 3.2a). Climatic and geological changes may be responsible for restricting seed flow among populations in southern Peru. A geological feature located perpendicularly to the Andes in southern Peru is the Fitzcarrald arch (Regard *et al.*, 2009). Even though previous studies have suggested that geological arches (e.g. Iquitos arch) co-incide with areas of high genetic differentiation among populations of small animals (Da Silva & Patton, 1998; Patton & Da Silva, 1998), it is hard to imagine that the current 600-m relief of the Fitzcarrald arch had been a physical barrier for western Amazonian tree species, many of which are distributed continuously across it. However we could expect that uplift of this arch during the Pliocene promoted changes in the environment and the landscape, and potentially restricted tropical rain forest in southern Peru (e.g.fossils of native bamboo indicate the potential occurrence of pre-Holocene bamboo forest in this region; Olivier *et al.*, 2009).

3.5.2. Genetic structure in the nuclear DNA

Nuclear and plastid DNA patterns are different in all species. In the case of *Jacaratia digitata* and *Ficus insipida*, no genetic differentiation was found among populations for the nuclear marker. The lesser variation found in ITS and the presence of single widespread haplotypes in western Amazonia are suggestive of effective nuclear gene flow via pollen. Pollen of *Jacaratia* and *Ficus insipida* is dispersed by sphingid moths (Bawa *et al.*, 1985a) and by tiny aganoid wasps (Machado *et al.*, 2001), respectively. Sphingid moths are large in size and have been shown to fly over 300 m between flowering trees in the Neotropics (*Pithecellobium elegans*; Chase *et al.*, 1996). Wasps that pollinate monoecious fig species such as *Ficus insipida* have been reported to travel over distances of up to 14 km in the Neotropics (Nason *et al.*, 1998) and up to more than 150 km in riparian vegetation of the Namibian desert (Ahmed *et al.*, 2009). In these two species, pollen dispersal may succeed even among geographically distant individuals indicating high effective population sizes and gene flow over large distances (Loveless & Hamrick, 1984). This pattern is also characteristic of *Cordia alliodora* (Rymer *et al.*, 2013) and *Ceiba pentandra* (Dick *et al.*, 2007), which share similar pollen dispersal syndromes (Table 3.5).

In the case of *Clarisia biflora* and *Poulsenia armata*, both species show geographically structured genetic variation for the nuclear DNA marker. Areas of high genetic differentiation are only congruent between plastid and nuclear markers for SAMOVA groups separating north-eastern populations of *Clarisia biflora*. With the lack of present physical barriers to gene flow among these SAMOVA groups, geographic genetic structure may represent historically isolated populations caused by the complex ecological configuration of western Amazonia and intra-specific adaptation to different environments. For example, *Clarisia biflora* occurs on fertile soils containing a high concentration of cations and bases in seasonally flooded forests and young terraces near main rivers in the north, and on less fertile soils in pre-montane and lowland terra firme forests in the rest of its range. In this case, populations of the north are consistently grouped into a SAMOVA group for both genetic markers indicating potential ecological specialization as an underlying causal factor. Specialization to divergent soil types matching genetic patterns has been reported in north-western Amazonia, especially for the generalist species *Protium subserratum* (Burseraceae). In this species, populations of white-sand soils were genetically differentiated from populations on clay and terrace soils (Fine *et al.*, 2013).

3.5.3. Long-term population stability and recent colonization

High haplotype and nucleotide diversities are found for most species in both DNA markers, with the exception of ITS in *Jacaratia digitata* and *Ficus insipida* (Table 3.2). This is indicative of populations having occupied western Amazonia for long periods of time, and confirmed by species crown nodes dating from the Late Miocene (c. 7-12 Ma). Although lineage divergence estimates have broad confidence intervals, minimum ages pre-date the Pleistocene suggesting that earlier events, rather than Pleistocene climatic changes underlie the initiation of genetic diversification among populations of these species in Amazonia (Rull, 2008; Hoorn *et al.*, 2010). Mean values of the most recent common ancestor of the extant populations coincides with the presence of Lake Pebas (6-23 Ma), a wetland system that dominated western Amazonia during the Miocene (Hoorn, 1996; Hoorn & Vonhof, 2006). It is possible that the species may have originated on small patches of dry land during the wetland period, and that the drainage of the lake may have favoured the diversification and spread of their lineages that are adapted to well-drained

soils (e.g. *Jacaratia digitata*, *Clarisia biflora*, *Poulsenia armata*, *Otoba parvifolia*) or to seasonally flooded areas (e.g. *Ficus insipida* and some populations of *Clarisia biflora*).

Although there is evidence for long-term population stability in western Amazonia, recent colonization of the south from the north is suggested for *Jacaratia digitata* and *Otoba parvifolia* with plastid lineages endemic to the south nested within lineages endemic to the north. Similarly, in *Ficus insipida*, several colonisation events from the north to the south are suggested by its phylogeographic pattern. This pattern supports the post-glacial rain forest expansion reported on the southern margin of the Amazon rain forest in Bolivia, based on pollen and charcoal records (Mayle *et al.*, 2000; Burbridge *et al.*, 2004). The authors of these studies suggest that changes in the position of the Intertropical Convergence Zone (ITCZ) determine a seasonally dry climate in south-western Amazonia, which has had latitudinal migrations explained by Milankovitch cycles. Therefore, historical changes in climate in this part of Amazonia may have favoured the expansion of rain forest during interglacial periods and the restriction of the tropical flora to northern regions during periods of longer seasonally co-inciding with glacial maxima.

3. 6. CONCLUSION

Genetic patterns among the five study species indicate that in some cases edaphic factors correlate with genetic variation. For example, the strong plastid genetic structure in *Jacaratia digitata* may reflect the scattered distribution of its preferred well-drained soils of high fertility in north-western Amazonia, and in *Clarisia biflora* north-south genetic changes co-incide with differences in edaphic preferences (seasonally flooded forest in the north and terra firme in the south). This potential role of edaphic factors underlying genetic variation within species (cf. Fine *et al.*, 2013) indicates the importance of collecting edaphic data as part of phylogeographic studies.

However, the fact that genetic breaks are not entirely congruent among all the species implies that a variety of strategies for occupying the complex environmental conditions of Amazonia may have contribute to the long historical persistence of rain forest species and the maintenance of the large diversity present in the basin. Future studies should sample

more species densely across their entire ranges to determine if similar genetic patterns are found in other widespread or restricted species and these studies should cover species with different biological and ecological traits.

Table 3.1. Relevant information for producing the dated phylogenies of lineage divergence for each study species. Mean root ages [and intervals] represent secondary calibration points provided by dated phylogenies of the families.

Family	Ingroup taxa	Outgroup taxa	GenBank accession	Root age [interval values] and reference
Caricaceae	*Jacaratia digitata*	*Vasconcellea candicans*	JX091986	18.8 Ma [14.4,23.7]
		Vasconcellea glandulosa	JX091991	Antunes Carvalho and Renner (2012)
		Vasconcellea sphaerocarpa	JX091993	
Moraceae	*Clarisia biflora*	*Clarisia racemosa* (E #)	n.a.	89.1 Ma [72.6,110.0]
	Ficus insipida	*Ficus abutilifolia*	EU213821	Zerega *et al.* (2005)
		Ficus bullenei	GQ982218	
		Ficus citrifolia	GQ982219	
		Ficus insipida[1] (MESO2)	GQ438204	
		Ficus insipida[1] (MESO6)	GQ438203	
		Ficus insipida[1] (MESO7)	GQ438019	
		Ficus insipida[1] (MESO8)	GQ438211	
		Ficus insipida[1] (MESO9)	GQ438156	
		Ficus insipida[1] (MESO10)	GQ438181	
		Ficus insipida[1] (MESO16)	GQ438186	
		Ficus popenoei	GQ982224	
		Ficus sycomorus	EU213825	
	Poulsenia armata	*Poulsenia armata*[1]	GQ982324	
Myristicaceae	*Otoba parvifolia*	*Compsoneura ulei*	EU090658	18.0 Ma [15.0,21.0]
		Horsfieldia basifissa	GQ248315	Doyle *et al.* (2004)
		Iryanthera sagotiana	GQ428668	
		Myristica fatua	GQ248350	
		Virola nobilis	GQ982402	

[1] samples of the study species from Central America

Table 3.2. Haplotype and nucleotide diversity for plastid (*trnH-psbA*) and nuclear (ITS) DNA sequences sampled for five widespread species in western Amazonia. Nuclear DNA sequences for *Otoba parvifolia* were not available. Number of populations, individuals, polymorphic sites, and haplotypes are provided.

DNA marker	Species	#Pop.	#Indiv.	#Poly.sites	#Hapl.	Hap.div ± SD	Nuc.div ± SD (%)
*trn*H-*psb*A	*Ficus insipida*	23	182	12	12	0.70 ± 0.05	0.37 ± 0.26
	Jacaratia digitata	17	121	32	6	0.55 ± 0.07	1.55 ± 0.83
	Clarisia biflora	19	124	15	16	0.92 ± 0.01	0.67 ± 0.40
	Poulsenia armata	12	61	11	7	0.75 ± 0.04	1.19 ± 0.66
	Otoba parvifolia	22	186	20	16	0.88 ± 0.02	1.22 ± 0.69
ITS	*Ficus insipida*	23	77	3	4	0.12 ± 0.07	0.00 ± 0.01
	Jacaratia digitata	11	12	3	3	0.50 ± 0.12	0.15 ± 0.13
	Clarisia biflora	19	76	20	27	0.92 ± 0.06	0.54 ± 0.33
	Poulsenia armata	12	49	16	7	0.76 ± 0.09	0.62 ± 0.38

Table 3.3. Analysis of molecular variance (AMOVA) based on pairwise differences of the nuclear (ITS) and plastid (*trnH-psbA*) DNA sequences sampled from five widespread species in western Amazonia. The analysis was run independently using all populations and using populations grouped by geographical region (north and south).

	*trn*H-*psb*A			ITS		
Groups	Among groups (%)	Among pops within groups (%)	Within populations (%)	Among groups (%)	Among pops within groups (%)	Within populations (%)
Ficus insipida						
All populations		66.94**	33.06NA		6.99NS	93.01NA
SAMOVA groups	69.46**	10.73**	19.81**	NA	NA	NA
North + South	-1.89**	68.52**	33.37NS	-3.23NS	8.54NS	94.69NS
Jacaratia digitata						
All populations		99.79**	0.21NA		100.00NS	0.00NA
SAMOVA groups	98.30**	1.62**	0.08**	NA	NA	NA
North + South	44.16**	55.68**	0.16*	32.02NS	67.98NS	0.00NS
Clarisia biflora						
All populations		91.20**	8.80NA		55.28**	44.72NA
SAMOVA groups	48.82**	43.88**	7.30**	60.76**	3.79**	35.45**
North + South	2.75**	88.56**	8.68NS	26.26**	33.73**	39.01*
Poulsenia armata						
All populations		97.85**	2.15NA		96.28**	3.72NA
SAMOVA groups	94.53**	3.84**	1.63**	91.96**	5.60**	2.44**
North + South	17.46**	80.57**	1.97NS	10.54**	85.93**	3.53NS
Otoba parvifolia						
All populations		92.44**	7.56NA			
SAMOVA groups	60.71**	32.87**	6.42**			
North + South	30.70**	62.86**	6.44**			

NA, not applicable; NS, not significant; *$p < 0.05$; ** $p < 0.005$.

Table 3.4. Physical and chemical properties for soil of each widespread species and 49 RAINFOR sites collected north- and south-western Amazonia. Mean values (± 95% confidence interval) are provided. SB: sum of base cations; ECEC: effective cation exchangeable capacity. An asterisk is given when the Kruskal-Wallis tests give significant difference in the different edaphic properties (Kruskal-Wallis test: df = 5, *p*-value < 0.05; Appendix S3.8)

Species	Region	*pH	Particles (%)			Cations ($cmol_c\ Kg^{-1}$)						
		H_2O	*Clay	*Silt	Sand	*Ca^{2+}	*Mg^{2+}	K^+	Na^+	*Al^{3+}	*SB	ECEC
Ficus insipida	North	5.7	21	30	49	4.15	0.98	0.16	0.03	0.40	5.32	5.72
		(0.8)	(9)	(6)	(12)	(1.33)	(0.34)	(0.05)	(0.01)	(0.68)	(1.64)	(1.20)
	South	5.6	17	40	43	3.70	1.19	0.12	0.02	0.24	5.03	3.90
		(0.3)	(8)	(12)	(18)	(2.01)	(0.47)	(0.04)	(0.01)	(0.45)	(2.43)	(1.93)
Jacaratia digitata	North	5.0	27	43	29	4.72	1.41	0.15	0.10	0.89	6.38	7.26
		(0.7)	(16)	(14)	(20)	(1.08)	(0.50)	(0.05)	(0.15)	(0.92)	(1.38)	(2.07)
	South	6.0	16	38	46	4.24	1.19	0.19	0.02	0.40	5.64	5.09
		(0.7)	(7)	(9)	(15)	(1.80)	(0.52)	(0.07)	(0.01)	(0.63)	(2.11)	(1.12)
Clarisia biflora	North	5.3	23	39	38	5.23	1.45	0.17	0.10	0.59	6.95	7.54
		(0.6)	(9)	(9)	(13)	(2.31)	(0.53)	(0.05)	(0.10)	(0.54)	(2.82)	(2.71)
	South	5.0	10	34	56	1.72	0.63	0.13	0.01	0.53	2.50	3.03
		(0.5)	(4)	(17)	(20)	(1.67)	(0.47)	(0.05)	(0.01)	(0.72)	(2.10)	(1.93)
Poulsenia armata	North	5.5	15	40	45	5.01	1.38	0.11	0.10	1.07	6.60	7.67
		(1.3)	(12)	(13)	(19)	(1.23)	(0.61)	(0.08)	(0.20)	(1.93)	(1.66)	(3.51)
	South	5.5	21	49	31	4.13	1.28	0.14	0.03	0.30	5.57	4.23
		(0.7)	(13)	(19)	(26)	(3.36)	(0.48)	(0.05)	(0.02)	(1.26)	(3.83)	(3.51)
Otoba parvifolia	North	5.3	22	37	41	3.72	1.09	0.12	0.07	0.84	4.99	5.83
		(0.5)	(9)	(5)	(11)	(1.23)	(0.43)	(0.04)	(0.06)	(0.51)	(1.57)	(1.5)
	South	5.4	20	46	34	4.31	1.49	0.14	0.03	0.10	5.96	4.36
		(0.4)	(11)	(17)	(22)	(2.58)	(0.51)	(0.05)	(0.01)	(0.22)	(2.95)	(2.30)
49 RAINFOR Sites	North	4.5	29	30	41	1.86	0.64	0.12	0.04	2.24	2.66	4.90
		(0.2)	(5)	(5)	(8)	(0.93)	(0.26)	(0.02)	(0.01)	(0.67)	(1.19)	(1.08)
	South	4.9	29	31	40	1.62	0.67	0.13	0.06	1.38	2.47	3.85
		(0.3)	(5)	(9)	(12)	(0.98)	(0.32)	(0.03)	(0.06)	(0.61)	(1.28)	(0.97)

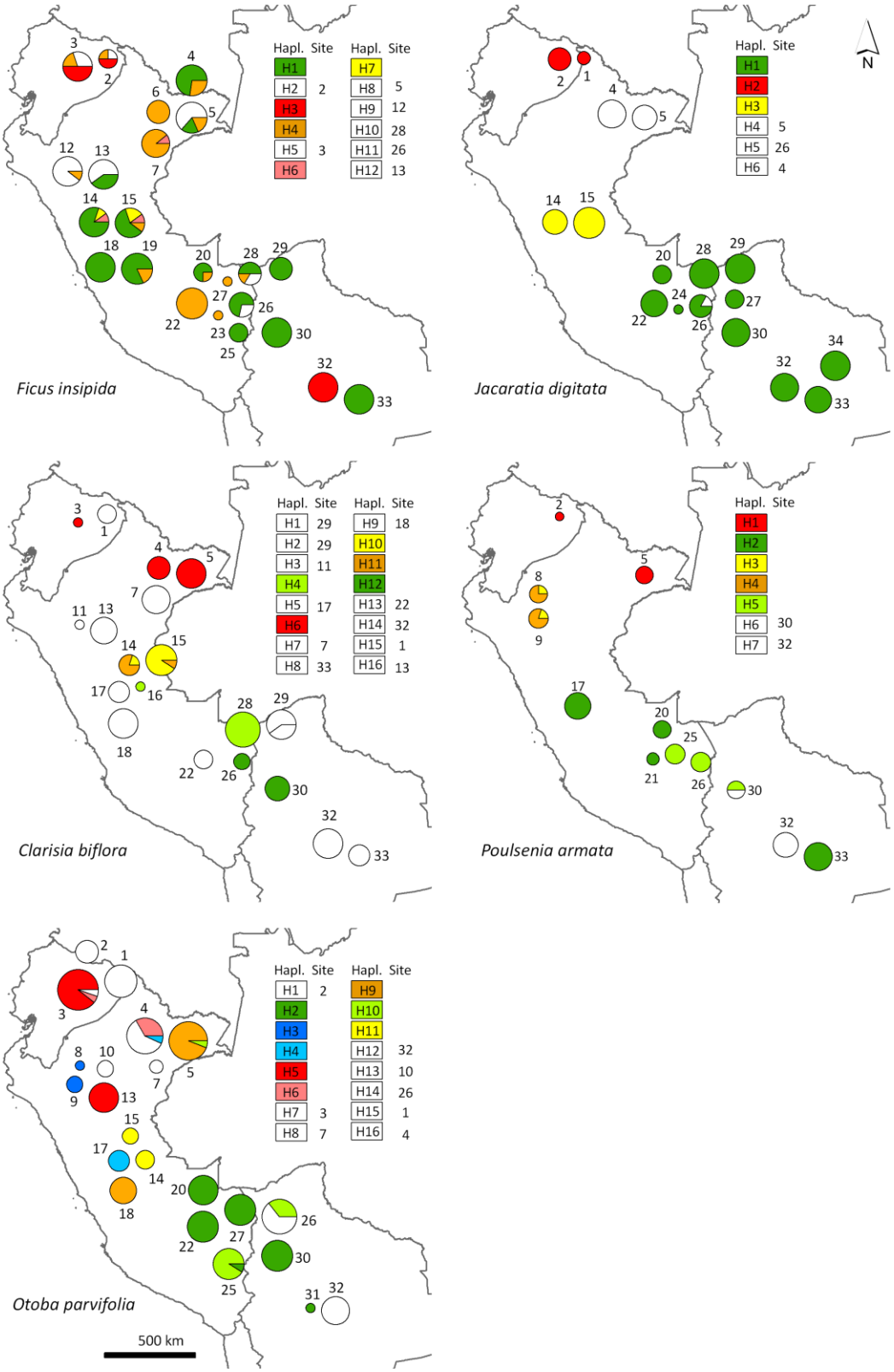
Table 3.5. Ecological traits and population genetic structure for different phylogeographic studies in Amazonia.

Species and family	Growth strategy	Floral sexuality[a]	Genetic marker	Pollen dispersal[a]	Seed Dispersal	Genetic structure		Reference
						Nuclear	Plastid	
Ceiba pentandra MALVACEAE	Light demanding	Hermaphrodite	nrDNA (ITS), cpDNA (*psb*B-*psb*F)	Vertebrates (bats) Insects (moths)	Wind and water	Low	Low	Dick *et al.* (2007)
Cordia alliodora BORAGINACEAE	Light demanding	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A), cpSSR	Insects (moths)	Wind	Low	Low	Rymer *et al.* (2013)
Jacaranda copaia[3] BIGNONIACEAE	Light demanding	Hermaphrodite	cpDNA (*trn*H-*psb*A, *trn*C-*ycf6*), nuSSR, cpSSR	Insects (large bees)	Wind	Low	Low	Scotti-Saintagne *et al.* (2013b)
Schizolobium parahyba FABACEAE	Light demanding	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A, *trn*L-*trn*F, *mat*K)	Insects (bees)	Wind	Low	High	Turchetto-Zolet *et al.* (2012)
Swietenia macrophylla MELIACEAE	Light demanding	Monoecious	cpSSR	Insects	Wind	?	High	Lemes *et al.* (2010)
Ficus insipida MORACEAE	Light demanding	Monoecious[1]	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Insects (wasps)	Vertebrates (fish, bats)	Low	Medium	This study

Species and family	Growth strategy	Floral sexuality[a]	Genetic marker	Pollen dispersal[a]	Seed Dispersal	Genetic structure		Reference
						Nuclear	Plastid	
Jacaratia digitata CARICACEAE	Light demanding	Dioecious	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Insects (moths)	Vertebrates (monkeys, birds)	Low	High	This study
Symphonia globulifera CLUSIACEAE	Shade tolerant	Hermaphrodite	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Vertebrates (hummingbirds)	Vertebrates (bats, birds, monkeys)	Low	Medium	Dick and Heuertz (2008)
Poulsenia armata MORACEAE	Shade tolerant	Monoecious[2]	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Insects (thrips)	Vertebrates (bats)	High	High	This study
Clarisia biflora MORACEAE	Shade tolerant	Dioecious	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	?	Vertebrates?	High	High	This study
Otoba parvifolia MYRISTICACEAE	Shade tolerant	Dioecious	nrDNA (ITS), cpDNA (*trn*H-*psb*A)	Insects (beetles?)	Vertebrates (bats)	?	High	This study

[a] Bawa *et al.* (1985a); Bawa *et al.* (1985b)
[1] male and female flowers are inside an enclosed receptacle called syconia
[2] separate male and female inflorescences
[3] only structure separating two subspecies but not within each clade

a)


Hapl. Site
H1
H2 2
H3
H4
H5 3
H6
H7
H8 5
H9 12
H10 28
H11 26
H12 13
Ficus insipida
N
Hapl. Site
H1
H2
H3
H4 5
H5 26
H6 4
Jacaratia digitata
Hapl. Site
H1 29
H2 29
H3 11
H4
H5 17
H6
H7 7
H8 33
H9 18
H10
H11
H12
H13 22
H14 32
H15 1
H16 13
Clarisia biflora
Hapl. Site
H1
H2
H3
H4
H5
H6 30
H7 32
Poulsenia armata
Hapl. Site
H1 2
H2
H3
H4
H5
H6
H7 3
H8 7
H9
H10
H11
H12 32
H13 10
H14 26
H15 1
H16 4
Otoba parvifolia
500 km

b)

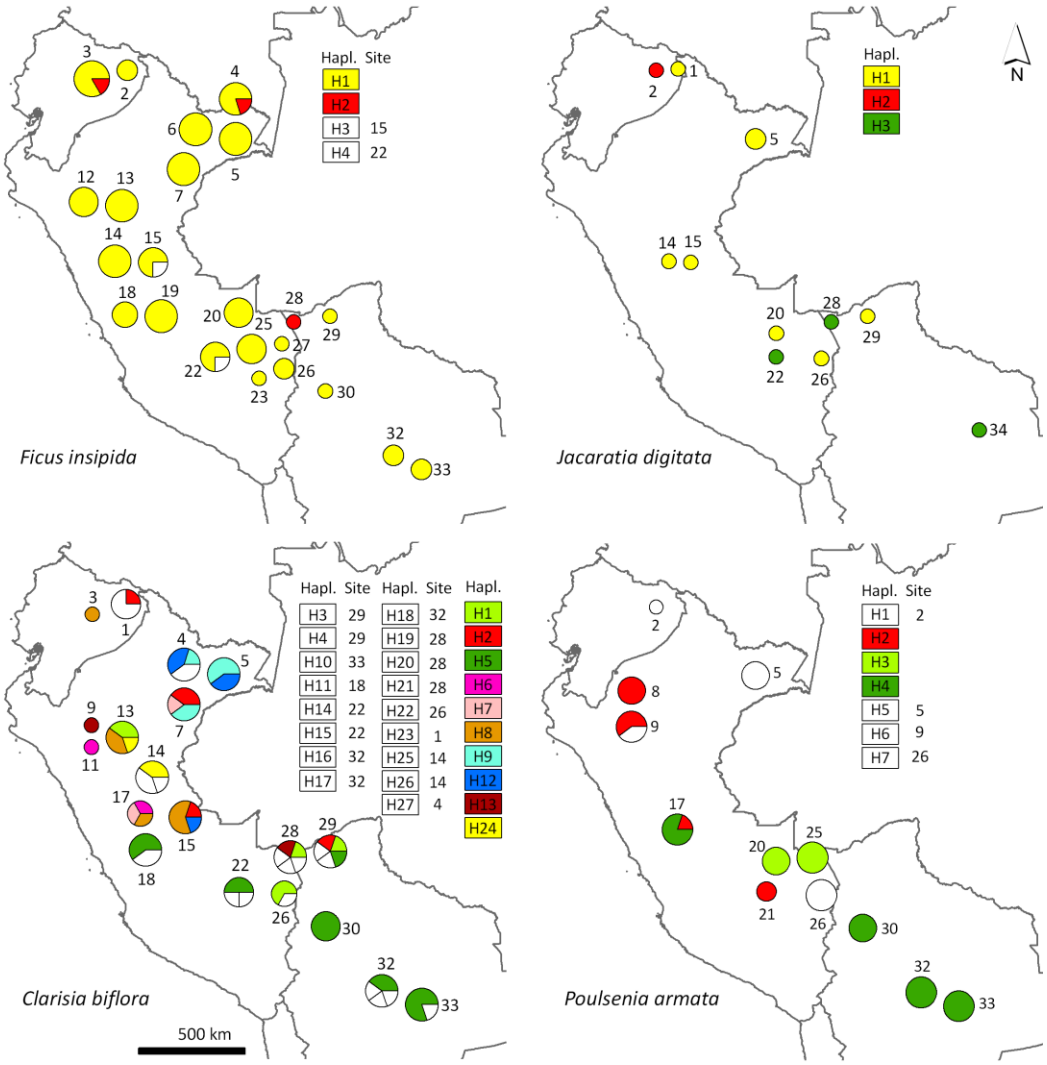
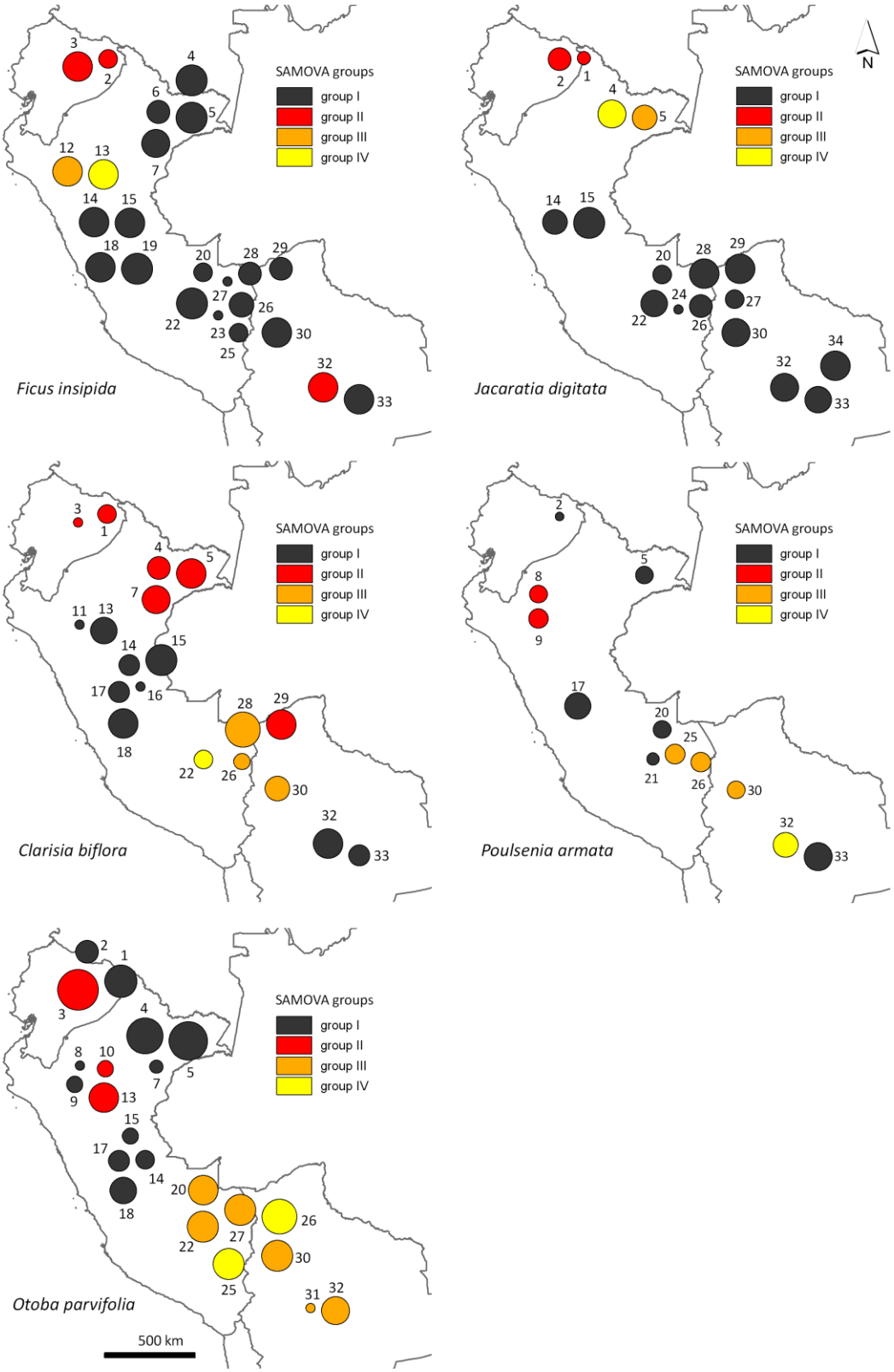


Figure 3.1. Distribution of haplotypes for populations of five widespread species sampled in western Amazonia. a) Plastid DNA sequences (*trn*H-*psb*A), and b) nuclear DNA sequences (ITS). Colours represent the haplotypes and these are not shared among species. Pie charts are labelled with population numbers as shown in Appendix S3.1. The size of the circles is proportional to sample size for each population. Haplotypes unique to single population are shown in white with site number given in the legend. Nuclear DNA sequences for *Otoba parvifolia* were not available.

a)


SAMOVA groups
group I
group II
group III
group IV
N
Ficus insipida
Jacaratia digitata
Clarisia biflora
Poulsenia armata
Otoba parvifolia
500 km

b)

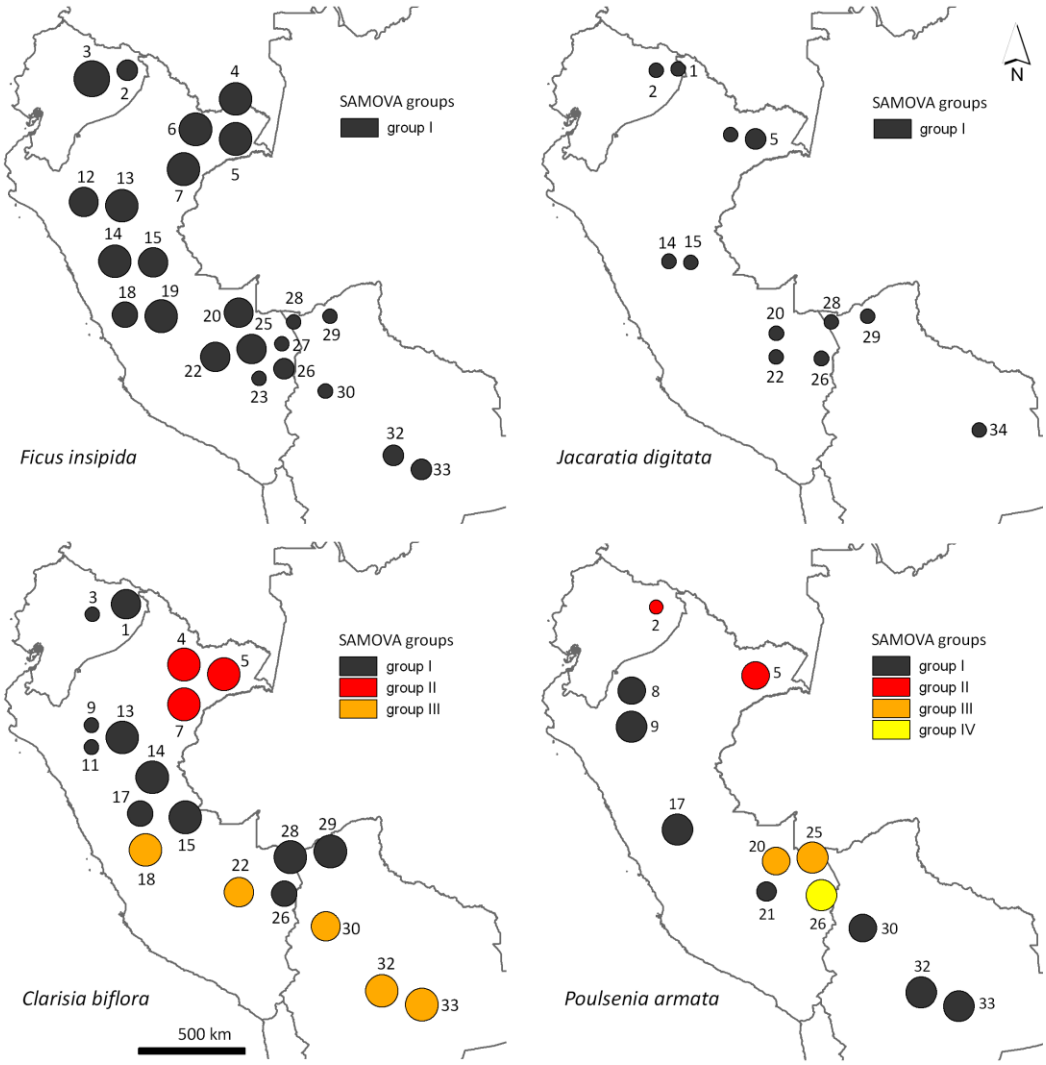


Figure 3.2. Groups of populations defined by the spatial analysis of molecular variance (SAMOVA) for five widespread species sampled in western Amazonia. Analysis was independently run for a) plastid (*trn*H-*psb*A) DNA sequences and b) nuclear (ITS) DNA sequences. Colours represent the SAMOVA groups. The size of the circles is proportional to sample size for each population that is labelled with population numbers as shown in Appendix S3.1. Nuclear DNA sequences for *Otoba parvifolia* were not available.

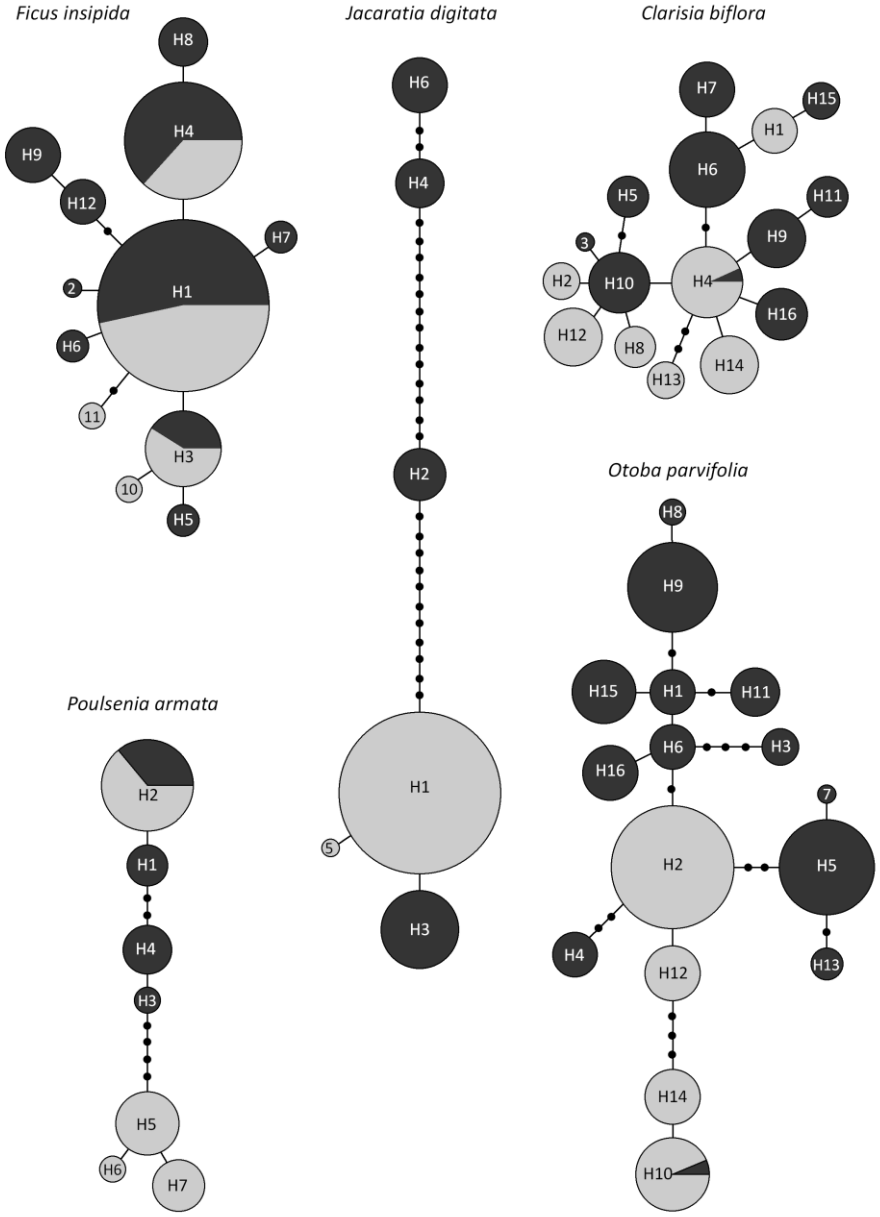


Figure 3.3. Haplotype networks of *trn*H-*psb*A sequences for populations of five widespread species sampled in western Amazonia. The size of the circles is proportional to sample size for each haplotype (n = 1 - 88 individuals). Colours represent the geographical regions: north-western Amazonia (dark grey), and south-western Amazonia (light grey). Missing haplotypes in the networks are shown as black dots.

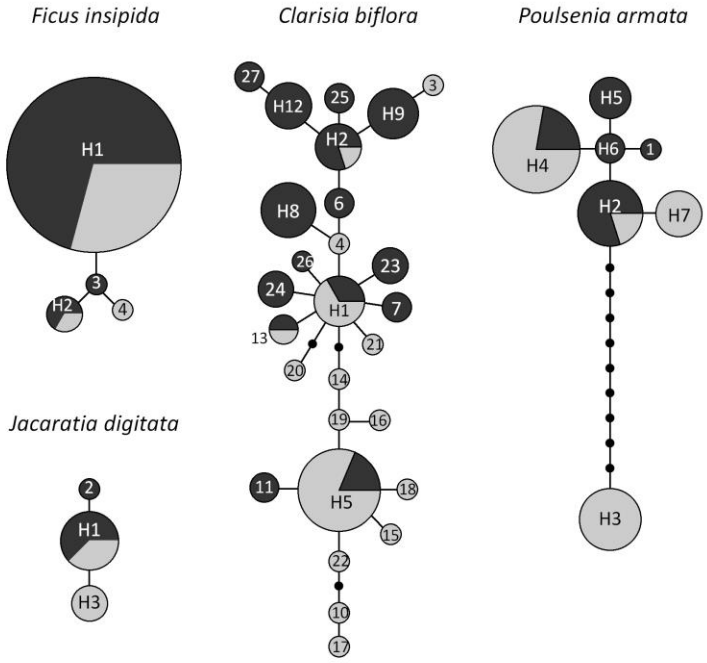


Figure 3.4. Haplotype networks of ITS sequences for populations of four widespread species sampled in western Amazonia. The size of the circles is proportional to sample size for each haplotype (n = 1 - 65 individuals). Colours represent the geographical regions: north-western Amazonia (dark grey), and south-western Amazonia (light grey). Missing haplotypes in the networks are shown as black dots. Nuclear DNA sequences for *Otoba parvifolia* were not available.

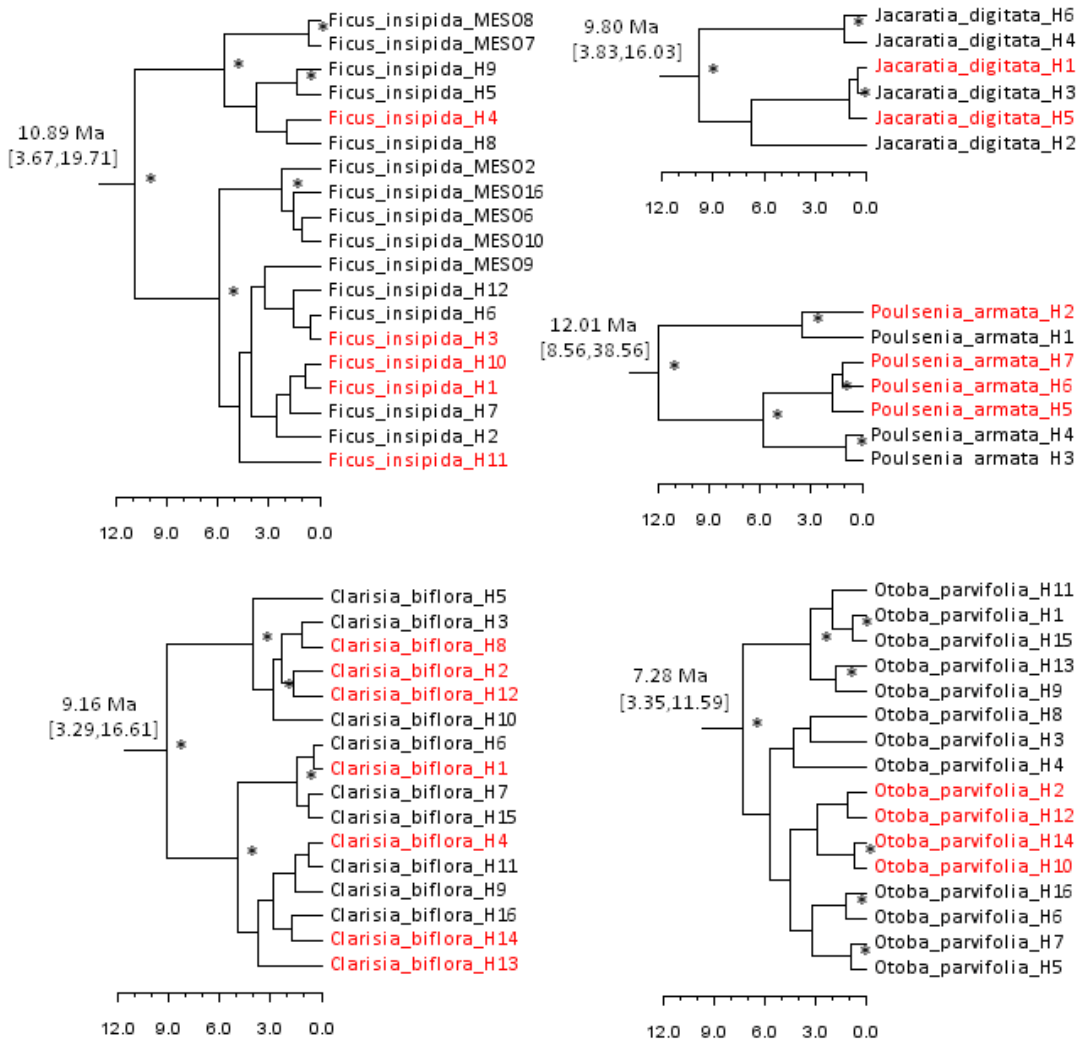


Figure 3.5. Lineage divergence dating for plastid haplotypes for five widespread species of western Amazonia. Mean divergence dates are given for each species with 95% highest posterior density values in brackets. Lineages occurring in the south are in red, although some of them could also occur in the north (see Figure 3.3 for detecting haplotypes occurring in both regions). Nodes with posterior probabilities ≥ 0.90 are indicated with an asterisk. Note that southern haplotypes are nested within lineages of the north in *Jacaratia digitata* and *Otoba parvifolia*, while for *Ficus insipida* several colonisation events from the north to the south are inferred.

Chapter 4. The uneven distribution of tree phylogenetic diversity across the Amazon rain forest

4. 1. ABSTRACT

This study aims to reveal the spatial variation in the distribution of phylogenetic diversity across the Amazon rain forest, using a large dataset of tree inventories. We expected that forest growing on the much older formations of the Guiana and Brazilian Shields would have accumulated a greater diversity of lineages and more distantly related species than forests elsewhere which should be dominated by more recent radiations, but we find that this is not so. Rather, phylogenetic diversity is unevenly distributed across the basin, as measured by both the residuals of phylogenetic diversity *sensu strictu* (PD) on species diversity and by the mean pairwise phylogenetic distance among unique taxa (MPDt). The most tree species diverse regions of Amazonia differ greatly in their phylogenetic patterns; communities of central Amazonia have lower phylogenetic diversity than expected from their taxon richness, indicating that communities are dominated by phylogenetic relatives compared to communities of north-western Amazonia which have more distant related species. In addition, a marked difference across the Amazon basin was found in the proportions of different angiosperm clades, with the greatest proportion of tree species of Magnoliids and Monocots in the west. Given that clades diverging earlier were also characteristic of pre-montane habitats, these results suggest that migration events from cooler environments at different geological times may have played an important role in the assemblage of the most phylogenetically diverse communities in lowland western Amazonia.

4. 2. INTRODUCTION

Biodiversity has traditionally been conceived of as the total number of species, and tropical rain forests are recognised as representing the greatest concentration of biodiversity on the planet. For example, Amazonia is well-known to have exceptionally high tree species diversity, especially in terra firme forests of western and central regions (Gentry, 1988b;

Ter Steege *et al.*, 2003). Although different species diversity indices can be used, these metrics do not include the genealogical relationship of taxa and so can potentially give similar values to communities regardless of whether they are dominated by phylogenetic relatives or by only distantly related species. As a result, some authors have suggested the use of phylogenetic diversity as a measure of the full spectrum of lineage diversity (Vane-Wright *et al.*, 1991; Faith, 1992) that can be estimated by the total branch length that separates taxa of a given community in a phylogenetic tree (Faith, 1992). Uncovering the spatial variation in the distribution of phylogenetic diversity is of particular relevance to conservation if the goal is to capture more fully the evolutionary processes determining species diversity such as speciation, migration and extinction. Furthermore, understanding the spatial distribution of phylogenetic diversity across a biome can provide insights into its biogeographical history. Although there is only a study that has attempt to measure phylogenetic diversity in Amazonia (Chave *et al.*, 2007), it was not possible to explore the basin extensively because the floristic dataset in use (Gentry, 1988a) is largely Andean and pre-Andean. Therefore, phylogenetic diversity across the most diverse ecosystem of the world remains largely unknown.

The huge spatial variability of ages in the geological sediments that underlie the Amazon basin needs to be accounted for in analysis of the distribution of species and phylogenetic diversity. The Guiana and Brazilian Shields located north and south of the eastern section of the Amazon River are the oldest formations of Pre-Cambrian origin, with highly weathered soils of low fertility (Quesada *et al.*, 2011). Between these shields (central and eastern Amazonia), sediments of the middle Tertiary and Cretaceous-Tertiary periods have been deposited and have been continuously weathered for more than 20 and 100 million years, respectively. By contrast, young and relative fertile sediments were deposited more recently in western Amazonia during the Pliocene uplift of the Andes (Hoorn *et al.*, 2010; Quesada *et al.*, 2011). The major geological event of the Andean orogeny was suggested by Gentry (1982) to have played an important role in promoting high species diversification near the Andes through the constant creation of new habitats and large-scale rearrangement of the complex, dissected landscapes. Some more recent phylogenetic evidence has supported this notion, with radiations of some Andean diverse genera apparently coinciding closely with the rapid uplift of the Andes (Richardson *et al.*, 2001;

Erkens *et al.*, 2007; Särkinen *et al.*, 2007). Although forests of the Guiana and Brazilian Shields contain rather lower species richness than western Amazonia (Ter Steege *et al.*, 2003), more stable conditions may have promoted the long-term accumulation and persistence of more lineages in the shields (Stebbins, 1974).

Broad gradients in climate are also characteristic of the Amazon rain forest. For example, dry season length varies across the basin with more seasonal climates in the southeast to almost no dry season in the northwest. Mean annual rainfall varies from 1600 mm in the southeast to more than 3000 mm in the northwest (Sombroek, 2001), and with seasonality sharply increasing again in the lowland tropics of the Orinoco basin and Caribbean watersheds. Within the most seasonal regions, two other forested biomes occur, tropical savannas and seasonally dry tropical forest (SDTF). SDTF occurs on highly fertile soils where the annual rainfall is less than 1600 mm, with at least 5-6 months receiving less than 100 mm (Gentry et al., 1995b). Savannas are found on poor soils, often oxisols, similar to the soils if large areas of Amazon rain forest, and with similar or slightly higher rainfall than SDTF (Sarmiento, 1992). The flora of SDTF is adapted to seasonal water stress with the vegetation mostly deciduous during dry season, while the savanna flora is adapted to fire with a grass dominated ground layer and sclerophyllous trees with evergreen leaves (Ratter *et al.*, 1997). SDTF contains low species diversity and clades of species found in this biome tend to show phylogenetic structure with sister species co-occurring in the same isolated geographic areas, indicating limited dispersal among disjunct SDTF nuclei (Pennington *et al.*, 2006b). Fire-tolerance traits in tropical savannas of Brazil have repeatedly evolved from lineages apparently originating in adjacent forest biomes (Simon *et al.*, 2009; Simon & Pennington, 2012).

Recent developments in applying standardized floristic sampling across the full extent of the Amazon rain forest (Malhi *et al.*, 2002; Phillips & Miller, 2002) and in estimating the phylogeny of angiosperms (Bremer *et al.*, 2009) have made it possible for researchers to probe the importance of phylogeny in structuring ecological communities (Webb *et al.*, 2002; Kembel & Hubbell, 2006; Emerson & Gillespie, 2008; Baraloto *et al.*, 2012). By using the phylogenetic diversity approach, it could be asked if the most species-rich Amazon communities also contain high phylogenetic diversity, or instead if the greatest phylogenetic diversity is located in the less diverse communities of geologically old

formations. This latter was the case in the Cape flora in South Africa where the species-rich communities had less phylogenetic diversity than would be expected from their taxon richness indicating a clustered phylogenetic structure compared to an over-dispersed structure in the species-poor communities (Forest *et al.*, 2007).

The aim of this study is to reveal the spatial variation in the distribution of phylogenetic diversity across the Amazon rain forest. Here, tree floristic data from a network of permanent plots are used (RAINFOR; Peacock *et al.*, 2007), representing the main ecological gradients in Amazon geology, soil fertility and climate (Sombroek, 2001; Quesada *et al.*, 2011). We tested several hypotheses using an integrated approach that includes the maximum age of geological formations and delimitation of communities by forest type. In terms of regional patterns, it is expected that communities of western Amazonia will show lower phylogenetic diversity than expected by their taxon diversity because of the recent radiation events in some species-rich genera driven by the Andean orogeny (Gentry, 1982) or other recent historical processes. In contrast, less species-rich communities of the Brazilian and the Guiana Shields that are geologically much older and which have had no direct impact from the Andean orogeny should contain higher phylogenetic diversity than expected by their taxon richness because they likely will have accumulated more lineages, and may have fewer recent species radiations (Stebbins, 1974). In addition, forest types with marked ecological constraints of nutrient and water supply within the Amazon rain forest are expected to have lower phylogenetic diversity than expected from their species richness, because only a few lineages will have successfully adapted to low-fertility soils or waterlogged conditions. A few plots available for tropical savannas and SDTF will be used and compared as extreme examples of ecological adaptations such as fire tolerance and extreme dry conditions.

4. 3. METHODS

4.3.1. Tree community plot data

A total of 283 floristic tree inventories of the RAINFOR forest plot network were compiled (Date of extraction: 28/01/2013; Lopez-Gonzalez *et al.*, 2011). For the purpose of this

study, only old-growth forest plots containing at least 65.5% of individuals identified to species (mean per plot ± 95% CI = 86.3 ± 1.1 %) were used (see Appendix S4.1). Plots representing successional vegetation and burnt and logged forests were excluded. Most plots cover one hectare of forest (mean size ± 95% CI = 1.1 ± 0.1 ha) in which arborescent palms and trees above 10 cm in diameter were identified to species. Each plot was treated as a community and classified into three main biomes: tropical rain forest (n = 267 plots), seasonally dry tropical forest (n = 11) and savannas (n = 5).

The 267 tropical rain forest plots include 254 from across the whole Amazon basin, and 13 from the northern Andes. These were further classified by the maximum age of the underlying geological formation and by forest type. The Guiana and Brazilian Shields represent the oldest geological formations of Pre-Cambrian origin in Amazonia (> 500 Ma), followed by formations of central and eastern Amazonia (20-100 Ma) located between the Shields; while areas near to the Andes (western Amazonia and northern Andes) are dominated by younger sediments (< 20 Ma; Quesada *et al.*, 2011) deposited mainly during the Pleistocene and the Holocene (Hoorn *et al.*, 2010). The following forest types were considered: montane forest (1650 - 3000 m a.s.l.), flooded forest (affected by the flooding of rivers or with a shallow water table), terra firme forest (interfluvial plain on clayed or brown-sand soils), and white-sand forest (on white-sand soils).

In total, the dataset included 183,908 individual trees sampled in nine South American countries (Colombia, Venezuela, Guiana, Surinam, French Guiana, Brazil, Ecuador, Peru, and Bolivia). To ensure a standardized nomenclature across plots, the Taxonomic Name Resolution Service version 3.0 was used (http://tnrs.iplantcollaborative.org; accessed on 01/03/2013). This source returns family information of taxa based on the most recent angiosperm phylogenetic classification (Bremer *et al.*, 2009) and accepted names for detected synonyms based on taxonomic databases such as TROPICOS of the Missouri Botanical Garden. After the exclusion of tree ferns and gymnosperms (0.8 % of individuals) and all trees not identified to species (13.6 %), the final dataset contained a total of 157,340 individuals, belonging to 3,868 species, 732 genera and 126 families of angiosperms.

4.3.2. Phylogenetic tree and diversity metrics

A phylogenetic tree of the whole species pool (Appendix S4.2) was generated using Phylomatic in PHYLOCOM version 4.2 (Webb *et al.*, 2008). This tool provides a phylogenetic hypothesis for the relationships among taxa by matching the list of taxa with up-to-date family and genus names, and tip labels of a provided megatree (Webb & Donoghue, 2005). In this case, the topology of R20120829.new provided at http://phylodiversity.net/phylomatic/ was used. An ultrametric phylogeny including branch length in millions of years (Ma) was obtained using bladj in PHYLOCOM. This command fixes the root node (angiosperms, 179 Ma) and other nodes to specified ages based on Wikström *et al.* (2001).

Two metrics were used to evaluate the evolutionary history present in communities, phylogenetic diversity *sensu strictu* (PD; Faith, 1992), and mean pairwise phylogenetic distance among unique taxa (MPDt; Webb, 2000; Webb *et al.*, 2002). While other phylogenetic diversity metrics exist (e.g. Helmus *et al.*, 2007; Cadotte *et al.*, 2010), these were chosen because of their simplicity and history of use in the literature (e.g. Forest *et al.*, 2007; Kembel, 2009; Gonzalez *et al.*, 2010; Fine & Kembel, 2011; Morlon *et al.*, 2011; Safi *et al.*, 2011). PD measures the sum of the phylogenetic branch lengths of all species occurring in a given community. Because this measure tends to correlate strongly with species diversity, it allows us to test if communities contain higher or lower PD than expected from their species diversity (Forest *et al.*, 2007). A lower (or higher) than expected PD for a given species richness indicates that taxa are clustered (or over-dispersed) in the phylogeny. MPDt measures the mean phylogenetic distance between all pairs of species, so it also includes information of how closely or distantly related the species are in a given community.

4.3.3. Data assessment and analysis

To minimize the effect of sampling effort (i.e. plot size), all calculations were carried out using a rarefaction procedure set for 100 runs that standardized all plots to the same number of individuals, i.e. the number of stems in the plot with the fewest number of individuals (n = 283 individuals). Species diversity for each plot was calculated using Fisher's alpha which represents the slope of the rank abundance distribution (Fisher *et al.*,

1943). Each taxon was classified into one of the three major angiosperm clades (Magnoliids, Monocots, and Eudicots) and the percentage of species and individuals in each major clade was estimated. Values of phylogenetic diversity for PD and MPDt were calculated using the package PICANTE (Kembel et al., 2010) in the R Statistical Software version 2.15.1.

Correlations were performed to assess the strength of the relationship between phylogenetic diversity metrics and two potential explanatory variables, species diversity and the percentage of species in each major angiosperm clade. To explore the spatial variation of the variables, these were also correlated to the geographical coordinates of each plot (latitude and longitude).

Because a high correlation between PD and species diversity was expected, the spatial patterns of the residuals from the linear regression of PD on species diversity was also provided ("PD residuals"; Forest *et al.*, 2007). Biomes and geological formations containing communities with higher or lower PD values than expected by their species diversity were tested using *t* test. Both “PD residuals” and MPDt were tested for significant difference using F-test and Tukey test.

Finally, we checked if there was any bias of the phylogenetic diversity metrics with respect to the percentage of unidentified individuals excluded per plot. Moreover, a sensitivity analysis was performed by checking the consistency of the patterns and by using rarefacted communities of 500 individuals for rain forest Amazonian plots.

4. 4. RESULTS

4.4.1. Major angiosperm clades and species diversity

Of the 267 tropical rain forest plots, 39.7 % were located in geologically old formations of the Guiana and Brazilian Shields, 15.4 % in geological formations of intermediate age in central and eastern Amazonia, and 44.9 % in young formations of western Amazonia and northern Andes. Most plots have between 400 and 700 stems per hectare (Table 4.1), with a mean value of 581 $\pm$ 19 stems ha^{-1} and a mean basal area of 28.1 $\pm$ 2.2 m^2 ha^{-1} ($\pm$ 95% CI).

Savannas and SDTF always have lower stem density and basal area than the overall mean value (Table 4.1).

The highest species diversity values were recorded in terra firme forest of intermediate and young geological formations (Table 4.2). Flooded forest communities in western and central Amazonia were more diverse than flooded and terra firme forests on the Guiana and Brazilian Shields, while the lowest diversity was found in white-sand forest of the Guiana Shield and Andean montane forest (Table 4.2). SDTF and savannas show intermediate values of species diversity, resembling values of flooded forests of central Amazonia and white-sand forest of the west. On average, 85.8 % of species per plot belong to Eudicots, 11.1 % to Magnoliids, and 3.2 % to Monocots. These percentages were similar when comparing number of individuals but with slight differences in Monocots and Eudicots (Table 4.2). Early diverging clades such as Magnoliids and Monocots tend to show higher percentage of species and individuals in young geological formations than in intermediate and old formations; while Eudicots show the opposite pattern. These large-scale patterns are conserved even for the different forest types of each region (Table 4.2). While SDTF shows the lowest value of Magnoliids and the greatest of Eudicots, the abundance of these clades in savannas is more similar to the values typical in the tropical rain forest biome.

4.4.2. Phylogenetic diversity metrics

Absolute values of phylogenetic diversity *sensu strictu* (PD) and mean pairwise phylogenetic distance among unique taxa (MPDt) vary among plots, with ranges of 860 - 5,282 Ma and 200 - 279 Ma, respectively. PD was greatest in terra firme forests of the youngest and intermediate aged geological formations, followed by the flooded forests on geologically young formations (Table 4.2). MPDt was highest in all forest types on young geological formations. While PD and MPDt values of savannas are similar to those of the tropical rain forest, seasonally dry tropical forests have overall the lowest values of both phylogenetic diversity metrics (Table 4.2).

Species diversity as measured by Fisher's alpha correlates strongly with PD ($r = 0.96$, $p < 0.001$; Figure 4.1a), following an exponential relationship, but a much weaker correlation was observed between Fisher's alpha and MPDt ($r = 0.41$, $p < 0.001$; Table 4.3). The

percentage of species in early diverging clades (= 1- Eudicots) correlates best with MPDt (r = 0.88, $p < 0.001$; Figure 4.1b), which is driven principally by Magnoliids (r = 0.89, $p < 0.001$) and to a lesser extent by Monocots (r = 0.28, $p < 0.001$). Conversely, the correlation of the percentage of species in major clades with PD was mostly weaker ($r_{1\text{-Eudicots}} = 0.48$, $r_{Magnoliids} = 0.48$, $r_{Monocots} = 0.28$, all $p < 0.001$; Table 4.3).

4.4.3. Spatial patterns

When the effect of species diversity on PD was controlled for, the spatial patterns of residuals of PD on species diversity ("PD residuals") resemble some general patterns of MPDt (Figure 4.2). "PD residuals" were fairly similar across most of the tropical rain forest biome (Figure 4.2c), although communities of western Amazonia and the northern Andes have higher PD values than expected from their species diversity and communities of central and eastern Amazonia have significantly lower PD values than expected (Figure 4.3). For MPDt, a strong gradient was observed from west to east with significantly higher values for tropical rain forest over young geological formations near to the Andes, followed by geological formations of central and eastern Amazonia, and the Guiana and Brazilian Shields (Figure 4.3). These spatial patterns are conserved among forest types of the tropical rain forest biome (Appendix S4.3 & Appendix S4.4).

While phylogenetic diversity metrics of savannas were rather similar to other communities in old geological formations, SDTF has a consistent low phylogenetic diversity for both metrics, and with significantly lower PD values than expected from their species diversity (Figure 4.3).

Finally, no correlation was found between the phylogenetic diversity metrics and the percentage of unidentified individuals per plot in the dataset, neither for PD (r = 0.01, $p = 0.90$) nor for MPDt (r = 0.03, $p = 0.61$; Appendix S4.5). Moreover, phylogenetic diversity patterns were similar when using a larger number of individuals per plot in Amazonia (n = 500; Appendix S4.6).

4. 5. DISCUSSION

4.5.1. Regional pattern of phylogenetic diversity

Overall the results show that the phylogenetic diversity of flowering trees is unevenly distributed across the Amazon rain forest. In particular, north-western and central Amazonia, both well-known for their exceptionally high tree species diversity (Ter Steege *et al.*, 2003), show remarkably different patterns of phylogenetic diversity. While communities of north-western Amazonia located on young geological formations have higher PD values than expected from their species diversity, communities of central Amazonia located on geological formations of intermediate age have significantly lower PD values than expected. Thus, communities of central Amazonia are dominated by phylogenetic relatives; that is, they contain species that are more closely related to one another than do the communities of north-western Amazonia.

The close correlation of PD with species diversity found across Amazonia is consistent with previous studies of grassland savannas in North America (Cadotte *et al.*, 2012) and of the Cape flora in South Africa (Forest *et al.*, 2007), and indicates that the two regions with high species diversity of Amazonia mentioned above also contain high absolute values of PD. However, when the effect of species diversity on PD was taken into account, it was found that the residual values were not remarkably different between the relatively species-poor communities of the Guiana and Brazilian Shields and the highly diverse communities of western Amazonia (Figure 4.3). Thus, the prediction that geologically older formations will contain a greater diversity of lineages than geologically younger formations is rejected.

Processes generating species diversity may differ among regions. While species-rich genera in western Amazonia are numerous and are found in distantly related clades (e.g. *Inga*, Fabaceae; *Miconia*, Melastomataceae; *Pouteria*, Sapotaceae; *Licania*, Chrysobalanaceae; *Protium*, Burseraceae*; Sloanea*, Elaeocarpaceae; *Swartzia*, Fabaceae; *Ficus*, Moraceae; *Guatteria*, Annonaceae; *Nectandra* and *Ocotea*, Lauraceae), in the Guiana and Brazilian Shields the species-rich genera are represented in fewer families mainly confined to the Eudicots (e.g. *Pouteria*, Sapotaceae; *Licania*, Chrysobalanaceae; and *Ocotea*, Lauraceae). The diversification of some species-rich genera such as *Inga* (Richardson et al., 2001) and *Guatteria* (Erkens *et al.*, 2007) have been related to the rapid uplift of the Andean

Cordillera (Gentry, 1982), which is reflected in their young crown ages (ca. 10 Ma and 11 Ma, respectively). However, the greater age of the clades that include *Licania* (ca. 46 Ma) and *Pouteria* (ca. 60 Ma) obtained in recent dated phylogenies of Chrysobalanaceae (Bardon *et al.*, 2013) and Sapotaceae (Richardson, pers. comm.) suggests that older historical events are responsible for the diversification within genera in the geological older formations of the Guiana and Brazilian Shields. Although ages of genera and species are not well-estimated in the phylogeny used in this study, phylogenetic studies of specific clades cited above may be indicating that processes operating over different timescales may have been responsible for diversification of taxa in different regions of Amazonia.

This study also shows a marked difference in the percentage of angiosperm clades across the Amazon rain forest, with a higher percentage of tree species of early diverging clades (Magnoliids and Monocots) in western Amazonia and the northern Andes compared to other regions and with the opposite pattern for Eudicots. The percentage of early diverging clades, especially of Magnoliids, best explained spatial differences in values of mean pairwise phylogenetic distance among unique taxa (MPDt). With more members from early diverging clades, communities of western Amazonia and the northern Andes also have more distantly related species and therefore higher MPDt than other regions. Therefore, the spatial variation of MPDt is largely a result of the unbalanced distribution of major clades across Amazonia. However, the use of MPDt can give us more insights into the potential sources of bias when using phylogenetic diversity metrics at large scales as in this study. Therefore, previous studies based on this metric should consider a re-evaluation of the results in order to assess if their general patterns and conclusions remain when controlling by the effect of early diverging clades.

4.5.2. The effect of forest type

Our analysis also demonstrates that phylogenetic diversity metrics vary among Amazon rain forest types. As expected, forests with high species diversity tend to have higher absolute values of PD than forests with low species diversity. However, similar values of residuals of PD on species diversity were found on different forest types, especially within each region. Although ecological constraints such as poor soils or persistent water-logging do reduce the number of genera and species in these communities, and this is reflected in

the *absolute* values of PD, the residuals of PD on species diversity did not differ for these forest types when compared to terra firme or montane forests within each region. In addition, species-rich (terra firme and flooded forest) and species-poor (montane and white-sand forest) forests of western Amazonia and the northern Andes alike have a high proportion of taxa found in early diverging clades, suggesting that the process(es) driving the regional pattern in young geological formations, especially for MPDt, have acted independently of forest type.

Both "PD residuals" and MPDt show similar values for tropical savannas and nearby communities of tropical rain forest in the Brazilian Shield, supporting the notion that adaptation to fire may have arisen locally (Simon *et al.*, 2009; Simon & Pennington, 2012). Conversely, these two metrics show the lowest values for SDTF communities compared to savannas and any other tropical rain forest type, emphasizing the high frequency of closely related species from few clades and the low frequency of distantly related species in these communities. Similar results have been obtained within a 16-ha forest plot in dry forest of Costa Rica in Central America, suggesting that species may generally tend to be more clustered in the phylogeny than expected by chance in this biome (Swenson & Enquist, 2009). In our SDTF communities, almost all arboreal species belong to the Eudicots (mean $\pm$ 95 % CI: 95.2 $\pm$ 2.5 %). Only a few genera of early diverging clades have been able to tolerate, evolve, and adapt to the seasonal drought that these systems experience (e.g. *Duguetia*, *Rollinia* - Annonaceae; and *Attalea*, *Syagrus* - Arecaceae). These results suggest that drought tolerance in tropical arboreal angiosperms has only evolved in particular clades, almost all confined to the Eudicots. However this interpretation remains tentative as only eleven SDTF plots were available.

Previous studies have indicated a strong habitat specialization in white-sand communities as indicated by the high number of individuals that belong to white-sand specialist species (Fine *et al.*, 2010), and by the distinct herbivore defences that these species have evolved to live in such poor-fertile soils (Fine *et al.*, 2004). Therefore, it was expected that white-sand forest would have a high frequency of closely related species, with the low species diversity being due to only a few lineages being adapted to low-fertility soil conditions. However, the results showed no significant difference for "PD residuals" or MPDt for white-sand forest compared to terra firme or flooded forest, either in western Amazonia

or in the Guiana Shield (Appendix S4.3). The 84.8 ± 0.7 % of the species in communities of terra firme, 86.4 ± 3.0 % in flooded forests, and 89.4 ± 4.4 % in white sand forest belong to Eudicots and the remaining to early diverging clades. Accounting for regional differences among these forest types, these values were lower than the values of SDTF. Our results suggest that adaptations to different environmental conditions within the rain forest biome (e.g. terra firme, white-sand forest and flooded forest) have appeared in a wide variety of lineages, and therefore that these edaphic challenges represent easier barriers for angiosperms to transcend than that of the seasonal drought of the SDTF.

4.5.3. Processes explaining spatial variation of phylogenetic diversity

Spatial variation in Amazon tree communities is a result of historical and ecological processes that affect communities in the Neotropics. For example, previous studies have suggested that spatial patterns in tree floristic composition (Ter Steege *et al.*, 2006; Honorio Coronado *et al.*, 2009), wood density (Baker *et al.*, 2004), total above biomass, productivity and mortality (Quesada *et al.*, 2012) are all influenced by differences in soil fertility and soil depth across Amazonia. Although soil properties are likely to play an important role in the assemblage of local Amazonian communities, other historical processes may be responsible for the uneven spatial variation of phylogenetic diversity demonstrated in this analysis.

Within the tropical rain forest biome, early diverging clades were best represented near to the Andean Cordillera. The adjacent montane forest also has a high presence of Magnoliids, suggesting a potentially recent floristic connection between lowland forests and the Andes. It has been suggested that reductions in temperature and atmospheric CO_2 during Quaternary glacial periods promoted the migration of montane taxa to the lowland forest (Van Der Hammen, 1974; Bush & Colinvaux, 1990; Colinvaux *et al.*, 1996; Cárdenas *et al.*, 2011). Such taxa may have included Magnoliid species and these recent migration events may have maintained the occurrence of these early diverging clades in lowland forests in western Amazonia. However, these lineages have a much deeper history in South America. Dated phylogenies of Arecaceae (Monocots), Annonaceae, and Lauraceae (both Magnoliids) indicate that these are of largely Laurasian origin and migrated into South America at various times throughout the Cenozoic (Chanderbali *et al.*, 2001; Richardson *et*

al., 2004) or even during the Late Cretaceous (Baker *et al.*, 2013). Our analysis was deliberately focused only on arboreal angiosperm species, excluding gymnosperms and ferns, as they are represented by few individuals in most of the montane plots and have a disproportionate impact on phylogenetic diversity measures (Faith *et al.*, 2004). As previous studies indicating elevated phylogenetic diversity in montane habitats in South America (Chave *et al.*, 2007) and in eastern Africa (Tallents *et al.*, 2005) have included ferns and/or gymnosperms, it is likely that montane forest in our dataset would show even higher values of MPDt, and probably a departure of residuals of PD on species diversity, if the arborescent ferns and gymnosperms that typically occur in montane habitats (Gentry *et al.*, 1995a; Dalling *et al.*, 2011) were included in the analysis.

Because phylogenetic diversity is so highly correlated with species richness, it has been suggested that this measure of biodiversity adds little information to what it is already know about the diversity of particular regions (Polasky *et al.*, 2001; Rodrigues & Gaston, 2002). However, this Amazon rain forest study provides independent confirmation of conclusions from a Southern African study that some areas hold more, or less, phylogenetic diversity than expected from their taxon richness alone (Forest *et al.*, 2007). In the Cape flora study, phylogenetically clustered assemblages of species were present in the species-rich communities of the west, whereas over-dispersed assemblages were a feature of the species-poor communities of the east. Within Amazonia, the residuals of PD on species diversity differs between the two Amazon regions with globally exceptionally diverse tropical rain forest communities - phylogenetically clustered assemblages are found in terra firme forests of central Amazonia, over-dispersed communities in the terra firme forest of north-western Amazonia. It appears therefore that for some of the world's most diverse regions that differences in PD are not simply predictable from patterns of species diversity. Regional conservation planning may thus need to explicitly account for phylogenetic diversity and its deviation from species diversity expectations.

4. 6. CONCLUSION

The present study has revealed an uneven spatial distribution of phylogenetic diversity across the Amazon rain forest for two metrics: residuals of PD on species diversity ("PD

residuals") and mean pairwise phylogenetic distance among unique taxa (MPDt). Contrasting patterns of "PD residuals" and MPDt were found between communities of the two most floristically diverse regions of Amazonia. Other predictions of significant differences among regions were mainly rejected indicating that high phylogenetic diversity is not only obtained by a slow accumulation of lineages in stable communities of old geological formations but also by potential historical migration events of early diverging lineages into younger formations near to the Andes. These results suggest that historical events may have played an important role in the assemblage of the most phylogenetically diverse communities of western Amazonia. Finally, studying large-scale patterns of phylogenetic diversity as in this study could contribute to preserving not only the full spectrum of lineage diversity but also the evolutionary processes that have determined the current extant biodiversity of Amazonian communities.

Table 4.1. General information of the floristic tree inventories (n = 283 RAINFOR plots). Tropical rain forest biome is classified based on maximum age of geological formations. Young ages are given to formations of western Amazonia and the northern Andes (< 20 Ma), intermediate ages to formations of central and eastern Amazonia (20-100 Ma) located between the Pre-Cambrian formations of the Guiana and Brazilian Shields (> 500 Ma; from Quesada *et al.*, 2011).

Biome (Max. geol. age)	Forest type	Nº of plots	Total area (ha)	Mean density (stems/ha)	Mean basal area (m^2/ha)	Total Nº of species	Total Nº of individuals
Tropical rain forest	Flooded	14	19	575	26.5	914	9,557
(< 20 Ma)	Montane	16	16	813	34.9	318	9,319
	Terra firme	86	95	575	26.6	2,191	46,753
	White sand	4	4	832	23.2	197	2,682
Tropical rain forest	Flooded	2	2	479	29.2	157	694
(20 - 100 Ma)	Terra firme	39	54	566	31.9	1,280	26,661
Tropical rain forest	Flooded	17	16	764	35.3	425	10,821
(> 500 Ma)	Terra firme	85	94	521	26.6	1,620	41,869
	White sand	4	4	676	29.9	92	2,354
Savanna	Savanna	5	4	523	14.0	95	1,848
SDTF	Dry forest	11	12	438	26.5	258	4,782
TOTAL		283	319	581	28.1	3,868	157,340

Table 4.2. Percentage of species and individuals in major clades including mean values of species diversity (Fisher's alpha) and phylogenetic diversity metrics. Phylogenetic diversity *sensu strictu* (PD), residuals from the linear regression of PD on species diversity (PDr), and mean pairwise phylogenetic distance among unique taxa (MPDt) are given in millions of years (Ma). Tropical rain forest biome is classified based on maximum age of geological formations (young: < 20 Ma; intermediate: 20-100 Ma, old: > 500 Ma; Quesada *et al.*, 2011). Values per plot were estimated using rarefaction procedure.

Biome (age)	Forest type	Mean nº of species & individuals (%)						Mean diversity values			
		Magnoliids		Monocots		Eudicots		Fisher's alpha	PD (Ma)	PDr (Ma)	MPDt (Ma)
		spp	ind	spp	ind	spp	ind				
Tr. rain forest	Flooded	16.3	15.5	5.5	18.4	78.2	66.1	38	4,083	107	260
(< 20 Ma)	Montane	12.3	10.4	0.9	0.8	86.8	88.8	9	2,174	18	255
	Terra firme	14.0	13.7	4.8	14.0	81.2	72.2	42	4,089	99	255
	White sand	10.2	4.8	4.6	4.6	85.1	90.6	16	2,829	132	254
Tr. rain forest	Flooded	9.5	4.9	0.4	0.1	90.1	94.9	34	3,476	-411	242
(20 - 100 Ma)	Terra firme	11.9	9.5	0.9	1.6	87.2	89.0	54	4,202	-198	248
Tr. rain forest	Flooded	4.9	3.7	2.4	3.9	92.7	92.4	12	2,369	78	238
(> 500 Ma)	Terra firme	9.7	9.9	2.9	5.6	87.4	84.5	25	3,307	-3	247
	White sand	6.3	1.2	0.0	0.0	93.7	98.8	6	1,587	-161	233
Savanna	Savanna	5.1	4.0	2.0	2.1	93.0	93.9	17	3,118	68	239
SDTF	Dry forest	1.9	0.6	2.9	3.5	95.2	95.9	11	2,212	-277	223
TOTAL		11.1	10.2	3.2	7.6	85.8	82.2	32	3,510	0	249

Table 4.3. Pearson correlation coefficients among nine test variables. *p*-values are given above the diagonal.

Test variables	PD	Log(SD)	PDres	MPDt	Magn	Mono	1 - Eudi	Lat	Lon
PD		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Log(Species diversity)	0.96		0.60	<0.001	<0.001	<0.001	<0.001	<0.001	0.06
PD residuals	0.28	0.03		<0.001	0.23	<0.001	<0.01	0.11	<0.01
MPDt	0.41	0.35	0.31		<0.001	<0.001	<0.001	0.19	<0.001
% Magnoliid species	0.48	0.48	0.07	0.89		0.03	<0.001	<0.01	<0.001
% Monocot species	0.28	0.20	0.27	0.28	0.13		<0.001	<0.01	<0.001
% 1 – Eudicot species	0.48	0.45	0.18	0.88	0.89	0.53		0.45	<0.001
Latitude	0.21	0.24	-0.09	0.08	0.16	-0.19	0.05		0.56
Longitude	-0.18	-0.11	-0.19	-0.49	-0.42	-0.37	-0.52	0.03	

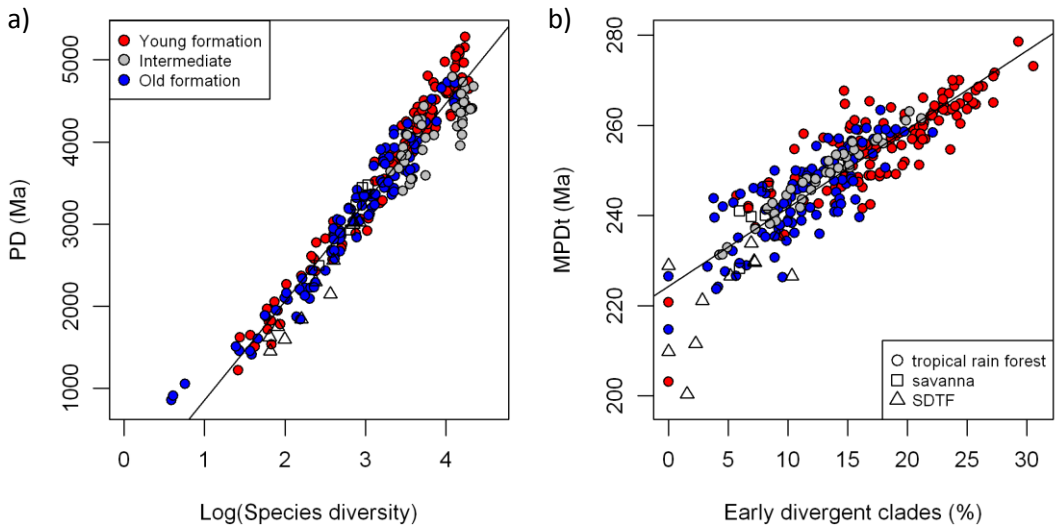


Figure 4.1. Relationship between phylogenetic diversity metrics and explanatory variables. (a) Correlation between phylogenetic *sensu strictu* (PD) and species diversity (r = 0.96, $p < 0.001$). (b) Correlation between mean pairwise phylogenetic distance among unique taxa (MPDt) and the percentage of species of early diverging clades (r = 0.88, $p < 0.001$) represented by Magnoliids and Monocots (= 1 - Eudicots). Symbols indicate different biomes and colours refer to the maximum age of geological formations within the tropical rain forest biome (young: < 20 Ma; intermediate: 20-100 Ma, old: > 500 Ma; Quesada *et al.*, 2011).

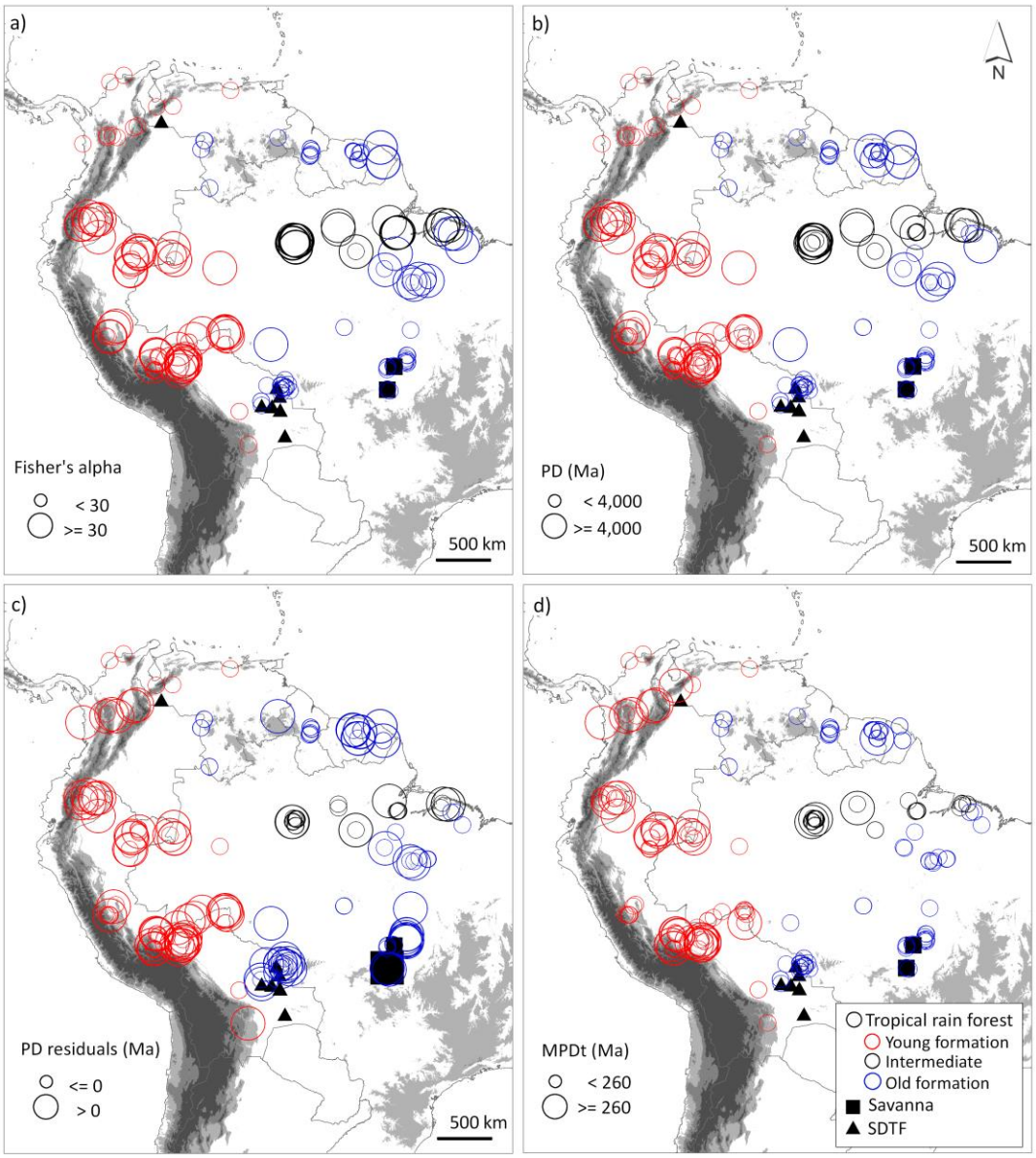


Figure 4.2. Spatial variation in the distribution of species diversity and phylogenetic diversity for 283 tree inventories in South America. (a) Species diversity measured as Fisher's alpha. (b) Phylogenetic diversity *sensu strictu* (PD). (c) Phylogenetic Residuals from the linear regression of PD on species diversity. (d) Mean pairwise phylogenetic distance among unique taxa. Colours refer to the maximum age of geological formations within the tropical rain forest biome. Young ages are given to formations of western Amazonia and the northern Andes (< 20 Ma), intermediate ages to formations of central and eastern Amazonia (20-100 Ma) located between the Pre-Cambrian formations of the Guiana and Brazilian Shields (> 500 Ma; from Quesada *et al.*, 2011).

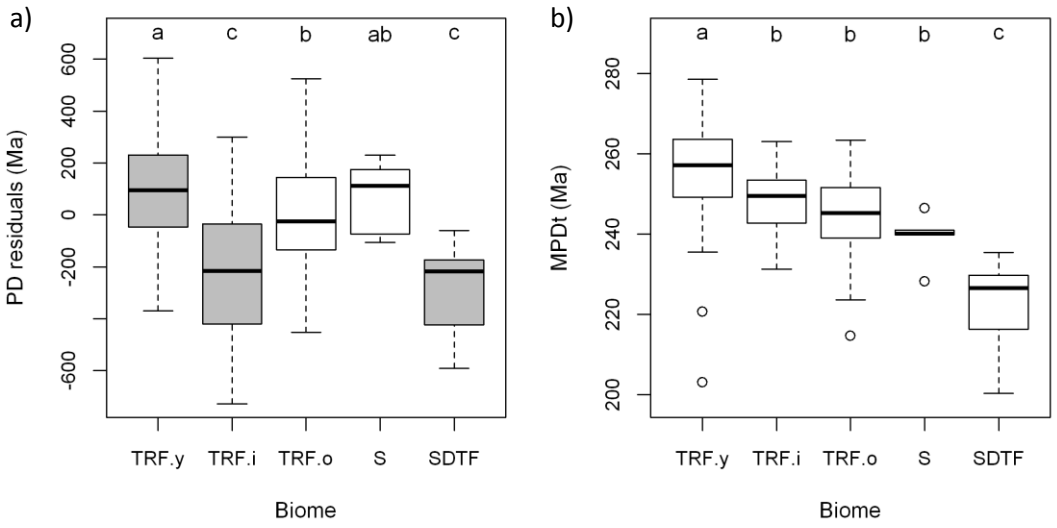


Figure 4.3. Biome comparison of phylogenetic diversity metrics. (a) Residuals of phylogenetic diversity *sensu strictu* on species diversity ("PD residuals"), and (b) mean pairwise phylogenetic distance among unique taxa (MPDt) are given in millions of years (Ma). Tropical rain forest biome is classified based on maximum age of geological formations (young: < 20 Ma; intermediate: 20-100 Ma, old: > 500 Ma; Quesada *et al.*, 2011). Grey boxplots contain communities with higher or lower PD values than expected by their species diversity. Letters indicate significant difference among mean values (Tukey's HSD, $p < 0.05$).

Chapter 5. Conclusions

5. 1. RESEARCH SYNTHESIS

This thesis investigates the importance of ecological factors and historical events in determining the spatial patterns in the distribution of species, population genetic structure, and phylogenetic diversity in the Amazon rain forest. The main objectives were to: 1) determine within-species genetic diversity and the effect of ecological and physical barriers to gene flow in a widespread neotropical species adapted to moist forest environments (chapter 2); 2) identify areas of genetic differentiation concordant among five widespread species in western Amazonia (chapter 3); and 3) investigate the spatial distribution of phylogenetic diversity of tree communities across Amazonia (chapter 4).

In chapter 2, I aimed to shed light on which species traits are associated with overcoming potential physical and ecological barriers to gene flow between Mesoamerica and Amazonia in rain forest trees. *Ficus insipida* subsp. *insipida* (Moraceae) was used as an exemplar taxon for widespread neotropical rain forest trees because it is distributed across the Andes and into Mesoamerica, is animal dispersed (like the majority of rain forest trees), and is confined to humid forest environments. It is a more appropriate representative of the rain forest tree flora than species that are more tolerant to drought and with wind-dispersed seeds that have been the focus of previous phylogeographic studies covering the area from Mesomerica to Amazonia. In chapter 3, I tested whether there are concordant patterns of genetic uniformity or geographic differentiation among five widespread species in western Amazonia, in order to establish if the patterns reflect the impact of Quaternary climate changes or more ancient geological events. In comparing the patterns of genetic structure of the study species with other published studies, the effect of species traits such as floral sexuality and dispersal mode was assessed. In chapter 4, I explored the distribution of phylogenetic diversity across the rain forests of the Amazon basin, using an integrated approach that included exploring the effects of forest type and the ages of geological formations. This permitted testing whether forests growing on the Guiana and Brazilian Shields have accumulated a higher phylogenetic diversity, that is a greater diversity of lineages and more distantly related species, as predicted by the age of these geological formations, and whether forests on the geologically newer soils of

western Amazonia might have lesser phylogenetic diversity because they are dominated by some clades that represent more recent radiations.

I used phylogeographic methods to assess the genetic patterns of widespread species in the first two chapters. Notably, these studies use a geographically extensive and thorough sampling scheme for the Neotropics (chapter 2) and for western Amazonia (chapter 3), compared to the more scattered sampling of previous large-scale phylogeographic studies. The emphasis on collecting material in the field was also a priority of my research project, partly because it also enabled observation of ecological variables. For example, I determined edaphic preferences (soil fertility) of each study species, and I was able to interpret genetic patterns in this context. Further, the fieldwork permitted observations of the forest types where the species were collected across western Amazonia, which also aided interpretation of genetic patterns (chapter 3). The final objective of investigating phylogenetic diversity across Amazonia is addressed using a large dataset of floristic inventories contributed by numerous colleagues. Here, the spatial distribution of phylogenetic diversity is explored across Amazonia in the context of the distinct geological histories and ecologies of different parts of the basin. Together the results of these three chapters demonstrate that both historical and ecological processes are important and have acted over different spatial scales to shape the current distribution of plants and communities in Amazonia. The key findings are described below.

5. 2. KEY RESEARCH FINDINGS

5.2.1. Seasonally dry vegetation in northern South America represents a barrier to migration for wet-adapted neotropical species (Objective 1; Chapter 2)

This chapter highlights the role of ecological traits in the historical biogeography of neotropical tree species. In comparing our results to published data for other species with similar distributions, we suggest that the seasonally dry vegetation in northern South America represents a barrier to migration for *Ficus insipida* subsp. *insipida* and other tree species that are confined to humid forest environments. The strong differentiation between Mesoamerican and Amazonian populations of *F. insipida* contrasts the weakly

differentiated phylogenetic structure found across the same area in tree species better adapted to survive seasonal drought.

5.2.2. Expansion of tropical rain forest in south-western Amazonia (Objective 1; Chapter 2)

Within Amazonia, diversification of plastid haplotypes of *F. insipida* indicates that a long evolutionary residence time must underlie the population differentiation that is found in Ecuador and northern Peru. However, the presence of genetically uniform populations, especially in south-western Amazonia, suggests recent colonization events which are consistent with independent palaoecological data that indicate rain forest expansion in this area since the last glacial maximum.

These two first results will be of broad interest to researchers both in highlighting key species traits that allow successful migration of rain forest trees, and for their insight into the history of the Amazon rain forest.

5.2.3. Patterns of population genetic structure in widespread western Amazonian species are explained by ecological factors and historical events (Objective 2; Chapter 3)

In this chapter, by identifying concordant patterns of population genetic structure among five widespread species I highlight the effect of ecological factors and historical events in determining the current distribution of tree species in western Amazonia. New soil analyses presented here show that these widespread species all occur on relatively fertile soils with high concentration of base cations and low concentration of aluminium. Geographic genetic structure in the plastid DNA is demonstrated for all species among both northern and southern populations of western Amazonia, and in some cases edaphic factors correlate with genetic variation. For example, the strong plastid genetic structure in *Jacaratia digitata* among its northern populations might be explained by limited seed dispersal among patches of relatively well-drained soils of high fertility to which the species is apparently restricted. In the case of *Clarisia biflora*, genetic structure seems to be correlated with different forest types: fertile soils in flooded areas in the north-western Amazonia and slightly less fertile soils of terra firme forest in the south.

Initial diversification of plastid haplotypes is indicated to have occurred for all species during the Late Miocene, a period that coincides with the presence of Lake Pebas, a wetland system that dominated western Amazonia. It is possible that the species may have originated on small patches of dry land during the wetland period, and that the drainage of the lake may have favoured the diversification of their lineages that are adapted to well-drained soils (e.g. *Jacaratia digitata*, *Clarisia biflora*, *Poulsenia armata*, *Otoba parvifolia*) or to seasonally flooded areas (e.g. *Ficus insipida* and some populations of *Clarisia biflora*). The presence of recent lineages restricted to some areas of the basin, especially in south-western Amazonia, also suggests the importance of more recent colonization events, which is consistent with the independent palaeoecological data that indicate rain forest expansion in this area since the last glacial maximum.

These results are an important contribution to the understanding of tree biodiversity of the Amazon rain forest, in which intraspecific genetic diversity has developed over millions of years and population genetic patterns indicate different responses of tropical rain forest trees to geological and climatic changes.

5.2.4. Tree phylogenetic diversity is unevenly distributed across the Amazon rain forest (Objective 3; Chapter 4)

Chapter 4 demonstrates that there is an uneven spatial distribution of phylogenetic diversity in Amazonian tree communities by using a large dataset of 283 floristic tree inventories. North-western forests tend to have greater phylogenetic diversity than expected from their taxon richness, indicating that their constituent species are distantly related in the phylogeny, compared to equally species-rich communities of central Amazonia which have more phylogenetically closely related lineages. In the case of the communities of the Brazilian and the Guiana Shields, no significant departure of phylogenetic diversity given expectations from species richness was found, indicating that these communities may contain equally closely and distantly related taxa. These results are perhaps unexpected when considering the context of the geological history of Amazonia. One might have anticipated that communities of western Amazonia, which contain more recent radiation events driven by the Andean orogeny (Gentry, 1982), would have shown more closely related taxa than communities of the Brazilian and the Guiana Shields that

are geologically much older and are more likely to have accumulated greater diversity of lineages (Stebbins, 1974). However, this prediction was not supported.

In contrast to the prediction of the geological age hypothesis, a marked difference was found in the proportion of different angiosperm clades, with the greatest proportion of tree species of early diverging clades (Magnoliids and Monocots) in the west. Given that clades diverging earlier were also characteristic of pre-montane habitats, these results suggest that migration events from cooler environments at different geological times may have played an important role in the assemblage of the phylogenetically diverse communities in lowland western Amazonia.

These results indicate that species diversity metrics cannot simply predict the phylogenetic diversity of communities in Amazonia and therefore that phylogenetic diversity should be explicitly accounted for in conservation priority setting if the aim is to preserve the evolutionary diversity of species and ecosystems.

5. 3. RESEARCH IMPLICATIONS

This research study has implications for other fields such species distribution modelling and for setting conservation priorities. The implications of my results for these disciplines are discussed below.

5.3.1. Species distribution modelling

Modelling of species distributions is usually based on records of species occurrence recorded on herbarium and museum specimens in combination with the environmental variables for the localities of these collections. In the tropics, accuracy of species distribution records is limited by the presence of large spatial gaps in botanical collections in regions such as Amazonia (Nelson *et al.*, 1990; Tobler *et al.*, 2007; Milliken *et al.*, 2010). Key environmental datasets are also limited by lack of data, for example precise understanding of neotropical climate patterns is limited by the lack of meteorological stations, which means climate data need to be greatly interpolated for areas between stations which are usually many hundreds of kilometres in Amazonia. Moreover, soil conditions are arguably still even more poorly mapped. Soil fertility also has an important

environmental role in determining the spatial distribution of neotropical tree species (e.g. Phillips *et al.*, 2003; Tuomisto *et al.*, 2003a; John *et al.*, 2007; Higgins *et al.*, 2011; see chapter 3), so soil conditions must be considered when determining the ecological niche of species in Amazonia.

In order to overcome the limitations of current sampling bias of plant collections across Amazonia, field sampling will be required to fill geographic gaps. Such field sampling was a priority in this study, resulting in the most thorough spatial sampling over a wide geographic scale in any plant phylogeography study in Amazonia. In order to cover some of the gaps in historical collections, collaborating with other researchers is also important. By fieldwork and collaboration, large-scale phylogeographic and species distribution modelling studies will adequately cover the large geographic and environmental space of tropical ecosystems such Amazonia, the Congo Basin and elsewhere.

A large soil dataset of good spatial coverage will be required to model soil conditions across the whole of the Amazon basin. Although this can be a great challenge, some research projects focused on inventory plots have contributed data for some sites (e.g. RAINFOR, PPBio, RBGE-UoLeeds project). Moreover, this study shows that collection of soil samples is feasible as part of a phylogeographic study, and that interpretation of genetic data in the context of edaphic variables can be illuminating.

5.3.2. Conservation priorities

My study has implications for conservation, in particular for maximizing the conservation attention given to tropical rain forest species and ecosystems. For example, areas of high genetic differentiation among populations, and therefore high genetic diversity, were detected in the comparative phylogeographic study and these might be considered for protection if conservation of the genetic diversity of Amazonian tree species is seen as priority. This genetic approach to conservation has been used in agroforestry where genetic diversity patterns are compared between cultivated stands and natural populations of tropical trees (e.g. *Inga edulis*; Hollingsworth *et al.*, 2005) in order to improve practices that promote the conservation of biodiversity and genetic resources (O'neill *et al.*, 2001; Dawson *et al.*, 2009). At a deeper phylogenetic level (e.g. chapter 4), it is being argued that conservation prioritisation of species and communities should take

into account their phylogenetic history. For example, the EDGE (evolutionarily distinct and globally endangered; Isaac *et al.*, 2007) initiative suggests conservation resources should be directed to threatened animal species that are phylogenetically isolated and therefore with few close relatives on the tree of life.

Species do not grow and reproduce in isolation, so efforts to including genetic and phylogenetic diversity information in conservation priorities are perhaps more practically applied at the community level. For example, conservation planning based upon species diversity metrics values communities similarly regardless of the total phylogenetic diversity of the species that they contain, and therefore do not prioritise evolutionary diversity in conservation (e.g. Forest *et al.*, 2007). In chapter 4, I show that Fisher's alpha, for example, does not predict the amount of closely or distantly related taxa in the phylogeny of communities across the world's largest and most species-rich tropical forest ecosystem. The two regions of Amazonia with most tree species have different phylogenetic diversity values than expected by their species richness.

In summary, uncovering the spatial variation in the distribution of genetic diversity of species and phylogenetic diversity of communities is of particular relevance to conservation if the goal is to capture the full, deep evolutionary heritage present in contemporary tropical ecosystems.

5. 4. FUTURE RESEARCH DIRECTIONS

5.4.1. Ecological barriers between Mesoamerica and Amazonia

This study found that seasonally dry vegetation in northern South America may represent a barrier to migration for wet-adapted neotropical species based upon the study of *Ficus insipida* (see chapter 2). One way of testing this scenario is by using seedling transplant experiments in areas of different seasonality in order to assess the survival and tolerance of tropical rain forest species to drought (e.g. study of *Piper* species in Costa Rica; Fleming, 1985). An alternative approach would be to use an *in situ* drought experiment (e.g. Da Costa *et al.*, 2010), but these both techniques require long-term monitoring and a significant number of replicates to test differences among treatment and control

experiments. Another way of testing the generality of this scenario would be to sample Mesoamerican populations of *Clarisia biflora*, *Poulsenia armata* and *Otoba parvifolia*, which, like *Ficus insipida*, are confined to ever-wet environments and have animal-dispersed seeds. If they show the same genetic structure between Mesoamerica and Amazonia as *F. insipida*, it would corroborate my inference that the seasonally dry vegetation of northern South America is a major barrier to the migration of rain forest trees; a barrier perhaps as significant as the Andes, which has received far greater attention in phylogenetic and phylogeographic studies (e.g. Dick *et al.*, 2003; Pirie *et al.*, 2006; Antonelli *et al.*, 2009; Cavers *et al.*, 2013).

5.4.2. The effect of edaphic factors on genetic patterns of widespread Amazonian species

In chapter 3, I showed that in some cases edaphic factors correlate with genetic variation. For example, the strong plastid genetic structure of *trn*H-*psb*A sequences in *Jacaratia digitata* may reflect the scattered distribution of its preferred high fertility, well-drained soils, and in *Clarisia biflora* north-south genetic changes co-incide with difference in edaphic preferences. An interesting question is if the evolution of the geomorphic environment is the main driver of phylogeographic patterns. This idea could be tested by comparing the genetic patterns among species with different soil preferences in Amazonia. One might predict that a strong genetic differentiation would occur in species restricted to particular environmental conditions compared to more weak differentiation in more generalist species tolerant to different environments. In a study of Burseraceae tree species in north-western Amazonia, Fine et al. (2013) found strong geographic structure in a species specialised on white-sand soils while the generalist species adapted to different soil conditions showed less structure.

5.4.3. Cryptic taxonomic diversity and hybridisation in Amazonia

In the tropics, traditional morphological taxonomic approaches may have been unable to detect biologically significant but morphologically cryptic diversity. Phylogenetic and phylogeographic studies of widespread species are relevant to the debate of the true number of tree species in neotropical rain forests (Hubbell *et al.*, 2008) because they can evaluate species definitions based upon traditional morphological taxonomy in the light of new DNA sequence data. For example, recent studies of the neotropical genus *Cedrela*

detected deep intraspecific divergence in *C. odorata* with three to four genetically distinct entities that were morphologically not distinguishable (Muellner *et al.*, 2009; Cavers *et al.*, 2013). Other examples of cryptic species in neotropical tree species include *Carapa guianensis* (Scotti-Saintagne *et al.*, 2013a), *Jacaranda copaia* (Scotti-Saintagne *et al.*, 2013b), and potentially *Jacaratia digitata* (see chapter 3). The degree of plastid sequence divergence found in *J. digitata* is remarkable and indicates genetically distinct lineages that were not recognised by morphological taxonomy. Future research is needed to assess the nature of *J. digitata* using plastid and nuclear markers available for the family Caricaceae (Antunes Carvalho & Renner, 2012).

Another area where genetic data can be of taxonomic use is in uncovering hybridisation. For example, in the study of *Carapa*, hybridization was reported between *C. guianensis* and *C. surinamensis* in which intermediate morphological and genetic types occur in overlapping populations (Scotti-Saintagne *et al.*, 2013a). In this present study, I reported that *Otoba glycycarpa* was not genetically distinct from *Otoba parvifolia* for the *trn*H-*psb*A marker (see chapter 3). Moreover, I also observed that within each population of these species, morphological characters were not consistently distinct, with some individuals showing intermediate morphological characters. Further research will be required to assess if these two described species hybridize. Hybridization can be tested by using a combination of plastid and nuclear markers to help determine parentage (e.g. *Leucaena*; Hughes *et al.*, 2002). If hybridisation is occurring, this could represent a useful example for studying the mechanisms of hybrid speciation, that is, the production of fertile hybrids that may be reproductively isolated from their parental species (Abbott & Rieseberg, 2001). The importance of hybridisation in the Amazonian flora is little known.

5.4.4. The recent southern expansion of Amazonia

At the current southern margin of the Amazon rain forest, palaeoecological studies of two lakes in Bolivia have suggested significant expansion of rain forest into dry forest and savanna in the past 600-3000 years (Mayle *et al.*, 2000; Burbridge *et al.*, 2004). Using a comparative phylogeographic study of tree species, my project has shown recent colonization of the south from the north for three of the five study species as evidenced by plastid lineages endemic to the south nested within lineages endemic to the north (see chapter 3). Although this is the first molecular study to explore the hypothesis of rain

forest expansion in south-western Amazonia, the use of more variable markers (e.g. microsatellites) will be required to capture in more detail the demographic imprint of this post-glacial event. Results from such studies will be useful for climate-vegetation models that aim to predict future changes over the southern portion of western Amazonia that was most strongly susceptible to climate change during the Last Glacial Maximum (Mayle *et al.*, 2004).

5.4.5. The distribution of phylogenetic diversity in neotropical forests

The research presented in chapter 4 about the spatial distribution of community phylogenetic diversity in Amazonia rejected the prediction that geologically older formations will contain a greater diversity of lineages than geologically younger formations. In particular, communities of western Amazonia and of the Guiana and Brazilian Shields have similar predicted phylogenetic diversity to that expected from their species diversity. An interesting research question could address if time of diversification of species-rich genera is different between these regions. For example, the diversification of some genera characteristic of western Amazonia such as *Inga* (Richardson et al., 2001) and *Guatteria* (Erkens *et al.*, 2007) has been related to the rapid uplift of the Andean Cordillera (Gentry, 1982), which is reflected in their young crown ages (ca. 10 Ma and 11 Ma, respectively). However, the greater age of clades characteristic of the Guiana and Brazilian Shields that include *Licania* (ca. 46 Ma) and *Pouteria* (ca. 60 Ma) obtained in recent dated phylogenies of Chrysobalanaceae (Bardon *et al.*, 2013) and Sapotaceae (Richardson, pers. comm.) suggests that older historical events may be responsible for the diversification within genera in the geologically older formations. Using this approach, new phylogenetic studies of other genera can help to understand the timescale of processes that may have been responsible for the diversification of taxa in different regions of Amazonia.

5. 5. SUMMARY

Ecological and molecular information on tree species and communities were used to explore how ecological factors and historical events have determined species distributions, population genetic structure, and phylogenetic diversity in the Amazon rain forest. Chapter

2 indicates that seasonally dry vegetation in northern South America represents a barrier to migration for *Ficus insipida* (Moraceae) and other wet-adapted Amazonian tree species with different plastid haplotypes restricted to Mesoamerica and Amazonia. Conversely, the ability to survive seasonal drought in other pioneer species may favour a weakly differentiated phylogeographic structure, with widespread haplotypes crossing this barrier. Chapter 3 explores patterns of population genetic structure in five widespread western Amazonian tree species. In some cases, such as *Jacaratia digitata* and *Clarisia biflora*, genetic patterns correlated with edaphic preferences. However, the fact that genetic breaks are not entirely congruent among all species suggests that tropical rain forest species respond differently to long-term geological and climatic changes. Despite this, some tentative generalisations emerge, notably long-term population stability with high genetic diversity and a strong genetic structure for plastid sequences across western Amazonia, and recent population expansions in the south-western Amazon. Chapter 4 explored patterns of phylogenetic diversity across Amazonia by using floristic inventories from the RAINFOR plot network. The results revealed that the communities of central Amazonia are dominated by phylogenetic close relatives compared to the communities of the north-west which tend to contain more distantly related species. An east-west gradient of early divergent angiosperm clades was found across Amazonia, with the greatest percentage of tree species of Magnoliids and Monocots in the west. Because clades diverging earlier were also characteristic of pre-montane habitats, these results suggest that migration events from cooler environments at different geological times played an important role in the assemblage of the most phylogenetically diverse communities in lowland western Amazonia.

The findings corroborate the notion that both ecological factors and historical events are important in determining species distribution and phylogenetic diversity of tropical tree communities. Regional differences in genetic structure among populations and phylogenetic diversity among communities should be taken into account in forest conservation planning and management, especially if the goal is to preserve more fully the evolutionary processes determining species diversity such as speciation, migration and extinction.

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Appendix A. Supporting information of chapter 2

Appendix S2.1. List of sampled sites for fresh and herbarium material of *Ficus insipida* subsp. *insipida* in Mesoamerica and Amazonia. Localities of samples taken from herbarium specimens are highlighted with an asterisk. The last four sites indicate localities of additional ITS sequences obtained from collaborators (see acknowledgements). Collector codes: Eurídice Honorio (EH), Kyle Dexter (KD), and Abel Monteagudo (AM).

Nº	Code	Region	Country	Population name	Lat	Long	Voucher (Herbarium code)	GenBank accession number (marker)
1	MEX	Mesoamerica	Mexico	Rio Grijalva*	16.56	-92.78	Accession # 1666497 (F)	
2	BEL	Mesoamerica	Belize	Roaring Creek*	12.23	-88.80	Accession # 1834951 (F)	
3	Ell	Mesoamerica	El Salvador	El Imposible	13.83	-89.95		GQ438036-438039, GQ438137-438140 (*trn*H-*psb*A)
4	Dei	Mesoamerica	El Salvador	Walter T. Deiniger	13.48	-89.27		GQ438028-438031, GQ438129-438132 (*trn*H-*psb*A)
5	Nan	Mesoamerica	El Salvador	Nancuchiname	13.34	-88.71		GQ438073-438076, GQ438173-438176 (*trn*H-*psb*A)
6	VoC	Mesoamerica	Nicaragua	Volcan Cosiguina	13.00	-87.63		GQ438105-438111, GQ438205-438211 (*trn*H-*psb*A)
7	Mir	Mesoamerica	Nicaragua	Miraflor	13.21	-86.34		GQ438069-438072, GQ438169-438172 (*trn*H-*psb*A)
8	ElO	Mesoamerica	Nicaragua	Zacatan	11.44	-85.94	Monica Poelchau s.n.	
9	HLl	Mesoamerica	Costa Rica	Hac. Los Inocentes	11.03	-85.50		GQ438051-438054, GQ438152-438155 (*trn*H-*psb*A)
10	RiT	Mesoamerica	Costa Rica	Rio Tempisquito	10.79	-85.55		GQ438093-438096, GQ438192-438196 (*trn*H-*psb*A)
11	CaN	Mesoamerica	Costa Rica	Cano Negro	10.89	-85.00		GQ438015-438018, GQ438116-438118 (*trn*H-*psb*A)
12	RiB	Mesoamerica	Costa Rica	Rio Bebedero	10.34	-85.21		GQ438187 (*trn*H-*psb*A)
13	RiN	Mesoamerica	Costa Rica	Rio Nacaome	10.18	-85.37		GQ438088-438092, GQ438188-438191 (*trn*H-*psb*A)
14	LaE	Mesoamerica	Costa Rica	La Ensenada	10.14	-85.04		GQ438059, GQ438160 (*trn*H-*psb*A)
15	Cur	Mesoamerica	Costa Rica	Curu	9.79	-84.93		GQ438024-438027, GQ438125-438128 (*trn*H-*psb*A)
16	CaB	Mesoamerica	Costa Rica	Cabo Blanco	9.59	-85.09	Monica Poelchau s.n.	
17	RSC	Mesoamerica	Costa Rica	Rio San Carlos	10.36	-84.51		GQ438097-438104, GQ438197-438204 (*trn*H-*psb*A)
18	Esp	Mesoamerica	Costa Rica	Esparza	10.02	-84.64		GQ438040-438042, GQ438141-438143 (*trn*H-*psb*A)
19	Jac	Mesoamerica	Costa Rica	Jaco	9.60	-84.62		GQ438055-438058, GQ438156-438159 (*trn*H-*psb*A)

Nº	Code	Region	Country	Population name	Lat	Long	Voucher (Herbarium code)	GenBank accession number (marker)
20	LaS	Mesoamerica	Costa Rica	La Selva	10.43	-84.01		GQ438060-438063, GQ438161-438164 (*trn*H-*psb*A)
21	EaU	Mesoamerica	Costa Rica	Earth University	10.18	-83.61		GQ438032-438035, GQ438133-438136 (*trn*H-*psb*A)
22	Car	Mesoamerica	Costa Rica	Carara	9.78	-84.61		GQ438019-438021, GQ438119-438121 (*trn*H-*psb*A)
23	MaA	Mesoamerica	Costa Rica	Manuel Antonio	9.38	-84.14		GQ438068, GQ438168 (*trn*H-*psb*A)
24	HaB	Mesoamerica	Costa Rica	Hacienda Baru	9.27	-83.88		GQ438047-438050, GQ438147-438151 (*trn*H-*psb*A)
25	Cah	Mesoamerica	Costa Rica	Cahuita	9.70	-82.88		GQ438012-438014, GQ438112-438115 (*trn*H-*psb*A)
26	PiB	Mesoamerica	Costa Rica	Piedras Blancas	8.70	-83.21		GQ438077-438079, GQ438177-438178 (*trn*H-*psb*A)
27	CeB	Mesoamerica	Panama	Cerro Batipa	8.39	-82.23		GQ438022-438023, GQ438122-438124 (*trn*H-*psb*A)
28	LaT	Mesoamerica	Panama	La Tronosa	7.37	-80.46		GQ438064-438067, GQ438165-438167 (*trn*H-*psb*A)
29	FtS	Mesoamerica	Panama	Ft. Sherman	9.33	-79.95		GQ438043-438046, GQ438144-438146 (*trn*H-*psb*A)
30	PLR	Mesoamerica	Panama	Pipeline Road	9.12	-79.72		GQ438080-438083, GQ438179-438183 (*trn*H-*psb*A)
31	PNM	Mesoamerica	Panama	Metropolitana	8.99	-79.54		GQ438084-438087, GQ438184-438186 (*trn*H-*psb*A)
32	JaS	Amazonia	Ecuador	Jatun Sacha	-1.07	-77.62	EH654, 702, 710-717 (HOXA)	
33	Bog	Amazonia	Ecuador	Bogi	-0.70	-76.48	AM19374, AM19649, 19650, 19563 (HOXA)	
34	Yan	Amazonia	Peru	Yanamono	-3.44	-72.85	EH977-986 (MOL)	
35	Mad	Amazonia	Peru	Madreselva	-3.62	-72.25	EH988-992, 1025, 1042, 1054-1056, KD9 (MOL)	
36	SaJ	Amazonia	Peru	San Jorge	-4.06	-73.20	EH913-918 (MOL)	
37	JeH	Amazonia	Peru	Jenaro Herrera	-4.90	-73.65	EH948, 954-960, 975 (MOL)	
38	Mar	Amazonia	Peru	Maray	-6.31	-76.66	EH1164,1169, 1170, 1172-1178 (MOL)	
39	Ura	Amazonia	Peru	Urahuacha	-6.47	-76.33	EH1127, 1132, 1135, 1138, 1139, 1142-1144, 1149, 1151 (MOL)	
40	vHu	Amazonia	Peru	von Humboldt	-8.83	-75.06	EH1219-1221, 1223-1226, 1233, 1235, 1236 (MOL)	
41	Mac	Amazonia	Peru	Macuya	-8.87	-75.01	EH1238-1241, 1248-1251, 1254, 1256 (MOL)	
42	LaG	Amazonia	Peru	La Genova	-11.09	-75.35	EH505-508, 547-552 (MOL)	
43	SaT	Amazonia	Peru	Santa Teresa	-11.17	-74.66	EH553, 556-565 (MOL)	
44	CoC	Amazonia	Peru	Cocha Cashu	-11.90	-71.36	KD167-170 (MOL)	
45	Qon	Amazonia	Peru	Qoñec	-12.90	-71.37	EH1294,1318-1325, 1335, 1341 (MOL)	
46	SaG	Amazonia	Peru	San Gaban	-13.50	-70.42	Miguel Luza 611	
47	Tam	Amazonia	Peru	Tambopata	-11.16	-68.71	EH1641-1645, KD3554-3556 (MOL)	
48	LoA	Amazonia	Peru	Los Amigos	-12.57	-70.10	KD45, 224, 259, 260 (MOL)	
49	LaP	Amazonia	Peru	Las Piedras	-12.06	-69.53	KD3974 (MOL)	

Nº	Code	Region	Country	Population name	Lat	Long	Voucher (Herbarium code)	GenBank accession number (marker)
50	Tah	Amazonia	Bolivia	Tahuamanu	-10.59	-68.98	EH1382, 1406-1410 (Herb. Cobija)	
51	Aba	Amazonia	Bolivia	Abaroa	-10.85	-66.53	EH1415, 1439, 1443-1446 (Herb. Cobija)	
52	Mai	Amazonia	Bolivia	Maije	-13.65	-66.32	EH1494-1503 (USZ)	
53	Sac	Amazonia	Bolivia	Sacta	-16.90	-63.22	EH1559, 1560, 1562-1568, 1570 (USZ)	
54	LaEn	Amazonia	Bolivia	La Envidia	-16.32	-62.41	EH1573, 1574, 1611,1612,1617-1622 (USZ)	
55	MX	Mesoamerica	Mexico	Mexico	16.56	-92.78	Otilene dos Anjos Santos 212 (Fsp319; INPA)	
56	CR	Mesoamerica	Costa Rica	Costa Rica*	10.59	-84.02	J-Y. Rasplus s.n. (Fsp293; INPA)	
57	BCI	Mesoamerica	Panama	Panama	9.16	-79.85	Chris Dick s.n. (bci609355, bci636150, bci646282, bci721338, bci749198)	
58	BR	Amazonia	Brazil	Brazil	-3.18	-60.18	Otilene dos Anjos Santos 177 (Fsp414; INPA)	

Appendix S2.2. Haplotypes and detected polymorphic sites for *trn*H-*psb*A and ITS found on populations of *Ficus insipida* of Mesoamerica and Amazonia. ID = insertion/deletion, NS = nucleotide substitution, IV = inversion.

	*trn*H-*psb*A (375 base pairs)																				*ITS* (635bp)				
	I	N	I	N	I	N	I	N	N	N	I	N	N	N	N	N	N	N	N	N	N	I	I	N	N
	D	S	V	S	D	S	D	S	S	S	D	S	S	S	S	S	S	S	S	S	S	D	D	S	S
	0	0	0	0	0	0	1	1	1	1	1	1	2	2	2	2	3	3	3	2	4	4	5	5
	4	5	6	7	7	8	0	1	2	2	3	6	4	4	5	6	0	2	4	2	0	0	1	8
Hapl.	5	0	0	5	6	7	0	1	0	2	5	5	5	8	8	7	0	5	4	4	6	7	2	0
H1	-	A	T	T	-	G	-	T	A	T	-	T	A	T	T	A	T	G	T	T	-	-	T	T
H2	-	A	T	T	-	G	-	T	A	T	-	C	A	T	T	A	T	G	C	G	-	-	T	T
H3	-	A	T	T	-	G	-	T	A	T	-	T	A	T	T	A	T	T	T	T	C	C	T	T
H4	A	A	T	T	A	G	-	T	A	T	-	T	A	T	T	G	T	G	T	T	C	C	T	C
H5	-	A	A	T	-	G	-	T	A	T	-	T	A	T	T	A	T	G	T	T	-	-	C	T
H6	-	A	T	T	-	G	-	T	A	A	-	C	A	T	T	A	T	G	C	T	C	-	T	T
H7	-	A	A	T	-	G	C	T	A	T	-	C	A	T	T	A	T	G	C					
H8	-	A	A	T	-	G	-	T	A	T	-	C	A	T	T	A	T	G	C					
H9	-	A	T	T	-	G	-	T	A	T	-	T	C	A	T	A	T	G	T					
H10	-	A	T	T	-	G	C	T	A	T	-	C	A	T	T	A	T	G	C					
H11	A	A	A	T	A	G	-	T	A	T	-	T	A	T	T	G	T	G	T					
H12	-	A	T	T	A	G	-	T	A	T	-	T	A	T	T	G	T	G	T					
H13	-	A	T	T	-	G	-	T	T	T	-	T	A	T	T	A	T	G	T					
H14	-	A	A	T	-	G	-	A	A	T	-	T	A	T	T	A	T	G	T					
H15	A	C	A	G	A	G	-	T	A	T	-	T	A	T	T	G	T	G	T					
H16	-	A	T	T	-	G	-	T	A	T	-	C	A	T	T	A	G	G	C					
H17	-	A	T	T	-	G	-	T	A	T	T	T	A	T	T	A	T	G	T					
H18	-	A	T	T	-	A	-	T	A	T	-	T	A	T	A	A	T	G	T					
H19	A	C	T	G	A	G	-	T	A	T	-	T	A	T	T	G	T	G	T					

Coded sites for *trn*H-*psb*A: ID045: -/ AAAAT, IV060: TTCTAT/ ATAGAA, ID076: -/ ATTTT, ID100: -/ CATTTT, ID135: -/ TATTTGTCTTTT. Excluded sites for *trn*H-*psb*A: ID171: poly-T, ID253: poly-A.

Appendix B. Supporting information of chapter 3

Appendix S3.1. Number of individuals of the plastid (*trnH-psbA*) and nuclear (ITS) DNA regions sampled for five widespread species in western Amazonia. Geographical region is indicated as north and south.

					Ficus insipida		*Jacaratia digitata*		*Clarisia biflora*		*Poulsenia armata*		*Otoba parvifolia*
Population name	Country	Region	Lat	Long	plastid	nuclear	plastid	nuclear	plastid	nuclear	plastid	nuclear	plastid
1. Tiputini	Ecuador	North	-0.64	-76.18			2	1	4	4			12
2. Bogi	Ecuador	North	-0.70	-76.48	4	2	6	1			1	1	6
3. Jatun Sacha	Ecuador	North	-1.07	-77.61	10	6			1	1			19
4. Yanamono	Peru	North	-3.44	-72.85	11	5	9		6	5			15
5. Madreselva	Peru	North	-3.62	-72.24	11	5	7	2	10	5	4	4	17
6. San Jorge	Peru	North	-4.06	-73.20	6	5							
7. Jenaro Herrera	Peru	North	-4.91	-73.71	9	5			9	5			2
8. Diamante	Peru	North	-5.75	-77.53							4	4	1
9. Oriente Nuevo	Peru	North	-5.79	-77.53						1	5	5	3
10. Santo Tomas	Peru	North	-5.98	-76.25									3
11. Betania	Peru	North	-6.17	-76.81					1	1			
12. Maray	Peru	North	-6.31	-76.66	10	4							
13. Urahuacha	Peru	North	-6.46	-76.33	10	5			8	5			10
14. von Humboldt	Peru	North	-8.83	-75.06	10	5	7	1	5	5			4

					Ficus insipida		*Jacaratia digitata*		*Clarisia biflora*		*Poulsenia armata*		*Otoba parvifolia*
Population name	Country	Region	Lat	Long	plastid	nuclear	plastid	nuclear	plastid	nuclear	plastid	nuclear	plastid
15. Macuya	Peru	North	-8.88	-75.01	10	4	11	1	11	5			3
16. Encanto	Peru	North	-9.26	-75.00					1				
17. Huampal	Peru	North	-10.17	-75.57					5	3	9	5	5
18. La Genova	Peru	North	-11.10	-75.35	10	3			10	5			8
19. Santa Teresa	Peru	North	-11.17	-74.66	11	5							
20. CochaCashu	Peru	South	-11.90	-71.36	4	4	4	1			4	4	10
21. Callanga	Peru	South	-12.81	-71.78							2	2	
22. Qonec	Peru	South	-12.90	-71.37	11	4	8	1	4	4			11
23. San Gaban	Peru	South	-13.50	-70.42	1	1							
24. Lechemayo	Peru	South	-13.20	-70.39			1						
25. Los Amigos	Peru	South	-12.57	-70.10	4	4					5	5	11
26. Tambopata	Peru	South	-12.97	-69.44	7	2	6	1	3	3	5	5	14
27. Las Piedras	Peru	South	-12.06	-69.53	1	1	4						11
28. Tahuamanu	Bolivia	South	-11.42	-69.02	6	1	10	1	14	5			
29. Abaroa	Bolivia	South	-11.17	-67.48	6	1	10	1	10	5			
30. Maije	Bolivia	South	-14.36	-67.68	10	1	9		7	4	4	4	11
31. Villa Tunari	Bolivia	South	-16.97	-65.42									1
32. Sacta	Bolivia	South	-17.10	-64.78	10	2	9		10	5	8	5	9
33. La Envidia	Bolivia	South	-17.69	-63.59	10	2	8		5	5	10	5	
34. Kenia	Bolivia	South	-16.01	-62.73			10	1					
TOTAL					182	77	121	12	124	76	61	49	186

Appendix S3.2. Details of individuals collected at each site indicating voucher information. Herbarium vouchers are deposited at local herbaria such as LOJA (EC: Ecuador), HOXA, MOL (PE: Peru), Herb. Cobija and USZ (BO: Bolivia).

Species	Location	Voucher number	Species	Location	Voucher number
C. biflora	Abaroa, BO	E. Honorio 1424	*C. biflora*	Madreselva, PE	E. Honorio 1048
C. biflora	Abaroa, BO	E. Honorio 1427	*C. biflora*	Madreselva, PE	E. Honorio 1050
C. biflora	Abaroa, BO	E. Honorio 1430	*C. biflora*	Madreselva, PE	E. Honorio 1051
C. biflora	Abaroa, BO	E. Honorio 1433	*C. biflora*	Madreselva, PE	E. Honorio 1052
C. biflora	Abaroa, BO	E. Honorio 1435	*C. biflora*	Madreselva, PE	E. Honorio 1057
C. biflora	Abaroa, BO	E. Honorio 1436	*C. biflora*	Madreselva, PE	E. Honorio 994
C. biflora	Abaroa, BO	E. Honorio 1437	*C. biflora*	Maije, BO	E. Honorio 1463
C. biflora	Abaroa, BO	E. Honorio 1438	*C. biflora*	Maije, BO	E. Honorio 1486
C. biflora	Abaroa, BO	E. Honorio 1440	*C. biflora*	Maije, BO	E. Honorio 1488
C. biflora	Abaroa, BO	E. Honorio 1441	*C. biflora*	Maije, BO	E. Honorio 1491
C. biflora	Betania, PE	E. Honorio 1677	*C. biflora*	Maije, BO	E. Honorio 1504
C. biflora	Encanto, PE	R. Zarate 15057	*C. biflora*	Maije, BO	E. Honorio 1505
C. biflora	Huampal, PE	E. Honorio 1347	*C. biflora*	Maije, BO	E. Honorio 1506
C. biflora	Huampal, PE	E. Honorio 1364	*C. biflora*	OrienteNuevo, PE	E. Honorio 1670
C. biflora	Huampal, PE	JL. Marcelo X.117	*C. biflora*	Qonec, PE	E. Honorio 1309
C. biflora	Huampal, PE	JL. Marcelo X.171	*C. biflora*	Qonec, PE	E. Honorio 1312
C. biflora	Huampal, PE	JL. Marcelo X.201	*C. biflora*	Qonec, PE	E. Honorio 1332
C. biflora	JatunSacha, EC	E. Honorio 706	*C. biflora*	Qonec, PE	E. Honorio 1344
C. biflora	JenaroHerrera, PE	E. Honorio 919	*C. biflora*	Sacta, BO	E. Honorio 1523
C. biflora	JenaroHerrera, PE	E. Honorio 920	*C. biflora*	Sacta, BO	E. Honorio 1548
C. biflora	JenaroHerrera, PE	E. Honorio 923	*C. biflora*	Sacta, BO	E. Honorio 1553
C. biflora	JenaroHerrera, PE	E. Honorio 931	*C. biflora*	Sacta, BO	E. Honorio 1554
C. biflora	JenaroHerrera, PE	E. Honorio 934	*C. biflora*	Sacta, BO	E. Honorio 1555
C. biflora	JenaroHerrera, PE	E. Honorio 938	*C. biflora*	Sacta, BO	E. Honorio 1556
C. biflora	JenaroHerrera, PE	E. Honorio 950	*C. biflora*	Sacta, BO	E. Honorio 1557
C. biflora	JenaroHerrera, PE	E. Honorio 952	*C. biflora*	Sacta, BO	E. Honorio 1558
C. biflora	JenaroHerrera, PE	E. Honorio 953	*C. biflora*	Sacta, BO	E. Honorio 1561
C. biflora	LaEnvidia, BO	E. Honorio 1584	*C. biflora*	Sacta, BO	E. Honorio 1569
C. biflora	LaEnvidia, BO	E. Honorio 1591	*C. biflora*	Tahuamanu, BO	E. Honorio 1371
C. biflora	LaEnvidia, BO	E. Honorio 1592	*C. biflora*	Tahuamanu, BO	E. Honorio 1373
C. biflora	LaEnvidia, BO	E. Honorio 1602	*C. biflora*	Tahuamanu, BO	E. Honorio 1377
C. biflora	LaEnvidia, BO	E. Honorio 1603	*C. biflora*	Tahuamanu, BO	E. Honorio 1378
C. biflora	LaGenova, PE	E. Honorio 503	*C. biflora*	Tahuamanu, BO	E. Honorio 1384
C. biflora	LaGenova, PE	E. Honorio 504	*C. biflora*	Tahuamanu, BO	E. Honorio 1385
C. biflora	LaGenova, PE	E. Honorio 517	*C. biflora*	Tahuamanu, BO	E. Honorio 1386
C. biflora	LaGenova, PE	E. Honorio 518	*C. biflora*	Tahuamanu, BO	E. Honorio 1387
C. biflora	LaGenova, PE	E. Honorio 521	*C. biflora*	Tahuamanu, BO	E. Honorio 1388
C. biflora	LaGenova, PE	E. Honorio 525	*C. biflora*	Tahuamanu, BO	E. Honorio 1389
C. biflora	LaGenova, PE	E. Honorio 529	*C. biflora*	Tahuamanu, BO	E. Honorio 1395
C. biflora	LaGenova, PE	E. Honorio 533	*C. biflora*	Tahuamanu, BO	E. Honorio 1403
C. biflora	LaGenova, PE	E. Honorio 536	*C. biflora*	Tahuamanu, BO	E. Honorio 1404
C. biflora	LaGenova, PE	E. Honorio 540	*C. biflora*	Tahuamanu, BO	E. Honorio 1414
C. biflora	Macuya, PE	E. Honorio 1245	*C. biflora*	Tambopata, PE	E. Honorio 1654
C. biflora	Macuya, PE	E. Honorio 1267	*C. biflora*	Tambopata, PE	E. Honorio 1655
C. biflora	Macuya, PE	E. Honorio 1271	*C. biflora*	Tambopata, PE	E. Honorio 1661
C. biflora	Macuya, PE	E. Honorio 1274	*C. biflora*	Tiputini, EC	A. Monteagudo 19672
C. biflora	Macuya, PE	E. Honorio 1282	*C. biflora*	Tiputini, EC	A. Monteagudo 19693
C. biflora	Macuya, PE	E. Honorio 1283	*C. biflora*	Tiputini, EC	A. Monteagudo 19729
C. biflora	Macuya, PE	E. Honorio 1284	*C. biflora*	Tiputini, EC	A. Monteagudo 19739
C. biflora	Macuya, PE	E. Honorio 1286	*C. biflora*	Urahuacha, PE	E. Honorio 1121
C. biflora	Macuya, PE	E. Honorio 1287	*C. biflora*	Urahuacha, PE	E. Honorio 1124
C. biflora	Macuya, PE	E. Honorio 1288	*C. biflora*	Urahuacha, PE	E. Honorio 1128
C. biflora	Macuya, PE	E. Honorio 1293	*C. biflora*	Urahuacha, PE	E. Honorio 1129
C. biflora	Madreselva, PE	E. Honorio 1026	*C. biflora*	Urahuacha, PE	E. Honorio 1136
C. biflora	Madreselva, PE	E. Honorio 1031	*C. biflora*	Urahuacha, PE	E. Honorio 1137
C. biflora	Madreselva, PE	E. Honorio 1039	*C. biflora*	Urahuacha, PE	E. Honorio 1141
C. biflora	Madreselva, PE	E. Honorio 1046	*C. biflora*	Urahuacha, PE	E. Honorio 1150

Species	Location	Voucher number
C. biflora	vonHumboldt, PE	E. Honorio 1213
C. biflora	vonHumboldt, PE	E. Honorio 1214
C. biflora	vonHumboldt, PE	E. Honorio 1218
C. biflora	vonHumboldt, PE	E. Honorio 1230
C. biflora	vonHumboldt, PE	E. Honorio 1231
C. biflora	Yanamono, PE	E. Honorio 1098
C. biflora	Yanamono, PE	E. Honorio 1104
C. biflora	Yanamono, PE	E. Honorio 1108
C. biflora	Yanamono, PE	E. Honorio 1112
C. biflora	Yanamono, PE	E. Honorio 1115
C. biflora	Yanamono, PE	E. Honorio 1119
F. insipida	Abaroa, BO	E. Honorio 1415
F. insipida	Abaroa, BO	E. Honorio 1439
F. insipida	Abaroa, BO	E. Honorio 1443
F. insipida	Abaroa, BO	E. Honorio 1444
F. insipida	Abaroa, BO	E. Honorio 1445
F. insipida	Abaroa, BO	E. Honorio 1446
F. insipida	Bogi, EC	A. Monteagudo 19374
F. insipida	Bogi, EC	A. Monteagudo 19563
F. insipida	Bogi, EC	A. Monteagudo 19649
F. insipida	Bogi, EC	A. Monteagudo 19650
F. insipida	CochaCashu, PE	K. Dexter 167
F. insipida	CochaCashu, PE	K. Dexter 168
F. insipida	CochaCashu, PE	K. Dexter 169
F. insipida	CochaCashu, PE	K. Dexter 170
F. insipida	JatunSacha, EC	E. Honorio 654
F. insipida	JatunSacha, EC	E. Honorio 702
F. insipida	JatunSacha, EC	E. Honorio 710
F. insipida	JatunSacha, EC	E. Honorio 711
F. insipida	JatunSacha, EC	E. Honorio 712
F. insipida	JatunSacha, EC	E. Honorio 713
F. insipida	JatunSacha, EC	E. Honorio 714
F. insipida	JatunSacha, EC	E. Honorio 715
F. insipida	JatunSacha, EC	E. Honorio 716
F. insipida	JatunSacha, EC	E. Honorio 717
F. insipida	JenaroHerrera, PE	E. Honorio 948
F. insipida	JenaroHerrera, PE	E. Honorio 954
F. insipida	JenaroHerrera, PE	E. Honorio 955
F. insipida	JenaroHerrera, PE	E. Honorio 956
F. insipida	JenaroHerrera, PE	E. Honorio 957
F. insipida	JenaroHerrera, PE	E. Honorio 958
F. insipida	JenaroHerrera, PE	E. Honorio 959
F. insipida	JenaroHerrera, PE	E. Honorio 960
F. insipida	JenaroHerrera, PE	E. Honorio 975
F. insipida	LaEnvidia, BO	E. Honorio 1573
F. insipida	LaEnvidia, BO	E. Honorio 1574
F. insipida	LaEnvidia, BO	E. Honorio 1611
F. insipida	LaEnvidia, BO	E. Honorio 1612
F. insipida	LaEnvidia, BO	E. Honorio 1617
F. insipida	LaEnvidia, BO	E. Honorio 1618
F. insipida	LaEnvidia, BO	E. Honorio 1619
F. insipida	LaEnvidia, BO	E. Honorio 1620
F. insipida	LaEnvidia, BO	E. Honorio 1621
F. insipida	LaEnvidia, BO	E. Honorio 1622
F. insipida	LaGenova, PE	E. Honorio 505
F. insipida	LaGenova, PE	E. Honorio 506
F. insipida	LaGenova, PE	E. Honorio 507
F. insipida	LaGenova, PE	E. Honorio 508
F. insipida	LaGenova, PE	E. Honorio 547
F. insipida	LaGenova, PE	E. Honorio 548
F. insipida	LaGenova, PE	E. Honorio 549
F. insipida	LaGenova, PE	E. Honorio 550
F. insipida	LaGenova, PE	E. Honorio 551
F. insipida	LaGenova, PE	E. Honorio 552
F. insipida	LasPiedras, PE	K. Dexter 3974
F. insipida	LosAmigos, PE	K. Dexter 224
F. insipida	LosAmigos, PE	K. Dexter 259
F. insipida	LosAmigos, PE	K. Dexter 260
F. insipida	LosAmigos, PE	K. Dexter 45
F. insipida	Macuya, PE	E. Honorio 1238
F. insipida	Macuya, PE	E. Honorio 1239
F. insipida	Macuya, PE	E. Honorio 1240
F. insipida	Macuya, PE	E. Honorio 1241
F. insipida	Macuya, PE	E. Honorio 1248
F. insipida	Macuya, PE	E. Honorio 1249
F. insipida	Macuya, PE	E. Honorio 1250
F. insipida	Macuya, PE	E. Honorio 1251
F. insipida	Macuya, PE	E. Honorio 1254
F. insipida	Macuya, PE	E. Honorio 1256
F. insipida	Madreselva, PE	E. Honorio 1025
F. insipida	Madreselva, PE	E. Honorio 1042
F. insipida	Madreselva, PE	E. Honorio 1054
F. insipida	Madreselva, PE	E. Honorio 1055
F. insipida	Madreselva, PE	E. Honorio 1056
F. insipida	Madreselva, PE	E. Honorio 988
F. insipida	Madreselva, PE	E. Honorio 989
F. insipida	Madreselva, PE	E. Honorio 990
F. insipida	Madreselva, PE	E. Honorio 991
F. insipida	Madreselva, PE	E. Honorio 992
F. insipida	Madreselva, PE	K. Dexter 9
F. insipida	Maije, BO	E. Honorio 1494
F. insipida	Maije, BO	E. Honorio 1495
F. insipida	Maije, BO	E. Honorio 1496
F. insipida	Maije, BO	E. Honorio 1497
F. insipida	Maije, BO	E. Honorio 1498
F. insipida	Maije, BO	E. Honorio 1499
F. insipida	Maije, BO	E. Honorio 1500
F. insipida	Maije, BO	E. Honorio 1501
F. insipida	Maije, BO	E. Honorio 1502
F. insipida	Maije, BO	E. Honorio 1503
F. insipida	Maray, PE	E. Honorio 1164
F. insipida	Maray, PE	E. Honorio 1169
F. insipida	Maray, PE	E. Honorio 1170
F. insipida	Maray, PE	E. Honorio 1172
F. insipida	Maray, PE	E. Honorio 1173
F. insipida	Maray, PE	E. Honorio 1174
F. insipida	Maray, PE	E. Honorio 1175
F. insipida	Maray, PE	E. Honorio 1176
F. insipida	Maray, PE	E. Honorio 1177
F. insipida	Maray, PE	E. Honorio 1178
F. insipida	Qonec, PE	E. Honorio 1294
F. insipida	Qonec, PE	E. Honorio 1318
F. insipida	Qonec, PE	E. Honorio 1319
F. insipida	Qonec, PE	E. Honorio 1320
F. insipida	Qonec, PE	E. Honorio 1321
F. insipida	Qonec, PE	E. Honorio 1322
F. insipida	Qonec, PE	E. Honorio 1323
F. insipida	Qonec, PE	E. Honorio 1324
F. insipida	Qonec, PE	E. Honorio 1325
F. insipida	Qonec, PE	E. Honorio 1335
F. insipida	Qonec, PE	E. Honorio 1341
F. insipida	Sacta, BO	E. Honorio 1559
F. insipida	Sacta, BO	E. Honorio 1560
F. insipida	Sacta, BO	E. Honorio 1562
F. insipida	Sacta, BO	E. Honorio 1563
F. insipida	Sacta, BO	E. Honorio 1564
F. insipida	Sacta, BO	E. Honorio 1565
F. insipida	Sacta, BO	E. Honorio 1566

Species	Location	Voucher number
F. insipida	Sacta, BO	E. Honorio 1567
F. insipida	Sacta, BO	E. Honorio 1568
F. insipida	Sacta, BO	E. Honorio 1570
F. insipida	SanGaban, PE	M. Luza 611
F. insipida	SanJorge, PE	E. Honorio 913
F. insipida	SanJorge, PE	E. Honorio 914
F. insipida	SanJorge, PE	E. Honorio 915
F. insipida	SanJorge, PE	E. Honorio 916
F. insipida	SanJorge, PE	E. Honorio 917
F. insipida	SanJorge, PE	E. Honorio 918
F. insipida	SantaTeresa, PE	E. Honorio 553
F. insipida	SantaTeresa, PE	E. Honorio 556
F. insipida	SantaTeresa, PE	E. Honorio 557
F. insipida	SantaTeresa, PE	E. Honorio 558
F. insipida	SantaTeresa, PE	E. Honorio 559
F. insipida	SantaTeresa, PE	E. Honorio 560
F. insipida	SantaTeresa, PE	E. Honorio 561
F. insipida	SantaTeresa, PE	E. Honorio 562
F. insipida	SantaTeresa, PE	E. Honorio 563
F. insipida	SantaTeresa, PE	E. Honorio 564
F. insipida	SantaTeresa, PE	E. Honorio 565
F. insipida	Tahuamanu, BO	E. Honorio 1382
F. insipida	Tahuamanu, BO	E. Honorio 1406
F. insipida	Tahuamanu, BO	E. Honorio 1407
F. insipida	Tahuamanu, BO	E. Honorio 1408
F. insipida	Tahuamanu, BO	E. Honorio 1409
F. insipida	Tahuamanu, BO	E. Honorio 1410
F. insipida	Tambopata, PE	E. Honorio 1641
F. insipida	Tambopata, PE	E. Honorio 1642
F. insipida	Tambopata, PE	E. Honorio 1643
F. insipida	Tambopata, PE	E. Honorio 1644
F. insipida	Tambopata, PE	E. Honorio 1645
F. insipida	Tambopata, PE	K. Dexter 3554
F. insipida	Tambopata, PE	K. Dexter 3555
F. insipida	Urahuacha, PE	E. Honorio 1127
F. insipida	Urahuacha, PE	E. Honorio 1132
F. insipida	Urahuacha, PE	E. Honorio 1135
F. insipida	Urahuacha, PE	E. Honorio 1138
F. insipida	Urahuacha, PE	E. Honorio 1139
F. insipida	Urahuacha, PE	E. Honorio 1142
F. insipida	Urahuacha, PE	E. Honorio 1143
F. insipida	Urahuacha, PE	E. Honorio 1144
F. insipida	Urahuacha, PE	E. Honorio 1149
F. insipida	Urahuacha, PE	E. Honorio 1151
F. insipida	vonHumboldt, PE	E. Honorio 1219
F. insipida	vonHumboldt, PE	E. Honorio 1220
F. insipida	vonHumboldt, PE	E. Honorio 1221
F. insipida	vonHumboldt, PE	E. Honorio 1223
F. insipida	vonHumboldt, PE	E. Honorio 1224
F. insipida	vonHumboldt, PE	E. Honorio 1225
F. insipida	vonHumboldt, PE	E. Honorio 1226
F. insipida	vonHumboldt, PE	E. Honorio 1233
F. insipida	vonHumboldt, PE	E. Honorio 1235
F. insipida	vonHumboldt, PE	E. Honorio 1236
F. insipida	Yanamono, PE	E. Honorio 977
F. insipida	Yanamono, PE	E. Honorio 978
F. insipida	Yanamono, PE	E. Honorio 979
F. insipida	Yanamono, PE	E. Honorio 980
F. insipida	Yanamono, PE	E. Honorio 981
F. insipida	Yanamono, PE	E. Honorio 982
F. insipida	Yanamono, PE	E. Honorio 983
F. insipida	Yanamono, PE	E. Honorio 984
F. insipida	Yanamono, PE	E. Honorio 985
F. insipida	Yanamono, PE	E. Honorio 986
F. insipida	Yanamono, PE	S. Patiño YAN02.124
J. digitata	Abaroa, BO	E. Honorio 1416
J. digitata	Abaroa, BO	E. Honorio 1417
J. digitata	Abaroa, BO	E. Honorio 1418
J. digitata	Abaroa, BO	E. Honorio 1419
J. digitata	Abaroa, BO	E. Honorio 1420
J. digitata	Abaroa, BO	E. Honorio 1421
J. digitata	Abaroa, BO	E. Honorio 1422
J. digitata	Abaroa, BO	E. Honorio 1423
J. digitata	Abaroa, BO	E. Honorio 1425
J. digitata	Abaroa, BO	E. Honorio 1426
J. digitata	Bogi, EC	A. Monteagudo 19254
J. digitata	Bogi, EC	A. Monteagudo 19255
J. digitata	Bogi, EC	A. Monteagudo 19404
J. digitata	Bogi, EC	A. Monteagudo 19495
J. digitata	Bogi, EC	A. Monteagudo 19496
J. digitata	Bogi, EC	A. Monteagudo 19535
J. digitata	CochaCashu, PE	K. Dexter 103
J. digitata	CochaCashu, PE	K. Dexter 104A
J. digitata	CochaCashu, PE	K. Dexter 127
J. digitata	CochaCashu, PE	K. Dexter 49
J. digitata	Kenia, BO	E. Honorio 1623
J. digitata	Kenia, BO	E. Honorio 1624
J. digitata	Kenia, BO	E. Honorio 1625
J. digitata	Kenia, BO	E. Honorio 1626
J. digitata	Kenia, BO	E. Honorio 1627
J. digitata	Kenia, BO	E. Honorio 1628
J. digitata	Kenia, BO	E. Honorio 1629
J. digitata	Kenia, BO	E. Honorio 1630
J. digitata	Kenia, BO	E. Honorio 1631
J. digitata	Kenia, BO	E. Honorio 1632
J. digitata	LaEnvidia, BO	E. Honorio 1604
J. digitata	LaEnvidia, BO	E. Honorio 1606
J. digitata	LaEnvidia, BO	E. Honorio 1607
J. digitata	LaEnvidia, BO	E. Honorio 1608
J. digitata	LaEnvidia, BO	E. Honorio 1610
J. digitata	LaEnvidia, BO	E. Honorio 1614
J. digitata	LaEnvidia, BO	E. Honorio 1615
J. digitata	LaEnvidia, BO	E. Honorio 1616
J. digitata	LasPiedras, PE	K. Dexter 4024
J. digitata	LasPiedras, PE	K. Dexter 4053
J. digitata	LasPiedras, PE	K. Dexter 4152
J. digitata	LasPiedras, PE	K. Dexter 4314
J. digitata	Lechemayo, PE	M. Luza 523
J. digitata	Macuya, PE	E. Honorio 1242
J. digitata	Macuya, PE	E. Honorio 1243
J. digitata	Macuya, PE	E. Honorio 1247
J. digitata	Macuya, PE	E. Honorio 1252
J. digitata	Macuya, PE	E. Honorio 1253
J. digitata	Macuya, PE	E. Honorio 1255
J. digitata	Macuya, PE	E. Honorio 1257
J. digitata	Macuya, PE	E. Honorio 1260
J. digitata	Macuya, PE	E. Honorio 1264
J. digitata	Macuya, PE	E. Honorio 1269
J. digitata	Macuya, PE	E. Honorio 1281
J. digitata	Madreselva, PE	E. Honorio 1000
J. digitata	Madreselva, PE	E. Honorio 1012
J. digitata	Madreselva, PE	E. Honorio 1036
J. digitata	Madreselva, PE	E. Honorio 1047
J. digitata	Madreselva, PE	E. Honorio 1049
J. digitata	Madreselva, PE	K. Dexter 64
J. digitata	Madreselva, PE	K. Dexter 90
J. digitata	Maije, BO	E. Honorio 1448
J. digitata	Maije, BO	E. Honorio 1450

Species	Location	Voucher number	Species	Location	Voucher number
J. digitata	Maije, BO	E. Honorio 1452	*O. parvifolia*	CochaCashu, PE	K. Dexter 132
J. digitata	Maije, BO	E. Honorio 1455	*O. parvifolia*	CochaCashu, PE	K. Dexter 133
J. digitata	Maije, BO	E. Honorio 1457	*O. parvifolia*	CochaCashu, PE	K. Dexter 24
J. digitata	Maije, BO	E. Honorio 1458	*O. parvifolia*	CochaCashu, PE	K. Dexter 25
J. digitata	Maije, BO	E. Honorio 1462	*O. parvifolia*	CochaCashu, PE	K. Dexter 26A
J. digitata	Maije, BO	E. Honorio 1467	*O. parvifolia*	CochaCashu, PE	K. Dexter 27
J. digitata	Maije, BO	E. Honorio 1473	*O. parvifolia*	CochaCashu, PE	K. Dexter 29
J. digitata	Qonec, PE	E. Honorio 1301	*O. parvifolia*	CochaCashu, PE	K. Dexter 31
J. digitata	Qonec, PE	E. Honorio 1306	*O. parvifolia*	CochaCashu, PE	K. Dexter 32
J. digitata	Qonec, PE	E. Honorio 1317	*O. parvifolia*	CochaCashu, PE	K. Dexter 33
J. digitata	Qonec, PE	E. Honorio 1336	*O. glycycarpa*	Diamante, PE	E. Honorio 1676
J. digitata	Qonec, PE	E. Honorio 1339	*O. glycycarpa*	Huampal, PE	E. Honorio 1355
J. digitata	Qonec, PE	E. Honorio 1340	*O. glycycarpa*	Huampal, PE	E. Honorio 1359
J. digitata	Qonec, PE	E. Honorio 1343	*O. glycycarpa*	Huampal, PE	E. Honorio 1360
J. digitata	Qonec, PE	E. Honorio 1346	*O. glycycarpa*	Huampal, PE	E. Honorio 1362
J. digitata	Sacta, BO	E. Honorio 1508	*O. glycycarpa*	Huampal, PE	E. Honorio 1363
J. digitata	Sacta, BO	E. Honorio 1512	*O. glycycarpa*	JatunSacha, EC	E. Honorio 569
J. digitata	Sacta, BO	E. Honorio 1515	*O. glycycarpa*	JatunSacha, EC	E. Honorio 574
J. digitata	Sacta, BO	E. Honorio 1517	*O. parvifolia*	JatunSacha, EC	E. Honorio 579
J. digitata	Sacta, BO	E. Honorio 1524	*O. glycycarpa*	JatunSacha, EC	E. Honorio 580
J. digitata	Sacta, BO	E. Honorio 1526	*O. parvifolia*	JatunSacha, EC	E. Honorio 582
J. digitata	Sacta, BO	E. Honorio 1531	*O. parvifolia*	JatunSacha, EC	E. Honorio 587
J. digitata	Sacta, BO	E. Honorio 1533	*O. parvifolia*	JatunSacha, EC	E. Honorio 589
J. digitata	Sacta, BO	E. Honorio 1536	*O. parvifolia*	JatunSacha, EC	E. Honorio 594
J. digitata	Tahuamanu, BO	E. Honorio 1375	*O. parvifolia*	JatunSacha, EC	E. Honorio 601
J. digitata	Tahuamanu, BO	E. Honorio 1390	*O. glycycarpa*	JatunSacha, EC	E. Honorio 607
J. digitata	Tahuamanu, BO	E. Honorio 1391	*O. parvifolia*	JatunSacha, EC	E. Honorio 615
J. digitata	Tahuamanu, BO	E. Honorio 1392	*O. glycycarpa*	JatunSacha, EC	E. Honorio 629
J. digitata	Tahuamanu, BO	E. Honorio 1393	*O. glycycarpa*	JatunSacha, EC	E. Honorio 636
J. digitata	Tahuamanu, BO	E. Honorio 1398	*O. glycycarpa*	JatunSacha, EC	E. Honorio 651
J. digitata	Tahuamanu, BO	E. Honorio 1399	*O. glycycarpa*	JatunSacha, EC	E. Honorio 676
J. digitata	Tahuamanu, BO	E. Honorio 1400	*O. glycycarpa*	JatunSacha, EC	E. Honorio 678
J. digitata	Tahuamanu, BO	E. Honorio 1401	*O. glycycarpa*	JatunSacha, EC	E. Honorio 688
J. digitata	Tahuamanu, BO	E. Honorio 1411	*O. glycycarpa*	JatunSacha, EC	E. Honorio 693
J. digitata	Tambopata, PE	E. Honorio 1648	*O. parvifolia*	JatunSacha, EC	E. Honorio 707
J. digitata	Tambopata, PE	E. Honorio 1650	*O. glycycarpa*	JenaroHerrera, PE	E. Honorio 963
J. digitata	Tambopata, PE	E. Honorio 1651	*O. glycycarpa*	JenaroHerrera, PE	E. Honorio 964
J. digitata	Tambopata, PE	E. Honorio 1657	*O. parvifolia*	LaGenova, PE	E. Honorio 511
J. digitata	Tambopata, PE	E. Honorio 1660	*O. parvifolia*	LaGenova, PE	E. Honorio 523
J. digitata	Tambopata, PE	K. Dexter 3565	*O. parvifolia*	LaGenova, PE	E. Honorio 532
J. digitata	Tiputini, EC	A. Monteagudo 19820	*O. parvifolia*	LaGenova, PE	E. Honorio 535
J. digitata	Tiputini, EC	A. Monteagudo s.n.	*O. parvifolia*	LaGenova, PE	E. Honorio 539
J. digitata	vonHumboldt, PE	E. Honorio 1202	*O. parvifolia*	LaGenova, PE	E. Honorio 541
J. digitata	vonHumboldt, PE	E. Honorio 1203	*O. parvifolia*	LaGenova, PE	E. Honorio 543
J. digitata	vonHumboldt, PE	E. Honorio 1204	*O. glycycarpa*	LaGenova, PE	E. Honorio 545
J. digitata	vonHumboldt, PE	E. Honorio 1209	*O. parvifolia*	LasPiedras, PE	K. Dexter 3912
J. digitata	vonHumboldt, PE	E. Honorio 1216	*O. parvifolia*	LasPiedras, PE	K. Dexter 3975
J. digitata	vonHumboldt, PE	E. Honorio 1229	*O. parvifolia*	LasPiedras, PE	K. Dexter 3985
J. digitata	vonHumboldt, PE	E. Honorio 1234	*O. parvifolia*	LasPiedras, PE	K. Dexter 3994
J. digitata	Yanamono, PE	E. Honorio 1059	*O. parvifolia*	LasPiedras, PE	K. Dexter 4001
J. digitata	Yanamono, PE	E. Honorio 1077	*O. parvifolia*	LasPiedras, PE	K. Dexter 4002
J. digitata	Yanamono, PE	E. Honorio 1082	*O. parvifolia*	LasPiedras, PE	K. Dexter 4007
J. digitata	Yanamono, PE	E. Honorio 1093	*O. parvifolia*	LasPiedras, PE	K. Dexter 4025
J. digitata	Yanamono, PE	E. Honorio 1105	*O. parvifolia*	LasPiedras, PE	K. Dexter 4055
J. digitata	Yanamono, PE	E. Honorio 1109	*O. parvifolia*	LasPiedras, PE	K. Dexter 4063
J. digitata	Yanamono, PE	E. Honorio 1111	*O. parvifolia*	LasPiedras, PE	K. Dexter 4334
J. digitata	Yanamono, PE	E. Honorio 1114	*O. parvifolia*	LosAmigos, PE	K. Dexter 100
J. digitata	Yanamono, PE	E. Honorio 1116	*O. parvifolia*	LosAmigos, PE	K. Dexter 101
O. parvifolia	Bogi, EC	A. Monteagudo 19253	*O. parvifolia*	LosAmigos, PE	K. Dexter 104B
O. parvifolia	Bogi, EC	A. Monteagudo 19388	*O. parvifolia*	LosAmigos, PE	K. Dexter 17
O. parvifolia	Bogi, EC	A. Monteagudo 19427	*O. parvifolia*	LosAmigos, PE	K. Dexter 203
O. glycycarpa	Bogi, EC	A. Monteagudo 19445	*O. parvifolia*	LosAmigos, PE	K. Dexter 226
O. parvifolia	Bogi, EC	A. Monteagudo 19481	*O. parvifolia*	LosAmigos, PE	K. Dexter 26B
O. glycycarpa	Bogi, EC	A. Monteagudo 19482	*O. parvifolia*	LosAmigos, PE	K. Dexter 83B

Species	Location	Voucher number
O. parvifolia	LosAmigos, PE	K. Dexter 95
O. parvifolia	LosAmigos, PE	K. Dexter 97
O. parvifolia	LosAmigos, PE	K. Dexter 98
O. parvifolia	Macuya, PE	E. Honorio 1289
O. parvifolia	Macuya, PE	E. Honorio 1291
O. parvifolia	Macuya, PE	E. Honorio 1292
O. parvifolia	Madreselva, PE	E. Honorio 1001
O. parvifolia	Madreselva, PE	E. Honorio 1002
O. glycycarpa	Madreselva, PE	E. Honorio 1004
O. parvifolia	Madreselva, PE	E. Honorio 1005
O. glycycarpa	Madreselva, PE	E. Honorio 1008
O. glycycarpa	Madreselva, PE	E. Honorio 1009
O. glycycarpa	Madreselva, PE	E. Honorio 1014
O. parvifolia	Madreselva, PE	E. Honorio 1015
O. glycycarpa	Madreselva, PE	E. Honorio 1018
O. parvifolia	Madreselva, PE	E. Honorio 1019
O. glycycarpa	Madreselva, PE	E. Honorio 1020
O. glycycarpa	Madreselva, PE	E. Honorio 1033
O. parvifolia	Madreselva, PE	E. Honorio 1035
O. parvifolia	Madreselva, PE	E. Honorio 1043
O. glycycarpa	Madreselva, PE	E. Honorio 1045
O. parvifolia	Madreselva, PE	E. Honorio 995
O. parvifolia	Madreselva, PE	K. Dexter 82A
O. glycycarpa	Maije, BO	E. Honorio 1451
O. glycycarpa	Maije, BO	E. Honorio 1454
O. glycycarpa	Maije, BO	E. Honorio 1459
O. glycycarpa	Maije, BO	E. Honorio 1460
O. glycycarpa	Maije, BO	E. Honorio 1464
O. glycycarpa	Maije, BO	E. Honorio 1465
O. glycycarpa	Maije, BO	E. Honorio 1477
O. glycycarpa	Maije, BO	E. Honorio 1479
O. glycycarpa	Maije, BO	E. Honorio 1482
O. glycycarpa	Maije, BO	E. Honorio 1485
O. glycycarpa	Maije, BO	E. Honorio 1487
O. glycycarpa	OrienteNuevo, PE	E. Honorio 1662
O. glycycarpa	OrienteNuevo, PE	E. Honorio 1664
O. glycycarpa	OrienteNuevo, PE	E. Honorio 1665
O. parvifolia	Qonec, PE	E. Honorio 1296
O. parvifolia	Qonec, PE	E. Honorio 1297
O. parvifolia	Qonec, PE	E. Honorio 1298
O. parvifolia	Qonec, PE	E. Honorio 1304
O. parvifolia	Qonec, PE	E. Honorio 1308
O. parvifolia	Qonec, PE	E. Honorio 1314
O. parvifolia	Qonec, PE	E. Honorio 1326
O. parvifolia	Qonec, PE	E. Honorio 1327
O. parvifolia	Qonec, PE	E. Honorio 1328
O. parvifolia	Qonec, PE	E. Honorio 1330
O. parvifolia	Qonec, PE	E. Honorio 1333
O. glycycarpa	Sacta, BO	E. Honorio 1514
O. glycycarpa	Sacta, BO	E. Honorio 1516
O. glycycarpa	Sacta, BO	E. Honorio 1518
O. glycycarpa	Sacta, BO	E. Honorio 1522
O. glycycarpa	Sacta, BO	E. Honorio 1525
O. glycycarpa	Sacta, BO	E. Honorio 1528
O. glycycarpa	Sacta, BO	E. Honorio 1534
O. glycycarpa	Sacta, BO	E. Honorio 1537
O. glycycarpa	Sacta, BO	E. Honorio 1545
O. glycycarpa	SantoTomas, PE	E. Honorio 1185
O. parvifolia	SantoTomas, PE	E. Honorio 1188
O. glycycarpa	SantoTomas, PE	E. Honorio 1190
O. glycycarpa	Tambopata, PE	E. Honorio 1633
O. glycycarpa	Tambopata, PE	E. Honorio 1637
O. glycycarpa	Tambopata, PE	E. Honorio 1638
O. glycycarpa	Tambopata, PE	E. Honorio 1639
O. glycycarpa	Tambopata, PE	E. Honorio 1640
O. parvifolia	Tambopata, PE	K. Dexter 3562
O. parvifolia	Tambopata, PE	K. Dexter 3592
O. parvifolia	Tambopata, PE	K. Dexter 3593
O. parvifolia	Tambopata, PE	K. Dexter 3596
O. parvifolia	Tambopata, PE	K. Dexter 3604
O. parvifolia	Tambopata, PE	K. Dexter 3625
O. parvifolia	Tambopata, PE	K. Dexter 3635
O. parvifolia	Tambopata, PE	K. Dexter 3740
O. parvifolia	Tambopata, PE	K. Dexter 3829
O. glycycarpa	Tiputini, EC	A. Monteagudo 19751
O. glycycarpa	Tiputini, EC	A. Monteagudo 19776
O. glycycarpa	Tiputini, EC	A. Monteagudo 19777
O. glycycarpa	Tiputini, EC	A. Monteagudo 19778
O. glycycarpa	Tiputini, EC	A. Monteagudo 19798
O. glycycarpa	Tiputini, EC	A. Monteagudo 19808
O. glycycarpa	Tiputini, EC	A. Monteagudo 19825
O. glycycarpa	Tiputini, EC	A. Monteagudo 19829
O. glycycarpa	Tiputini, EC	A. Monteagudo 19832
O. glycycarpa	Tiputini, EC	A. Monteagudo 19850
O. glycycarpa	Tiputini, EC	A. Monteagudo 19867
O. glycycarpa	Tiputini, EC	A. Monteagudo 19893
O. parvifolia	Urahuacha, PE	E. Honorio 1120
O. parvifolia	Urahuacha, PE	E. Honorio 1123
O. parvifolia	Urahuacha, PE	E. Honorio 1126
O. parvifolia	Urahuacha, PE	E. Honorio 1130
O. parvifolia	Urahuacha, PE	E. Honorio 1131
O. parvifolia	Urahuacha, PE	E. Honorio 1133
O. parvifolia	Urahuacha, PE	E. Honorio 1134
O. parvifolia	Urahuacha, PE	E. Honorio 1147
O. parvifolia	Urahuacha, PE	E. Honorio 1148
O. parvifolia	VillaTunari, BO	K. Dexter 4409
O. parvifolia	vonHumboldt, PE	E. Honorio 1198
O. parvifolia	vonHumboldt, PE	E. Honorio 1206
O. parvifolia	vonHumboldt, PE	E. Honorio 1228
O. parvifolia	vonHumboldt, PE	E. Honorio 1232
O. glycycarpa	Yanamono, PE	E. Honorio 1058
O. parvifolia	Yanamono, PE	E. Honorio 1060
O. glycycarpa	Yanamono, PE	E. Honorio 1063
O. glycycarpa	Yanamono, PE	E. Honorio 1066
O. parvifolia	Yanamono, PE	E. Honorio 1067
O. glycycarpa	Yanamono, PE	E. Honorio 1073
O. parvifolia	Yanamono, PE	E. Honorio 1074
O. parvifolia	Yanamono, PE	E. Honorio 1083
O. glycycarpa	Yanamono, PE	E. Honorio 1084
O. glycycarpa	Yanamono, PE	E. Honorio 1087
O. glycycarpa	Yanamono, PE	E. Honorio 1091
O. parvifolia	Yanamono, PE	E. Honorio 1095
O. glycycarpa	Yanamono, PE	E. Honorio 1096
O. parvifolia	Yanamono, PE	E. Honorio 1100
O. glycycarpa	Yanamono, PE	E. Honorio 1101
P. armata	Bogi, EC	A. Monteagudo 19523
P. armata	Callanga, PE	W. Farfan 1626
P. armata	Callanga, PE	W. Farfan CA1250.213
P. armata	CochaCashu, PE	K. Dexter 105
P. armata	CochaCashu, PE	K. Dexter 6
P. armata	CochaCashu, PE	K. Dexter 7
P. armata	CochaCashu, PE	K. Dexter 8
P. armata	Diamante, PE	E. Honorio 1672
P. armata	Diamante, PE	E. Honorio 1673
P. armata	Diamante, PE	E. Honorio 1674
P. armata	Diamante, PE	E. Honorio 1675
P. armata	Huampal, PE	E. Honorio 1349
P. armata	Huampal, PE	E. Honorio 1350

Species	Location	Voucher number	Species	Location	Voucher number
P. armata	Huampal, PE	E. Honorio 1351	*P. armata*	Madreselva, PE	K. Dexter 140
P. armata	Huampal, PE	E. Honorio 1353	*P. armata*	Madreselva, PE	K. Dexter 65
P. armata	Huampal, PE	E. Honorio 1354	*P. armata*	Maije, BO	E. Honorio 1466
P. armata	Huampal, PE	E. Honorio 1356	*P. armata*	Maije, BO	E. Honorio 1475
P. armata	Huampal, PE	E. Honorio 1358	*P. armata*	Maije, BO	E. Honorio 1480
P. armata	Huampal, PE	E. Honorio 1361	*P. armata*	Maije, BO	E. Honorio 1507
P. armata	Huampal, PE	JL. Marcelo X.245	*P. armata*	OrienteNuevo, PE	E. Honorio 1666
P. armata	LaEnvidia, BO	E. Honorio 1572	*P. armata*	OrienteNuevo, PE	E. Honorio 1667
P. armata	LaEnvidia, BO	E. Honorio 1576	*P. armata*	OrienteNuevo, PE	E. Honorio 1668
P. armata	LaEnvidia, BO	E. Honorio 1577	*P. armata*	OrienteNuevo, PE	E. Honorio 1669
P. armata	LaEnvidia, BO	E. Honorio 1581	*P. armata*	OrienteNuevo, PE	E. Honorio 1671
P. armata	LaEnvidia, BO	E. Honorio 1583	*P. armata*	Sacta, BO	E. Honorio 1520
P. armata	LaEnvidia, BO	E. Honorio 1588	*P. armata*	Sacta, BO	E. Honorio 1529
P. armata	LaEnvidia, BO	E. Honorio 1590	*P. armata*	Sacta, BO	E. Honorio 1535
P. armata	LaEnvidia, BO	E. Honorio 1594	*P. armata*	Sacta, BO	E. Honorio 1539
P. armata	LaEnvidia, BO	E. Honorio 1595	*P. armata*	Sacta, BO	E. Honorio 1543
P. armata	LaEnvidia, BO	E. Honorio 1596	*P. armata*	Sacta, BO	E. Honorio 1549
P. armata	LosAmigos, PE	K. Dexter 218	*P. armata*	Sacta, BO	E. Honorio 1550
P. armata	LosAmigos, PE	K. Dexter 240	*P. armata*	Sacta, BO	E. Honorio 1551
P. armata	LosAmigos, PE	K. Dexter 241	*P. armata*	Tambopata, PE	K. Dexter 3566
P. armata	LosAmigos, PE	K. Dexter 85	*P. armata*	Tambopata, PE	K. Dexter 3576
P. armata	LosAmigos, PE	K. Dexter 93	*P. armata*	Tambopata, PE	K. Dexter 3631
P. armata	Madreselva, PE	E. Honorio 1017	*P. armata*	Tambopata, PE	K. Dexter 3642
P. armata	Madreselva, PE	K. Dexter 137	*P. armata*	Tambopata, PE	K. Dexter 3836

Appendix S3.3. Haplotypes and polymorphic sites of the plastid (*trnH-psbA*) and nuclear (ITS) sequences sampled from five widespread species in western Amazonia. ID = insertion/deletion, NS = nucleotide substitution, IV = inversion.

	trnH-psbA												ITS		
	I	N	I	N	I	N	N	N	I	N	N	N	I	I	N
Ficus insipida	D	S	V	S	D	S	S	S	D	S	S	S	D	D	S
	0	0	0	0	0	0	1	1	1	2	2	3	4	4	5
	4	5	6	7	7	8	1	2	3	5	6	2	0	0	8
	5	0	0	5	6	7	1	0	5	8	7	5	6	7	0
H1	-	A	T	T	-	G	T	A	-	T	A	G	-	-	T
H2	-	A	T	T	-	G	T	A	-	T	A	T	C	C	T
H3	A	A	T	T	A	G	T	A	-	T	G	G	C	C	C
H4	-	A	A	T	-	G	T	A	-	T	A	G	C	-	T
H5	A	A	A	T	A	G	T	A	-	T	G	G			
H6	-	A	T	T	A	G	T	A	-	T	G	G			
H7	-	A	T	T	-	G	T	T	-	T	A	G			
H8	-	A	A	T	-	G	A	A	-	T	A	G			
H9	A	C	A	G	A	G	T	A	-	T	G	G			
H10	-	A	T	T	-	G	T	A	T	T	A	G			
H11	-	A	T	T	-	A	T	A	-	A	A	G			
H12	A	C	T	G	A	G	T	A	-	T	G	G			

	N	N	N	N	N	I	I	I	N	N	N	N	I	I	I	I	N	N	N	N	N	N	N	I	N	N	I	N	N	I	N	I	N	N	N
	S	S	S	S	S	V	D	D	S	S	S	S	D	V	V	D	S	S	S	S	S	S	S	V	S	S	D	S	S	D	S	D	S	S	S
Jacaratia digitata	0	0	0	0	0	0	1	1	1	1	1	1	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	4	5	5	5	1	2	4
	0	0	4	4	8	8	3	4	7	7	8	9	0	2	6	6	4	6	6	7	7	8	9	0	2	6	6	8	9	0	1	2	6	1	2
	4	7	0	5	2	3	2	7	1	4	7	3	7	1	0	7	5	0	8	4	9	1	0	0	0	4	9	6	5	5	7	0	1	7	4
H1	C	C	G	C	C	C	-	-	T	T	A	G	-	A	A	-	T	T	A	C	G	A	C	G	A	T	A	G	T	-	G	G	C	C	G
H2	C	C	G	A	A	T	-	-	T	A	A	T	-	A	A	G	T	G	C	C	T	A	G	T	A	T	-	G	A	-	G	-	C	C	T

H3	C	C	G	C	C	C	-	-	T	T	A	G	-	A	A	-	T	T	A	T	G	A	C	G	A	T	A	G	T	-	G	G
H4	T	T	G	C	A	T	A	A	G	A	C	T	T	T	T	-	G	G	C	C	T	A	C	G	C	G	-	G	A	-	T	G
H5	C	C	T	C	C	C	-	-	T	T	A	G	-	A	A	-	T	T	A	C	G	A	C	G	A	T	A	G	T	-	G	G
H6	T	T	G	C	A	T	A	A	G	A	C	T	T	T	T	-	G	G	A	C	T	C	C	G	C	G	-	T	A	T	T	G

H3	T	T	G
H4			
H5			
H6			

Clarisia biflora	N	I	N	N	N	I	N	N	N	N	N	N	N	N	N
	S	V	S	S	S	D	S	S	S	S	S	S	S	S	S
	0	0	1	1	1	1	1	2	2	2	2	2	3	3	3
	1	8	2	3	4	5	9	2	2	3	8	8	1	2	3
	1	7	9	7	5	7	5	0	9	1	3	4	8	2	2
H1	T	T	A	C	T	A	A	C	T	C	T	G	A	C	A
H2	T	A	A	A	T	A	A	C	G	C	T	A	A	A	A
H3	T	A	A	C	T	A	A	C	G	C	A	A	A	A	A
H4	T	T	A	C	T	A	A	C	G	C	T	A	A	A	A
H5	T	A	A	C	T	A	G	C	G	G	T	A	A	A	A
H6	T	T	A	C	T	A	A	C	T	C	T	A	A	C	A
H7	T	T	A	C	T	A	A	C	T	C	T	A	A	C	C
H8	T	A	C	C	T	A	A	C	G	C	T	A	A	A	A
H9	G	T	A	C	T	A	A	C	G	C	T	A	A	A	A
H10	T	A	A	C	T	-	A	C	G	C	T	A	A	A	A
H11	T	T	A	C	T	-	A	C	G	C	T	A	A	A	A
H12	T	A	A	C	G	A	A	C	G	C	T	A	A	A	A
H13	T	T	A	C	G	A	A	C	G	C	T	A	T	A	C
H14	T	T	C	C	T	A	A	C	G	C	T	A	A	A	A
H15	T	T	A	C	T	A	A	C	T	C	T	C	A	C	A
H16	T	T	A	C	T	A	A	T	G	C	T	A	A	A	A
H17															
H18															
H19															
H20															
H21															

Clarisia biflora	N	N	N	N	N	N	N	N	N	N	N	N	N	N	I	N	N	N	N	N
	S	S	S	S	S	S	S	S	S	S	S	S	S	S	D	S	S	S	S	S
	0	0	0	0	1	1	1	1	3	4	4	4	4	4	4	4	4	4	5	5
	4	5	7	7	5	6	7	8	9	1	1	1	2	3	4	4	8	8	2	2
	2	4	1	3	6	7	7	8	8	2	4	9	5	5	2	8	2	3	3	5
H1	C	T	G	T	T	A	T	C	C	T	C	C	A	C	-	A	T	C	T	C
H2	C	T	G	T	C	G	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H3	C	C	G	T	T	G	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H4	C	T	G	T	T	G	T	C	C	T	C	C	A	C	G	A	T	C	T	C
H5	C	T	A	T	T	A	T	C	C	C	C	C	A	C	G	G	T	G	T	C
H6	C	T	G	T	C	G	T	C	C	T	C	C	A	C	G	A	T	C	T	C
H7	C	T	G	T	T	A	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H8	C	T	G	T	T	A	T	C	C	T	C	C	A	C	G	A	T	C	T	C
H9	C	C	G	T	C	G	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H10	T	T	A	T	T	A	T	C	C	T	C	C	A	C	G	G	T	G	T	A
H11	C	T	A	T	T	A	T	C	C	C	C	C	A	C	G	G	C	G	T	C
H12	C	T	G	T	T	G	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H13	C	T	G	T	T	A	T	C	C	T	C	C	G	C	-	A	T	C	T	C
H14	C	T	A	T	T	A	T	C	C	T	C	C	A	C	-	A	T	G	T	C
H15	C	T	A	T	T	A	T	C	C	C	T	C	A	C	G	G	T	G	T	C
H16	C	T	A	T	T	A	T	C	C	C	C	A	A	C	G	A	T	G	T	C
H17	T	T	A	T	T	A	T	C	C	T	C	C	A	C	G	A	T	G	T	A
H18	C	T	G	T	T	A	T	C	C	C	C	C	A	C	G	G	T	G	T	C
H19	C	T	A	T	T	A	T	C	C	C	C	C	A	C	G	A	T	G	T	C
H20	C	T	G	T	T	A	C	T	C	T	C	C	A	C	-	A	T	C	T	C
H21	C	T	G	T	T	A	T	C	C	T	C	C	A	T	G	A	T	C	T	C

H22	C	T	A	T	T	A	T	C	C	T	C	C	A	C	G	G	T	G	T	C
H23	C	T	G	T	T	A	T	C	C	T	T	C	A	C	G	A	T	C	T	C
H24	C	T	G	T	C	A	T	C	C	T	C	C	A	C	G	A	T	C	T	C
H25	C	T	G	T	C	A	T	C	A	T	C	C	A	C	G	A	T	C	T	C
H26	C	T	G	C	T	A	T	C	C	T	C	C	A	C	G	A	T	C	T	C
H27	C	T	G	T	T	G	T	C	A	T	C	C	A	C	G	A	T	C	C	C

	N	N	I	N	N	N	N	N	N	N	N
	S	S	V	S	S	S	S	S	S	S	S
Poulsenia armata	0	1	1	1	1	1	3	3	3	3	3
	9	0	0	1	6	8	0	2	3	4	5
	7	2	3	4	9	8	9	0	3	4	4
H1	T	G	A	A	T	T	A	T	G	T	T
H2	T	G	A	G	T	T	A	T	G	T	T
H3	C	C	T	A	T	T	A	T	G	T	A
H4	C	C	T	A	T	T	A	T	G	T	T
H5	C	G	T	A	A	G	C	T	G	G	A
H6	C	G	T	A	A	G	C	T	A	G	A
H7	C	G	T	A	A	G	C	G	G	G	A

	N	I	N	N	N	N	I	N	I	N	N	N	N	N	N	N
	S	D	S	S	S	S	V	S	D	S	S	S	S	S	S	S
Poulsenia armata	0	0	0	0	1	1	1	1	3	3	4	4	4	5	5	6
	5	6	8	9	3	6	8	9	9	9	1	2	2	4	9	1
	5	4	4	3	6	9	6	2	5	6	9	0	1	9	1	8
H1	T	-	C	T	A	C	C	T	-	A	T	C	G	A	C	T
H2	T	-	C	C	A	C	C	T	C	A	T	C	G	A	C	C
H3	G	T	A	C	A	T	T	C	-	G	C	C	T	C	T	C
H4	T	-	C	C	A	C	C	T	-	A	T	C	G	A	C	C
H5	T	-	C	C	T	C	C	T	-	A	T	C	G	A	C	T
H6	T	-	C	C	A	C	C	T	-	A	T	C	G	A	C	T
H7	T	-	C	C	A	C	C	T	-	A	T	T	G	A	C	C

	I	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	V	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Otoba parvifolia	0	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	3	3	3	3
	7	1	2	2	3	4	4	4	1	2	3	3	3	5	8	0	0	0	1	1
	6	9	4	5	7	4	5	6	3	8	3	5	6	5	5	3	4	9	0	1
H1	C	G	T	T	A	A	A	A	T	C	A	T	T	C	A	A	A	T	G	A
H2	G	G	T	T	A	A	A	A	T	C	A	T	T	A	A	A	A	T	T	A
H3	G	G	A	T	A	A	A	A	T	C	T	T	G	C	A	A	A	G	G	A
H4	G	G	T	T	A	A	C	T	T	C	A	T	T	C	A	A	A	T	T	A
H5	G	G	T	T	A	A	A	A	T	C	A	T	T	C	C	A	A	T	T	T
H6	C	G	T	T	A	A	A	A	T	C	A	T	T	C	C	A	A	T	T	T

H7	G	G	T	T	A	A	A	A	T	C	A	T	T	C	A	A	A	T	G	A
H8	G	G	T	T	C	A	A	A	T	C	A	T	T	C	C	A	A	T	T	T
H9	G	G	T	T	A	A	A	A	T	A	A	T	T	C	A	A	A	T	T	A
H10	C	G	T	T	A	A	A	A	T	A	A	T	T	C	A	A	A	T	T	A
H11	G	G	T	G	A	C	C	A	T	A	A	T	T	A	A	C	C	T	T	A
H12	C	G	T	T	A	A	A	A	A	C	A	T	T	C	A	A	A	T	T	A
H13	G	G	T	T	A	A	A	A	T	C	A	T	T	A	A	C	A	T	T	A
H14	C	G	T	T	A	A	A	A	T	C	A	G	T	C	C	A	A	T	T	T
H15	G	G	T	G	A	C	C	A	T	A	A	T	T	A	A	C	A	T	T	A
H16	C	G	T	T	A	A	A	A	T	C	A	T	T	T	A	A	A	T	G	A
H17	G	T	T	T	A	A	A	A	T	C	A	T	T	C	A	A	A	T	G	A

Coded sites:

Ficus insipida, ID045: -/ AAAAT, IV060: TTCTAT/ ATAGAA, ID076: -/ ATTTT, ID135: -/ TATTTGTCTTTT

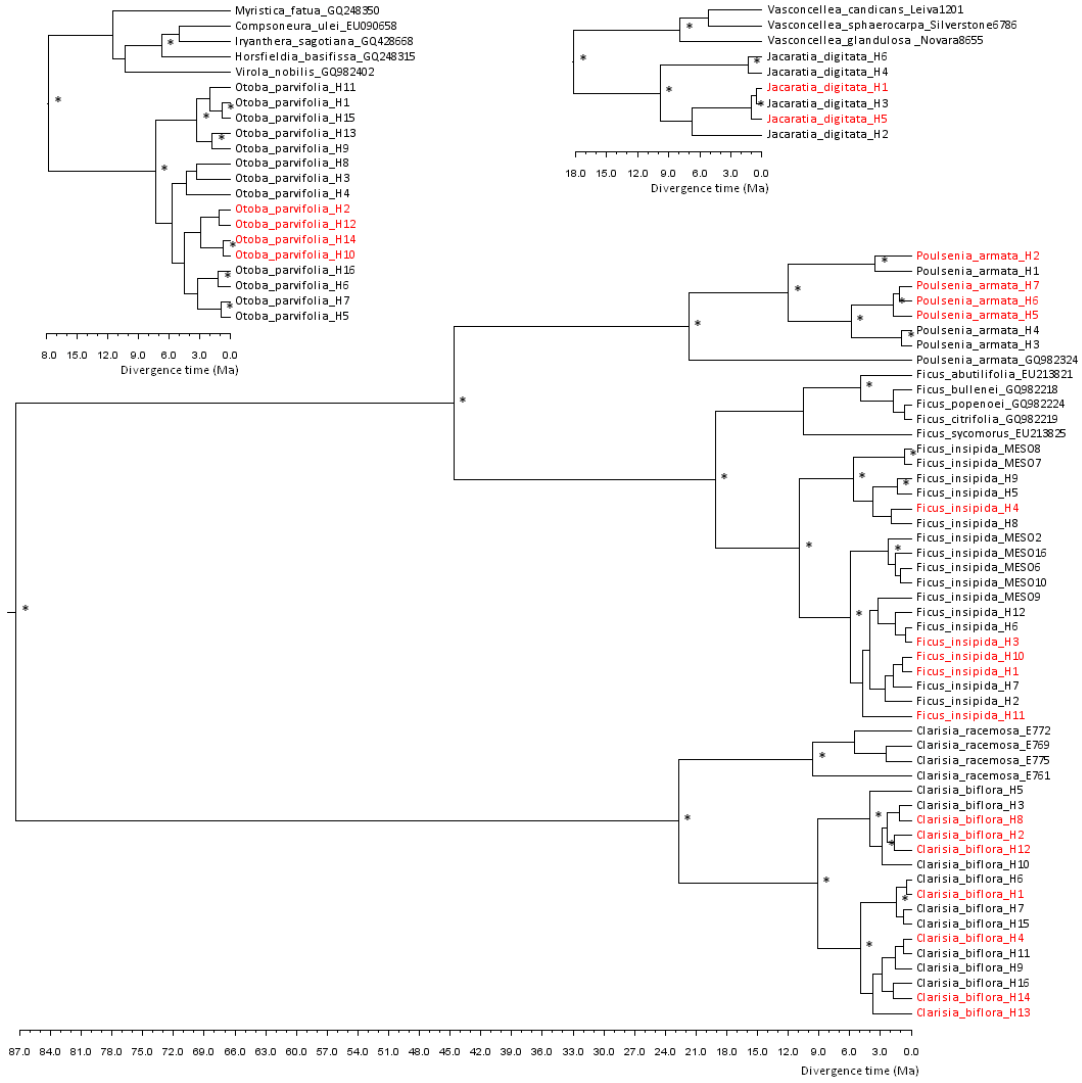
Jacaratia digitata, IV083: CAACCTTCTTGATAGAACAAGAAAGTTTA/TAAACTTTCTTGTTCTATCAAGAAGGTTG, ID132: ATTTAG/-, ID147: ATTTAG/-, ID207: TTCATAAATTTT/-, IV221: AACAAAAGTTTTTATGCTCTCAACATAAAATAAGAAA/ TTTCTTATTTTATGTTGAGAGCATAAAAACTTTTGTT, IV260: AAAAAAT/ TAAAAAA, ID267: GTTATTA/-, ID469: AATTTAT/-, ID505: TTAAATA/-, ID 520: GTATAAAAT/-

Clarisia biflora, IV87: TGAT/ATCA, ID 157: AGTCTTTTACTTAAGATAACGTCTTTTTT/-

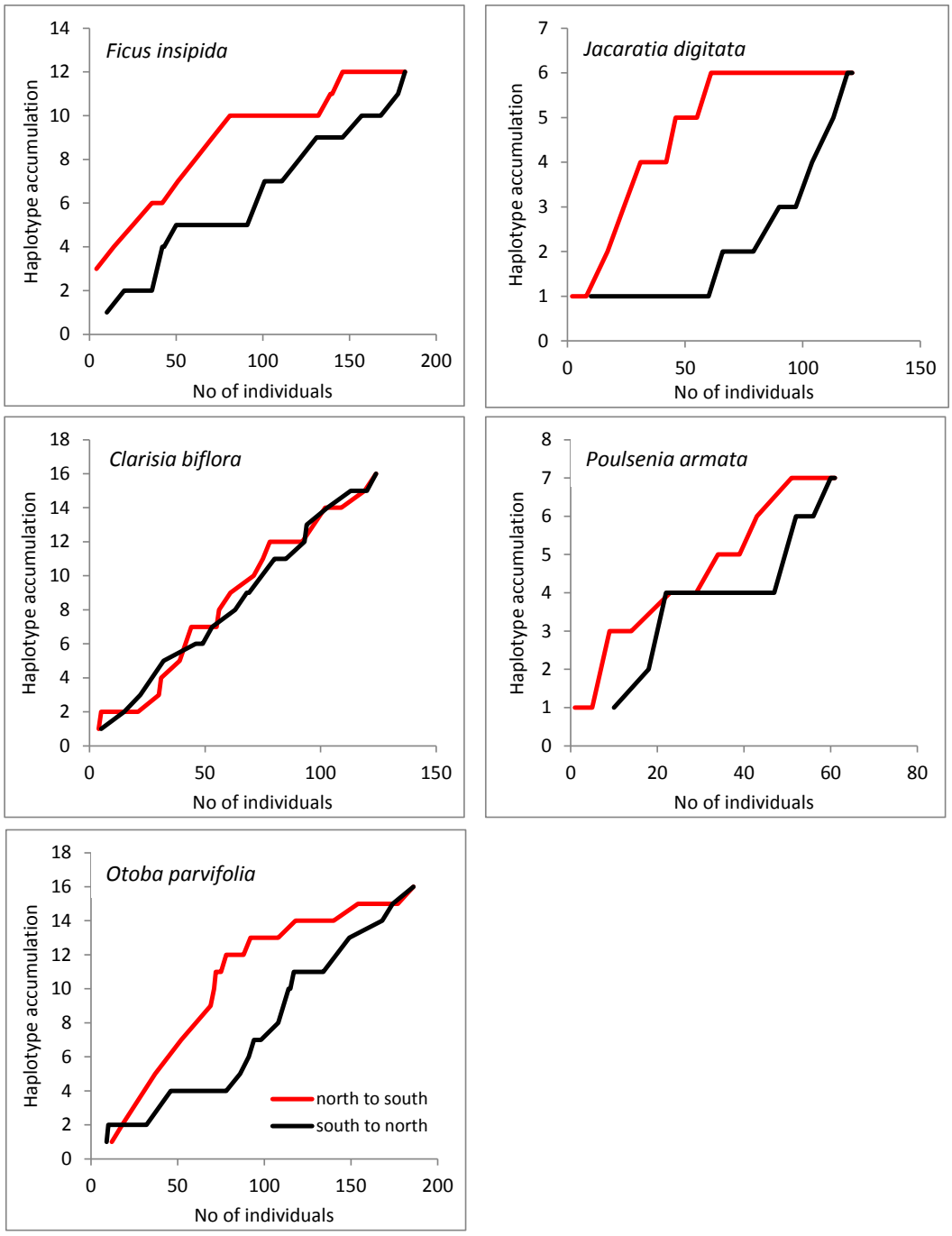
Poulsenia armata, IV103: ATGAA/ TTCTAT

Otoba parvifolia, IV076: CTTC/GAAG

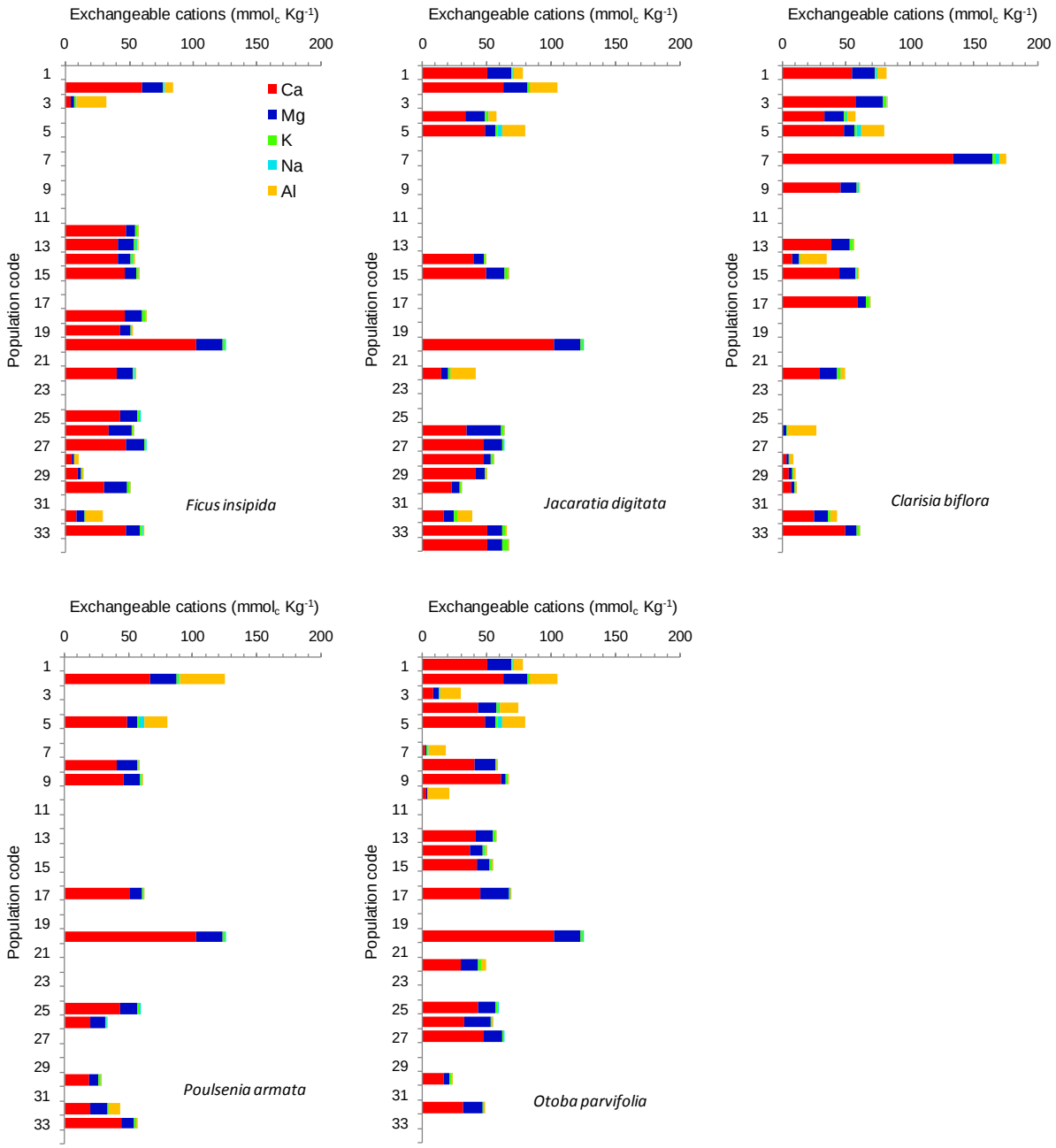
Appendix S3.4. Dated phylogenies for five study species including outgroup taxa. Lineages of the study species occur in the south are in red, although some of them could also occur in the north (see Figure 3.3). Nodes with posterior probabilities ≥ 0.90 are indicated with an asterisk.



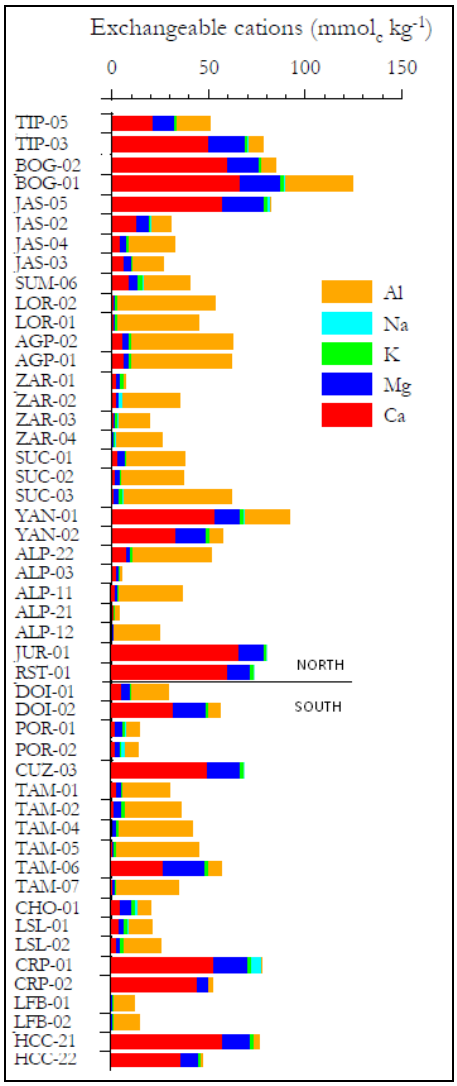
Appendix S3.5. Plastid haplotype accumulation by increasing number of individuals for each species across Amazonia. The accumulation of haplotypes is calculated from north to south (red) and from south to north (black). Note how the number of plastid haplotypes clearly accumulates more rapidly from north to south in four of the five species, illustrating the concentration of haplotypes clearly in the north, and by extension the apparent more recent population expansion in the south.

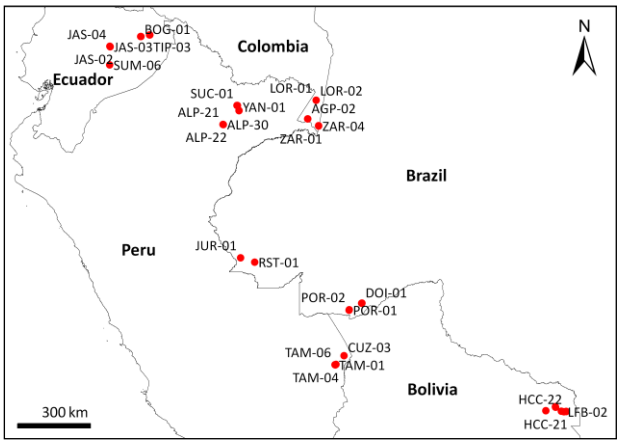


Appendix S3.6. Concentration of exchangeable cations for five widespread species across western Amazonia. Population codes as shown in Appendix S3.1 and these are ordered from north to south. Units: 1 $mmol_c\ Kg^{-1}$ = 10 $cmol_c\ Kg^{-1}$

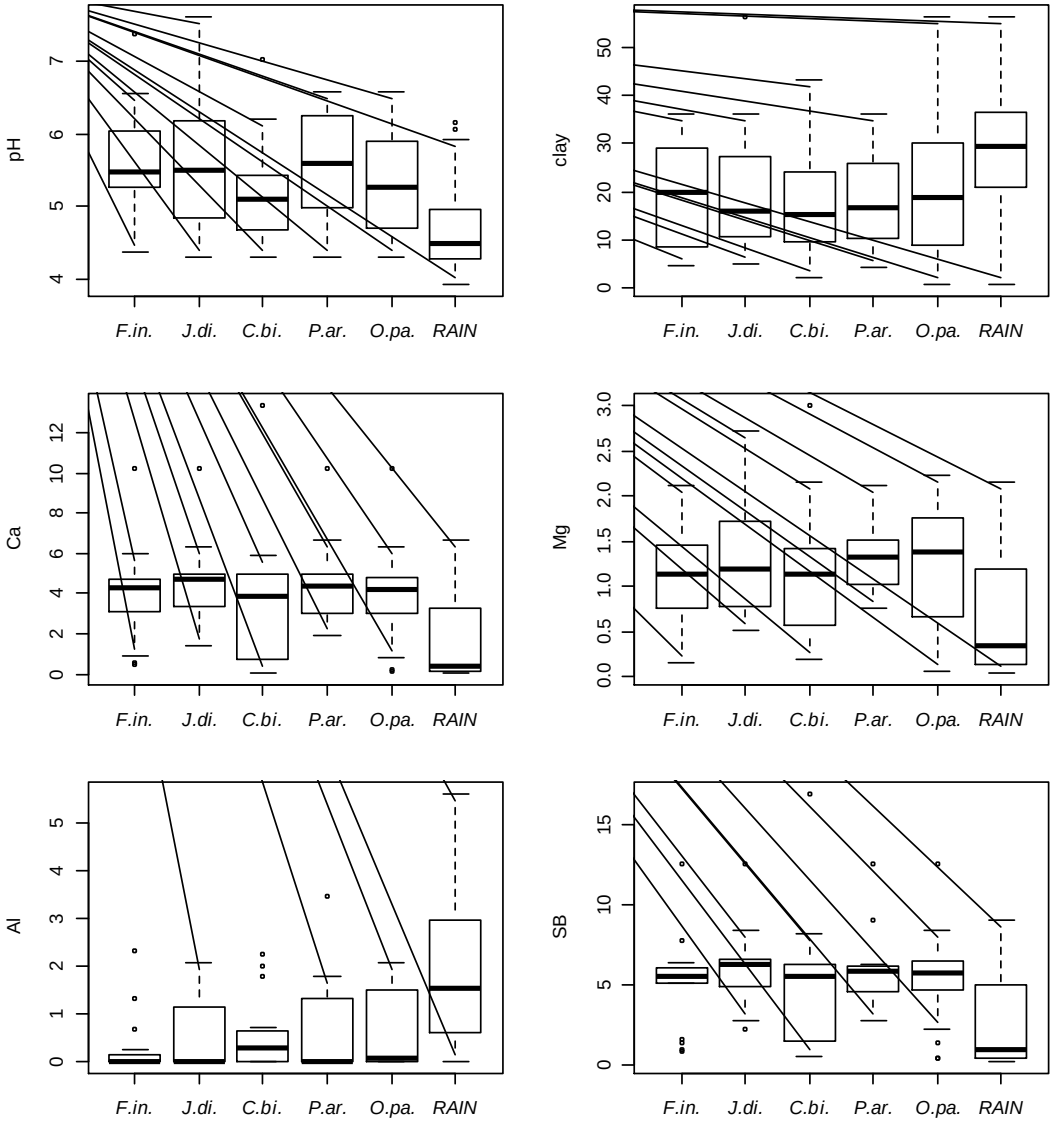


Appendix S3.7. Concentration of exchangeable cations for different sites across western Amazonia based on soil analysis of RAINFOR permanent plots (from Quesada *et al.*, 2010). The north-south regional division used in the chapter is indicated in the graph. Geographical location of each plot is indicated in the map.

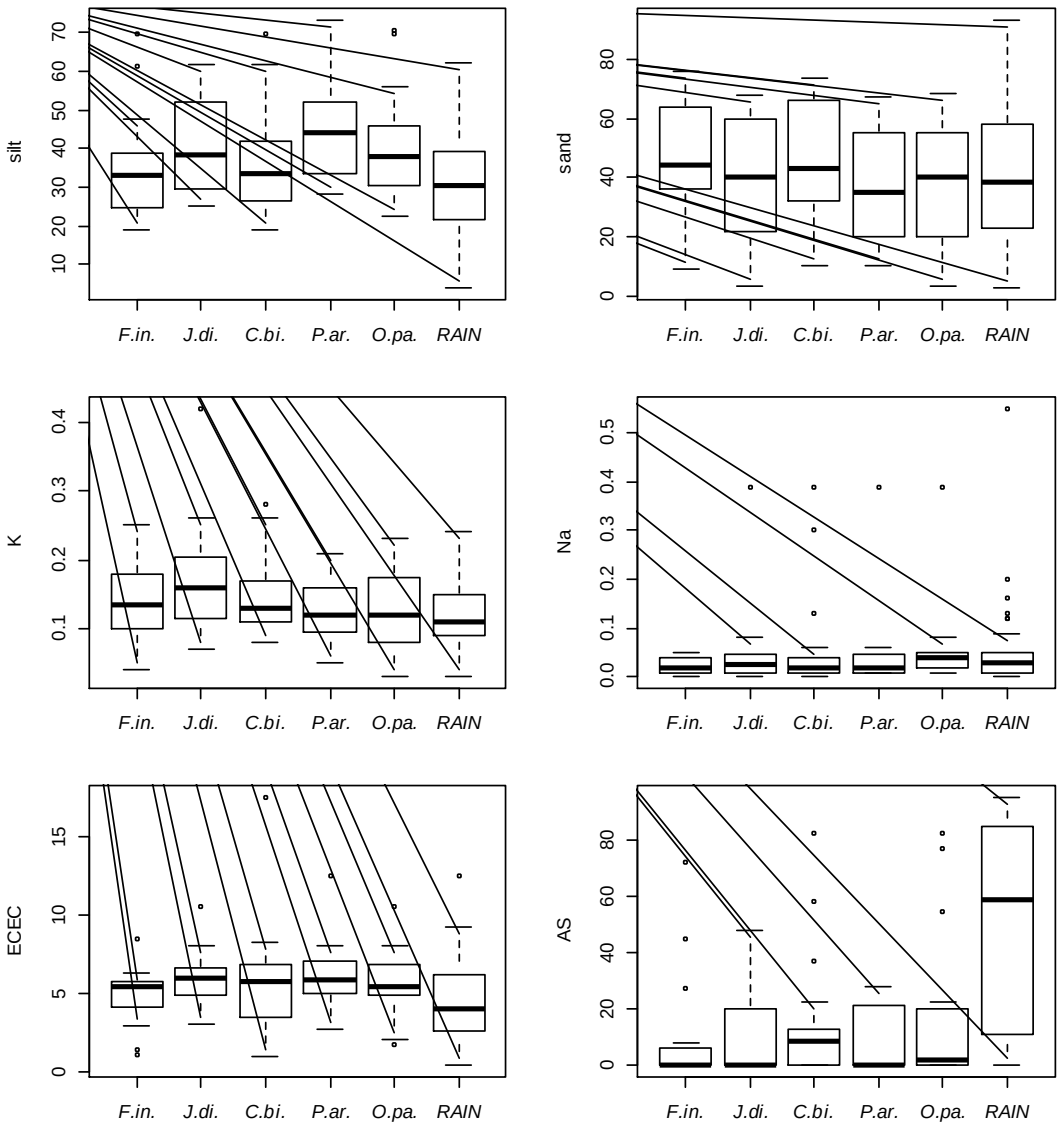




Appendix S3.8. Comparison of physical and chemical properties for soil of each widespread species and RAINFOR sites. Units: Particle sizes in %, cations, sum of bases, and ECEC in $cmol_c\ Kg^{-1}$, and aluminium saturation in %.



Appendix S3.8 [continued]



Appendix C. Supporting information of chapter 4

Appendix S4.1. Floristic tree inventories (n = 283 plots) compiled from RAINFOR forest plot network. Total number of angiosperm individuals, and proportion of individuals identified to species are provided. Regions: Brazilian Shield (BSh), central Amazonia (CA), eastern Amazonia (EA), Guiana Shield (GSh), Northern Andes (NSA), north-western Amazonia (NWA), south-western Amazonia (SWA).

Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	BSh	Flooded	Bolivia	HCC-11	-13.91	-60.82	1.0	582	67.4
Trop. rain forest	BSh	Flooded	Bolivia	HCC-12	-13.91	-60.82	1.0	725	66.9
Trop. rain forest	BSh	Flooded	Bolivia	LGB-01	-14.80	-60.39	1.0	656	76.4
Trop. rain forest	BSh	Flooded	Bolivia	LSL-01	-14.40	-61.14	1.0	501	82.6
Trop. rain forest	BSh	Flooded	Bolivia	LSL-02	-14.40	-61.14	1.0	631	81.3
Trop. rain forest	BSh	Flooded	Bolivia	NCR-01	-14.64	-61.16	1.0	566	75.1
Trop. rain forest	BSh	Flooded	Bolivia	NCR-02	-14.71	-61.15	1.0	590	75.3
Trop. rain forest	BSh	Flooded	Brazil	NXV-06	-14.72	-52.36	1.4	719	97.8
Trop. rain forest	BSh	Flooded	Brazil	PEA-01	-12.15	-50.83	0.4	330	100.0
Trop. rain forest	BSh	Flooded	Brazil	PEA-02	-12.32	-50.74	0.4	461	100.0
Trop. rain forest	BSh	Flooded	Brazil	PEA-03	-12.38	-50.89	1.0	1579	99.9
Trop. rain forest	BSh	Flooded	Brazil	PEA-04	-12.42	-50.71	1.0	1278	100.0
Trop. rain forest	BSh	Flooded	Brazil	PEA-05	-11.90	-50.75	1.0	966	99.8
Trop. rain forest	BSh	Flooded	Brazil	PEA-06	-11.92	-50.71	1.0	922	99.6
Trop. rain forest	BSh	Flooded	Brazil	PEA-07	-12.48	-50.90	1.0	472	100.0
Trop. rain forest	BSh	Flooded	Brazil	PEA-08	-12.54	-50.74	1.0	426	100.0
Trop. rain forest	BSh	Terra firme	Bolivia	BBC-01	-14.30	-60.53	1.0	532	81.0
Trop. rain forest	BSh	Terra firme	Bolivia	BBC-02	-14.30	-60.53	1.0	543	81.0
Trop. rain forest	BSh	Terra firme	Bolivia	CHO-01	-14.39	-61.15	1.0	729	86.0
Trop. rain forest	BSh	Terra firme	Bolivia	CHO-02	-14.34	-61.16	1.0	544	74.6
Trop. rain forest	BSh	Terra firme	Bolivia	HCC-21	-14.53	-60.74	1.0	529	90.9
Trop. rain forest	BSh	Terra firme	Bolivia	HCC-22	-14.53	-60.73	1.0	613	85.6
Trop. rain forest	BSh	Terra firme	Bolivia	HCC-23	-14.56	-60.75	1.0	712	86.5
Trop. rain forest	BSh	Terra firme	Bolivia	HCC-24	-14.57	-60.75	1.0	616	83.3
Trop. rain forest	BSh	Terra firme	Bolivia	KEN-01	-16.02	-62.73	1.0	450	99.8
Trop. rain forest	BSh	Terra firme	Bolivia	LCA-13	-15.68	-62.78	1.0	423	82.7
Trop. rain forest	BSh	Terra firme	Bolivia	LCA-16	-15.68	-62.78	1.0	385	74.5
Trop. rain forest	BSh	Terra firme	Bolivia	LCA-29	-15.68	-62.77	1.0	394	84.5
Trop. rain forest	BSh	Terra firme	Bolivia	LCA-30	-15.68	-62.77	1.0	417	85.9
Trop. rain forest	BSh	Terra firme	Bolivia	LFB-01	-14.58	-60.83	1.0	564	89.7
Trop. rain forest	BSh	Terra firme	Bolivia	LFB-02	-14.58	-60.83	1.0	536	92.0
Trop. rain forest	BSh	Terra firme	Bolivia	MVE-01	-15.01	-61.13	1.0	567	89.9
Trop. rain forest	BSh	Terra firme	Bolivia	SRQ-01	-14.40	-62.30	1.0	291	97.9
Trop. rain forest	BSh	Terra firme	Brazil	ALF-01	-9.60	-55.94	1.0	514	93.0
Trop. rain forest	BSh	Terra firme	Brazil	ALF-02	-9.58	-55.92	1.0	537	96.3
Trop. rain forest	BSh	Terra firme	Brazil	AMD-01	-1.83	-46.75	1.0	536	92.5
Trop. rain forest	BSh	Terra firme	Brazil	AMD-02	-1.83	-46.75	1.0	453	94.3
Trop. rain forest	BSh	Terra firme	Brazil	ARA-01	-4.82	-52.52	1.0	439	80.4
Trop. rain forest	BSh	Terra firme	Brazil	ASR-01	-4.76	-52.60	1.0	455	88.6
Trop. rain forest	BSh	Terra firme	Brazil	CAR-01	-5.58	-49.72	1.0	482	84.6

Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	BSh	Terra firme	Brazil	CPP-01	-1.84	-47.10	1.0	477	93.9
Trop. rain forest	BSh	Terra firme	Brazil	CPP-02	-1.84	-47.10	1.0	465	97.4
Trop. rain forest	BSh	Terra firme	Brazil	CRG-01	-5.90	-50.13	1.0	438	91.3
Trop. rain forest	BSh	Terra firme	Brazil	FLO-01	-12.81	-51.85	1.0	604	97.7
Trop. rain forest	BSh	Terra firme	Brazil	FLO-02	-12.76	-51.88	1.0	484	96.1
Trop. rain forest	BSh	Terra firme	Brazil	LFA-01	-5.85	-50.48	1.0	460	95.4
Trop. rain forest	BSh	Terra firme	Brazil	MCP-01	-5.88	-50.47	1.0	536	86.2
Trop. rain forest	BSh	Terra firme	Brazil	MRB-01	-5.73	-49.05	2.0	1035	97.2
Trop. rain forest	BSh	Terra firme	Brazil	MRB-02	-5.72	-49.03	2.0	1084	96.6
Trop. rain forest	BSh	Terra firme	Brazil	MRB-03	-5.70	-490.00	2.0	997	94.4
Trop. rain forest	BSh	Terra firme	Brazil	NXV-02	-14.70	-52.35	1.0	564	99.8
Trop. rain forest	BSh	Terra firme	Brazil	NXV-04	-14.70	-52.35	0.5	372	89.2
Trop. rain forest	BSh	Terra firme	Brazil	NXV-09	-14.69	-52.35	0.5	359	98.3
Trop. rain forest	BSh	Terra firme	Brazil	ODE-01	-3.48	-51.67	3.0	1413	70.8
Trop. rain forest	BSh	Terra firme	Brazil	RBR-01	-11.00	-61.95	1.0	560	85.2
Trop. rain forest	BSh	Terra firme	Brazil	RIA-01	-2.90	-46.15	4.0	1937	93.3
Trop. rain forest	BSh	Terra firme	Brazil	SAA-01	-9.79	-50.43	1.0	509	90.6
Trop. rain forest	BSh	Terra firme	Brazil	SMT-02	-12.82	-51.77	1.0	444	100.0
Trop. rain forest	BSh	Terra firme	Brazil	SNP-01	-6.04	-50.15	1.0	382	87.4
Trop. rain forest	BSh	Terra firme	Brazil	TAN-02	-13.09	-52.38	1.0	476	90.3
Trop. rain forest	BSh	Terra firme	Brazil	TAN-03	-12.82	-52.36	1.0	589	87.4
Trop. rain forest	BSh	Terra firme	Brazil	TAN-04	-12.92	-52.37	1.0	578	95.7
Trop. rain forest	BSh	Terra firme	Brazil	VCR-04	-14.83	-52.17	1.0	459	96.5
Trop. rain forest	CA	Terra firme	Brazil	BDF-01	-2.34	-60.10	2.0	1330	91.5
Trop. rain forest	CA	Terra firme	Brazil	BDF-03	-2.42	-59.85	1.0	592	83.3
Trop. rain forest	CA	Terra firme	Brazil	BDF-04	-2.43	-59.85	1.0	590	90.5
Trop. rain forest	CA	Terra firme	Brazil	BDF-05	-2.43	-59.85	1.0	652	91.7
Trop. rain forest	CA	Terra firme	Brazil	BDF-06	-2.41	-59.86	3.0	1896	90.5
Trop. rain forest	CA	Terra firme	Brazil	BDF-07	-2.40	-59.90	1.0	638	87.3
Trop. rain forest	CA	Terra firme	Brazil	BDF-08	-2.40	-59.90	1.0	592	86.0
Trop. rain forest	CA	Terra firme	Brazil	BDF-09	-2.40	-59.85	1.0	575	86.3
Trop. rain forest	CA	Terra firme	Brazil	BDF-10	-2.39	-59.86	2.0	1241	84.9
Trop. rain forest	CA	Terra firme	Brazil	BDF-11	-2.38	-59.85	3.0	1826	89.9
Trop. rain forest	CA	Terra firme	Brazil	BDF-12	-2.39	-59.85	2.0	1211	88.9
Trop. rain forest	CA	Terra firme	Brazil	BDF-13	-2.40	-59.91	9.0	5192	84.1
Trop. rain forest	CA	Terra firme	Brazil	BDF-14	-2.36	-59.97	1.0	685	90.7
Trop. rain forest	CA	Terra firme	Brazil	BNT-01	-2.64	-60.16	1.0	567	69.1
Trop. rain forest	CA	Terra firme	Brazil	BNT-04	-2.63	-60.15	1.0	612	70.1
Trop. rain forest	CA	Terra firme	Brazil	BNT-05	-2.63	-60.17	1.0	560	67.3
Trop. rain forest	CA	Terra firme	Brazil	BNT-07	-2.63	-60.17	1.0	643	65.6
Trop. rain forest	CA	Terra firme	Brazil	TEM-01	-2.97	-59.90	1.0	576	93.1
Trop. rain forest	CA	Terra firme	Brazil	TEM-02	-2.93	-59.95	1.0	599	92.8
Trop. rain forest	CA	Terra firme	Brazil	TEM-03	-2.41	-59.90	1.0	674	88.4
Trop. rain forest	CA	Terra firme	Brazil	TEM-04	-2.43	-59.79	1.0	581	90.2
Trop. rain forest	CA	Terra firme	Brazil	TEM-05	-2.62	-60.21	1.0	612	94.9
Trop. rain forest	CA	Terra firme	Brazil	TEM-06	-2.60	-60.11	1.0	693	91.2
Trop. rain forest	EA	Flooded	Brazil	TEC-01	-1.71	-51.46	1.0	501	70.1
Trop. rain forest	EA	Flooded	Brazil	TEC-06	-1.73	-51.43	1.0	456	75.2
Trop. rain forest	EA	Terra firme	Brazil	CAX-01	-1.74	-51.46	1.0	515	86.6
Trop. rain forest	EA	Terra firme	Brazil	CAX-02	-1.74	-51.46	1.0	523	74.4
Trop. rain forest	EA	Terra firme	Brazil	GMT-01	-1.11	-47.80	1.0	505	89.5
Trop. rain forest	EA	Terra firme	Brazil	JBU-01	-1.14	-47.70	1.0	456	91.2
Trop. rain forest	EA	Terra firme	Brazil	JRI-01	-0.89	-52.19	1.0	599	77.0
Trop. rain forest	EA	Terra firme	Brazil	PPB-01	-1.18	-47.32	1.0	415	94.9
Trop. rain forest	EA	Terra firme	Brazil	PPB-02	-1.18	-47.32	1.0	497	89.5
Trop. rain forest	EA	Terra firme	Brazil	PPB-03	-1.18	-47.32	1.0	440	97.3

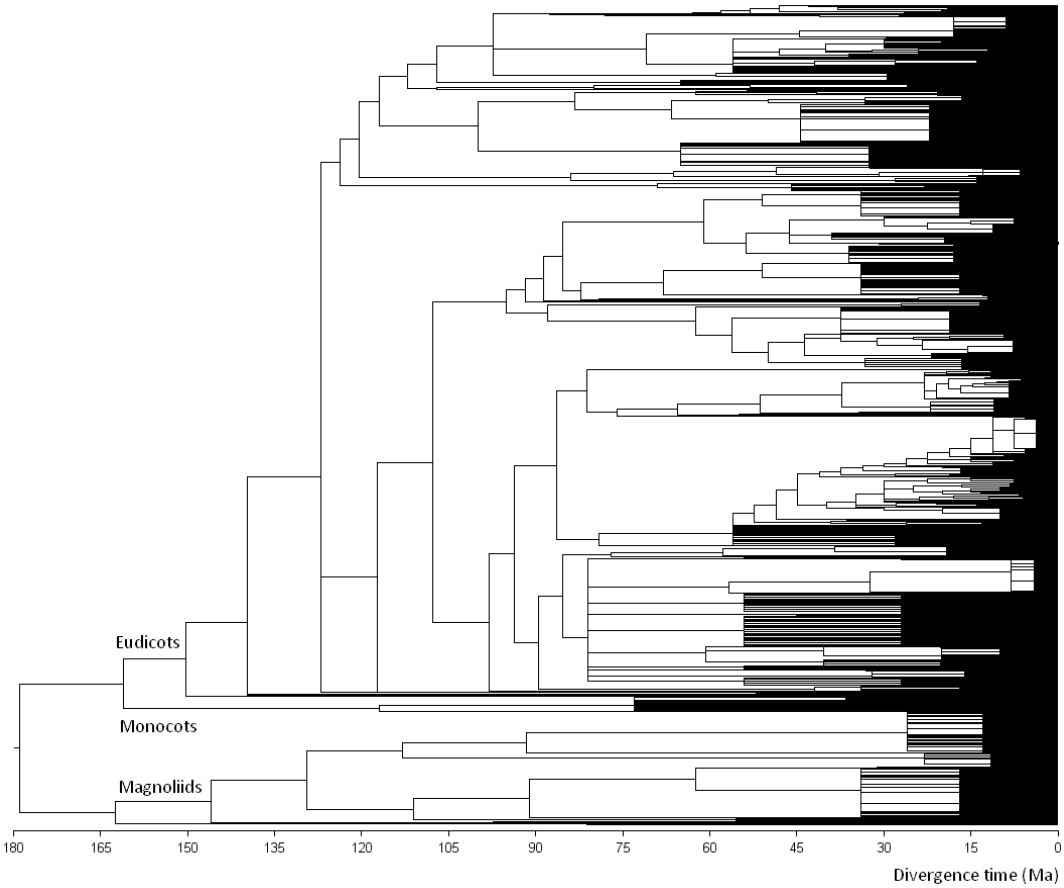
Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	EA	Terra firme	Brazil	PTB-01	-1.17	-56.41	1.0	438	95.7
Trop. rain forest	EA	Terra firme	Brazil	PTB-02	-1.48	-56.39	1.0	502	97.4
Trop. rain forest	EA	Terra firme	Brazil	SRT-01	-1.46	-47.92	1.0	503	93.6
Trop. rain forest	EA	Terra firme	Brazil	TAP-01	-3.31	-54.94	1.0	561	77.2
Trop. rain forest	EA	Terra firme	Brazil	TAP-02	-3.31	-54.95	1.0	479	81.0
Trop. rain forest	EA	Terra firme	Brazil	TAP-03	-3.31	-54.94	1.0	525	77.0
Trop. rain forest	EA	Terra firme	Brazil	TEC-04	-1.75	-51.52	1.0	474	76.4
Trop. rain forest	EA	Terra firme	Brazil	TEC-05	-1.78	-51.59	1.0	510	67.3
Trop. rain forest	GSh	Flooded	Fr. Guiana	PAR-21	5.28	-52.92	1.0	590	87.6
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-02	4.09	-52.67	1.0	512	90.8
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-11	4.08	-52.68	1.0	524	94.1
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-12	4.08	-52.68	1.0	475	81.7
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-15	4.08	-52.68	1.0	482	83.8
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-17	4.08	-52.68	1.0	572	81.3
Trop. rain forest	GSh	Terra firme	Fr. Guiana	NOU-18	4.08	-52.68	1.0	567	81.8
Trop. rain forest	GSh	Terra firme	Fr. Guiana	PAR-20	5.28	-52.92	1.5	927	88.3
Trop. rain forest	GSh	Terra firme	Fr. Guiana	PAR-22	5.28	-52.92	1.5	968	82.2
Trop. rain forest	GSh	Terra firme	Fr. Guiana	PAR-23	5.28	-52.92	2.0	499	86.4
Trop. rain forest	GSh	Terra firme	Guyana	FMH-01	5.17	-58.69	1.0	455	95.8
Trop. rain forest	GSh	Terra firme	Guyana	FMH-02	5.17	-58.69	1.0	353	92.4
Trop. rain forest	GSh	Terra firme	Guyana	IWO-03	4.53	-58.78	1.0	563	75.0
Trop. rain forest	GSh	Terra firme	Guyana	IWO-11	4.62	-58.72	1.0	445	78.4
Trop. rain forest	GSh	Terra firme	Guyana	IWO-12	4.73	-58.72	1.0	450	77.1
Trop. rain forest	GSh	Terra firme	Guyana	IWO-22	4.62	-58.72	1.0	443	84.0
Trop. rain forest	GSh	Terra firme	Guyana	PIB-05	5.02	-58.62	1.0	470	86.8
Trop. rain forest	GSh	Terra firme	Guyana	PIB-06	5.01	-58.62	1.0	494	93.5
Trop. rain forest	GSh	Terra firme	Guyana	PIB-12	5.03	-58.60	1.0	407	84.5
Trop. rain forest	GSh	Terra firme	Suriname	BBS-04	4.97	-55.18	1.0	465	77.2
Trop. rain forest	GSh	Terra firme	Suriname	BBS-05	4.99	-55.20	1.0	515	82.7
Trop. rain forest	GSh	Terra firme	Suriname	BBS-07	4.92	-55.13	1.0	458	80.3
Trop. rain forest	GSh	Terra firme	Suriname	BBS-08	4.93	-55.14	1.0	555	75.7
Trop. rain forest	GSh	Terra firme	Suriname	LMS-04	4.25	-54.73	1.0	524	67.0
Trop. rain forest	GSh	Terra firme	Suriname	LMS-05	4.25	-54.73	1.0	981	76.8
Trop. rain forest	GSh	Terra firme	Suriname	LMS-06	4.26	-54.78	1.0	477	68.3
Trop. rain forest	GSh	Terra firme	Suriname	LMS-07	4.27	-54.78	1.0	476	74.2
Trop. rain forest	GSh	Terra firme	Suriname	LMS-08	4.27	-54.75	1.0	489	72.8
Trop. rain forest	GSh	Terra firme	Suriname	NMS-01	4.78	-54.62	1.0	475	69.1
Trop. rain forest	GSh	Terra firme	Suriname	NMS-03	4.82	-54.60	1.0	497	76.3
Trop. rain forest	GSh	Terra firme	Suriname	NMS-04	4.93	-54.52	1.0	739	78.3
Trop. rain forest	GSh	Terra firme	Suriname	NMS-05	4.93	-54.52	1.0	810	80.2
Trop. rain forest	GSh	Terra firme	Suriname	NMS-06	4.83	-54.61	1.0	607	68.7
Trop. rain forest	GSh	Terra firme	Venezuela	ELD-01	6.10	-61.39	1.0	576	94.6
Trop. rain forest	GSh	Terra firme	Venezuela	PTA-01	5.11	-67.74	1.0	500	89.6
Trop. rain forest	GSh	Terra firme	Venezuela	PTA-02	5.84	-67.45	1.0	436	95.4
Trop. rain forest	GSh	Terra firme	Venezuela	PTA-03	5.11	-67.74	1.0	593	94.4
Trop. rain forest	GSh	Terra firme	Venezuela	PTA-04	5.84	-67.49	1.0	407	87.0
Trop. rain forest	GSh	Terra firme	Venezuela	SCR-05	1.93	-67.04	1.0	681	93.1
Trop. rain forest	GSh	White sand	Guyana	FMH-03	5.18	-58.70	1.0	635	91.0
Trop. rain forest	GSh	White sand	Guyana	IWO-09	4.61	-58.73	1.0	675	70.2
Trop. rain forest	GSh	White sand	Guyana	IWO-21	4.63	-58.74	1.0	563	90.2
Trop. rain forest	GSh	White sand	Venezuela	SCR-04	1.93	-67.04	1.0	829	95.8
Trop. rain forest	NSA	Flooded	Colombia	AMA-02	5.58	-77.50	1.0	453	81.9
Trop. rain forest	NSA	Montane	Colombia	BET-01	6.92	-73.30	1.0	936	91.0
Trop. rain forest	NSA	Montane	Colombia	BET-02	6.92	-73.30	1.0	889	84.0
Trop. rain forest	NSA	Montane	Colombia	DIV-01	7.05	-73.02	1.0	824	76.9
Trop. rain forest	NSA	Montane	Colombia	MTV-01	6.28	-75.51	1.0	1050	92.8

Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	NSA	Montane	Colombia	SSE-01	6.10	-75.51	1.0	891	69.0
Trop. rain forest	NSA	Montane	Venezuela	SEU-03	8.64	-71.41	1.0	676	89.5
Trop. rain forest	NSA	Terra firme	Colombia	ECE-01	10.68	-75.27	1.0	352	95.2
Trop. rain forest	NSA	Terra firme	Colombia	KAL-01	11.24	-74.14	1.0	363	78.5
Trop. rain forest	NSA	Terra firme	Colombia	PTN-01	6.12	-74.67	1.0	540	66.1
Trop. rain forest	NSA	Terra firme	Colombia	SRF-01	6.27	-75.09	0.9	632	72.3
Trop. rain forest	NSA	Terra firme	Venezuela	CAI-01	8.70	-70.07	1.5	424	94.1
Trop. rain forest	NSA	Terra firme	Venezuela	CLA-03	10.01	-65.32	0.5	302	98.3
Trop. rain forest	NWA	Flooded	Colombia	ZAR-02	-4.00	-69.90	1.0	624	73.2
Trop. rain forest	NWA	Flooded	Ecuador	JAS-05	-1.06	-77.62	1.0	557	86.2
Trop. rain forest	NWA	Flooded	Ecuador	TIP-03	-0.64	-76.15	1.0	444	67.6
Trop. rain forest	NWA	Flooded	Peru	JEN-13	-4.92	-73.54	1.0	643	70.3
Trop. rain forest	NWA	Flooded	Peru	SUC-03	-3.25	-72.92	1.0	599	98.7
Trop. rain forest	NWA	Flooded	Peru	YAN-01	-3.43	-72.84	1.0	567	95.8
Trop. rain forest	NWA	Montane	Peru	PNY-01	-10.38	-75.47	1.0	580	87.4
Trop. rain forest	NWA	Montane	Peru	PNY-02	-10.30	-75.61	1.0	653	68.8
Trop. rain forest	NWA	Terra firme	Brazil	JAM-01	-4.67	-66.17	4.0	3118	82.4
Trop. rain forest	NWA	Terra firme	Colombia	AGP-01	-3.72	-70.31	1.0	625	93.8
Trop. rain forest	NWA	Terra firme	Colombia	AGP-02	-3.72	-70.30	1.0	584	90.1
Trop. rain forest	NWA	Terra firme	Colombia	LOR-01	-3.06	-69.99	1.0	604	88.2
Trop. rain forest	NWA	Terra firme	Colombia	LOR-02	-3.06	-69.99	1.0	578	87.2
Trop. rain forest	NWA	Terra firme	Colombia	ZAR-03	-3.99	-69.90	1.0	692	67.1
Trop. rain forest	NWA	Terra firme	Colombia	ZAR-04	-3.99	-69.91	1.0	652	68.3
Trop. rain forest	NWA	Terra firme	Ecuador	BOG-01	-0.70	-76.48	1.0	544	80.1
Trop. rain forest	NWA	Terra firme	Ecuador	BOG-02	-0.70	-76.47	1.0	614	78.5
Trop. rain forest	NWA	Terra firme	Ecuador	JAS-02	-1.07	-77.62	1.0	724	89.4
Trop. rain forest	NWA	Terra firme	Ecuador	JAS-03	-1.08	-77.61	1.0	648	91.0
Trop. rain forest	NWA	Terra firme	Ecuador	JAS-04	-1.07	-77.61	1.0	794	69.8
Trop. rain forest	NWA	Terra firme	Ecuador	JUY-01	-2.13	-76.20	1.0	620	65.5
Trop. rain forest	NWA	Terra firme	Ecuador	PAY-01	-0.45	-77.03	1.0	653	67.8
Trop. rain forest	NWA	Terra firme	Ecuador	SHI-01	-1.02	-76.98	1.0	628	94.1
Trop. rain forest	NWA	Terra firme	Ecuador	SUM-01	-0.60	-77.63	0.8	572	77.6
Trop. rain forest	NWA	Terra firme	Ecuador	TIP-01	-0.66	-76.40	1.0	566	78.4
Trop. rain forest	NWA	Terra firme	Ecuador	TIP-02	-0.63	-76.14	1.0	502	82.9
Trop. rain forest	NWA	Terra firme	Peru	ALP-01	-3.95	-73.43	1.0	612	92.3
Trop. rain forest	NWA	Terra firme	Peru	ALP-02	-3.95	-73.44	1.0	593	92.6
Trop. rain forest	NWA	Terra firme	Peru	CDM-01	-10.33	-75.30	1.0	520	72.5
Trop. rain forest	NWA	Terra firme	Peru	IND-01	-3.52	-72.85	1.0	488	71.1
Trop. rain forest	NWA	Terra firme	Peru	JEN-11	-4.88	-73.63	1.0	600	85.3
Trop. rain forest	NWA	Terra firme	Peru	MSH-01	-3.78	-73.50	1.0	813	92.4
Trop. rain forest	NWA	Terra firme	Peru	PNY-03	-10.31	-75.29	1.0	797	75.9
Trop. rain forest	NWA	Terra firme	Peru	PNY-04	-10.34	-75.25	1.0	542	83.0
Trop. rain forest	NWA	Terra firme	Peru	PNY-05	-10.35	-75.25	1.0	573	88.7
Trop. rain forest	NWA	Terra firme	Peru	PNY-06	-10.36	-75.25	1.0	481	82.3
Trop. rain forest	NWA	Terra firme	Peru	PNY-07	-10.35	-75.26	1.0	536	84.0
Trop. rain forest	NWA	Terra firme	Peru	RCS-01	-9.47	-74.77	1.0	639	66.4
Trop. rain forest	NWA	Terra firme	Peru	RCS-05	-9.62	-74.93	1.0	595	89.1
Trop. rain forest	NWA	Terra firme	Peru	SUC-01	-3.25	-72.91	1.0	594	92.9
Trop. rain forest	NWA	Terra firme	Peru	SUC-02	-3.25	-72.90	1.0	591	94.9
Trop. rain forest	NWA	Terra firme	Peru	SUC-04	-3.25	-72.89	1.0	614	93.2
Trop. rain forest	NWA	Terra firme	Peru	SUC-05	-3.26	-72.89	1.0	561	90.6
Trop. rain forest	NWA	Terra firme	Peru	YAN-02	-3.43	-72.84	1.0	599	94.0
Trop. rain forest	NWA	White sand	Colombia	ZAR-01	-4.01	-69.91	1.0	872	73.6
Trop. rain forest	NWA	White sand	Peru	ALP-30	-3.95	-73.43	1.0	504	93.5
Trop. rain forest	NWA	White sand	Peru	ALP-40	-3.94	-73.44	1.0	1209	73.6
Trop. rain forest	NWA	White sand	Peru	JEN-12	-4.90	-73.63	1.0	744	91.3

Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	SWA	Flooded	Peru	AGJ-01	-11.89	-71.36	2.0	1314	93.6
Trop. rain forest	SWA	Flooded	Peru	LAS-02	-12.57	-70.09	1.0	596	86.9
Trop. rain forest	SWA	Flooded	Peru	MNU-01	-11.89	-71.41	2.3	1305	93.7
Trop. rain forest	SWA	Flooded	Peru	MNU-05	-11.88	-71.41	2.0	1252	93.2
Trop. rain forest	SWA	Flooded	Peru	MNU-06	-11.89	-71.40	2.3	1155	95.1
Trop. rain forest	SWA	Flooded	Peru	TAM-03	-12.84	-69.28	1.0	650	95.4
Trop. rain forest	SWA	Flooded	Peru	TAM-06	-12.84	-69.30	1.0	544	93.6
Trop. rain forest	SWA	Montane	Peru	ESP-01	-13.18	-71.59	1.0	709	82.1
Trop. rain forest	SWA	Montane	Peru	TRU-01	-13.11	-71.61	1.0	647	79.1
Trop. rain forest	SWA	Montane	Peru	TRU-02	-13.11	-71.60	1.0	743	77.4
Trop. rain forest	SWA	Montane	Peru	TRU-03	-13.11	-71.60	1.0	561	75.4
Trop. rain forest	SWA	Montane	Peru	TRU-04	-13.11	-71.59	1.0	888	70.8
Trop. rain forest	SWA	Montane	Peru	TRU-05	-13.09	-71.57	1.0	1001	82.1
Trop. rain forest	SWA	Montane	Peru	TRU-06	-13.08	-71.57	1.0	862	73.4
Trop. rain forest	SWA	Montane	Peru	WAY-01	-13.19	-71.59	1.0	1100	88.1
Trop. rain forest	SWA	Terra firme	Bolivia	BEE-01	-16.53	-64.58	1.0	574	87.1
Trop. rain forest	SWA	Terra firme	Bolivia	BEE-05	-16.53	-64.58	1.0	544	94.7
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-01	-10.07	-65.89	1.0	451	82.5
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-02	-10.05	-65.89	1.0	403	73.0
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-04	-10.31	-65.55	1.0	493	69.6
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-05	-10.03	-65.63	1.0	456	74.3
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-07	-9.91	-65.74	1.0	485	75.3
Trop. rain forest	SWA	Terra firme	Bolivia	MBT-08	-9.94	-65.75	1.0	442	74.4
Trop. rain forest	SWA	Terra firme	Bolivia	MTG-01	-19.27	-63.83	1.0	535	82.6
Trop. rain forest	SWA	Terra firme	Bolivia	RET-05	-10.97	-65.72	1.0	597	83.9
Trop. rain forest	SWA	Terra firme	Bolivia	RET-06	-10.97	-65.72	1.0	524	87.0
Trop. rain forest	SWA	Terra firme	Bolivia	RET-08	-10.97	-65.72	1.0	526	89.2
Trop. rain forest	SWA	Terra firme	Bolivia	RET-09	-10.97	-65.72	1.0	474	90.5
Trop. rain forest	SWA	Terra firme	Brazil	DOI-01	-10.57	-68.32	1.0	479	77.7
Trop. rain forest	SWA	Terra firme	Brazil	FEC-01	-10.07	-67.62	1.0	521	70.2
Trop. rain forest	SWA	Terra firme	Brazil	POR-01	-10.82	-68.77	1.0	558	79.9
Trop. rain forest	SWA	Terra firme	Brazil	POR-02	-10.80	-68.77	1.0	535	75.9
Trop. rain forest	SWA	Terra firme	Peru	ALM-01	-11.80	-71.47	2.0	1226	89.5
Trop. rain forest	SWA	Terra firme	Peru	BAR-01	-11.90	-71.42	1.0	473	92.8
Trop. rain forest	SWA	Terra firme	Peru	CAG-01	-12.18	-69.05	1.1	663	92.8
Trop. rain forest	SWA	Terra firme	Peru	CAL-01	-12.80	-71.78	1.0	630	72.4
Trop. rain forest	SWA	Terra firme	Peru	CAL-02	-12.81	-71.78	1.0	848	71.1
Trop. rain forest	SWA	Terra firme	Peru	CBP-01	-12.39	-69.31	0.9	537	97.0
Trop. rain forest	SWA	Terra firme	Peru	CJC-04	-12.66	-69.08	0.7	562	93.4
Trop. rain forest	SWA	Terra firme	Peru	CLT-01	-12.82	-69.35	0.9	581	94.3
Trop. rain forest	SWA	Terra firme	Peru	CUZ-01	-12.54	-69.06	1.0	516	98.6
Trop. rain forest	SWA	Terra firme	Peru	CUZ-02	-12.54	-69.06	1.0	553	98.2
Trop. rain forest	SWA	Terra firme	Peru	CUZ-03	-12.53	-69.05	1.0	497	97.0
Trop. rain forest	SWA	Terra firme	Peru	CUZ-04	-12.54	-69.05	1.0	558	94.1
Trop. rain forest	SWA	Terra firme	Peru	INF-01	-12.73	-69.70	1.3	810	82.3
Trop. rain forest	SWA	Terra firme	Peru	MNU-03	-11.90	-71.40	2.0	1281	93.6
Trop. rain forest	SWA	Terra firme	Peru	MNU-04	-11.90	-71.40	2.0	1196	92.9
Trop. rain forest	SWA	Terra firme	Peru	MNU-08	-12.00	-71.24	2.0	1153	96.2
Trop. rain forest	SWA	Terra firme	Peru	MNU-09	-12.04	-71.21	2.0	971	97.4
Trop. rain forest	SWA	Terra firme	Peru	PAK-01	-11.94	-71.28	1.0	575	89.6
Trop. rain forest	SWA	Terra firme	Peru	RPA-01	-12.39	-69.36	1.0	494	76.9
Trop. rain forest	SWA	Terra firme	Peru	RPI-01	-12.36	-69.23	1.0	552	86.4
Trop. rain forest	SWA	Terra firme	Peru	RTH-01	-11.37	-69.66	1.0	525	93.1
Trop. rain forest	SWA	Terra firme	Peru	TAM-01	-12.84	-69.29	1.0	566	95.6
Trop. rain forest	SWA	Terra firme	Peru	TAM-02	-12.83	-69.29	1.0	600	93.0
Trop. rain forest	SWA	Terra firme	Peru	TAM-05	-12.83	-69.27	1.0	532	93.6

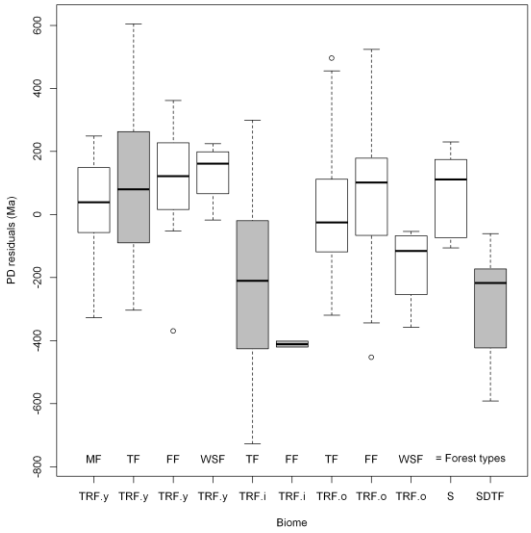
Biome	Region	Forest type	Country	Plot code	Lat (°S)	Long (°W)	Area (ha)	Nº Ind.	ID (%)
Trop. rain forest	SWA	Terra firme	Peru	TAM-07	-12.83	-69.26	1.0	549	89.3
Trop. rain forest	SWA	Terra firme	Peru	TAM-08	-12.83	-69.27	1.0	532	93.0
Trop. rain forest	SWA	Terra firme	Peru	TMP-01	-13.13	-69.57	2.3	1306	81.5
Savanna	BSh	Savanna	Brazil	LET-01	-14.71	-52.35	1.0	375	100.0
Savanna	BSh	Savanna	Brazil	NXV-01	-14.71	-52.35	1.0	341	99.7
Savanna	BSh	Savanna	Brazil	NXV-03	-14.71	-52.35	0.5	320	99.7
Savanna	BSh	Savanna	Brazil	NXV-05	-14.71	-52.35	0.5	439	99.3
Savanna	BSh	Savanna	Brazil	SMT-01	-12.82	-51.77	1.0	380	99.5
SDTF	BSh	Dry forest	Bolivia	ACU-01	-15.25	-61.25	1.0	479	95.4
SDTF	BSh	Dry forest	Bolivia	ACU-02	-15.25	-61.24	1.0	406	90.6
SDTF	BSh	Dry forest	Bolivia	ALV-01	-16.12	-61.89	1.0	479	98.5
SDTF	BSh	Dry forest	Bolivia	ALV-02	-16.08	-61.89	1.0	423	98.3
SDTF	BSh	Dry forest	Bolivia	CRP-01	-14.54	-61.50	1.0	456	95.8
SDTF	BSh	Dry forest	Bolivia	CRP-02	-14.54	-61.50	1.0	464	92.7
SDTF	BSh	Dry forest	Bolivia	KEN-02	-16.02	-62.73	1.0	396	98.7
SDTF	BSh	Dry forest	Bolivia	OTT-01	-16.39	-61.21	1.0	422	95.7
SDTF	BSh	Dry forest	Bolivia	OTT-03	-16.42	-61.19	1.0	252	98.8
SDTF	SWA	Dry forest	Bolivia	TUC-01	-18.52	-60.81	1.0	758	98.5
SDTF	NSA	Dry forest	Venezuela	BAC-01	7.46	-71.01	1.5	432	95.1

Appendix S4.2. Phylogenetic tree for the whole species pool for 283 floristic inventories compiled from RAINFOR dataset. This phylogeny reflects the most current understanding of the phylogenetic relationship among families for angiosperms (Bremer *et al.*, 2009), but the resolution among genera or species is poor, i.e. lower taxa are represented as polytomies in the phylogeny, for example, with identical node age for congeneric species.

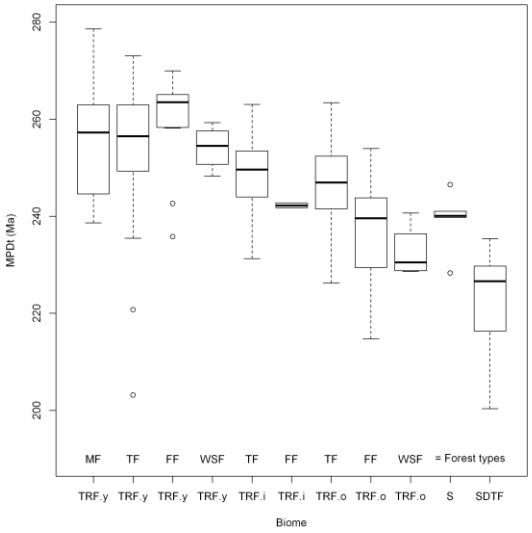


Appendix S4.3. Biome and forest type comparison of phylogenetic diversity metrics. a) Residuals of phylogenetic diversity *sensu strictu* on species diversity ("PD residuals"), and b) mean pairwise phylogenetic distance among unique taxa (MPDt) are given in millions of years (Ma). Tropical rain forest biome is classified by forest type and maximum age of geological formations (young: < 20 Ma; intermediate: 20-100 Ma, old: > 500 Ma; Quesada *et al.*, 2011). Grey boxplots contain communities with higher or lower PD values than expected by their species diversity. Forest types: Montane (MF), terra firme (TF), flooded (FF), and white-sand (WSF) forest.

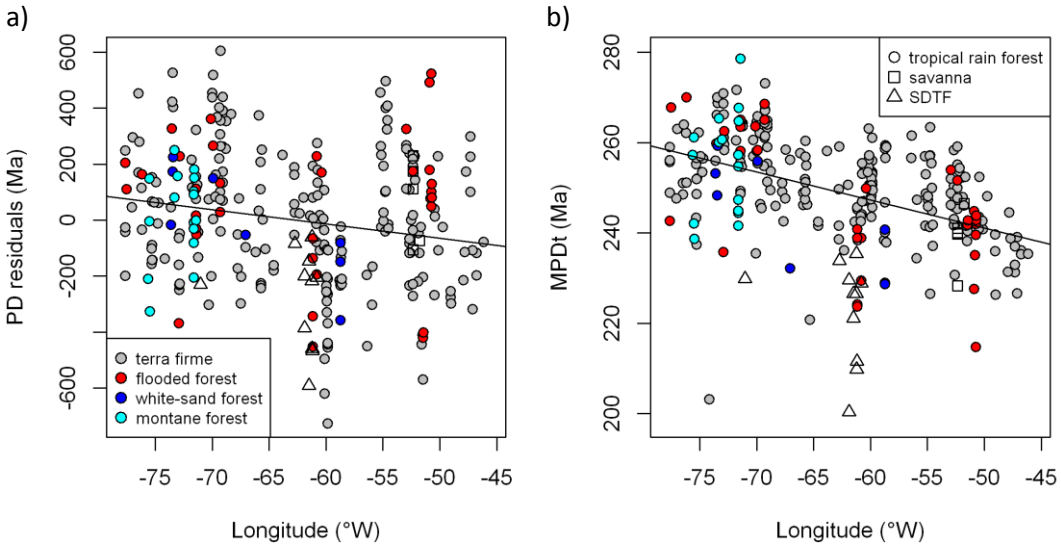
a)



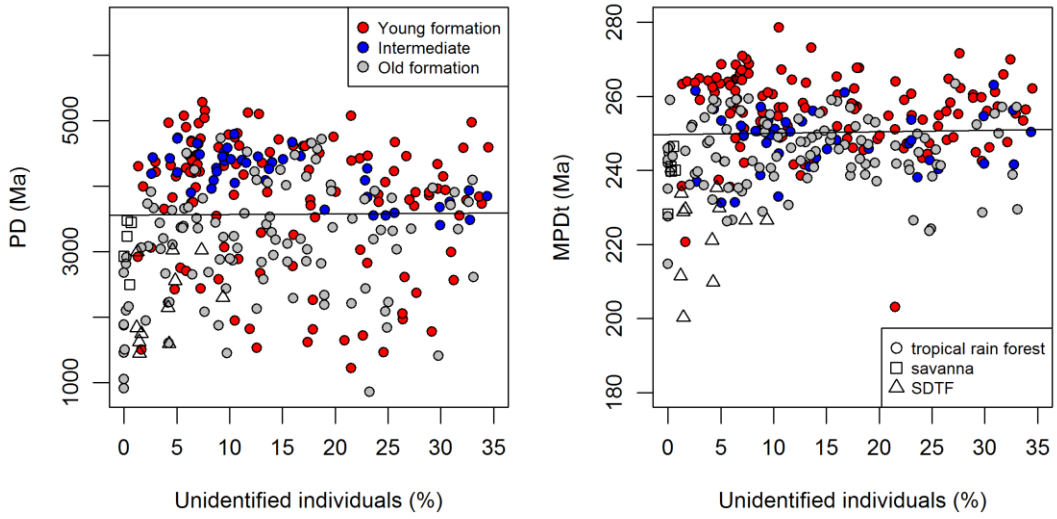
b)



Appendix S4.4. Spatial variation in the distribution of phylogenetic diversity metrics for 283 tree inventories by forest type. (a) Residuals from the linear regression of PD on species diversity ("PD residuals"), and (b) mean pairwise phylogenetic distance among unique taxa (MPDt) are given in millions of years (Ma). Symbols indicate different biomes and colours refer to forest types within the tropical rain forest.



Appendix S4.5. Lack of correlation between phylogenetic diversity metrics and the percentage of unidentified individuals excluded for each plot. Linear regression fits are provided for tropical rain forest plots (r_{PD} = 0.01, p = 0.90; r_{MPDt} = 0.03, p = 0.61).



Appendix S4.6. Sensitivity analysis of phylogenetic diversity metrics for the tropical rain forest biome calculated using different number of individuals per plot. Values calculated using rarefaction procedure to 283 and 503 individuals are compared. The regression fit (solid line) and the 1:1 line (thin line) are similar, indicating that general patterns are conserved irrespective of the number of individuals used per plot in the analysis.