PHYLOGENETIC ANALYSES OF ANDEAN AND AMAZONIAN TREE COMMUNITIES IN ECUADOR

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PHYLOGENETIC ANALYSES OF ANDEAN AND AMAZONIAN TREE COMMUNITIES IN ECUADOR

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Thesis Abstract

The forests of Ecuador are known for their high levels of diversity and endemism, classifying the country as a biodiversity hotspot. Both the western Amazon and Andean montane forests are richly populated with tropical tree species that have been little studied in a community phylogenetic context. The implementation of elevational transects and trait based analyses having proven useful in gaining a better understanding of how environmental factors are affecting the tree community structure in these habitats. The goal of this research was to evaluate the magnitude of DNA barcode diversity among Amazonian and Andean tree species. Specifically, the objectives were to (1) evaluate community phylogenetic structure and correlate phylogenetic analyses with diversity metrics among Andean tree species along an elevational gradient at Siempre Verde Reserve, Ecuador, and to (2) construct a tropical tree community phylogeny using DNA barcodes and to test for phylogenetic signal in the occurrence of phytochemicals among tree species within Yasuní National Park, Ecuador. In the montane forest at Siempre Verde, 595 individuals were tagged, collected and identified, comprising 36 families, 53 genera, and 88 species. Analyses revealed that species richness was decreasing with elevation but the number of stems of common species was increasing causing phylogenetic clumping at higher elevations. Evidence implies that habitat filtering of species due to cloud inundation is behind this observed pattern contributing to the community structure. In the upland Amazonian forest of Yasuní, 337 common tree species making up 181 genera and 56 families were sent for sequencing, and the trait distribution of phytochemical presence was determined. Metrics of phylogenetic trait distribution all supported a random distribution of the medicinal trait within the Yasuní tree community. In the future, having higher sequence recovery and resolution along with complete floristic sampling will improve statistical power and the ability to detect fine scale

community structure patterns in both of these forests. Studies like this, which include taxonomic, functional, and phylogenetic diversity, will allow for more comparisons to better understand these unique biodiversity hotspots.

Index Words: Ecuador, DNA barcode, tree, community phylogenetics

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"If you see further than others, it is by standing on the shoulders of giants." – Isaac Newton

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Chapter I

Phylogenetic Analysis of Andean Tree Communities along an Elevational Gradient in Ecuador

Abstract

Montane forests are known for their high levels of diversity and endemism. However, limited research has been conducted in these forest due to the intense amount of effort necessary to gather the data. Using elevational gradients in these forests is imperative to gain a better understanding of how environmental conditions are affecting the community structure. A combination of traditional diversity metrics (Shannon's and Simpson's indices) and phylogenetic analyses can potentially shed light on patterns generated by abiotic and/or biotic factors due to elevation in montane forests. The goal of this research was to evaluate the magnitude of genetic diversity among Andean tree species along an elevational gradient at Siempre Verde Reserve, Ecuador. Specifically, the aim was to evaluate community phylogenetic structure and correlate phylogenetic analyses with diversity metrics to shed light on patterns of community structure. Along a transect, 595 individuals were tagged, collected and identified. They comprised 36 families, 53 genera, and 88 species. Of these individuals, 152 were sequenced for the *rbc*L and *mat*K gene regions. In summary, species richness was decreasing with elevation but the number of stems of common species was increasing. Hence, at higher elevations there are fewer species, but more individuals of the same species. This study showed significant clumping at the highest two plots within the transect for all three metrics (PD, MPD, and MNTD) tested. This correlates with the Shannon's and Simpson's diversity findings, that there are more closely related species at higher elevations. Research has linked phylogenetic clumping with habitat filtering. This process seems plausible for this transect as diversity peaks at mid-elevations, where clouds begin

to inundate the forest, causing vast difference in habitat above and below this elevation. Since only four species span the entirety of the transect, abiotic stress could be the limiting factor in species distributions and a main contributor to the community structure. In conclusion, plant communities are constructed in non-random ways along this elevational gradient. To completely grasp the biodiversity changes along elevational gradients, taxonomic diversity, functional diversity, and phylogenetic diversity must all be considered in the future.

Introduction

Montane cloud forests are found on tropical mountains that are frequently inundated with clouds (Bruijnzeel and Veneklaas 1998). Besides cloud cover, tropical montane forests are also known for their unique vegetation. These forests are recognized for their low canopy height, multi-stemmed trees, and high epiphyte abundance (Fahey et al. 2015). Research has shown that although montane forests have lower species diversity compared to tropical forests at lower elevations, they possess higher levels of endemism (Lieberman et al. 1996; Clark et al. 2015), due in part to the environmental conditions where tropical montane forests are found. Although environmental conditions such as temperature, humidity, wind, and soil nutrients are all known to vary with elevation (Young and Keating 2001; Fahey et al. 2015), relatively few studies have shown how vegetation structure is affected by varying environmental conditions across elevational gradients in montane tropical forests systems.

Historically, research along elevation gradients has been limited due to the intense amount of effort necessary to gather the data. It has been estimated that as much as 75 percent of the planimetric area of montane forest occurs on slopes of > 27° (Clark et al. 2015). Studying plants along elevational gradients is becoming increasingly important as approximately 25 percent of the land surface on the Earth is covered with mountains. These mountains are home to at least a third of all terrestrial plant species and supply half of the Earth's human population with water (Körner 2007). Tropical elevational studies are also imperative as these areas contain high biodiversity and endemism, much of which has yet to be explored. For example, endemism in the biodiverse country of Ecuador has been estimated for varying altitudes: 13% up to 900 meters above sea level, 39% between 900 and 3,000 meters above sea level, and 40% at 3,000 meters above sea level (Young and Keating 2001; Rios et al. 2007). Recently, interest has been

shown in studying tropical plants along elevational gradients because these areas will likely show large effects from global warming. However, knowledge on montane forest composition, structure, functional processes, and ecosystem development remains limited (Chain-Guadarrama et al. 2012; Asner et al. 2014; Clark et al. 2015).

Traditionally, forest vegetation is described using diversity metrics such as alpha diversity (species richness) and beta diversity (species turnover). These metrics, along with overall species composition, have been shown to be useful in understanding the structure, composition and diversity of plant species along elevational gradients (Pausas and Austin 2001; Kessler et al. 2009; Thinh et al. 2015). For example, alpha diversity has been shown to decrease with increasing elevation (Gentry 1988; Lieberman et al. 1996; Ashton 2003; Homeier et al. 2010) and beta diversity has been shown to decrease with increasing elevation (Condit et al. 2002; Kraft et al. 2011; Swenson et al. 2011). Interestingly, a number of abiotic factors such as temperature, precipitation, cloud cover, soil nutrients, and light availability have been shown to cause shifts in community structure (Gentry 1988; Ashton 2003; Barone et al. 2008; Homeier et al. 2010). Körner (2007) has divided abiotic factors affecting community structure into two categories: those physically tied to elevation like temperature and atmospheric pressure and those not generally tied to elevation, such as hours of sunshine, wind, and geology. Although competition has also been shown to determine community makeup (Barone et al. 2008), most studies suggest that there are multiple factors affecting community structure along elevational gradients that have to be accounted for (Bruijnzeel and Veneklaas 1998).

DNA barcoding has been widely used to address questions in ecology, evolution, and conservation biology (Losos 1996; Hebert et al. 2003; Valentini et al. 2008; Chen et al. 2010; Erickson et al. 2014; Muscarella et al. 2014). For plants, a DNA barcode can be generated in a

rapid, accurate and cost-effective manner from a short standardized sequence of DNA from the chloroplast genome (Newmaster et al. 2013, Kress et al. 2015). Many factors that could complicate taxonomic identifications, like the age of a specimen, whether it is sterile, or only having a small amount of plant material available have little bearing on the ability of DNA barcoding to identify a species. Commonly, DNA barcodes include the phylogenetically conserved coding region, *rbc*L, combined with the more rapidly evolving gene region, *mat*K (Kress et al. 2009; Hollingsworth et al. 2011; Saslis-Lagoudakis et al. 2011). This universal multi-locus DNA barcode has been shown to align taxa at both higher and lower taxonomic levels (CBOL 2009), making it an ideal tool to investigate phylogenetic relationships and community dynamics, particularly across environmental gradients.

Most community-level analyses have relied on previously published taxon-specific phylogenies, which often lack DNA sequence data (Kress et al. 2009; Kress et al. 2015). DNA barcode phylogenies have the advantages of being able to resolve species-level relationships and provide estimates of evolutionary distances and relationships between species within a phylogeny (Erickson et al. 2014). These phylogenies have been known to reveal aspects of biodiversity that are not normally observable by merging understandings of ecology, evolution, and biogeography in plant communities (Losos 1996; Kesanakurti et al. 2011, Liu et al. 2014, Braukmann et al. 2017). Given the success of using DNA barcodes to build tropical forest community phylogenies (Kress et al. 2009, Kress et al. 2010, Muscarella et al. 2014), there is potential for their use in this research to highlight patterns previously seen and/or currently unknown when compared to diversity metric values.

One country that is ideal for studying plant diversity and dynamics along elevational gradients is Ecuador. Ecuador has one of the greatest densities of species per area of any country

on Earth: it occupies only 0.2% of the Earth's land mass but possesses 10% of its plant species (Rios et al. 2007). Approximately 20,000 plant species reside in Ecuador, with at least 4,000 of them endemic (Myers 1988). The Andes Mountains run from north to south through Ecuador, greatly contributing to its high plant diversity and making them ideal sites to study tree community structure (Girardin et al. 2013; Asner et al. 2014). The montane forests of the Andes are known for their high species richness, especially for vascular plants (Mosandle and Günter 2008; Hutter et al. 2013). Unfortunately, tropical montane forests suffer high rates of deforestation. For example, in 1995, it was estimated that Andean montane forests had already been reduced in size by 90 percent (Homeier et al. 2010). Even though high species diversity exists in the remote montane forests of the Ecuadorian Andes, few studies have investigated the forest structure using diversity metrics and phylogenetic patterns generated by elevation. It seems likely that a better understanding of the factors affecting plant community structure along elevation gradients in tropical montane forest ecosystems can be accomplished by combining comparative analyses of diversity measures with phylogenetic analyses based on DNA barcoding.

Goal and Objectives

The goal of this research was to evaluate the magnitude of genetic diversity among Andean tree species. Specifically, the aim was to evaluate community phylogenetic structure across an elevation gradient and correlate phylogenetic analyses with diversity metrics.

Methods

Study Site

This study was conducted at the Siempre Verde Reserve in the Imbabura Province of northern Ecuador between 2014 and 2016. Siempre Verde is located in the western foothills of the Cotacachi volcano in Andean forest in the eastern most portion of the Intag River Valley. It covers an area of 334 hectares (3.34 km^2) and has an elevation range from 2300 to 3300 meters above sea level. The Reserve is found within the coordinates: North: 00°22'38" and South: 00°21'30"; East: 78°24'09" and West: 78°25'37" (Fig. 1) (Reynolds, 2011). At Siempre Verde, the rainy season begins around October and ends in June. The area receives around 2,532 mm of annual rainfall with the heaviest rains happening between January and April. The driest months are usually between July and September. The temperature at the preserve has vast ranges due to the steep elevation cline. At an intermediate elevation, the temperature ranges from ~6.4°C to 24.2°C. At the top of the mountain, cooler temperatures are observed as the range is ~4.5°C to 18°C. On average, the temperature is ~15.11°C throughout the entirety of the reserve (Reynolds 2011). According to the General Soil Map of Ecuador, the soil at Siempre Verde is allophanic, loam to silty loam and deeply rich in organic material. The soil is of medium fertility and has an acidic pH with low base saturation (20-100%). (PRONAREG 1984). These soils are the result of slow weathering of volcanic ash and glass, especially at high elevations in the tropical Andes (Reynolds 2011). Permission to carry out research in Ecuador and at Siempre Verde Reserve was given by the Ministerio de Ambiente (MAE-DNB-CM-2015-0031).

Sampling Design

A transect was established that included 15 plots that were 5 m x 50 m (0.025 ha each) with the long side perpendicular to the slope. The plots were at 100 m intervals spanning from 2,437 to 3,334 m asl (Fig 1). For simplicity, plots will be referenced by their elevation rounded to the nearest 50 m. Within each plot, every tree and tree fern with a diameter at breast height of ≥ 5 cm was measured, collected, and tagged with a numbered aluminum plate (Paz et al. in prep). Collections were doused in alcohol to control for pests, identified and herbarium specimens were deposited into the Herbario-QCA at Pontificia Universidad Católica del Ecuador (APG 2009; Paz et al. in prep). A DNA voucher was taken off the herbarium samples for 152 specimens.

DNA isolation, PCR amplification, and Sequencing

DNA extraction, PCR amplification, and sequencing were conducted at the Canadian Center for DNA Barcoding, Biodiversity Institute of Ontario, Canada, following their protocols with adaptations by Maria Kuzmina (CCDB Protocols DNA Extraction; CCDB Protocols PCR Amplification; CCDB Protocols Sequencing) (Appendix). Two coding gene regions of the chloroplast genome were sequenced: the phylogenetically conserved ribulose-bisphosphate/carboxylase large subunit (*rbc*L) gene region and the more rapidly evolving region, maturase-K (*mat*K) gene region. Forward and reverse primers were sequenced for each gene region: rbcLa-F/rbcLa-R and matK-xf/matK-MALP (Table 1). An attempt to substitute unsuccessful sequences with those publicly available on BOLD and GenBank was ineffective for all but four samples. After publication, these sequences will be publicly available within the Barcode of Life Datasystems (BOLD) (www.boldsystems.org) (Ratnasingham and Herbert 2007)

Data Analysis

Community Structure

To better understand the community structure along the transect at Siempre Verde, two diversity indices were used: Shannon's index and Simpson's index. Both of these diversity indices are widely used, making future comparisons probable (Nagendra 2002; Morris et al. 2014).

To understand alpha diversity trends along the transects, Shannon's diversity index (Shannon and Weaver, 1949) was calculated. The relation between Shannon's diversity indices and elevation was determined using a regression analysis at the species, genus, and family levels. Shannon's index was calculated using the following formula:

$$H' = -\sum_{i=1}^{S} p_i \ ln \, p_i$$

where, S is total number of species, genera, or families in the community (richness) and p_i is the proportion of S made up of the i^{th} species, genus, or family (Kappelle et al. 1995). This metric stresses richness and responds strongest to changes in importance of the rarest species, genera, or families in the community (Nagendra 2002; Morris et al. 2014; Thinh et al. 2015).

Simpson's diversity index (Simpson 1949) was also calculated for each plot along the transect at the species, genera, and family levels. The index was calculated using the following formula:

$$D = 1 - \frac{\sum n(n-1)}{\mathrm{N}(\mathrm{N}-1)}$$

where D is the diversity index, n is the number of individuals of a particular species, and N is the number of individuals of all species (Shaheen et al. 2011). In this formula, Simpson's diversity was subtracted from one indicating that the greater the value, the greater the diversity. Simpson's

index of diversity was measured because it stresses the lopsidedness of abundance values and puts greater emphasis on evenness and dominance in the data set. It responds most strongly to changes in proportional abundance in species, genera, or families that are the most common and gives the probability that two randomly selected individuals will be same (Nagendra 2002; Morris et al. 2014; Thinh et al. 2015). Regression analyses were executed to determine any correlation between Simpson's Index of diversity and changes in elevation.

For all regression analyses, JMP® version 11.2.0 was used and significance was determined at $P < 0.05$ in all cases.

Phylogenetic Analysis

After sequences for each species were obtained, alignments and phylogenies were constructed using Geneious version 9.1 (http://www.geneious.com; Kearse et al. 2012; Muscarella et al. 2014). The *rbc*L and *mat*K genes were aligned separately using Multiple Alignment and Fast Fourier Transform (MAAFT v. 7) (Katoh et al. 2002) default settings. These alignments were then concatenated into a supermatrix. A phylogeny was generated using maximum likelihood (ML) methods, executed in Geneious using Randomized Axelerated Maximum Likelihood (RAxML v. 7.2.8) (Stamatakis et al. 2006). *Ginkgo biloba* served as the outgroup (Kress et al. 2009) and nucleotide substitution was modeled using the GTR+GAMMA model, with substitution rates estimated independently for each gene (Saslis-Lagoudakis et al. 2011; Muscarella et al. 2014). The rapid bootstrapping algorithm was implemented in order to search for the best scoring ML tree after node support was evaluated using 1000 bootstrap runs (Stamatakis et al. 2008).

Phylogenetic Structure Analysis

All phylogenetic analyses were estimated within the Picante package (Kembel et al. 2010) of the R programming language. Three metrics were assessed in this study, phylogenetic distance (PD) (Faith 1992), mean pairwise distance (MPD) (Webb 2000), and mean nearest taxon distance (MNTD) (Webb 2000). Traditional calculations of these metrics do not account for species abundance, which can be problematic since species are rarely equally abundant. For this reason, all formulas were weighted by species abundance in order to relay important ecological information. Therefore, the PD calculation used in this study is based on the Weighted Faith's Index equation:

$$Weighted\ Faith\ PD = n\ x\ \frac{\sum_i^n l_i \bar{A}_i}{\sum_i^n \bar{A}_i}$$

where n is the number of branches in the phylogenetic tree, the length of the ith branch is l_i, and $\bar{A}_i$ is the average abundance of all species subtended by that branch of the phylogeny (Swenson 2014). PD values are often determined because they can be correlated with species richness in a system as adding a species to the system would add at minimum a terminal branch to the phylogeny altering the PD value (Erickson et al. 2014).

The MPD metric obtains a pair-wise phylogenetic distance (distance matrix) across all pairs of taxa in a community and gives an estimate of the overall divergence of taxonomic clades present and is calculated as:

$$Abundance\ weighted\ MPD = \frac{\sum_i^n \sum_j^n \delta_{i,j} f_i f_j}{\sum_i^n \sum_j^n f_i f_j},\ where\ i \neq j$$

where there are n species in the community, δ is the phylogenetic distance matrix, $\delta_{i,j}$ is the phylogenetic distance between species i and j, and f represents the frequency of the abundances of species. It can be considered to be a "basal" metric of phylogenetic diversity as it captures the

overall phylogenetic dissimilarity of the taxa in a sample. MPD does not detect finer scale phylogenetic patterns that may be present (Erickson et al. 2014; Swenson 2014).

The last metric estimated was MNTD. It provides an average of the distances between each species and its nearest phylogenetic neighbor in the community. It quantifies the degree that a community may be a set of closely related species versus a heterogeneous set of taxa from disparate taxonomic clades (Erickson et al. 2014).

$$Abundance\ weighted\ MNTD = \frac{\sum_i^n \min \delta_{i,j} f_i}{n}, wehre\ i \neq j$$

where there are n species in the community, $\delta_{i,j}$ is the phylogenetic distance between species i and j, and $\min\delta_{i,j}$ is the minimum phylogenetic distance between species i and all other species in the community (i.e., the nearest neighbor distance). The variable f_i (frequency) was included to indicate the abundance of species i in the community (Swenson 2014).

As the raw values of MP, MPD, and MNTD give no means of standardized comparisons between communities. Null models were implemented so that standardized effect sizes (S.E.S.) could be determined.

$$Standardized\ effect\ size = \frac{observed - \overline{null}}{sd(null)}$$

This calculation removes any directional bias associated with the decreases in variance in the expected values with increasing species richness (Swenson 2014). For MPD and MNTD, positive S.E.S. values (obs.z > 0) and high quantiles (obs.p > 0.95) indicate phylogenetic evenness, or a greater phylogenetic distance among co-occurring species than expected. Negative S.E.S. values (obs.z < 0) and low quantiles (obs.p < 0.05) indicate phylogenetic clustering, or smaller phylogenetic distances among co-occurring species than expected (Kembel 2010; Saslis-Lagoudakis et al. 2011, Muscarella et al. 2014). Positive values of S.E.S. indicate a higher than

average expected value and a negative S.E.S. values indicated a lower than average expected value. The category of null model used in this research was constrained randomization of the phylogenetic data where information in the community data matrix is preserved. The other option would have been to randomize the community data matrix, but when research questions are focused around the idea of comparing phylogenetic diversity between communities, construction of random communities is unwanted (Swenson 2014). For the models used here, the total abundance of species with and across communities, the occupancy rates of species across communities, the species alpha and beta diversity and patterns of dispersal limitation are all fixed. For the PD, MPD, and MNTD metrics, the 'taxa.labels' null model was used with 999 runs and 1000 iterations to determine standardized effect sizes (Saslis-Lagoudakis et al. 2011; Erickson et al. 2014; Muscarella et al. 2014).

All community structure and phylogenetic analyses were performed on the data set in two ways. First, calculations were based on all plots separately (n=15). Secondly, calculations were performed with the plots grouped into three elevation classifications: low, medium, and high. Specifically, plots 1-5 with an elevation range of 3,100-3,334 m asl were designated as high, plots 6-11 with an elevation range of 3,700-3,100 m asl were designated as medium, and plots 12-15 with an elevation range of 2,437-2,700 were designated as low. These separate analyses were done in order to determine if patterns were stronger when plots were grouped versus treated separately.

Results

Forest Composition

Within the transect, 595 individuals were tagged, collected and identified. These comprise 36 families, 53 genera, and 88 species (Fig. 2, Table S1). These data were then partitioned into the low, medium, and high elevation groups. There were 157 individuals comprised of 23 families, 34 genera, and 42 species in the low elevation group. In the medium elevation group, there were 186 individuals, with 29 families, 34 genera, and 48 species. Lastly, in the high elevation group, there were 252 individuals having 20 families, 24 genera, and 35 species. Nine families make up 74.5% of the individuals of the entire transect, while 10 genera make up 67.2% (Table 2). By far, the two most abundant species in the transect are *Cyathea cf. frigida* (n=60) and *Weinmannia rollottii* (n=75). When the data set was divided up into the low, medium, and high groupings, the distribution of families and genera was partitioned by elevation (Table 2). The similarity of species composition among the three elevational zones decreased with increasing distance (Fig. 2, Table S1). We found that high and low elevations share 6.5% of species, high and middle elevation share 19.3% of species, and middle and low elevation share 22.2% of species. Four species can be found in all three elevational zones: *Cyathera cf. frigida*, *Geissanthus ecuadorensis*, *Palicourea amethystina*, and *Gordonia fruticosa*. Plot number 10 at elevation 2,820 m asl had the highest number of families (n=17) and species (n=23) of all the plots. It also tied with plot 12 for the highest number of genera (n=18).

Community Structure Analysis

The Shannon diversity index, H', was calculated for each individual plot and when plots were grouped by elevation range: low medium, and high. Calculations were done at the species,

genus, and family level. For each plot considered separately, H' ranged from 1.81 to 2.94, 1.53 to 2.73, and 1.52 to 2.59 for species, genera, and families, respectively (Table 4). When plots were clumped into elevation zones, H' ranged from 2.72 to 3.34, 2.23 to 3.05, and 2.15 to 2.62 for species, genera, and families, respectively (Table 4). The average H' for the entire transect was 2.33, 2.18, and 2.05 at the species, genus, and family levels. The highest H' value was seen for plot 10 at all hierarchies. Bivariate correlations were performed between Shannon's diversity values and elevation at the species, genus, and family levels. At the species, genus and family levels, a trend was seen for decreasing Shannon's diversity with elevation (Species: $F_{1,13}$=6.335, p=0.026; Genus: $F_{1,13}$=11.424, p=0.005; Family: $F_{1,13}$=5.606, p=0.030; Fig 4A).

Bivariate correlations were also performed between Shannon's diversity values and elevation with plots group into elevation zones. These analyses were performed at the species, genus, and family levels. The same relationship was seen for all three groupings, decreasing Shannon's diversity with increasing elevation (Species: $F_{1,1}$=2.699, p=0.348, Genus: $F_{1,1}$=17.956, p=0.148; Family: $F_{1,1}$=4.022, p=0.206, Fig. 4B).

The Simpson's diversity index, D, was calculated for each individual plot and the grouped plots. D ranged from 0.79 to 0.96, 0.68 to 0.96, and 0.68 to 0.93 for species, genera, and families, respectively (Table 4). When plots were clumped into elevation zones, D ranged from 0.88 to 0.95, 0.81 to 0.94, and 0.81 to 0.90 for low, medium, and high groups, respectively (Table 4). The average D for the entire transect was 0.90, 0.87, and 0.85 at the species, genus, and family levels. Bivariate correlations were performed between Simpson's diversity values and elevation at the species, genus, and family levels. At the species, genus, and family levels, a trend was seen for decreasing Simpson's Index of diversity with elevation (Species: $F_{1,13}$=2.768, p=0.120; Genus: $F_{1,13}$=4.507, p=0.054; Family: $F_{1,13}$=2.149, p=0.167; Fig. 5A).

As with Shannon's diversity, bivariate correlations were also performed between Simpson's diversity values and elevation with plots grouped into elevation zones. The same relationship was seen for all three hierarchies, decreasing Simpson's diversity with increasing elevation. (Species: $F_{1,1}$=6.277, p=0.242; Genus: $F_{1,1}$=9.705, p=0.198; Family: $F_{1,1}$=12.255, p=0.177, Fig. 5B).

Sequence Recovery

Of the 595 individuals present in the transect, 152 were hapharzardly selected and sent for sequencing (Table S2). There were 33 families, 45 genera, and 70 species within the sequenced data set. Sequence recovery was low: only 40% and 27% of the samples that were sent for sequencing returned high quality sequences for *rbc*L and *mat*K, respectively. Of the 61 *rbc*L sequences recovered, 10 were uni-directional, whereas 8 of the 41 *mat*K sequences were uni-directional. Of the 64 successfully sequenced individuals, 38 (59.4%) had both *rbc*L and *mat*K sequences and 23 (35.9%) had only a *rbc*L sequence. Only three individuals had a *mat*K sequence without also having a *rbc*L sequence (*Nectandra cf. obtusata*, *Siparuna piloso-lepidota*, and *Viburnum urbanii*). The addition of uni-directional sequences to the data set added two more unique species (*Piper puraceanum* and *Weinmannia mariquitae*). Originally, unsuccessful sequences were going to be substituted with sequences from BOLD and/or GenBank (Clark et al. 2016), but no sequences were available from these databases.

Phylogenetic Analysis

A phylogenetic tree of all successfully sequenced individuals was constructed (Fig. 3). The consensus tree from rapid bootstrapping found 90.3% of all nodes were supported by 50% or

greater. Also, phylogenetic trees of each gene region were separately constructed: *rbc*L (Fig. S1) and *mat*K (Fig. S2). Rapid bootstrapping found 64.7% and 86.4% of all nodes for *rbc*L and *mat*K phylogenetic trees were supported by 50% or greater, respectively. The *rbc*L and *mat*K phylogenetic trees do not include replicate sequences of individuals, with the individual having sequences of the highest quality chosen.

Phylogenetic Structure Analysis

For phylogenetic analyses, 23 families, 29 genera, and 38 species (out of 70 possible species) were included (Fig. S3, Table S2). For these species, 23 (60.5%) had both *rbc*L and *mat*K sequences, 14 (36.8%) had just a *rbc*L sequence, and only one (2.6%) species had just a *mat*K sequence. Following analyses protocols, only one individual per species was included in the phylogeny for analyses. The community data set takes into account the abundance of each species in the transect. All analyses were conducted for the plots separately and then with plots grouped by low, medium, and high designations. The phylogenetic tree used for the analysis was constructed containing only one representative from each species (Fig. S4). The consensus tree from rapid bootstrapping found 91.7% of all nodes were supported by 50% or greater.

For the three different phylogenetic diversity metrics, observed values were compared to null model calculations to determine significance. Results where each plot is considered separately can be seen in Table 3. Significant differences from random were detected for each metric in a variety of plots (Table S3-S5 for PD, MPD, and MNTD, respectively). All significant metrics detected were consistent with phylogenetic clustering (niche similarity). There was no evidence of significant phylogenetic evenness. The metrics were again calculated after the plots in the data set had been divided into low, medium, and high categories (Table 3). The only

significant results were from the MPD calculation at medium and high elevation categories. Again, the significance that was detected was consistent with phylogenetic clustering and there was no evidence of significant phylogenetic evenness (Table S3-S5 for PD, MPD, and MNTD, respectively.

Discussion

Forest Composition

The forest composition found at Siempre Verde is in general agreement with comparative studies conducted in montane forest habitats. For example, a previous study found that South American montane forests are typically dominated by species of *Weinmannia, Schefflera, Miconia* and *Myrcianthes* (Jørgensen and León-Yánez 1999). At the study site, we found that each of these genera, excluding *Schefflera*, were found to be among the most diverse within the transect (Table 2). When the data set was divided into the three elevational zones (low, medium, high), they differed greatly in the composition of individuals at the genus and family levels, in agreement with previous findings (Table 2) (Young and Keating 2001; Homeier et al. 2010; Chain-Guadarrama et al. 2012; Girardin et al. 2013). For example, the number of species (35) and families (19) in the high elevation plots were in-line with similar studies conducted along elevational gradients in the forests at Pashochoa volcano, Ecuador, where the number of species and families in high elevation plots were 32 and 21, respectively (Valencia and Jørgensen 1992). Furthermore, Gentry (1988) found that *Aquifoliaceae* and *Theaceae* become more abundant at high elevations, a result that matches this study's findings (Table 2). Only four species in the dataset were distributed across every plot in the transect, the vast majority of species had restricted ranges (see Lieberman et al. 1996 for a similar finding). These results suggest that

factors related to elevation play an important role in determining the composition of tropical montane forests where abiotic/biotic factors may be limiting species distributions within the transect (Homeier et al. 2010; Chain-Guadarrama et al. 2012).

Numerous suggestions have been made as to which factors most affect forest composition along elevational gradient; some of the primary abiotic factors suspected of causing community structure shifts are temperature, precipitation, cloud cover, soil nutrients, and light availability (Gentry 1988; Ashton 2003; Barone et al. 2008; Homeier et al. 2010). The main biotic factor that has been shown to determine community makeup is competition (Barone et al. 2008), although most studies suggest that there are multiple factors affecting community structure along elevational gradients that have to be accounted for (Bruijnzeel and Veneklaas 1998; Körner 2007). Based on the data, determining the causes of observed patterns is difficult because of the lack of environmental data needed to parse out which factors primarily control plant diversity at Siempre Verde.

Although abiotic or biotic factors at the study site were not directly measured, analyses showed the potential of one of these factors to affect plant distribution along the transect. Two of the mid-elevational plots (Plot 10 and 12) had the highest number of genera (n=18) among all plots (Table 4). Other studies along elevational gradients have similar findings where one plot, not located at the lowest elevation, exceeds all others in diversity (Lieberman et al. 1996). One potential cause for the increased diversity at these plot deals with cloud cover. Cloud cover is known to saturate forest causing a decrease in temperature and an increase in precipitation and overall moisture (Bruijnzeel and Veneklaas 1998; Barone et al. 2008; Girardin et al. 2013; Fahey et al. 2015). This has led many to refer to plots located where clouds move into the forest as "mid-elevation bulges" as the highest diversity is often seen at these intermediate elevation plots

(Girardin et al. 2013; Hutter et al. 2013; Clark et al. 2015). This diversity is often attributed to species reaching adaptation limits where species of lower elevation cannot adapt to increased precipitation and decreased temperature levels and species of higher elevations cannot adapt to increased temperature and decreased precipitation at lower elevations (Körner 2007). At these mid-elevations, a mixture of species can be seen at the limits of their niches, increasing diversity. Future analysis of soil nutrients and collection of long-term climatic data will aid in the evaluation of which factor(s) contribture most to the forest composition of Siempre Verde.

Sequence Recovery

Recovery of sequences was low compared to similar studies. In tropical and temperate forests, other studies have successfully sequenced between 85-93% of samples for *rbc*L and between 69-75% of samples for *mat*K (Kress et al. 2009; Kress et al. 2010; Burgess, et al. 2011; Muscarella et a. 2014). In this study, however, only 40% of *rbc*L and 27% of matK sequences were successful. An increase in recovery percentage for *rbc*L over *mat*K can be attributed to its shorter length, making it easier to be obtained from degraded DNA. It is believed that the majority of failure is due to herbarium specimen preservation techniques. DNA samples were taken from herbarium specimens that had been preserved in alcohol. It is known that alcohol quickly degrades the quality of DNA. In the future, DNA vouchers will be taken from fresh collections and dried in silica gel until DNA extraction.

When attempting to substitute unsuccessful sequences with those from BOLD and/or GenBank, only four species from the study site have *rbc*L or *mat*K sequences that are publicly available. After publication, the addition of the sequences obtained from this study to BOLD and

GenBank for public use will greatly contribute to future research and growing DNA barcode libraries.

Community Structure

Analysis showed that Shannon's diversity decreased with increasing elevation at the species, genus, and family levels (Fig. 4A). This trend is also visible when plots are grouped into elevational zones (Fig. 4B). This tendency of decreasing richness has been shown along elevation gradients in different forest types around the world (Gentry 1988, Kappelle et al. 1995, Givnish 1999, Ashton 2003, Homeier et al. 2010, Swenson et al. 2011, Clark et al. 2015). A similar relationship was also found between Simpson's diversity and elevation, as elevation increased, Simpson's diversity decreased at the species, genus, and family levels (Fig 5A). As with Shannon's diversity, the same relationship can be seen when plots are grouped by elevational zone (Fig. 5B). At all levels and within all plots, Simpson's diversity values were very high, comparable with other elevation gradient studies (Shaheen et al. 2011). The lowest value from any plot at the species level is 0.79 and 0.68 at the genus and family levels (Table 4). When plots were grouped by elevation, the lowest value was 0.80 (Table 4). Since Simpson's diversity was subtracted from one, the greater the value, the greater the diversity. Use of these diversity metrics showed that elevation plays a strong role in the structure and composition of forests along elevational gradients.

Phylogenetic Relationships

A phylogeny was constructed with all samples sequenced from the transect, including replication at the species level from different plots (Fig. 3). This phylogeny highlights potential

taxonomic identification issues, cryptic speciation, or lack of sequence resolution. An example of sequence resolution issues can be seen for *Ocotea sericea*. *Ocotea sericea* (T13_125) and *Ocotea sericea* (T15_27) are not identical matches on the phylogeny. One factor contributing to this difference is that *Ocotea sericea* (T13_125) has both *rbc*L and *mat*K sequences where *Ocotea sericea* (T15_27) only has a *rbc*L sequence. An example of potential cryptic speciation can be seen in four *Asteraceae sp.* samples that separate into potentially two separate species. *Asteraceae sp.* (T9_242) and *Asteraceae sp.* (T10_202) are identical sequences on the phylogeny. Also, *Asteraceae sp.* (T15_05) and *Asteraceae sp.* (T15_40) are shown as identical sequences on the phylogeny. When looking at *Oreopanax palamophyllus* and *Oreopanax grandifolius*, potential misidentification seems logical. *Mat*K and *rbc*L sequences were obtained for both species but they are still seen as identical on the phylogeny. These issues can be rectified in a variety of ways. Taxonomic identification issues can be reviewed by reexamining herbarium samples. Where sequence resolution is lacking, obtaining sequences for *rbc*L and *mat*K gene regions, as well as potentially adding a third gene region has been shown to correct resolution issues in phylogenies (Muscarella et a. 2014). Where cryptic speciation could be a factor, sequencing more individuals could shed light on divergent species.

Phylogenetic Structure

For the three different phylogenetic diversity metrics, phylogenetic distance (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD), observed values were compared to null model calculations to determine significance. These types of analyses focus on the rationale that some community assembly mechanisms favor co-existence of closely-related species, whereas others favor co-existence of distantly related species (Eiserhardt et al. 2013).

Significant differences from random were detected for each metric in a variety of plots (Table 3). All significance detected was consistent with phylogenetic clustering, meaning that individuals are not distributed evenly across the phylogeny and that they are more closely related than random. Plots 1, 2 and 10 were the only plots to show significance for all three metrics. Also, only MPD at medium and high elevations showed significance when plots were grouped by elevation (Table 3). Clumping of individuals has been hypothesized to be evidence for the influence of habitat filtering on community structure (Kress et al. 2009; Eiserhardt et al. 2013; Erickson et al. 2014; Boyle and Adamowicz 2015). This seems plausible for this transect as diversity peaks at mid-elevations (plot 10) where clouds begin to inundate the forest causing vast differences in habitat above and below this elevation. Since only four species span the entirety of the transect, abiotic stress could be the limiting factor in species distribution and main contributor to the community structure. Other studies offer evidence to why nonrandom patterns may not have been recovered in this research. One study suggests that poorly resolved phylogenies tend to reduce statistical power for detecting patterns of community structure (Muscarella et al. 2014). In the future, having both *rbc*L and *mat*K sequences as well as potentially adding a third intergenic spacer region sequence, for all individuals used for phylogenetic structure analyses will improve resolution and statistical power. Also, the addition of species trait information could help determine processes underlying variation along elevational gradients (Muscarella et al. 2014).

Synthesis

From this research, a few major patterns can be seen in the data. Both Shannon's diversity and Simpson's diversity decrease with increasing elevation (Fig 4)(Fig 5). Since

Shannon's diversity focuses on species richness and Simpson's diversity has its focus on the lopsidedness of abundance, determining the relationship between these similar trends is important. To investigate this further, the number of stems was analyzed with increasing elevation (Girardin et al. 2013). The number of stems was found to increase, but not significantly. In summary, species richness decreases with elevation but the number of stems of common species increases. Hence, at higher elevations there are fewer species, but more individuals of the same species.

In agreement with this information, significant clumping was found at the highest two plots within the transect for all three metrics tested (Table 3). Hence, there are more closely related species at higher elevations. Diversity peaks at mid-elevations (plot 10) for this transect, with only four species spanning the entirety of the transect. Determining the reason for this community structure relies on interpretation of the importance of abiotic and biotic factors in the community. In conclusion, plant communities are constructed in non-random ways along elevational gradients.

Conclusion

This research shows that in order to maintain long-term biodiversity, many elements need to be preserved including genetic and environmental diversity. While diversity metrics continue to be used to describe the composition of an environment with a single number, the addition of genetic diversity values can aid in an overall analysis of the habitat for better conservation awareness (Valentini et al. 2008). No matter the technique used for measurement, the tropical Andes stand out as an international diversity hotspot (Girardin et al. 2013; Luebert and Weigend 2014; Hughes 2016).

To aid in a better global understanding of tropical forests along elevational gradients, broader relationships should be analyzed in these areas that take into account climate, geology, soils and vegetation. It is the expectation that as more plants are DNA barcoded around the world, comparative measures of phylogenetic diversity will become standard metrics for biodiversity assessment (Dick and Kress et al. 2009; Kress et al. 2015). These DNA barcodes will add value to DNA barcode libraries that support future research endeavors. To completely grasp the biodiversity along elevational gradients, taxonomic diversity, functional diversity, and phylogenetic diversity must all be considered in the future.

Chapter II

Evaluation of the phylogenetic relationship between phytochemical presence and genetic diversity in tropical tree species

Abstract

Only a small percentage of the world's flora has been adequately analyzed to determine its chemical composition. Analyzing the chemical content of plants seems an overwhelming task, with around 300,000 higher plant species on Earth. Treating phytochemical content as a trait measurement and combining this information with DNA barcode genetic sequences has the potential to lead to a better understanding of the distribution of medicinal plants. The western upland Amazon of Ecuador is considered a biodiversity hotspot for both plants and animals and is home to Yasuní National Park, where many medicinal plants with their phytochemicals are waiting to be discovered. Using a combination of indigenous guidance and prior chemical component knowledge, predictions can be made about which plants in Ecuadorian forest are medicinal. The goal of this research was to evaluate the phylogenetic relationship between phytochemical presence and phylogenetic dispersion in tropical tree species of Yasuní National Park, Ecuador. Specifically, this study aimed to construct a tropical tree community phylogeny using DNA barcodes and to test for phylogenetic signal in the occurrence of phytochemicals. Within Yasuní, 337 common tree species making up 181 genera and 56 families were sequenced. Of these individuals, 248 species were successfully sequenced for the *rbc*L and/or *mat*K gene regions with 67 of these classified as medicinal. Mean pairwise distance (MPD), mean nearest taxa distance (MNTD), and Fritz and Purvis' D statistic support a random distribution of the medicinal trait within the phylogeny. In the future, having both *rbc*L, *mat*K and potentially a

third intergenic spacer region sequences, as well as a complete sampling of all tree species in Yasuní National Park will improve resolution, statistical power, and the ability to detect fine scale trait distribution patterns. It is hoped that complete tree phylogenies will be constructed for Yasuní so comparative studies can be initiated in order to better conserve this unique biodiversity hotspot.

Introduction

In many world cultures the health benefits of medicinal plants have been recognized for centuries (Shanley and Luz 2003; Sharma and Sarkar 2013). Approximately one in four plant species have been used in some form of traditional medicine (Saslis-Lagoudakis et al. 2011), although only a small percentage of the world's flora has been thoroughly assessed for their medicinal components (Saslis-Lagoudakis et al. 2012). Many of the health benefits attributed to medicinal plants are the result of synergistic effects among naturally occurring, non-nutritive secondary metabolites more commonly referred to as phytochemicals (Paranhos et al. 2005). As a result of diverse adaptive responses to insect herbivory, phytochemicals often display chemical and/or structural complexity (Larsen et al. 2010; Fine et al. 2014). The on-going evolutionary arms race between plants and their insect herbivores has not only produced diverse phytochemistry but has also contributed to a staggering amount of biological diversity (García-Robledo et al. 2013). Given that there are approximately 300,000 plant species worldwide, discovering plant species with potential health benefits and subsequently analyzing them for their phytochemical content is an overwhelming task.

Tropical forests may be an ideal place to search for medicinal plants. Due to the response to herbivory pressures imposed by insects, leaves of tropical forests have been shown to have both higher overall levels of defenses and a greater variety of defenses when compared to their temperate counterparts (Coley and Barone 1996). About 170,000 species of vascular plants, or 68% of the known plant species on the planet, can be found in the tropical forests of South America, Africa, and Asia (Rios et al. 2007). Within a single hectare of tropical forest, as many as 900 vascular plants have been found (Dick and Kress 2009). The Amazon rainforest, the largest rainforest on Earth, is a biodiversity hotspot with many medicinal plants and their

phytochemicals waiting to be discovered (Davis and Yost 1983a; Bennett 1992). Unfortunately, the Amazon is being degraded at a faster rate than the discovery of plants and animals. In 2009, over half of the estimated 11,000 Amazonian trees species were at risk of extinction (Gonzalez et al. 2009).

Treating phytochemical content as a trait measurement and combining this information with genetic analyses could guide future investigations in the discovery of phytochemicals within certain clades of tropical plant community phylogenies. Previous studies that have combined phylogenetic and phytochemical analyses have shown that there is a strong phylogenetic signal in the distribution of chemical constituents in plants, but chemical data is largely unavailable for most tropical plant species (Saslis-Lagoudakis et al. 2011). Notably, the use of functional trait analyses to predict phylogenetic signals is often time consuming and expensive when trying to directly determine the chemical content of plants. An alternative approach to directly sampling the chemical content of each species to be incorporated in a phylogenetic analysis is to use prior research on the presence/absence of chemical/medicinal properties of these species. Although there are numerous phytochemical studies focused on certain plant genera or families (Larsen et al. 2010; Newmaster and Ragupathy 2010; Courtois et al. 2012), the phylogenetic signal of phytochemical composition in tropical plant communities has rarely been evaluated.

DNA barcoding has been widely used as a tool for investigation in ecology, evolution, and conservation biology (Losos 1996; Hebert et al. 2003; Valentini et al. 2008; Chen et al. 2010; Erickson et al. 2014; Muscarella et al. 2014). For plants, a DNA barcode consists of a short standardized sequence of DNA from the chloroplast genome that can be generated in a rapid, accurate and cost-effective manner (Newmaster et al. 2013, Kress et al. 2015). Many factors that could complicate taxonomic identifications, such as the age of a specimen, the

absence of flower, or small size, have little bearing on the ability of DNA barcoding to identify a species. DNA barcodes usually include a phylogenetically conserved coding region (*rbc*L) with the addition of one or more rapidly evolving gene regions such as *mat*K or t*rn*H-*psb*A (CBOL 2009; Kress et al. 2009; Saslis-Lagoudakis et al. 2011). Typically, the conserved locus will easily align taxa at higher taxonomic levels whereas the hyper-variable regions will align sequences that are more closely related at lower taxonomic levels (Hollingsworth et al. 2011). Combined with trait data, phylogenies based on DNA barcodes not only have the potential to signal medicinal plants in a plant community but may also be able to provide insights into the evolutionary relationships of phytochemical adaptations among species.

Most community-level analyses have relied heavily on previously published taxon-specific phylogenies (Kress et al. 2015). Such studies, however, often lack DNA sequence data and are not able to truly show evolutionary relationships between species (Kress et al. 2009). DNA barcode phylogenies have the advantage in their ability to resolve relationships at the species-level and provide estimates of evolutionary distances that connect clades within the phylogeny (Erickson et al. 2014). For example, on Barro Colorado Island, a phylogeny was assembled for 296 woody plant species using three traditional markers, *rbc*L, *mat*K, and *trn*H-*psb*A (Kress et al. 2015) and in Puerto Rico, 136 species were DNA barcoded with *rbc*L, *mat*K, and *trn*H-*psb*A (Kress et al. 2010). Another major phylogenetic study was conducted on 1347 tree species across 15 forest dynamic plots in the ForestGEO network using the same three loci as the previous two studies mentioned (Erickson et al. 2014). Many aspects of ecology, evolution, and biogeography patterns, not well understood, were revealed from these large scale community phylogenetic studies (Losos 1996; Muscarella et al. 2014). Given the extensive and successful use of DNA barcoding to build tropical forest community phylogenies, the technique

shows tremendous potential to elucidate phylogenetic patterns of phytochemical dispersion among tropical plant communities.

Goal and Objectives

The goal of this research was to investigate the relation between phytochemical presence and its phylogenetic dispersion in tropical tree species. Specifically, the objectives are to 1) construct a tropical tree community phylogeny using DNA barcodes and 2) test for phylogenetic signal in the occurrence of phytochemicals among taxa.

Methods

Study Site

The research is focused in Yasuní National Park that is located where the foothills of the Andes Mountains meet the Amazon in eastern Ecuador. The topography ranges from completely flat to steeply dissected as the proportion of sand in the surface soil has been shown to vary from 11 to 51%. The soil has been tentatively classified as Ultisols and Inceptisols, derived from young deposits of Andean alluvium (Pitman et al. 2001; Valencia et al 2004a). This habitat is home to the greatest species richness of amphibian, birds, mammals and trees of anywhere on Earth at local scales, $\leq$100 km^2 (Bass et al. 2010). Yasuní National Park is 9,820 km^2 of western Amazonian forest situated at 76° 24′ 1.8′′ W; 0° 40′ 16.7′′ S along the border between Ecuador and Peru (Fig. 6) (Kraft and Ackerly 2010). The forest of Yasuní are dominated by a few common species and many rare species, mainly composed of evergreen lowland wet forest (Pitman et al. 2001; Valencia et al. 2004a). The canopy varies between 15 and 30 meters tall with some emergent trees reaching 40 and up to 50 meters (Valencia et al. 2004a).

Yasuní has been classified as having an aseasonal climate, but variation in monthly rainfall amount is seen (Valencia et al. 2004; Kraft and Ackerly 2010). The annual rainfall is approximately 3200 mm year-round with the wettest months being April-May and October-November (Pitman et al. 2001; Valencia et al. 2004a). Humidity averages around 80-94% throughout the year (Valencia et al. 2004). The mean shade temperature is approximately 23 °C with temperatures never dropping below 10 °C (Pitman et al. 2001). In full sun, the average temperature is around 35 °C (Valencia et al. 2004). All elevations are less than 500 meters, with a landscape dominated by terra firme forest (Valencia et al. 2004; Kraft and Ackerly 2010). Permission to carry out research in Ecuador and at Yasuní National Park was given by the Ministerio de Ambiente (permit #).

Sampling Design

Previous analyses indicate that there are 337 common tree species (>100 individuals within 50-ha) in Yasuní National Park (Table S6, Pérez et al. 2014). These species were chosen as the data set for this research and provide a base-line for future efforts to DNA barcode the majority of the tree species of the Yasuní region. DNA vouchers were collected from herbarium specimens housed in Herbario-QCA at Pontificia Universidad Católica del Ecuador, in the herbarium at the Yasuní Research Station, or from freshly collected specimens.

DNA isolation, PCR amplification, and Sequencing

DNA extraction, PCR amplification, and sequencing were conducted at the Canadian Center for DNA Barcoding, Biodiversity Institute of Ontario, Canada following their protocols with adaptations by Maria Kuzmina (CCDB Protocols DNA Extraction; CCDB Protocols PCR

Amplification; CCDB Protocols Sequencing) (Appendix). The chloroplast gene regions ribulose-bisphosphate/carboxylase large subunit (*rbc*L) and maturase-K (*mat*K) were sequenced using forward and reverse primers: rbcLa-F/rbcLa-R and matK-xf/matK-MALP (Table 1). After publication, all sequences will be publicly available on the Barcode of Life Datasystem (BOLD) (www.boldsystems.org) (Ratnasingham and Herbert 2007). Supplemental sequences for taxa that were unable to be sequenced were obtained from BOLD and GenBank.

Data Analysis

Trait Collection

Information on medicinal value for species included in this study was compiled from prior publications and local compendia of traditional medicine. All of the original data for these resources was collected from within present day Yasuní National Park or within directly comparable forest type. Due to the difficulty in obtaining chemical component data for large samples of plants, the use of prior literature is a common procedure to identify medicinal plant species (Newmaster and Ragupathy 2010; Saslis-Lagoudakis et al. 2011; Saslis-Lagoudakis et al. 2012). Five sources of information were used in this research including four published manuscripts, Davis and Yost 1983, Schultes and Raffauf 1990, Cerón and Montalvo 1998, Rios et al. 2007, and unpublished data from direct interviews with members of the Waorani community within the immediate area of the study site. Interviews were conducted during three separate collecting trips, with four consecutive days of morning and afternoon interviews per trip. All interviews were conducted along established trails near the Yasuní Research Station or at the Waorani village. Participants in the interviews were both male and female spanning ages between 25 and 65 years old. Plants of any ethnobotanical use were pointed out by the

interviewee then recorded and identified. This data set was trimmed to only include plants of medicinal value to address the questions of this research.

Phylogenetic Analysis

After sequences were obtained, alignments and phylogenies were constructed using Geneious version 9.1 (http://www.geneious.com, Kearse et al. 2012; Muscarella et al. 2014). The *rbc*L and *mat*K genes were aligned separately using Multiple Alignment that uses Fast Fourier Transform (MAAFT v. 7) (Katoh et al. 2002) default settings. These alignments were then concatenated into an alignment supermatrix. A phylogeny was generated using maximum likelihood (ML) methods, executed in Geneious using Randomized Axelerated Maximum Likelihood (RAxML v. 7.2.8) (Stamatakis 2006). *Ginkgo biloba* served as the outgroup in agreement with a prior publication (Kress et al. 2009). Nucleotide substitution was modeled using the GTR+GAMMA model, with substitution rates estimated independently for each gene (Saslis-Lagoudakis et al. 2011; Muscarella et al. 2014). The rapid bootstrapping algorithm was implemented in order to search for the best scoring ML tree after node support was evaluated using 1000 bootstrap runs (Stamatakis et al. 2008).

Phylogenetic Structure Analysis

Phylogenetic analyses were estimated using multiple metrics in order to get a complete picture of the phylogenetic distribution of medical plants within the data set. First, phylogenetic analyses were estimated within the Picante package (Kembel et al. 2010) of the R programming language. Two metrics were assessed in this package, mean pairwise distance (MPD) (Webb 2000) and mean nearest taxon distance (MNTD) (Webb 2000). The MPD metric obtains a pair-

wise phylogenetic distance (distance matrix) across all pairs of taxa in a community and gives an estimate of the overall divergence of taxonomic clades present.

$$MPD = \frac{\sum_i^n \sum_j^n \delta_{i,j}}{n}, where\ i \neq j$$

where there are n species in the community or sample, δ is the phylogenetic distance matrix, $\delta_{i,j}$ is the phylogenetic distance between species i and j. It can be considered to be a "basal" metric of phylogenetic diversity as it captures the overall phylogenetic dissimilarity of the taxa in a sample. MPD does not detect finer scale phylogenetic patterns that may be present (Erickson et al. 2014; Swenson 2014). The second metric estimated in the Picante package was MNTD. It provides an average of the distances between each species and its nearest phylogenetic neighbor in the community. It quantifies the degree that a community may be a set of closely related species versus a heterogeneous set of taxa from disparate taxonomic clades (Erickson et al. 2014).

$$MNTD = \frac{\sum_i^n \min \delta_{i,j}}{n}, wehre\ i \neq j$$

where there are n species in the community, $\delta_{i,j}$ is the phylogenetic distance between species i and j, and $\min\delta_{i,j}$ is the minimum phylogenetic distance between species i and all other species in the community (i.e. the nearest neighbor distance) (Swenson 2014).

For both metrics, the raw values give no means of standardized comparisons between communities. Null models were implemented so that standardized effect sizes (S.E.S.) could be determined.

$$Standardized\ effect\ size = \frac{observed - \overline{null}}{sd(null)}$$

This calculation removes any directional bias associated with the decreases in variance in the expected values with increasing species richness (Swenson 2014). For MPD and MNTD,

positive S.E.S. values (obs.z > 0) and high quantiles (obs.p > 0.95) indicate phylogenetic evenness, or a greater phylogenetic distance among co-occurring species than expected. Negative S.E.S. values (obs.z < 0) and low quantiles (obs.p < 0.05) indicate phylogenetic clustering, or smaller phylogenetic distances among co-occurring species than expected (Kembel 2010; Saslis-Lagoudakis et al. 2011, Muscarella et al. 2014). Positive values of S.E.S. indicate a higher than average expected value and a negative S.E.S. values indicated a lower than average expected value. The category of null model used in this research was constrained randomization where the row and column sums are fixed in the presence-absence community data matrix in all randomizations (Swenson 2014). For the MPD, and MNTD metrics, the 'taxa.labels' null model was used with 999 runs and 1000 iterations to determine standardized effect sizes (Saslis-Lagoudakis et al. 2011;Erickson et al. 2014, Muscarella et al. 2014).

The last metric analyzed in this study was Fritz and Purvis' *D* statistic (Fritz and Purvis 2010) to measure phylogenetic signal in a binary trait using the Caper package (Orme et al. 2013) of the R programming language. When you estimate the *D* value, it tests for significant departure from both random association and clumping expected under Brownian Motion evolution. When the estimate for $D=1$, the distribution of binary traits is said to be random with respect to the phylogeny. When the estimate for $D=0$, the distribution is expected under Brownian motion. *D* can also be estimated as less than zero or greater than one indicating phylogenetic clumping or over-dispersion, respectively (Fritz and Purvis 2010; Weber and Keeler 2013).

Results

Forest Composition

Within this data set, there are 337 tree species, from 181 genera and 56 families (Table S6). The most abundant genus was *Inga*, while the most abundant family was Fabaceae. There are 61 (33.5%) polytypic genera with an average of 1.86 species per genus. There are 18 (32.1%) monotypic families in the data set.

Sequence Recovery

With the addition of publicly available sequences from GenBank and BOLD, 148 (43.9%) of the species had both *rbc*L and *mat*K sequences and 84 (24.9%) had only a *rbc*L sequence (Fig. 7). Only 16 (4.7%) of the individuals had a *mat*K sequence without also having a *rbc*L sequence. Uni-directional sequences were used when they were of high quality; 9 uni-directional *rbc*L and 49 uni-directional *mat*K sequences. Public databases contributed 43 (12.8%) *rbc*L sequences and 50 (14.8%) *mat*K sequences (Fig. 7). The source of outside sequences from BOLD and/or GenBank can be seen in Table S6. There were 90 individuals that failed sequencing and did not have publicly available sequences.

Trait Designation

The designation of medicinal or non-medicinal assigned to each species and the source(s) that confirm the information are seen in Table S6. Overall, 88 (26.1%) species were designated as medicinal and 249 (73.9%) were designated as not being noted for their medicinal composition. After accounting for sequence failures, there were 67 medicinal and 181 non-medicinal species in the molecular data set (Total = 248 taxa used in the phylogenetic analysis).

Phylogenetic Analysis

A phylogenetic tree of all successful sequenced individuals was constructed (Fig. 8). This tree includes 49 families, 151 genera, and 248 species distributed across six main clades (Fig. S5-S10). The consensus tree from rapid bootstrapping found 62.6% of all nodes were supported by 50% or greater. Monophyly was supported for only 17.9% of genera.

Phylogenetic Structure Analysis

Values for mean pairwise distance, mean nearest taxon distance, and Fritz and Purvis' D are given in Table 5. Both MPD and MNTD were significantly different from random, with positive S.E.S. values (obs.z > 0) and high quantiles (obs.p > 0.95) with values indicating phylogenetic evenness (Table S7). The D value was significantly different from 0, but not significantly different from 1 indicative of random trait distribution (Table S7).

Discussion

Sequence Recovery

Recovery of sequences in the current study was relatively low compared to that of other floras that have been screened with DNA barcodes (Fig. 7). In the current study, sequences were successfully recovered from 56% and 34% of samples for *rbc*L and *mat*K, respectively. This compares to 85-93% (*rbc*L) and 69-75% (*mat*K) recovery rates for fresh samples taken from taxa in tropical (Kress et al. 2009; Kress et al. 2010; Muscarella et a. 2014) and temperate (Burgess et al. 2011) forests, although recovery of the *rbc*L gene region was also more successful than *mat*K in the current study. The reason for the low sequencing success found in this study is likely attributable to the quality of the samples used, where the majority of DNA vouchers were taken

from herbarium specimens with an average collection year of 2007. This is also a likely explanation for why the *rbc*L gene region was more successful than *mat*K due to its shorter length, which makes it easier to capture from degraded DNA compared to previous studies that were mainly sampling fresh tissue. Previous research has shown that there is generally a decrease in sequence recoverability with the age of herbarium specimen (especially after 10 years) and that ~74% and 53% recovery rates of *rbc*L and *mat*K, respectively could be expected from herbarium specimens (de Vere et al. 2012). Given the time it takes to process a sample from collection to sequence, additional effort to obtain fresh material, especially for the specimens with failed sequences, should be considered for future research at Yasuni.

BOLD and/or GenBank contributed 93 sequences (43 *rbc*L and 50 *mat*K) to missing data in this research (Fig. 7). Many other studies use publicly available sequences in order to replace failures that occur during the sequencing process in order to have the most complete data set of the community (Saslis-Lagoudakis et al. 2012; Erickson et al. 2014; Muscarella et al. 2014). However, there were still sequences obtained that are not available in these databases. After publication, the addition of the sequences obtained from this study to BOLD and GenBank for public use will greatly contribute to future research and growing DNA barcode libraries.

Trait Designation

Only 19.9% (67) of the species were classified as medicinal. In the literature, there is some dispute about the accurate number of medicinal plants in this area. These results are in-line with surveys by Davis and Yost (1983a) who believed they had sampled over 80% of all plants used by Waorani Indian tribes in this same region and found that only 35 species were used medicinally. However, it has also been estimated that there are between 1,300 -1,550 medicinal

plants in this region (Schultes 1979; Schultes and Raffauf. 1990). It seems likely that the results reflect the relatively low sample size (248) represented in the molecular data set compared to the actual flora of Yasuní National Park (over 1,400 tree species) or the Amazon forest (approximately 11,000 tree species) (Dick and Kress 2009; Gonzalez et al. 2009). Regardless of this limitation, the base estimate certainly represents the potential phytochemical diversity in the Yasuni National Park and underscores the need for further collections in the area.

Phylogenetic Analysis

The phylogenetic tree produced included 248 species (Fig. 8). The consensus tree from rapid bootstrapping found 62.6% of all nodes were supported by 50% or greater. This number is closely comparable with 68% of all nodes in a 523 species phylogeny from Puerto Rico and with 65% of all nodes in 296 species from Panama (Kress et al. 2009; Muscarella et al. 2014). However, 87% of genera were monophyletic while this research supported monophyly for only 17.9% of genera (Muscarella et al. 2014). When conducting phylogenetic analyses, it is best to have all monophyletic taxa to decrease errors in conclusions drawn from phylogenetic relationships (Losos 1996). Increased sampling will correct instances of paraphyletic groups in future work.

The phylogenic analysis has illuminated potential taxonomic or sequencing errors in the dataset. For example, three *Protium* species were sequenced, but two (*Protium nodulosum* and *Protium sagotianum*) were found clumped together and the other (*Protium guianense*) was in a completely separate clade (Fig. 9A). In a less severe instance, two *Geonoma* species (*Geonoma cf. aspidiifolia* and *Geonoma* maxima) were spread apart within a clade (Fig. 9B). Such discrepancies are likely due to taxonomic errors during initial identification of the plant

specimens. Alternatively, examples of potential sequencing errors come from the genera *Sterculia* and *Piper*. *Sterculia frondosa* has *rbc*L and *mat*K sequences from GenBank, but *Sterculia tessmannii* had a *rbc*L sequence that we obtained. These two species diverge greatly in their location on the phylogeny, but *Sterculia tessmannii* is within the same clade as all other genera in the Malvaceae family (Fig. 10A). In the *Piper* instance, *Piper arboreum* and *Piper augustum* each have only one gene region sequenced, both obtained from GenBank, and divergent in the phylogeny (Fig. 10B). Such discrepancies could be due to the fact that GenBank lacks regulation of available sequences allowing erroneous sequences to become publicly available. In the future, the identity of sequences obtained from GenBank should be confirmed prior to inclusion in studies

Phylogenetic Structure Analysis

For all of the phylogenetic analyses performed, mean pairwise distance (MPD), mean nearest taxon distance (MNTD), and Fritz and Purvis's D statistic were compared to null model calculations to determine significance. These types of analyses focus on the rationale that some assembly mechanisms favor co-existence of closely-related species, whereas others favor co-existence of distantly related species (Eiserhardt et al. 2013). Significant differences from random were detected for all three metrics, (Table 5 and S7) consistent with phylogenetic evenness meaning there was greater phylogenetic distance among co-occurring species than expected. This result was surprising as previous research has shown that medicinal properties in plants tend to be clumped within genera and families (Lukhoba et al. 2006; Larsen et al. 2010; Saslis-Lagoudakis et al. 2012). One instance of random distribution was found in the *Pterocarpus* species (Saslis-Lagoudakis et al. 2011). However, these metrics tend to rely heavily

on sampling and the phylogenetic relationship between samples. There were instances within the phylogeny that supported the results of a random distribution and signs of medicinal trait clumping that could potentially be significant if the sample size was increased. The Meliaceae clade supported the results of a random distribution of the medicinal trait (Fig. 11). When comparing the Myristicaceae and Annonaceae clades, all species in the Myristicaceae clade are medicinal, but there is a random distribution within the Annonaceae clade (Fig. 12). With more sampling within the Myristicaceae family, a clumping pattern of the medicinal trait could be potentially obtained. Lastly, the Moraceae clade shows a distribution of medicinal species that appears less than random (Fig. 13).

The estimate for the Fritz and Purvis' D statistic was found to be significantly different than 0 but not significantly different than 1 (Table 5 and S7). For this statistic, the closer the value is to 1, the more likely the trait is distributed randomly within the phylogeny. The result indicates that medicinal plants are randomly distributed throughout the phylogeny. This research is the first to access phylogenetic signal of medicinal presence/absence using the D statistic, with only a few comparable studies in plants. For example, the D statistic has revealed that the phylogenetic signal of plant exudates clusters according to Brownian expectations (Whitfeld et al. 2012), and the phylogenetic distribution of extra-floral nectaries has a moderate level of phylogenetic signal (Weber and Keeler 2013). Although using the D statistic to measure phylogenetic signal of medicinal presence/absence is a novel application, it can reduce cost and time of determining the exact quantity of phytochemicals in every plant species and can be integrated into future phylogenetic studies of tree communities.

When considering the results of all three metrics, medicinal properties appear to be randomly distributed within the Yasuní tree community. These metrics are largely determined by

density of sampling and the phylogenetic relationship between samples. After accounting for sequencing failures, the data set had very few instances where multiple species within genera and/or multiple genera within families were included. For example, 4 out 6 *Eugenia* species, 9 out of 10 *Miconia* species, 7 out 18 Malvaceae species, and 5 out of 9 Myristicaceae species were not included in phylogenetic analyses due to lack of sequences. Studies suggest that incompletely sampled communities and poorly resolved phylogenies tend to reduce statistical power for detecting patterns of community structure (Muscarella et al. 2014; Swenson and Umaña 2014). In the future, having both *rbc*L, *mat*K and potentially a third intergenic spacer region sequences, as well as a complete sampling of all tree species in Yasuní National Park will improve resolution, statistical power, and the ability to detect fine scale trait distribution patterns.

Conclusion

Tropical forests are home to the largest variety of plant and animal life of anywhere on the planet. However, some of the most diverse places like Yasuní National Park have had little research completed. Previous work has set a precedent on the type of analyses that can be completed at Yasuní and this research is a step forward in gaining an understanding of tree community ecology at Yasuní and its contribution to global plant biodiversity knowledge. In the future, it is anticipated that more research will combine multiple fields of study including taxonomic, phylogenetic, and ethnobotanical information in order to provide new perspectives to these fields.

This research will also facilitate the building of a DNA barcode sequence library that will enable future barcoding applications. It is the expectation that as more DNA barcode libraries are populated with species from around the world, comparative measures of phylogenetic diversity

will become standard metrics for conservation assessment (Dick and Kress 2009; Kress et al. 2015). It is hoped that within the near future, complete tree phylogenies will be constructed for Yasuní so comparative studies can be initiated in order to better conserve this unique biodiversity hotspot.

References

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161:105-121.

Ashton, P. A. 2003. Floristic zonation of tree communities on wet tropical mountains revisited. Perspect. Plant Ecol. Evol. Syst. 6:87-104.

Asner, G. P., C. B. Anderson, R. E. Martin, D. E. Knapp, R. Tupayachi, F. Sinca, and Y. Malhi. 2014. Landscape-scale changes in forest structure and functional traits along an Andes-to-Amazon elevation gradient. Biogeosciences. 11:843-956.

Barone, J. A., J. Thomlinson, P. A. Cordero, and J. K. Zimmerman. 2008. Metacommunity structure of tropical forest along an elevation gradient in Puerto Rico. J. Trop. Ecol. 24:1-10.

Bass, M. S., M. Finer, C. N. Jenkins, H. Kleft, D. F. Cisneros-Heredia, S. F. McCracken, N. C. A. Pitman, P. H. English, K. Swing, G. Villa, A. Di Fiore, C. C. Voigt, and T. H. Kunz. 2010. Global conservation significance of Ecuador's Yasuní National Park. PLoS ONE 5:e8767.

Bennett, B. C. 1992. Plants and people of the Amazonian rainforest. BioScience 42:599-607.

Boyle, E. E. and S. J. Adamowicz. 2015. Community phylogenetics: Assessing tree reconstruction methods and the utility of DNA barcodes. PLoS ONE 10:e0126662.

Braukmann, T. W. A., M. L. Kuzmina, J. Sills, E. V. Zakharov, P. D. N. Hebert. 2017. Testing the efficacy of DNA barcodes for identifying the vascular plants of Canada. PLoS ONE 12:e0169515.

Bruijnzeel, L. A. and E. J. Veneklaas. 1998. Climatic conditions and tropical montane forest productivity: the fog has not lifted yet. Ecology 79:3-9.

Burgess, K. S., A. J. Fazekas, P. R. Kesanakurti, S. W. Graham, B. C. Husband, S. G. Newmaster, D. M. Percy, M. Hajibabaei, and S. C. H. Barrett. 2011. Discriminating plant species in a local temperate flora using the *rbc*L + *mat*K DNA barcode. Methods Ecol. Evol. 2:333-340.

CBOL Plant Working Group: P. M. Hollingsworth, L. L. Forrest, J. L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M. W. Chase, R. S. Cowan, D. L. Erickson, A. J. Fazekas, S. W. Graham, K. E. James, K-J. Kim, W. J. Kress, H. Schneider, J. van AlphenStahl, S. C. H. Barrett, C. van den Berg, D. Bogarin, K. S. Burgess, K. M. Cameron, M. Carine, J. Chacón, A. Clark, J. J. Clarkson, F. Conrad, D. S. Devey, C. S. Ford, T. A. J. Hedderson, M. L. Hollingsworth, B. C. Husband, L. J. Kelly, P. R. Kesanakurti, J. S. Kim, Y-D. Kim, R. Lahaye, H-L. Lee, D. G. Long, S. Madriñán, O. Maurin, I. Meusnier, S. G. Newmaster, C-W. Park, D. M. Percy, G. Petersen, J. E. Richardson, G. A. Salazar, V. Savolainen, O. Seberg, M. J. Wilkinson, D-K. Yi, and D. P. Little. 2009. A DNA barcode for land plants. Proc. Natl. Acad. Sci. U.S.A. 106:12794-12797.

CCDB Protocols, DNA Extraction. Prepared by, Ivanova, N., M. Kuzmina, and A. Fazekas. Available online: http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_DNA_Extraction-Plants.pdf (accessed on 13 October 2016).

CCDB Protocols, PCR Amplification. Prepared by, Kuzmina, M. and N. Ivanova. Available online: http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_Amplification-Plants.pdf (accessed on 13 October 2016).

CCDB Protocols, Sequencing. Prepared by, Kuzmina, M. and N. Ivanova. Available online: http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets-Plants.pdf (accessed on 13 October 2016).

Cerón, C. E. and C. Montalvo. 1998. Ethnobotany of the Huaorani and Quehueiri-Ono-Ecuador Napo. Ediciones Abya-Yala, Quito, Ecuador.

Chain-Guadarrama, A., B. Finegan, S. Vilchez, and F. Casanoves. 2012. Determinants of rain-forest floristic variation on an altitudinal gradient in southern Costa Rica. J. Trop. Ecol. 28:463-481.

Chen, S., H. Yao, J. Han, C. Liu, J. Song, L. Shi, Y. Zhu, X. Ma, T. Gao, X. Pang, K. Luo, Y. Li, X. Li, X. Jia, Y. Lin, and C. Leon. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLoS ONE 5:e8613.

Clark, D. B., J. Hurtado, and S. S. Saatchi. 2015. Tropical rain forest structure, tree growth and dynamics along a 2700-m elevational transect in Costa Rica. PLoS ONE 10:e0122905.

Clark, K., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2016. GenBank. Nucleic Acids Res. 44:D67-D72.

Coley, P. and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. Annu. Rev. Ecol. Evol. Syst. 27:305-335.

Condit, R. N. Pitman, E. G. Leigh Jr., J. Chave, J. Terborgh, R. B. Foster, P. Núñez V., S. Aguilar, R. Valencia, G. Villa, H. C. Muller-Landau, E. Losos, and S. P. Hubbell. 2002. Beta-Diversity in Tropical Forest Trees. Science 295:666-669.

Courtois, E. A., C. Baraloto, C. E. T. Paine, P. Petronelli, P. Blandinieres, D. Stien, E. Höuel, J. Bessiere, and J. Chave. 2012. Differences in volatile terpene composition between the bark and leaves of tropical tree species. Phytochemistry, 82:81-88.

Davis, W. and J. Yost. 1983. The ethnobotany of the Waorani of Eastern Ecuador. Botanical Museum Leaflets, Harvard University. 29:159-217.

Davis, W. and J. Yost. 1983a. The ethnomedicine of the Waorani of Amazonian Ecuador. J. Ethnopharm. 9:273-297.

de Vere, N. T. C. G. Rich, R. Ford, S. A. Trinder, C. Long, C. W. Moore, D. Satterthwaite, H. Davies, J. Allainguillaume, S. Ronca, T. Tatarinova, H. Garbett, K. Walker, and M. J. Wilkinson. 2012. DNA barcoding the native flowering plants and conifers of Wales. PLoS ONE 7:e37945.

Dick, C. and W. J. Kress. 2009. Dissecting tropical plant diversity with forest plots and a molecular toolkit. BioScience 59:745-755.

Dunning, L. T. and V. Savolainen. 2010. Broad-scale amplification of *matK* for DNA barcoding plants, a technical note. Bot. J. Linn. Soc. 164:1-9.

Eiserhardt, W. L., J. Svenning, F. Borchsenius, T. Kristianses, and H. Balslev. 2013. Separating environmental and geographical determinants of phylogenetic community structure in Amazonian plants (*Arecaceae*). Bot. J. Linn. Soc. 171:244-259.

Erickson, D. L., F. A. Jones, N. G. Swenson, N. Pei, N. Bourg, W. Chen, S. J. Davies, X. Ge, Z. Hao, R. W. Howe, C. L. Huang, A. Larson, S. Lum, J. A. Lutz, K. Ma, M. Meegaskumbura, X. Mi, and J. D. Parker. 2014. Comparative evolutionary diversity and phylogenetic structure across multiple forest dynamics plots: a mega-phylogeny approach. Front. Genet. 5:358.

Fahey, T. J., R. E. Sherman, and E. V. J. Tanner. 2015. Tropical montane cloud forest: environmental drivers of vegetation structure and ecosystem function. J. Trop. Ecol. 1-13.

Faith, D. P. 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61:1-10.

Fine, P. V. A., F. Zapata, and D. C. Daly. 2014. Investigating processes of neotropical rain forest tree diversification by examining the evolution and historical biogeography of the Protieae (Burseraceae). Evolution, 68:1988–2004.

Ford, C. S., K. L. Ayres, N. Toomey, N. Haider, J. V. A. Stahl, L. J. Kelly, N. Wikström, P. M. Hollingsworth, R. J. Duff, S. B. Hoot, R. S. Cowan, M. W. Chase, and M. J. Wilkinson. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. Bot. J. Linn. Soc. 159:1-11.

Fritz, S. A. and A. Purvis. 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. Conserv. Biol. 24:1042-1051.

García-Robledo, C., D. L. Erickson, C. L. Staines, T. L. Erwin, and W. J. Kress. 2013. Tropical plant–herbivore networks: reconstructing species interactions using DNA barcodes. PLoS ONE, 8:e52967.

Gentry, A. H. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. Ann. MO. Bot. Gard. 75:1-34.

Girardin, C. A. J., W. Farfan-Rios, K. Garcia, K. J. Feeley, P. M. Jørgensen, A. A. Murakami, L. C. Pérez, R. Seidel, N. Paniagua, A. F. Fuentes, C. Maldonado, M. Silman, N. Salinas, C. Reynel, A. D. Neill, M. Serrano, C. J. Caballero, M. Torre, J. M. Macías, T. J. Killen, and Y. Malhi. 2013. Spatial patterns of above ground structure, biomass and composition in a network of six Andean elevation transect. Plant Ecol. Divers. 7:161-171.

Gonzalez, M. A., C. Baraloto, J. Engel, S. A. Mori, P. Pétronelli, B. Riéra, A. Roger, C. Thébaud, and J. Chave 2009. Identification of Amazonian Trees with DNA Barcodes. PLoS ONE, 4:e7483.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003. Biological identification through DNA barcodes. Proc. Biol. Sci. 270:313-321.

Hollingsworth, P., S. W. Graham, and D. P. Little. 2011. Choosing and using a plant DNA barcode. PLoS ONE 6:e19254.

Homeier, J., S. W. Breckle, S. Günter, R. T. Rollenbeck, and C. Leuschner. 2010. Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. Biotropica 42:140-148.

Hughes, C. E. 2016. The tropical Andean plant diversity powerhouse. New Phytol. 210:1430-1442.

Hutter, C. R., J. M. Guayasamin and J. J. Wiens. 2013. Explaining Andean megadiversity: the evolutionary and ecological causes of glass frog elevational richness patterns. Ecol. Lett. 16:1135-1144.

JMP®, Version 11.2.0. SAS Institute Inc., Cary, NC, 1989-2007.

Kappelle, M., J. V. Uffelen, A. M. Cleef. 1995. Altitudinal zonation of montane *Quercus* forests along two transects in Chirripó National Park, Costa Rica. Vegetatio 119:119-153.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30:3059-3066.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28:1647-1649.

Kembel, S. 2010. An introduction to the picante package. http://picante.r-forge.r-project.org/picante-intro.pdf. Last accessed October 13, 2016.

Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics. 26:1463-1464.

Kesanakurti, P. R., A. J. Fakekas, K. S. Burgess, D. M. Percy, S. G. Newmaster, S. W. Graham, S. C. H. Barrett, M. Hajibabaei, and B. C. Husband. 2011. Spatial patterns of plant diversity below-ground as revealed by DNA barcoding. Mol. Ecol. 20:1289-1302.

Kessler, M., S. Abrahamczyk, M. Bos, D. Buchori, D. D. Putra, S. R. Gradstein, P. Höhn, J. Kluge, F. Orend, R. Pitopang, S. Saleh, C. H. Schulze, S. G. Spron, I. Steffan-Dewenter, S. S. Tjitrosoedirdjo, and T. Tscharntke. 2009. Alpha and beta diversity of plants and animals along a tropical land-use gradient. Ecol. Appl. 19:2142-2156.

Körner, C. 2007. The use of 'altitude' in ecological research. Trends Ecol. Evol. 22:569-574.

Kraft, N. J. B. and D. D. Ackerly. 2010. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. Ecol. Monogr. 80:401-422.

Kraft, N. J. B., L. S. Comita, J. M. Chase, N. J. Sanders, N. G. Swenson, T. O. Crist, J. C. Stegen, M. Vellend, B. Boyle, M. J. Anderson, H. V. Cornell, K. F. Davies, A. L. Freestone, B. D. Inouye, S. P. Harrison, and J. A. Myers. 2011. Disentangling the drivers of β diversity along latitudinal and elevational gradients. Science 333:1755-1758.

Kress, W. J., D. L. Erickson, F. A. Jones, N. G. Swenson, R. Perez, O. Sanjur, and E. Bermingham. 2009. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci. U.S.A. 106:18621–18626.

Kress, W. J., D. L. Erickson, N. G. Swenson, J. Thompson, M. Uriarte, and J. K. Zimmerman. 2010. Advances in the use of DNA barcodes to build a community phylogeny for tropical trees in a Puerto Rican forest dynamics plot. PLoS ONE. 5:e15409.

Kress, W. J., C. García-Robledo, M. Uriarte, and D. L. Erickson. 2015. DNA barcodes for ecology, evolution, and conservation. Trends Ecol. Evol. 30:25–35.

Larsen, M. M., A. Adsersen, A. P. Davis, M. Dolores Lledó, A. K. Jäger, and N. Rønsted. 2010. Using a phylogenetic approach to selection of target plants in drug discovery of acetylcholinesterase inhibiting alkaloids in Amaryllidaceae tribe Galantheae. Biochem. Syst. Ecol. 38:1026-1034.

Levin, R. A., W. L. Wagner, P. C. Hoch, M. Nepokroeff, J. C. Pires, E. A. Zimmer, and K. J. Sytsma. 2003. Family-level relationships of Onagraceae based on chloroplast *rbc*L and *ndh*F data. Am. J. Bot. 90:107-115.

Lieberman, D., M. Lieberman, R. Peralta, and G. S. Hartshorn. 1996. Tropical forest structure and composition on a large-scale altitudinal gradient in Costa Rica. J. Ecol. 84:137-152.

Liu, J., H-F. Yan, S. G. Newmaster, N. Pei, S. Ragupathy, and X-J. Ge. 2014. The use of DNA barcoding as a tool for the conservation biogeography of subtropical forests in China. Divers. Distrib. 21:188-199.

Losos, J. B. 1996. Phylogenetic perspectives on community ecology. Ecology. 77:1344-1354.

Luebert F. and M. Weigend. 2014. Phylogenetic insights into Andean plant diversification. Front. Ecol. Evol. 2:27.

Lukhoba, C. W., M. S. J. Simmonds, and A. J. Paton. 2006. *Plectranthus*: A review of ethnobotanical uses. J. Ethnopharm. 103:1–24.

Ministerio de Agricultura y Ganaderia: Programa Nacional de Regionalizacion Agraria (PRONAREG). 1984. Soil map. 1:50,000. Quito: Instituto Geografico Militar.

Morris, E. K., T. Caruso, F. Buscot, M. Fischer, C. Hancock, T. S. Maier, T. Meiners, C. Müller, E. Obermaier, D. Prati, and S. A. Socher. 2014. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecol. Evol. 4: 3514-3524.

Mosandle, R. and S. Günter. 2008. Sustainable management of tropical mountain forests in Ecuador. Pp. 177-193 *in* S. R. Gradstein, J. Homeier, and D. Gansert, eds. The tropical mountain forest - patterns and processes in a biodiversity hotspot. Biodiversity and Ecology Series 2. University Press, Göttingen, Germany.

Muscarella, R., M. Uriarte, D. L. Erickson, N. G. Swenson, J. K. Zimmerman, and W. J. Kress. 2014. A well-resolved phylogeny of the trees of Puerto Rico based on DNA barcode sequence data. PLoS ONE 9:e112843.

Myers, N. 1988. Threatened biotas: "Hot Spots" in tropical forests. Environmentalist 8:187-208.

Nagendra, H. 2002. Opposite trends in response for the Shannon and Simpson indices of landscape diversity. Appl. Geogr. 22:175-186.

Newmaster, S. G., M. Grguric, D. Shanmughanandhan, S. Ramalingam, and S. Ragupathy. 2013. DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine 11:222.

Newmaster, S. G. and S. Ragupathy. 2010. Ethnobotany genomics - discovery and innovation in a new era of exploratory research. J. Ethnobio. Ethnomed. 6:2-11.

Orme, C. D. L., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse 2013. Caper: comparative analyses of phylogenetics and evolution in R. R package version 0.5.2. http://CRAN.R-project.org/package=caper.

Paranhos, J. T., V. Fragoso, A. T. Henriques, A. G. Ferreira, and A. G. Fett-Neto. 2005. Regeneration of *Psychotria umbellata* and production of the analgesic indole alkaloid umbellatine. Tree Physiol. 25:251-255.

Pausas, J. G. and M. P. Austin. 2001. Patterns of plant species richness in relation to different environments: an appraisal. J. Veg. Sci. 12:153-166.

Paz, R. A. J., A. J. Pérez, R. Valencia, J. A. Barone, K. S. Burgess. In prep. Floristic composition, structure and diversity along an elevational gradient in an Andean forest of Northern Ecuador.

Pérez, A. J., C. Hernández, H. Romero-Saltos, and R. Valencia. 2014. Árboles emblemáticos de Yasuní, Ecuador. Publicaciones del Herbario QCA. Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito.

Pitman, N. C. A., J. W. Terborgh, M. R. Silman, P. Núñez, D. A. Neill, C. E. Cerón, W. A. Palacios, and M. Aulestia. 2001. Dominance and distribution of tree species in upper Amazonian terra firme forests. Ecology. 82:2101-2117.

Ratnasingham, S. and P. D. N. Hebert. 2007. BOLD: The barcode of life data system (www.barcodinglife.org). Mol. Ecol. Notes. 7:355-364.

Reynolds, A. 2011. Siempre Verde bosque y vegetacion protector. 1-46.

Rios, M., M. J. Koziol, H. B. Pedersen, and G. Granda, eds. 2007. Plantas útiles del Ecuador: aplicaciones, retos y perspectivas/Useful plants of Ecuador: Applications, challenges, and perspectives. Ediciones Abya-Yala, Quito, Ecuador.

Saslis-Lagoudakis, C. H., B. B. Klitgaard, F. Forest, L. Francis, V. Ssavolainen, E. M. Williamson, and J. A. Hawkins. 2011. The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: An example from *Pterocarpus* (Leguminosae). PLoS ONE 6:e22275.

Saslis-Lagoudakis, C. H., V. Savolainen, E. M. Williamson, F. Forest, S. J. Wagstaff, S. R. Baral, M. F. Watson, C. A. Pendry, and J. A. Hawkins. 2012. Phylogenies reveal predictive power of traditional medicine in bioprospecting. Proc. Natl. Acad. Sci. U. S. A. 109:15835-15840.

Schultes, R. E. 1979. The Amazonia as a source of new economic plants. Econ. Bot. 33:259-266.

Schultes, R. E. and R. F. Raffauf. 1990. The healing forest: Medicinal and toxic plants of the northwest Amazonia. Dioscorides Press, Portland, Oregon, USA.

Shaheen, H., S. M. Khan, D. M. Harper, Z. Ullah, and R. A. Qureshi. 2011. Species diversity, community structure, and distribution patterns in Western Himalayan alpine pastures of Kashmir, Pakistan. Mt. Res. Dev. 32:153-159.

Shanley, P. and L. Luz. 2003. The impacts of forest degradation on medicinal use and implications for health care in eastern Amazonia. BioScience 53:573-584.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication. Illinois: University of Illinois Press.

Sharma, V. and I. N. Sarkar. 2013. Bioinformatics opportunities for identification and study of medicinal plants. Brief Bioinform. 14:238–250.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22:2688-2690.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57:758-771.

Swenson, N. G. 2014. Functional and phylogenetic ecology in R. Springer, New York, USA.

Swenson, N. G. and M. N. Umaña. 2014. Phylofloristics: an example from the Lesser Antilles. J. Plant Ecol. 7:166-175.

Swenson, N. G., P. Anglada-Cordero, and J. A. Barone. 2011. Deterministic tropical tree community turnover: evidence from patterns of functional beta diversity along an elevational gradient. Proc. R. Soc. B. 278:877-884.

Thinh, N. V., R. Mitlöhner, and N. V. Bich. 2015. Comparison of floristic composition in four sites of a tropical lowland forest on the North-Central coast of Vietnam. J. Nat. Sci. 1:e144.

Valencia, R. and P. M. Jørgensen. 1992. Composition and structure of a humid montane forest on the Pasochoa volcano, Ecuador. Nord. J. Bot. 12:239-247.

Valencia, R., R. Condit, R. B. Foster, K. Romoleroux, G. V. Muñoz, J. C. Svenning, E. Magård, M. Bass, E. C. Losos, and H. Balslev. 2004a. Yasuní forest dynamics plot, Ecuador. pages 609-620 in E. C. Losos and E. G. Leigh, Jr., editors. Tropical forest diversity and dynamism: Findings from a large scale plot network. University of Chicago Press, Chicago, USA.

Valencia, R., R. B. Foster, G. Villa, R. Condit, J. C. Svenning, C. Hernández, K. Romolerouz, E. Losos, E. Magård, and H. Balslev. 2004. Tree species distributions and local habitat variation in the Amazon: large forest plot in eastern Ecuador. J. Ecol. 92:214-229.

Valentini, A., F. Pompanon, and P. Taberlet. 2008. DNA barcoding for ecologists. Trends Ecol. Evol. 24:110-117.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: An example for rainforest trees. Am. Nat. 156:145-155.

Weber, M. G. and K. H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. Ann. Bot. 111:1251-1261.

Whitfeld, T. J. S., V. Novotny, S. C. Miller, J. Hrcek, P. Klimes, and G. D. Weiblen. 2012. Predicting tropical insect herbivore abundance from host plant traits and phylogeny. Ecology. 93:211-223.

Young, K. R. and P. L. Keating. 2001. Remnant forests of Volcan Cotacachi, northern Ecuador. Arct., Antarc., Alp. Res. 33:165-172.

Figures

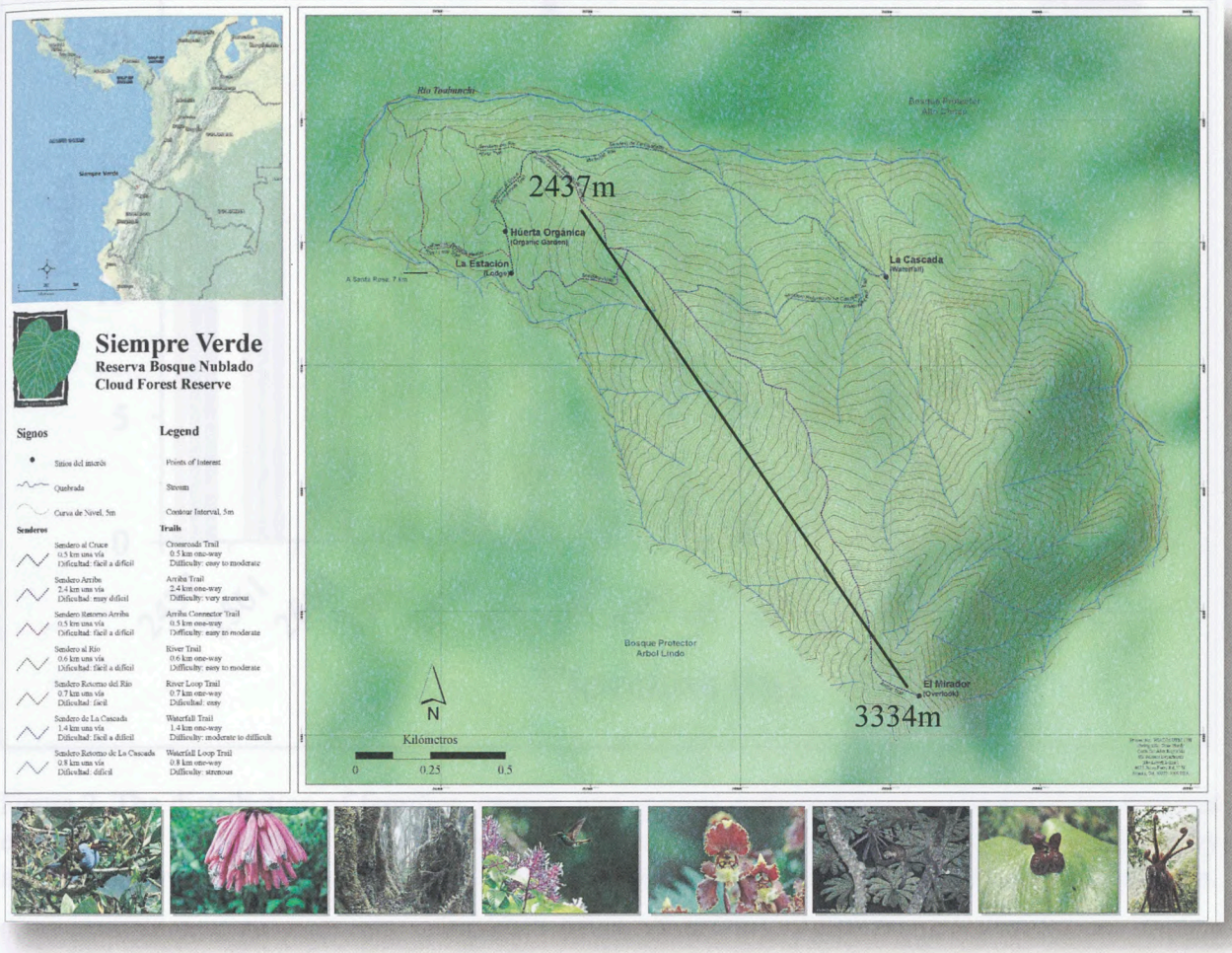


Figure 1. Topographic map of the study site at Siempre Verde Reserve, Imbabura Province, Ecuador. The black line shows the transect established within the preserve that runs from 2,437 m to 3,334 m above sea level (Reynolds 2011).

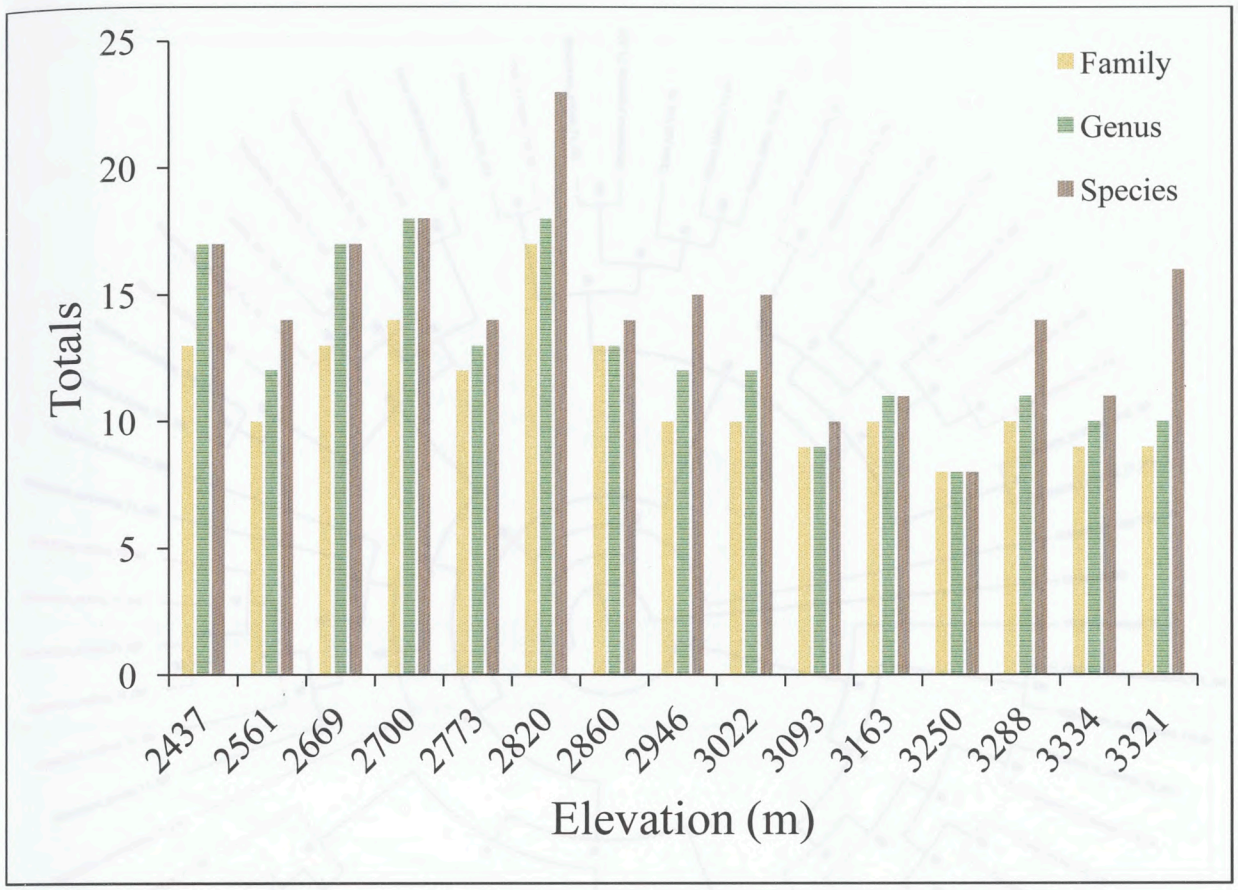


Figure 2. The distribution of families, genera, and species collected from each plot in the transect established at the Siempre Verde Reserve, Imbabura Province, Ecuador. The plots are ordered by increasing elevation where plot 1 is at the highest elevation and plot 15 is at the lowest elevation.

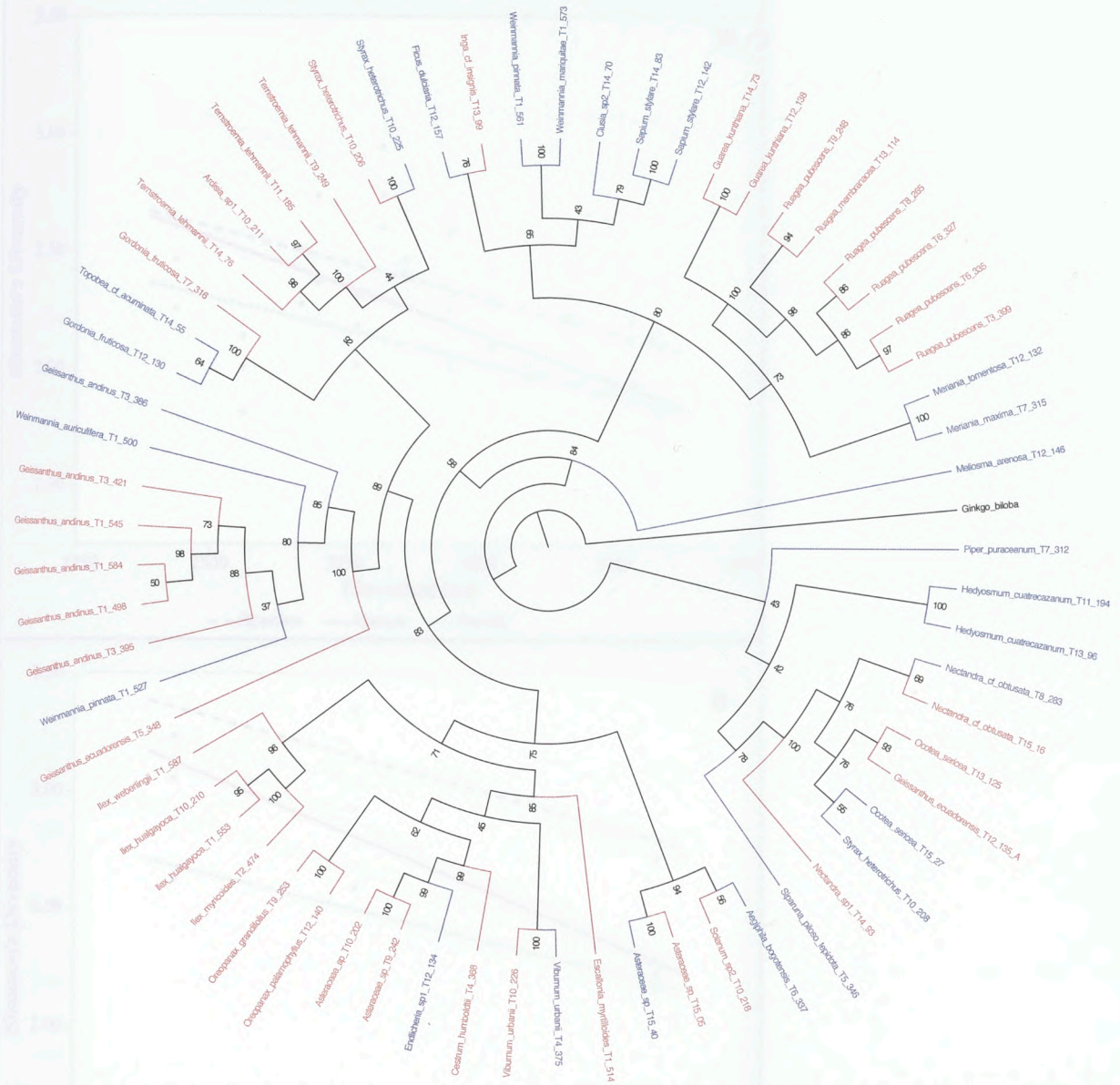


Figure 3. Phylogenetic tree of taxa successfully sequenced in the transect at the Siempre Verde Reserve, Imbabura Province, Ecuador. Bootstrap values based on maximum likelihood are reported at each node. The transect (T) number and plant number are indicated beside each species name. Taxa are colored coded by sequence obtained: red (*rbc*L and *mat*K), blue (*rbc*L), and purple (*mat*K).

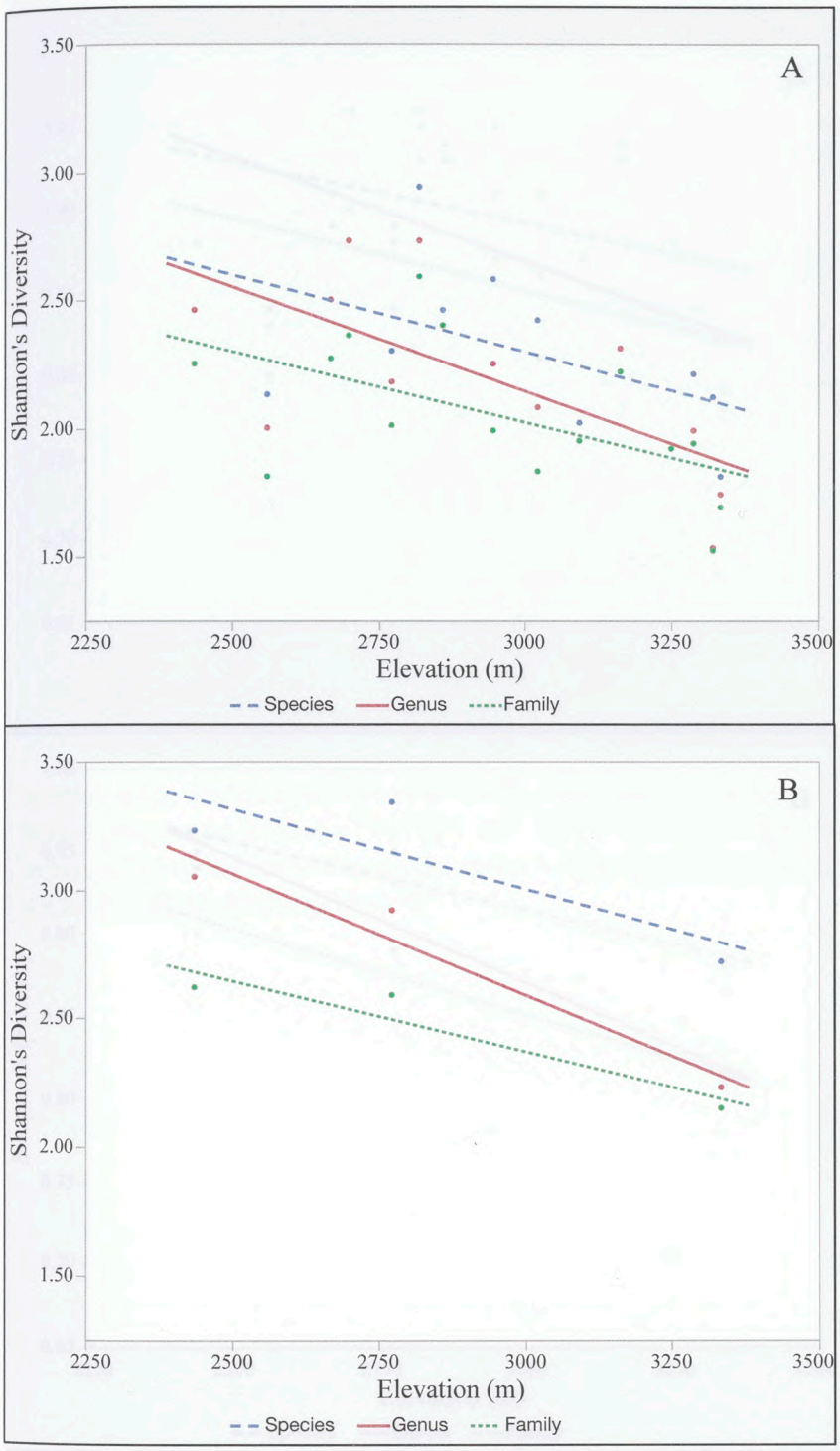


Figure 4. Relationship between Shannon's Diversity and elevation (A) at the species (R^2=0.328, p=0.026), genus (R^2=0.468, p=0.005), and family (R^2=0.301, p=0.030) levels for each plot along the transect and (B) when plots are group into elevation zones at the species (R^2=0.729, p=0.348), genus (R^2=0.947, p=0.148), and family (R^2=0.899, p=0.206) levels along the transect.

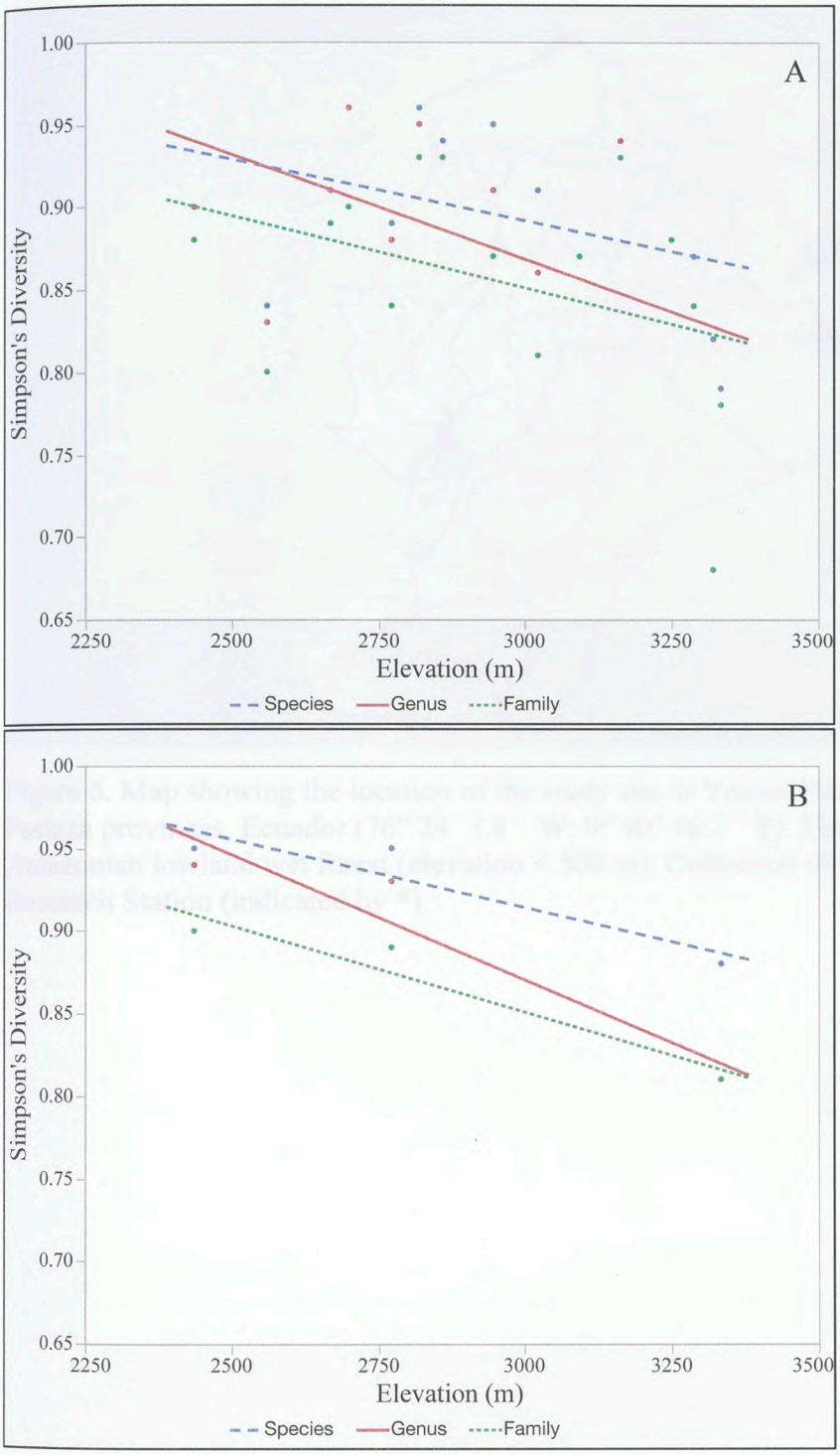


Figure 5. Relationship between Simpson's Diversity and elevation (A) at the species (R^2=0.176, p=0.120), genus (R^2=0.257, p=0.054), and family (R^2=0.142, p=0.167) levels for each plot along the transect and (B) when plots are grouped into elevation zones at the species (R^2=0.863, p=0.242), genus (R^2=0.907, p=0.198), and family (R^2=0.925, p=0.177) levels along the transect.

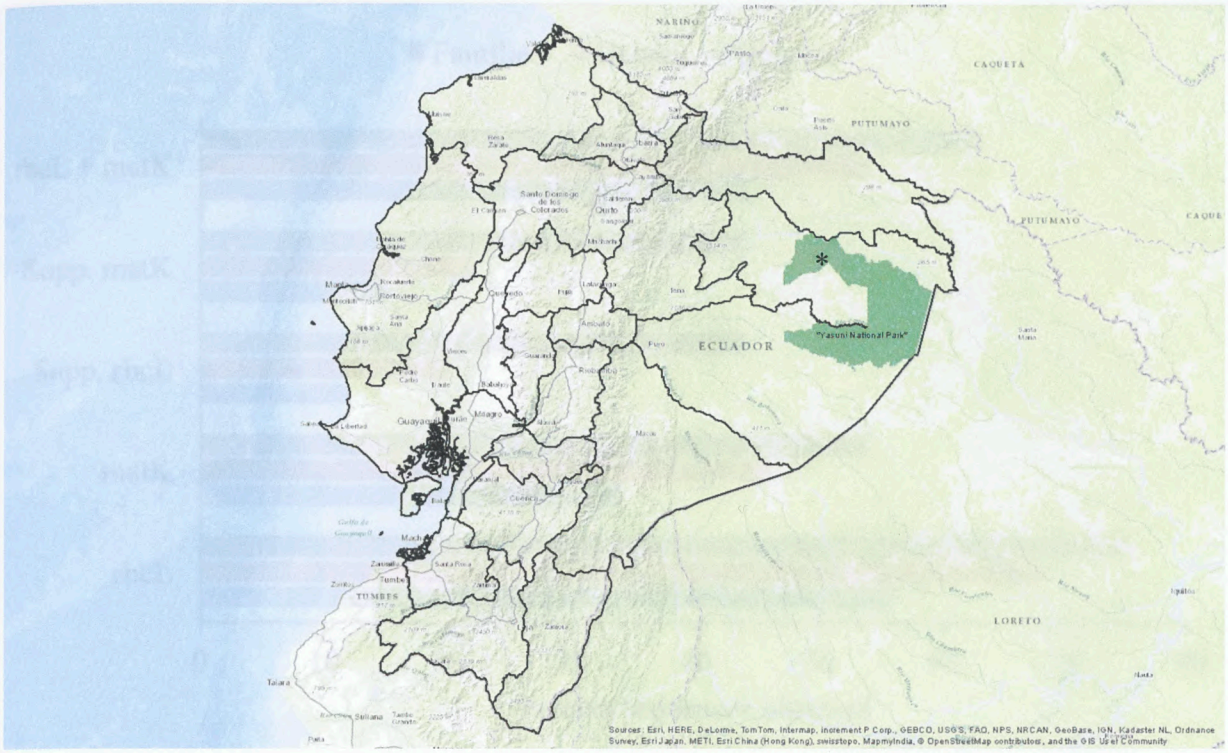


Figure 6. Map showing the location of the study site at Yasuní National Park, in Orellana and Pastaza provinces, Ecuador (76° 24′ 1.8′′ W; 0° 40′ 16.7′′ S). The park is comprised of western Amazonian lowland wet forest (elevation < 500 m). Collection sites were near the Yasuní Research Station (indicated by *).

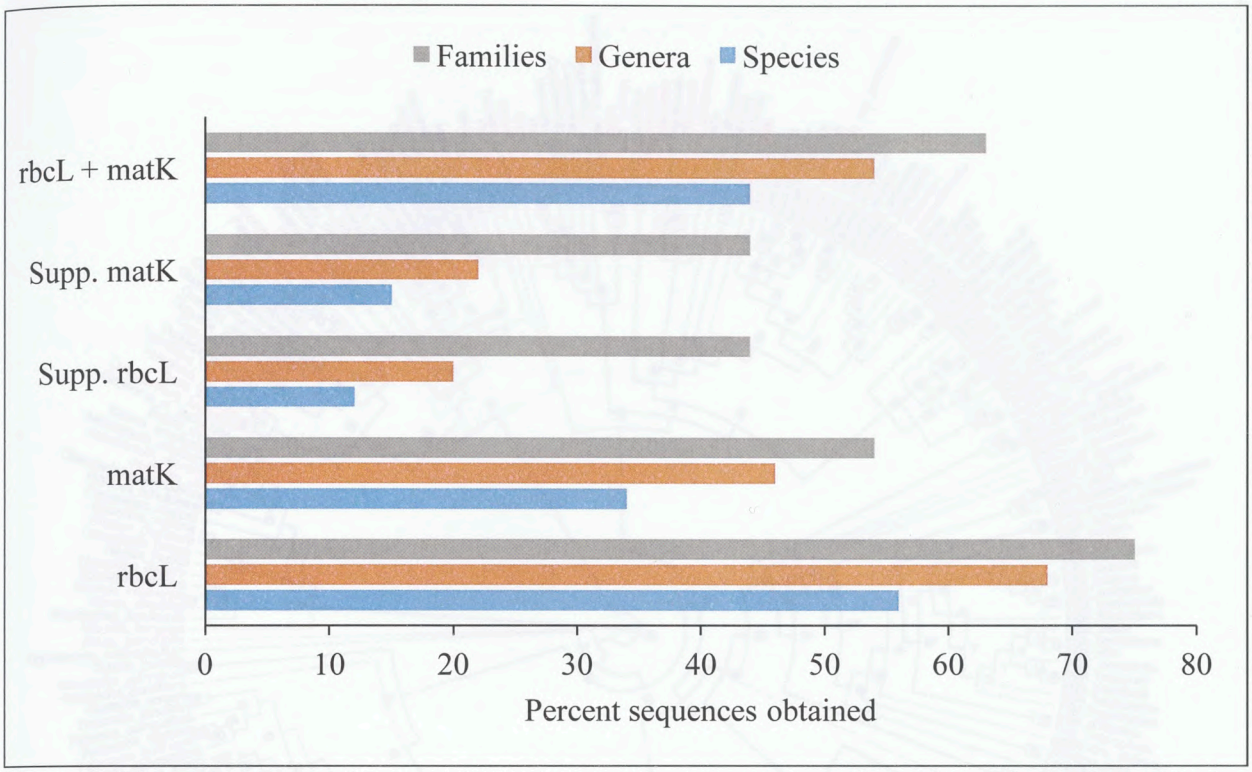


Figure 7. The percentage of taxa with DNA barcode sequences used in this study. Indicated is the percent sequence recovery for *rbc*L and *mat*K sequences. The percentage of supplemented (Supp.) *rbc*L and *mat*K sequences obtained from GenBank or the Biodiversity of Life Data systems (BOLD) and the percentage of taxa that had both *rbc*L + *mat*K sequences.

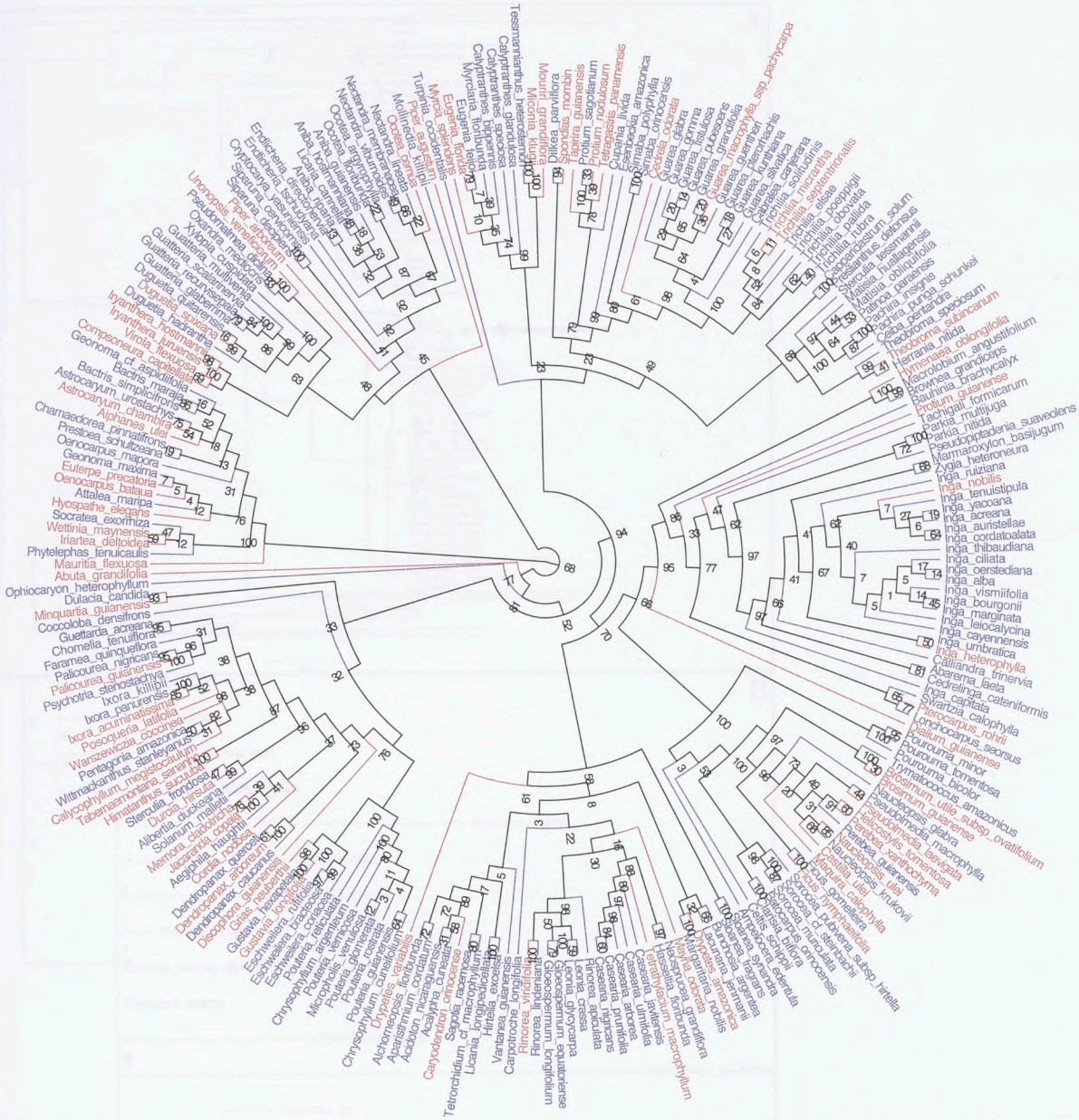


Figure 8. Phylogenetic tree of 248 taxa successfully sequenced at Yasuní National Park, Ecuador. Bootstrap values based on maximum likelihood are reported at each node. Species are colored by whether they are classified as medicinal (red) or not (purple).

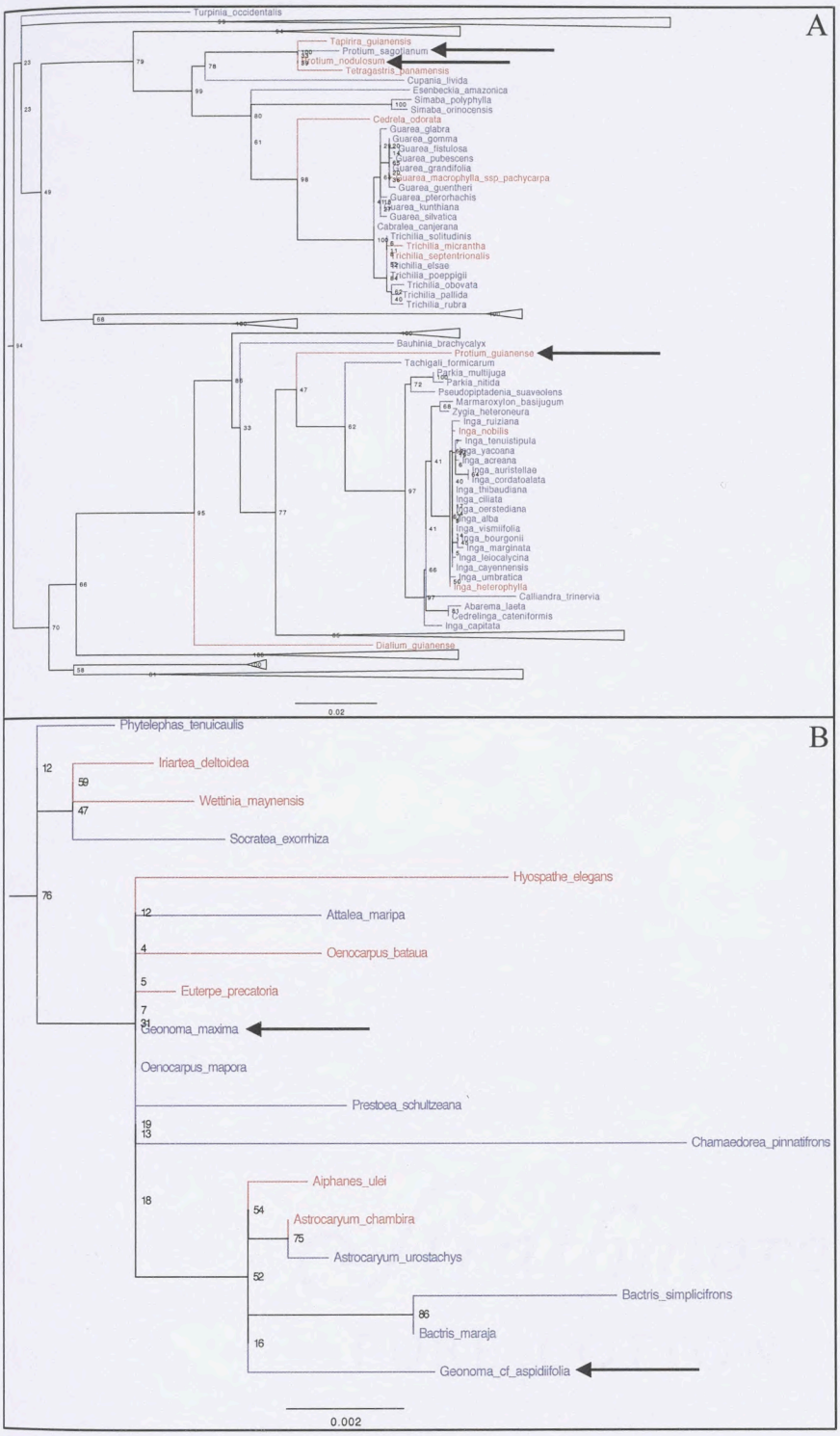


Figure 9. Potential taxonomic errors detected in the phylogenetic analysis. (A) Three *Protium* species were sequenced, but two (*Protium nodulosum* and *Protium sagotianum*) were found clumped together and the other (*Protium guianense*) was in a separate clade. (B) Two *Geonoma* species (*Geonoma cf. aspidiifolia* and *Geonoma* maxima) were spread apart within a clade.

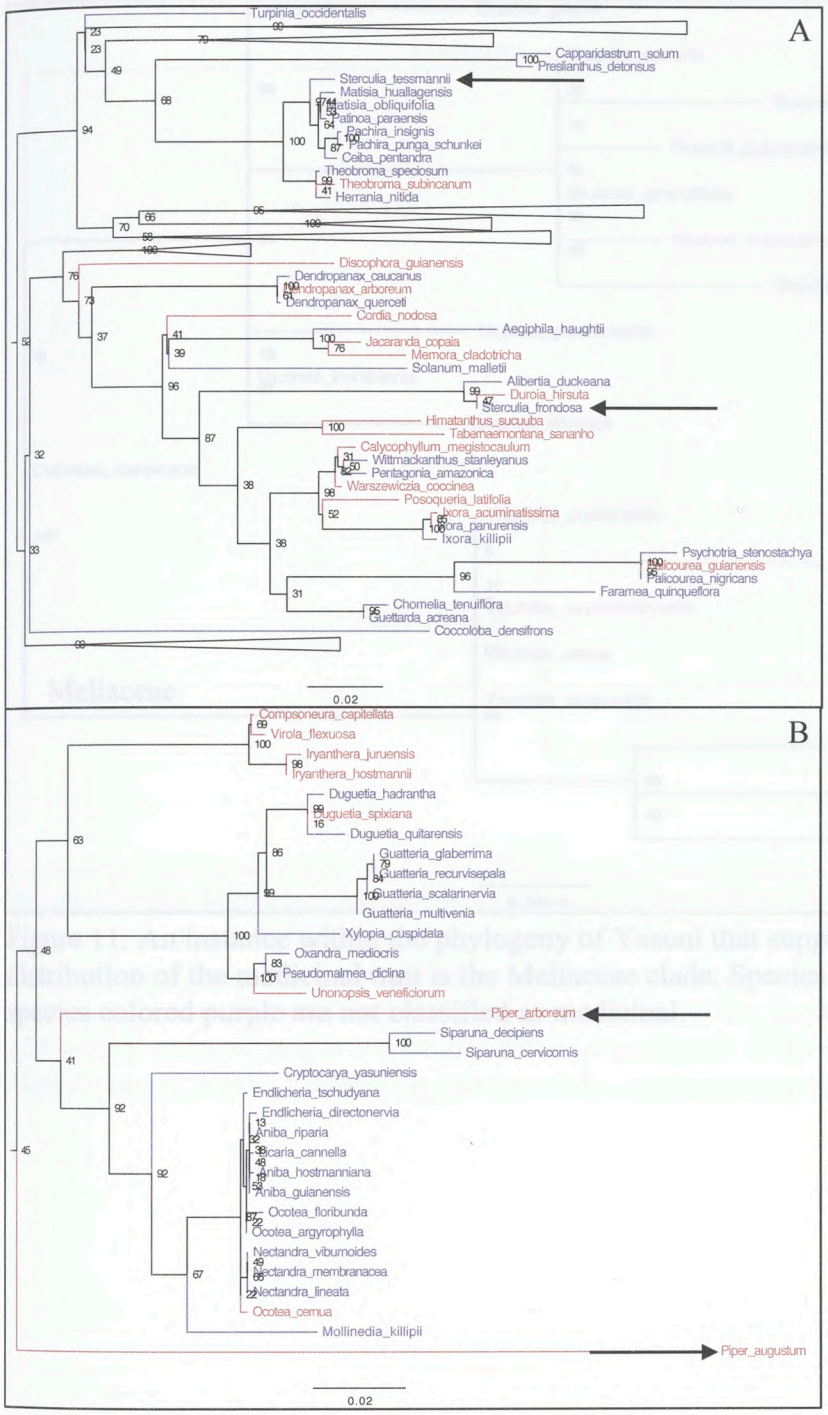


Figure 10. Potential sequencing errors detected in the phylogenetic analysis. (A) *Sterculia frondosa* has *rbc*L and *mat*K sequences from GenBank, but *Sterculia tessmannii* had a *rbc*L sequence that was obtained for this study. These two species diverge greatly in their location on the phylogeny, but *Sterculia tessmannii* is within the same clade as all other genera in the Malvaceae family. (B) *Piper arboreum* and *Piper augustum* each have only one gene region sequenced, both obtained from GenBank, and they are divergent in the phylogeny.

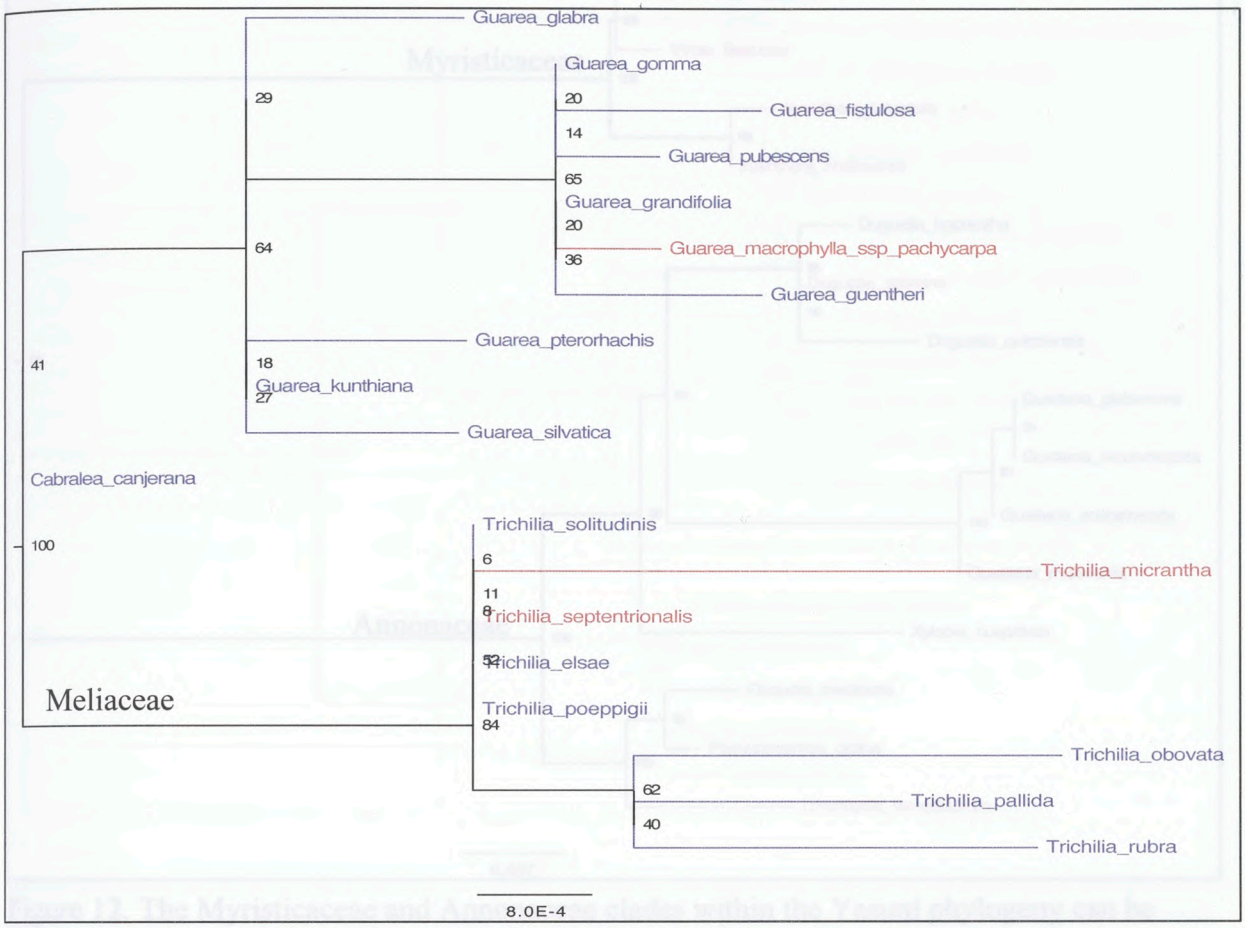


Figure 11. An instance within the phylogeny of Yasuní that supports the results of a random distribution of the medicinal trait is the Meliaceae clade. Species colored red are medicinal and species colored purple are not classified as medicinal.

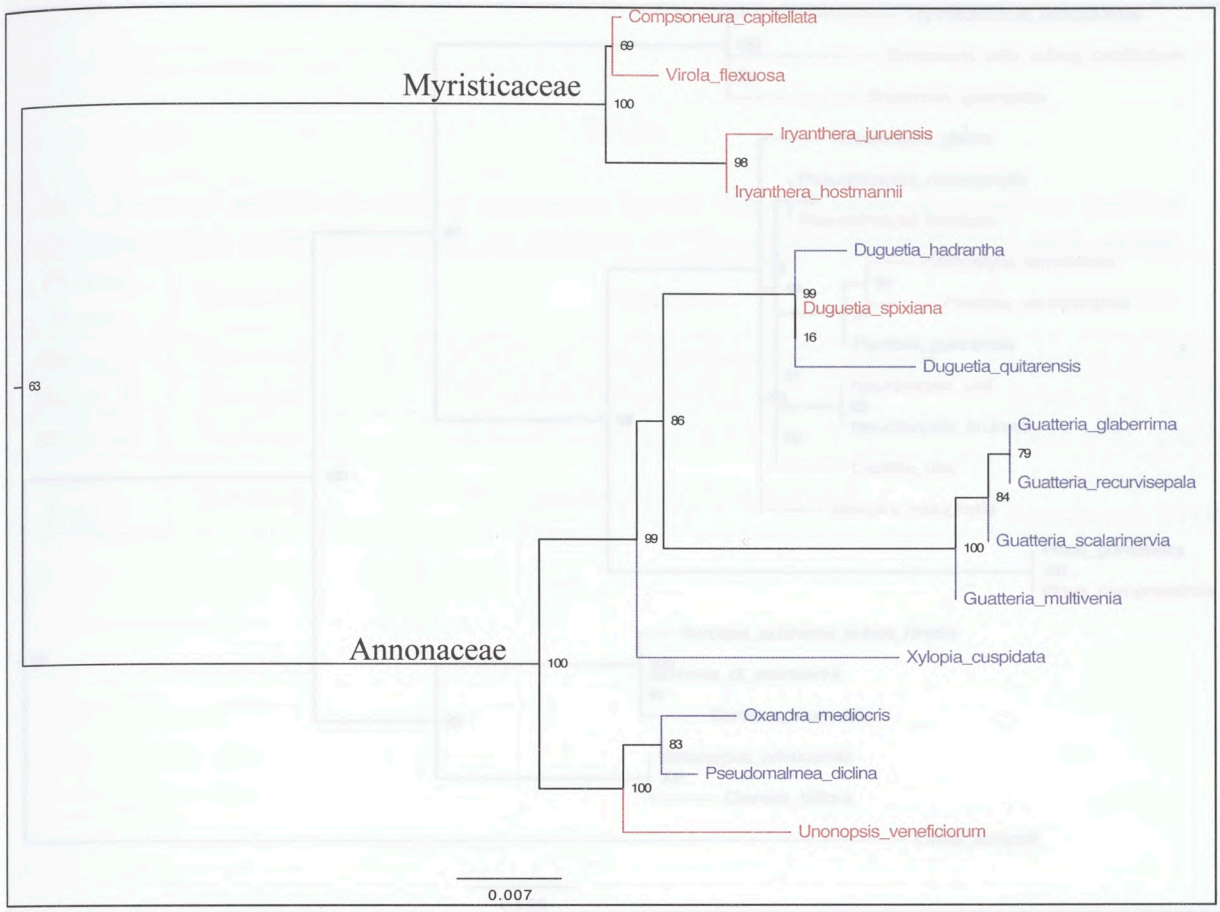


Figure 12. The Myristicaceae and Annonaceae clades within the Yasuní phylogeny can be compared to show different distributions of the medicinal trait. When comparing the Myristicaceae and Annonaceae clades, all species in the Myristicaceae clade are medicinal and there is a random distribution within the Annonaceae clade. Species colored red are medicinal and species colored purple are not classified as medicinal. With more sampling within the Myristicaceae family, a clumping pattern of the medicinal trait could be potentially obtained.

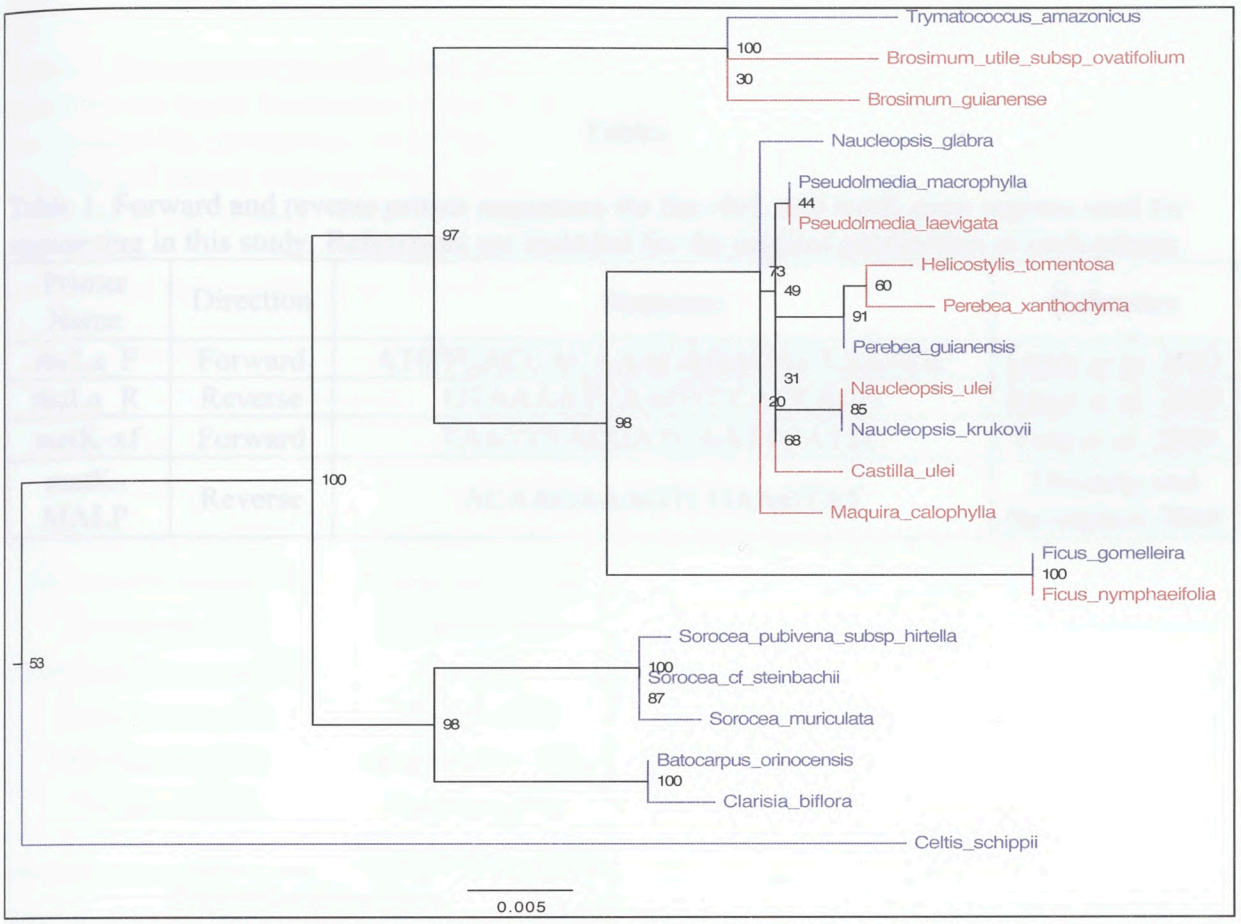


Figure 13. The Moraceae clade within the Yasuní phylogeny shows a distribution of medicinal species that appears less than random. To address this distribution, species currently not designated as medicinal should be tested as the phylogeny seems to indicate medicinal presence in this family. Species colored red are medicinal and species colored purple are not classified as medicinal.

Tables

Table 1. Forward and reverse primer sequences for the *rbc*L and *mat*K gene regions used for sequencing in this study. References are included for the original publication of each primer.

Primer Name	Direction	Sequence	Reference
rbcLa_F	Forward	ATGTCACCACAAACAGAGACTAAAGC	Levin et al. 2003
rbcLa_R	Reverse	GTAAAATCAAGTCCACCRCG	Kress et al. 2009
matK-xf	Forward	TAATTTACGATCAATTCATTC	Ford et al. 2009
matK-MALP	Reverse	ACAAGAAAGTCGAAGTAT	Dunning and Savolainen 2010

Table 2. Most diverse plant families and genera in the entire transect and when plots are grouped by elevation (n >20). These families and genera make up 74.5% and 67.2% of all individuals within the transect, respectively. The exact number of individuals within these designations is in parentheses.

Families	Genera
Total Transect	
Aquifoliaceae (25)	*Cyathea* (60)
Cunoniaceae (112)	*Faramea* (33)
Cyatheaceae (60)	*Freziera* (29)
Melastomataceae (74)	*Geissanthus* (23)
Myrtaceae (27)	*Gordonia* (29)
Pentaphylacaceae (35)	*Ilex* (25)
Primulaceae (27)	*Miconia* (38)
Rubiaceae (54)	*Myrcianthes* (27)
Theaceae (29)	*Topobea* (24)
	Weinmannia (112)
High Elevation Plots	
Aquifoliaceae (24)	*Cyathea* (21)
Cunoniaceae (97)	*Freziera* (29)
Cyatheaceae (21)	*Geissanthus* (20)
Primulaceae (21)	*Ilex* (24)
Pentaphylacaceae (29)	*Weinmannia* (97)
Medium Elevation Plots	
Cyatheaceae (26)	*Cyathea* (26)
Melastomataceae (48)	*Miconia* (29)
Low Elevation Plots	
Myrtaceae (22)	*Myrcianthes* (22)
Rubiaceae (33)	

Table 3. Values for three phylogenetic diversity metrics, phylogenetic distance (PD), mean pairwise distance (MPD), and mean nearest taxon distance (MNTD), are given for each plot (1-15) and for the groups of plots at low, medium, and high elevations. For each metric, 999 randomizations were used to assess departure from random. Significant differences from random are in bold. The * denotes a significant clustering pattern ($p < 0.05$).

Plot	PD	MPD	MNTD
1	**0.440***	**0.096***	**0.021***
2	**0.219***	**0.031***	**0.019***
3	0.306	**0.082***	0.065
4	0.209	0.060	0.120
5	0.422	0.144	0.115
6	0.551	0.156	0.144
7	0.404	0.141	0.135
8	0.392	0.123	0.084
9	0.364	0.082	0.071
10	**0.554***	**0.102***	**0.046***
11	0.397	0.094	**0.040***
12	0.790	0.164	0.096
13	0.570	0.139	0.059
14	0.529	0.154	0.114
15	0.506	0.170	0.146
Elevation Groups			
Low	0.994	0.169	0.054
Medium	1.283	**0.120***	0.069
High	0.727	**0.111***	0.061

Table 4. Shannon's Diversity (H') and Simpson's Diversity (D) for each plot (1-15) of the transect and plots grouped by elevation (low, medium, and high) calculated at the species, genera, and family levels. The elevation of each plot, as well as the number of stems, species, genera, and families, are given.

Plot	Elevation (m)	No. of stems	No. of species	H' species	No. of genera	H' genus	No. of families	H' family	D species	D genus	D family
1	3321	100	16	2.12	10	1.53	9	1.52	0.82	0.68	0.68
2	3334	51	11	1.81	10	1.74	9	1.69	0.79	0.78	0.78
3	3288	63	14	2.21	11	1.99	10	1.94	0.87	0.84	0.84
4	3250	17	8	1.92	8	1.92	8	1.92	0.88	0.88	0.88
5	3163	21	11	2.31	11	2.31	10	2.22	0.94	0.94	0.93
6	3093	21	10	2.02	9	1.95	9	1.95	0.87	0.87	0.87
7	3022	35	15	2.42	12	2.08	10	1.83	0.91	0.86	0.81
8	2946	24	15	2.58	12	2.25	10	1.99	0.95	0.91	0.87
9	2860	23	14	2.46	13	2.40	13	2.40	0.94	0.93	0.93
10	2820	44	23	2.94	18	2.73	17	2.59	0.96	0.95	0.93
11	2773	39	14	2.30	13	2.18	12	2.01	0.89	0.88	0.84
12	2700	29	18	2.73	18	2.73	14	2.36	0.96	0.96	0.90
13	2669	33	17	2.50	17	2.50	13	2.27	0.91	0.91	0.89
14	2561	42	14	2.13	12	2.00	10	1.81	0.84	0.83	0.80
15	2437	53	17	2.46	17	2.46	13	2.25	0.90	0.90	0.88
Low	2437-2700	157	42	3.23	34	3.05	23	2.62	0.95	0.94	0.90
Medium	2773-3100	186	48	3.34	34	2.92	28	2.59	0.95	0.93	0.89
High	3163-3334	252	35	2.72	24	2.23	20	2.15	0.88	0.81	0.81

Table 5. Values for two phylogenetic diversity metrics (mean pairwise distance (MPD), and mean nearest taxon distance (MNTD) and Fritz and Purvis' D are given below. For each metric, 999 randomizations were used to assess departure from random. Significant differences from random are indicated with *. Both MPD and MNTD have p >0.95 indicating phylogenetic evenness. The observed D value is reported in the D column. D was shown to be significantly different than 0, but not significantly different than 1.

	Values
MPD	0.204*
MNTD	0.047*
D	0.881
p (D > 0)	<0.001
p (D < 1)	0.073

Supplementary Figures

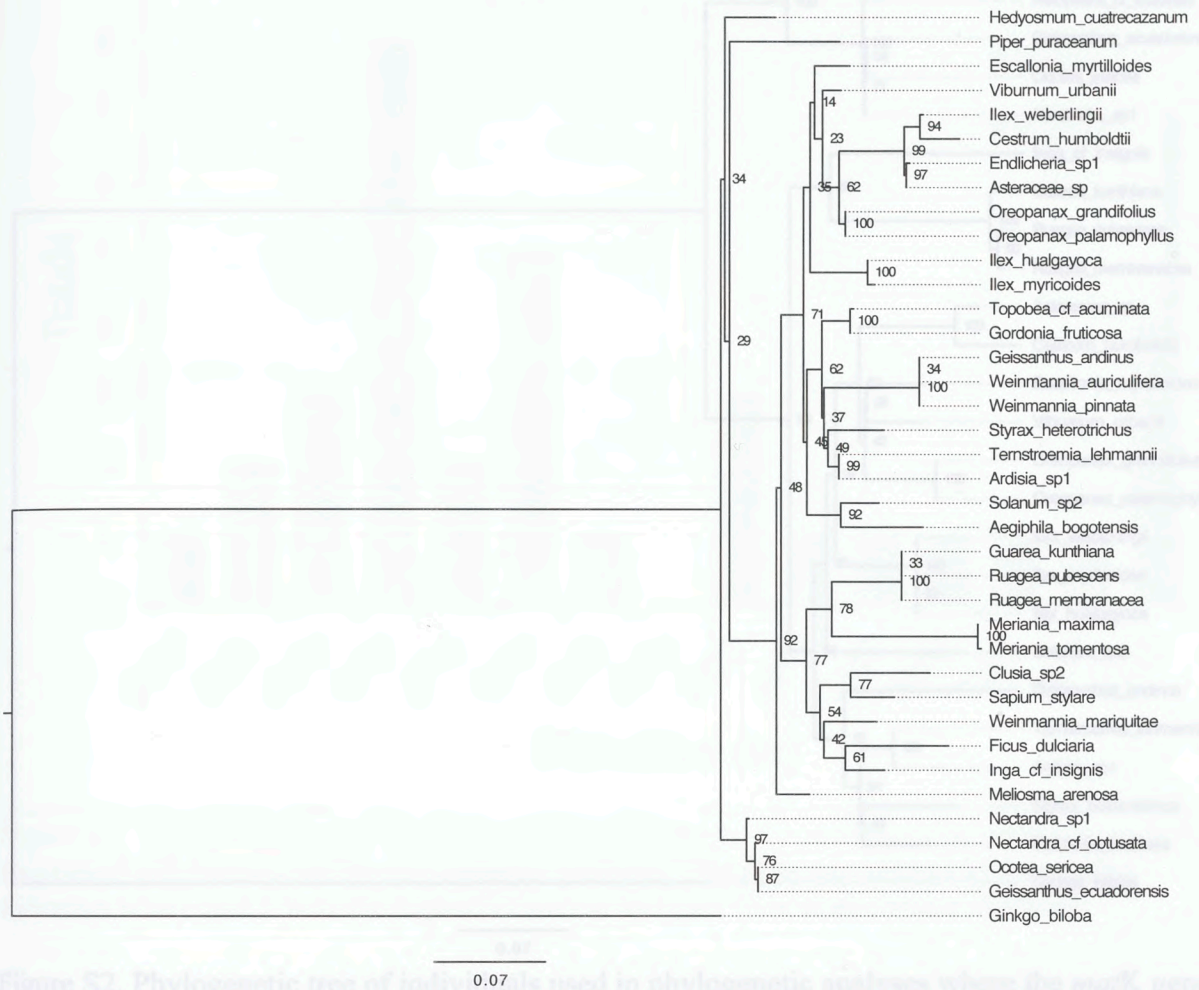


Figure S1. Phylogenetic tree of individuals used in phylogenetic analyses where the *rbc*L gene region was successfully sequenced. Bootstrap values based on maximum likelihood are reported at each node.

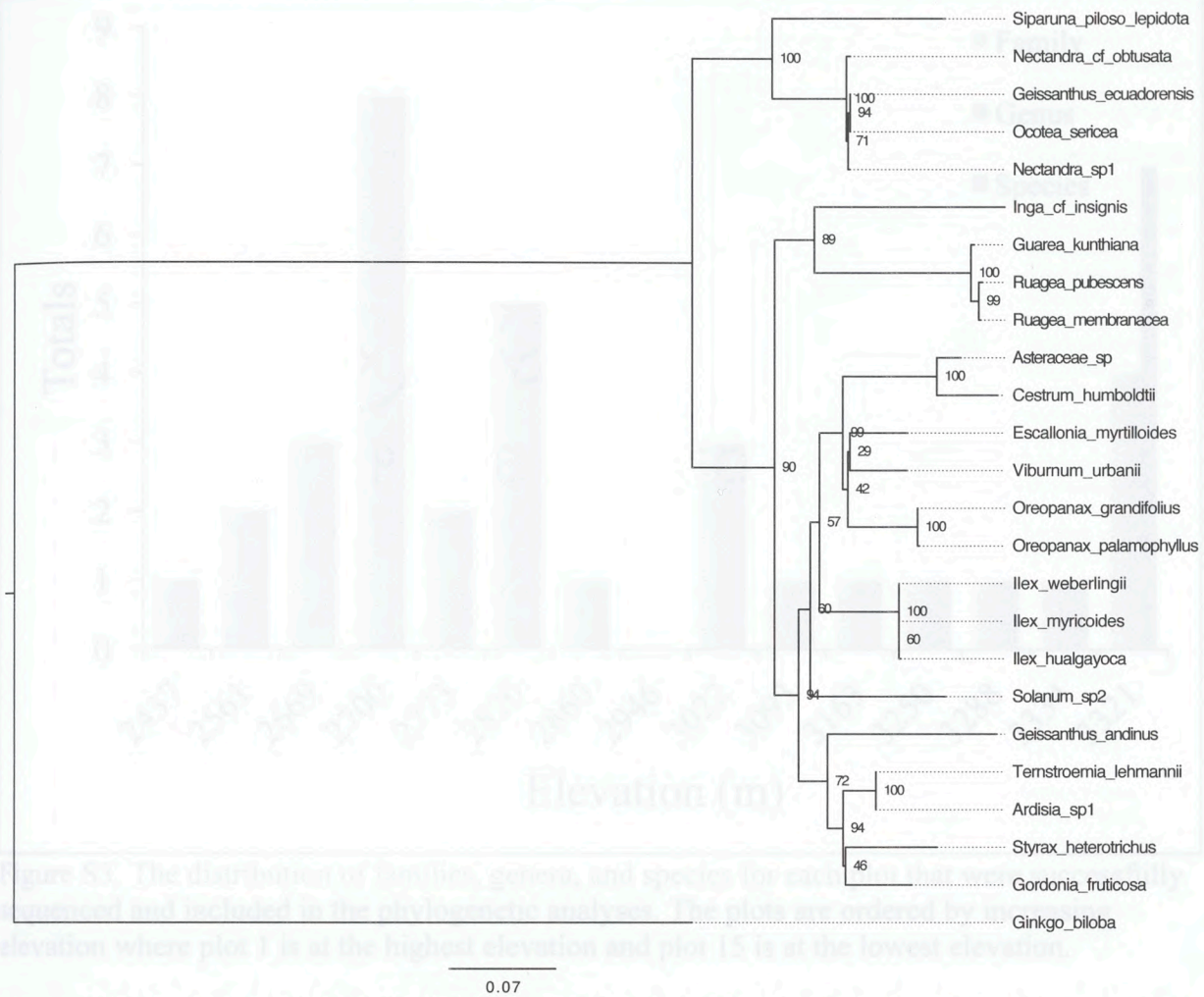


Figure S2. Phylogenetic tree of individuals used in phylogenetic analyses where the *mat*K gene region was successfully sequenced. Bootstrap values based on maximum likelihood are reported on the nodes.

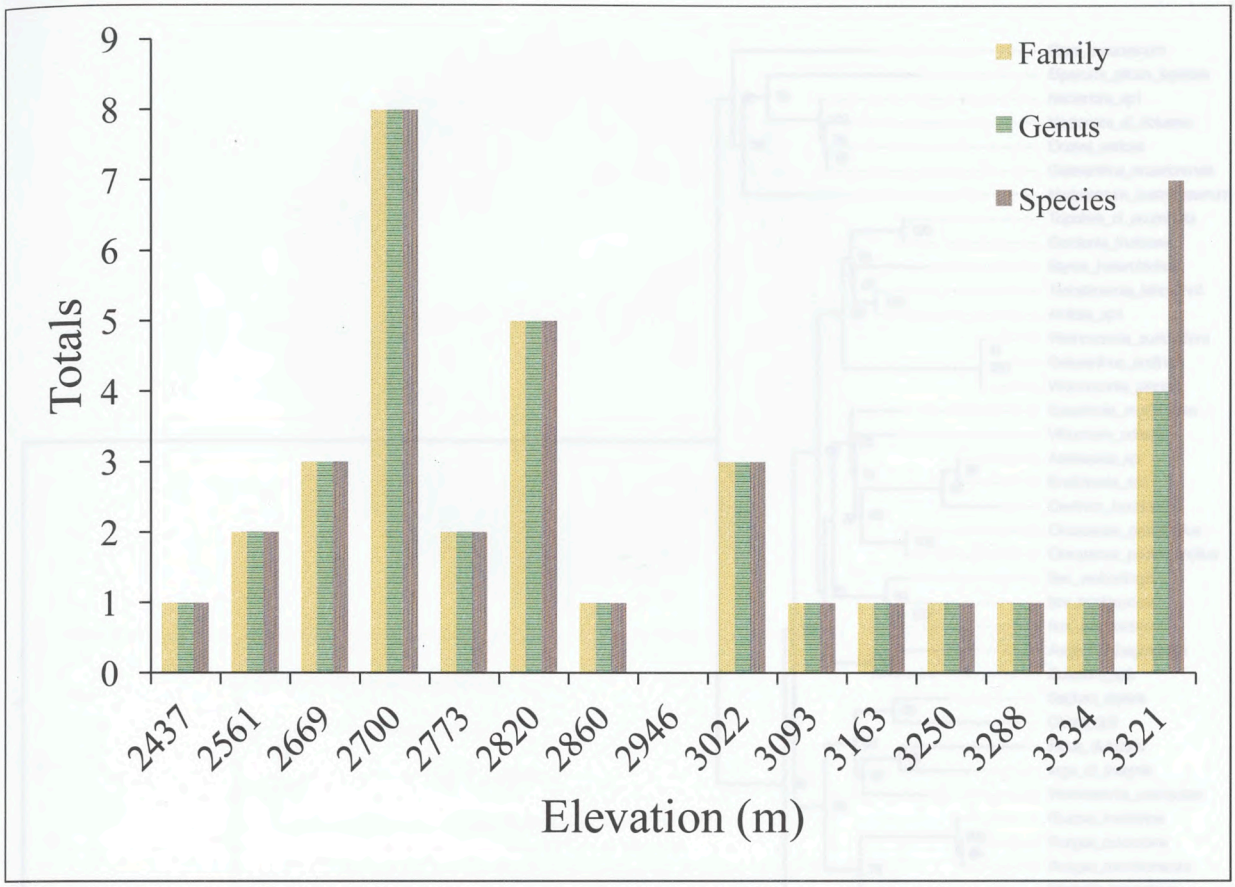


Figure S3. The distribution of families, genera, and species for each plot that were successfully sequenced and included in the phylogenetic analyses. The plots are ordered by increasing elevation where plot 1 is at the highest elevation and plot 15 is at the lowest elevation.

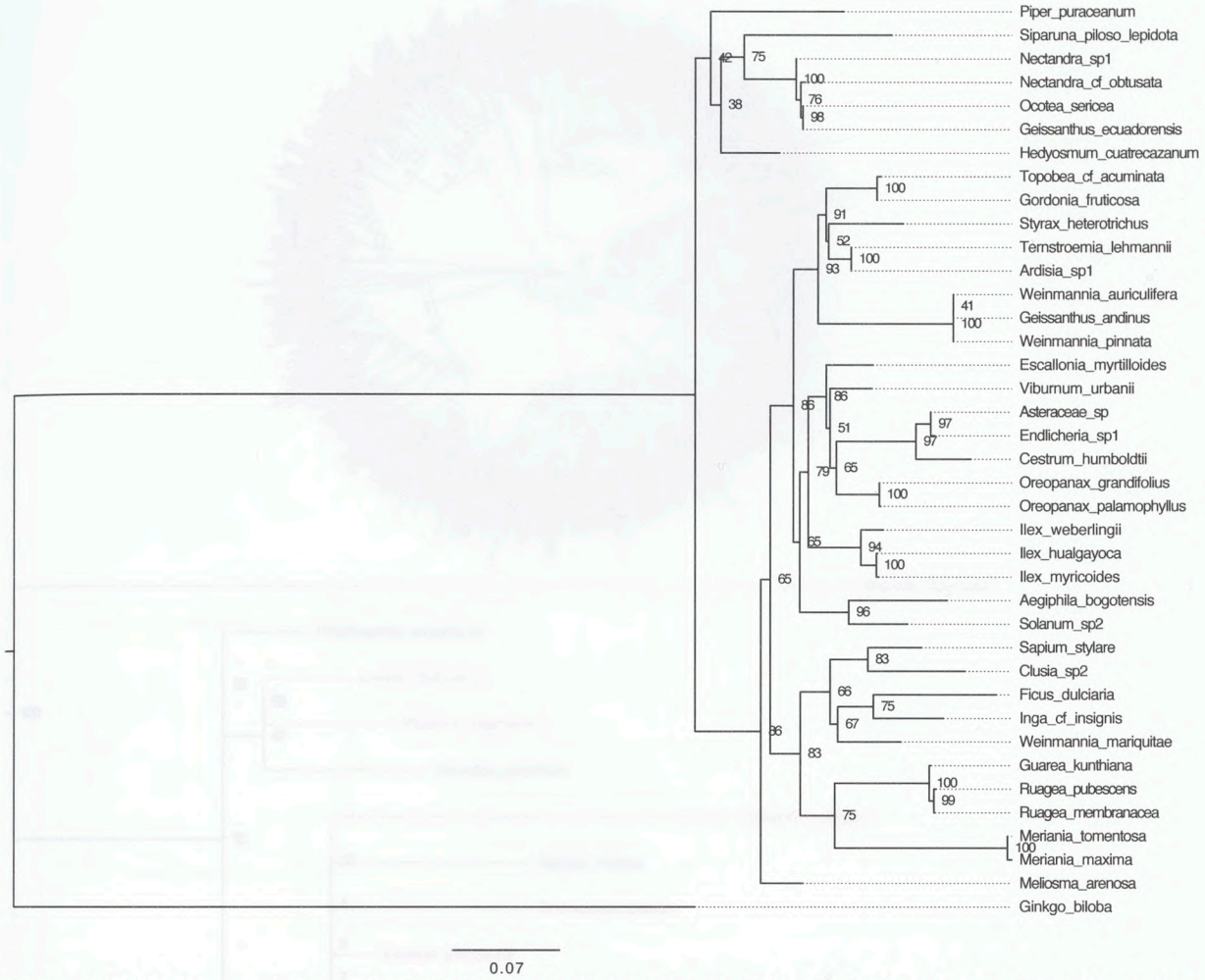


Figure S4. Phylogenetic tree of all individuals that were used in phylogenetic analyses from the transect at Siempre Verde Reserve, Imbabura Province, Ecuador. Bootstrap values based on maximum likelihood are reported at the nodes.

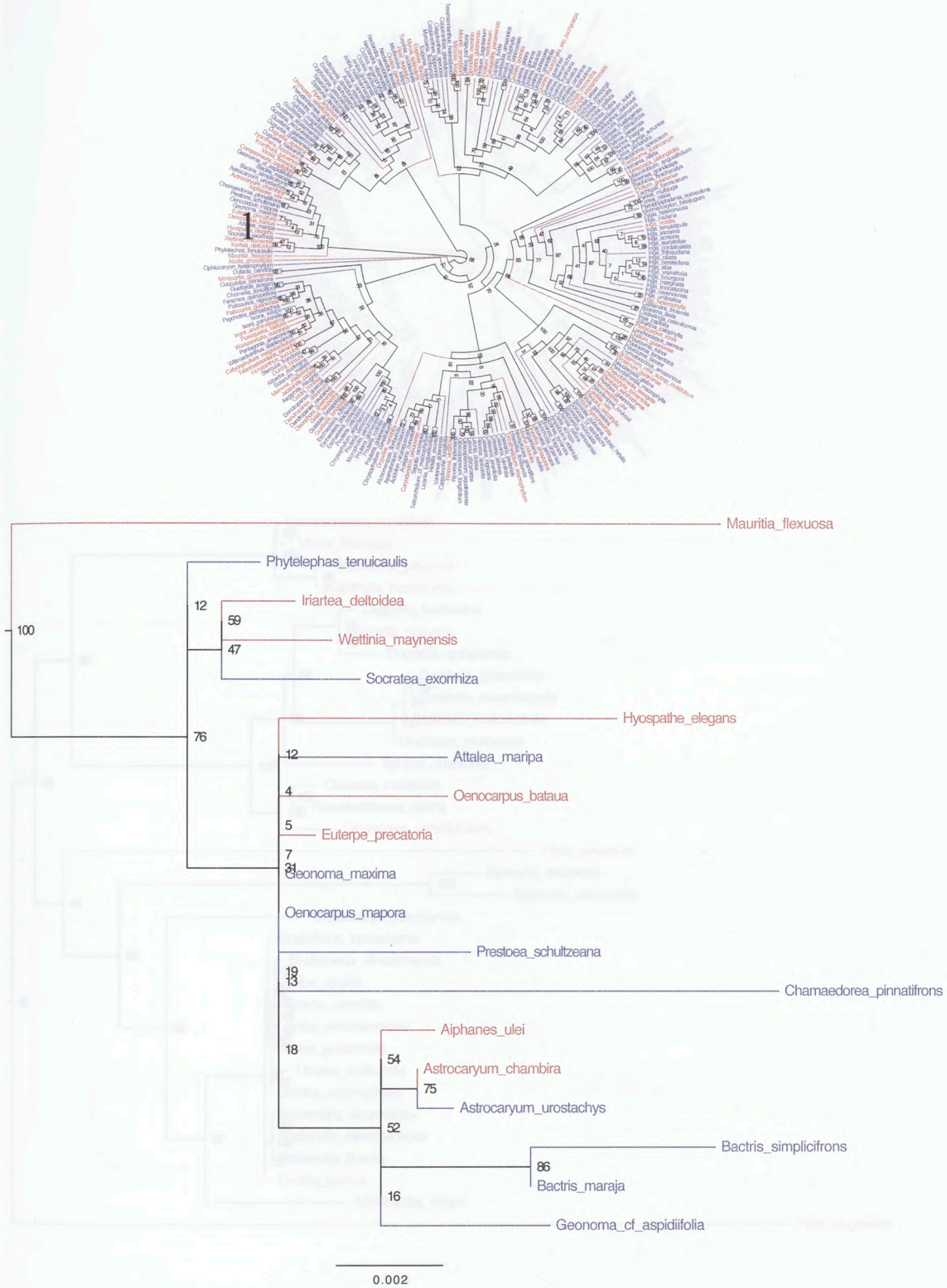


Figure S5. One of six major clades that makes up the phylogenetic tree of all successful sequenced individuals from Yasuní (Fig. 8). This clade is comprised of 19 species all within the Arecaceae family.

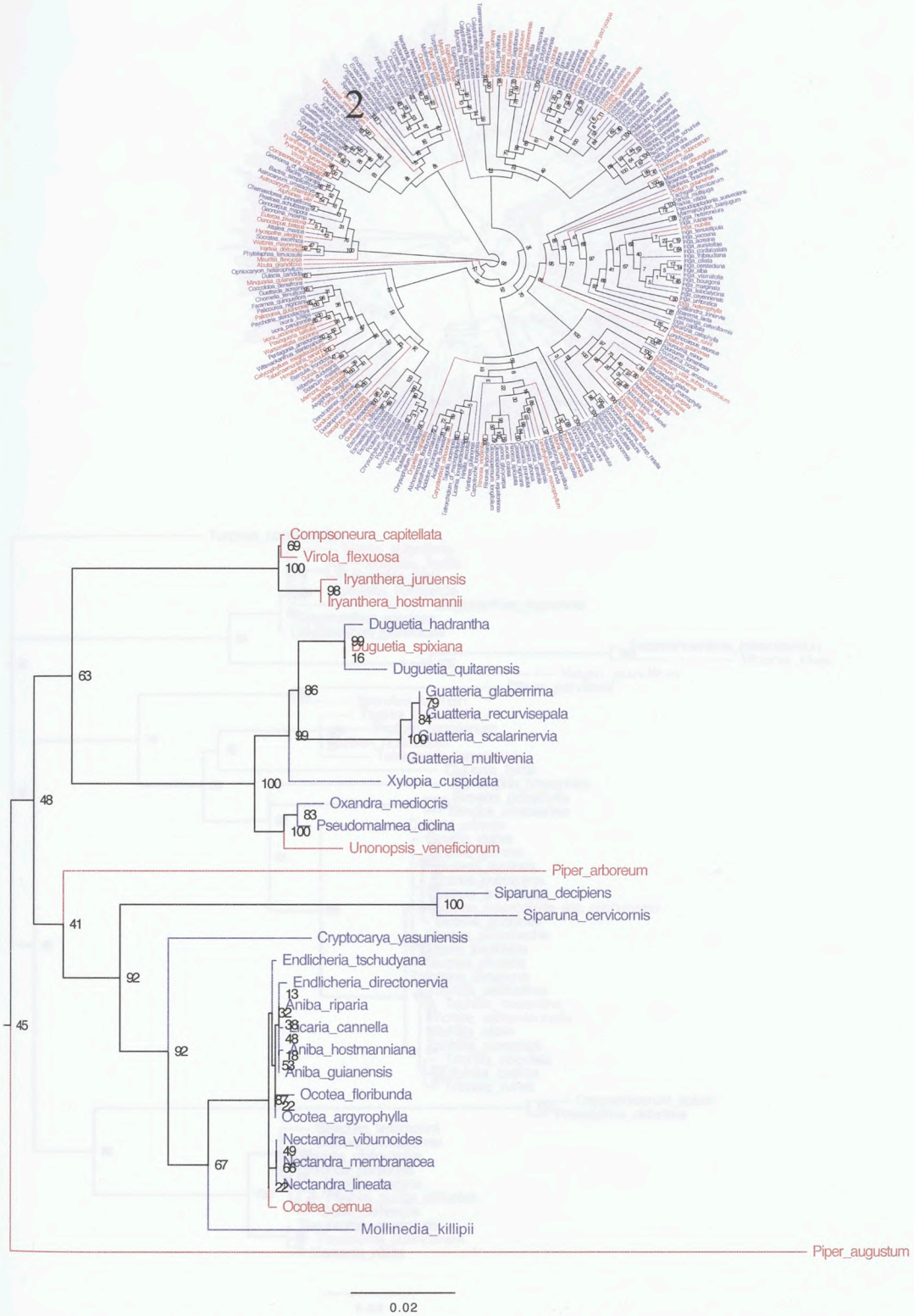
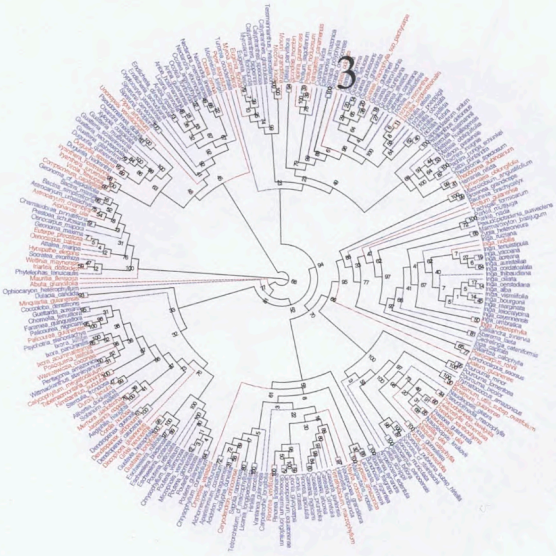


Figure S6. One of six major clades that makes up the phylogenetic tree of all successfully sequenced individuals from Yasuní (Fig. 8). This clade is comprised of the Myristicaceae, Annonaceae, Piperaceae, Siparunaceae, Lauraceae, Monimaceae families.



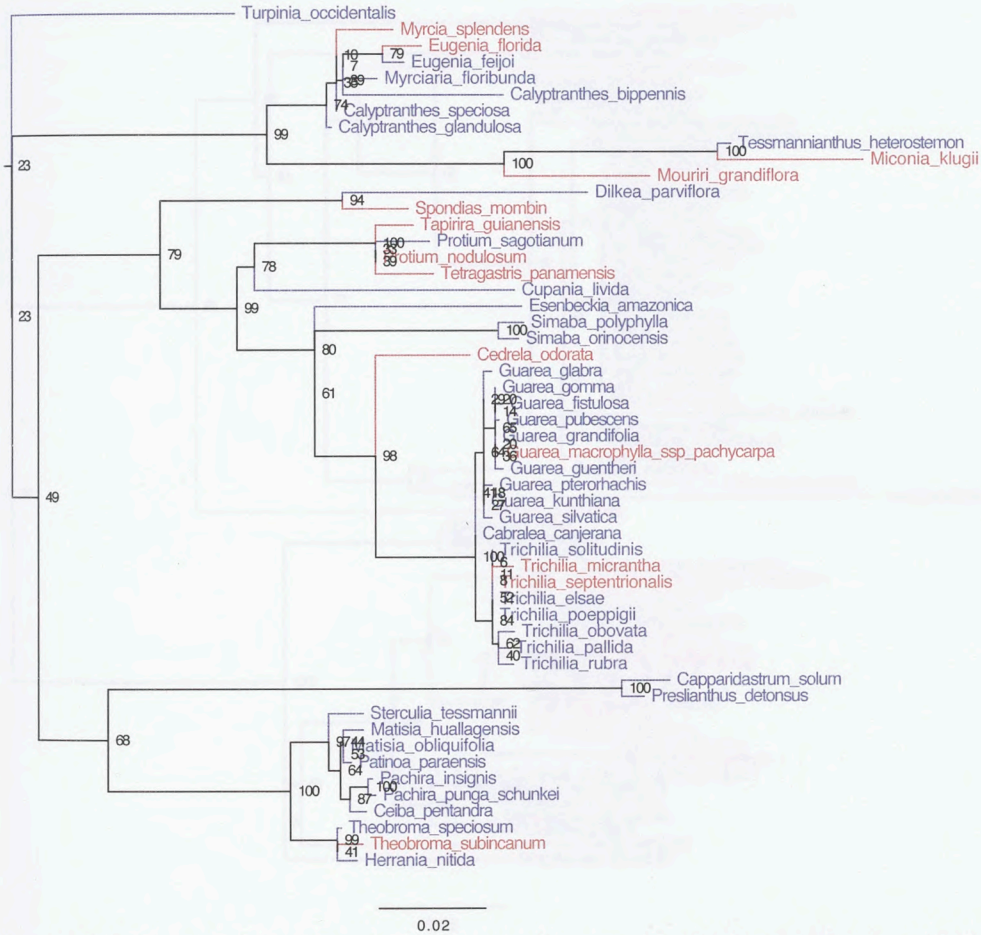


Figure S7. One of six major clades that makes up the phylogenetic tree of all successfully sequenced individuals from Yasuní (Fig. 8). This clade is comprised of the Staphyleaceae, Myrtaceae, Melastomataceae, Passifloraceae, Anacardiaceae, Burseraceae, Sapindaceae, Rutaceae, Simaroubaceae, Meliaceae, Capparaceae, and Malvaceae families.

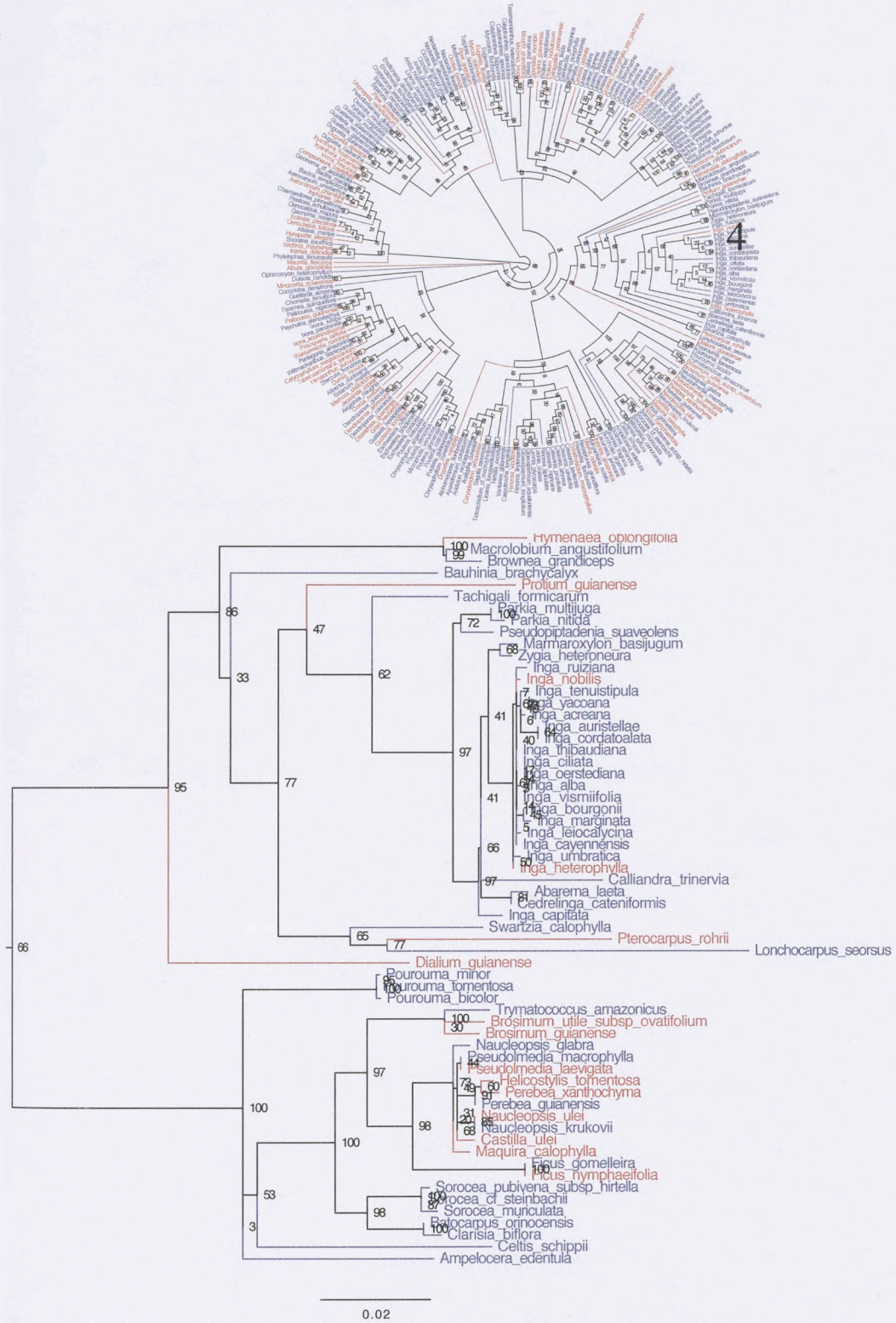
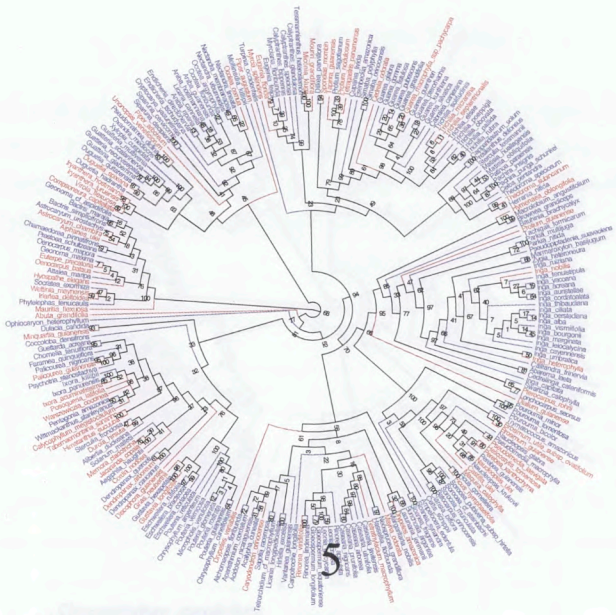


Figure S8. One of six major clades that makes up the phylogenetic tree of all successfully sequenced individuals from Yasuní (Fig. 8). This clade is comprised of Fabaceae, Urticaceae, Moraceae, Cannabaceae, and Ulmaceae families.



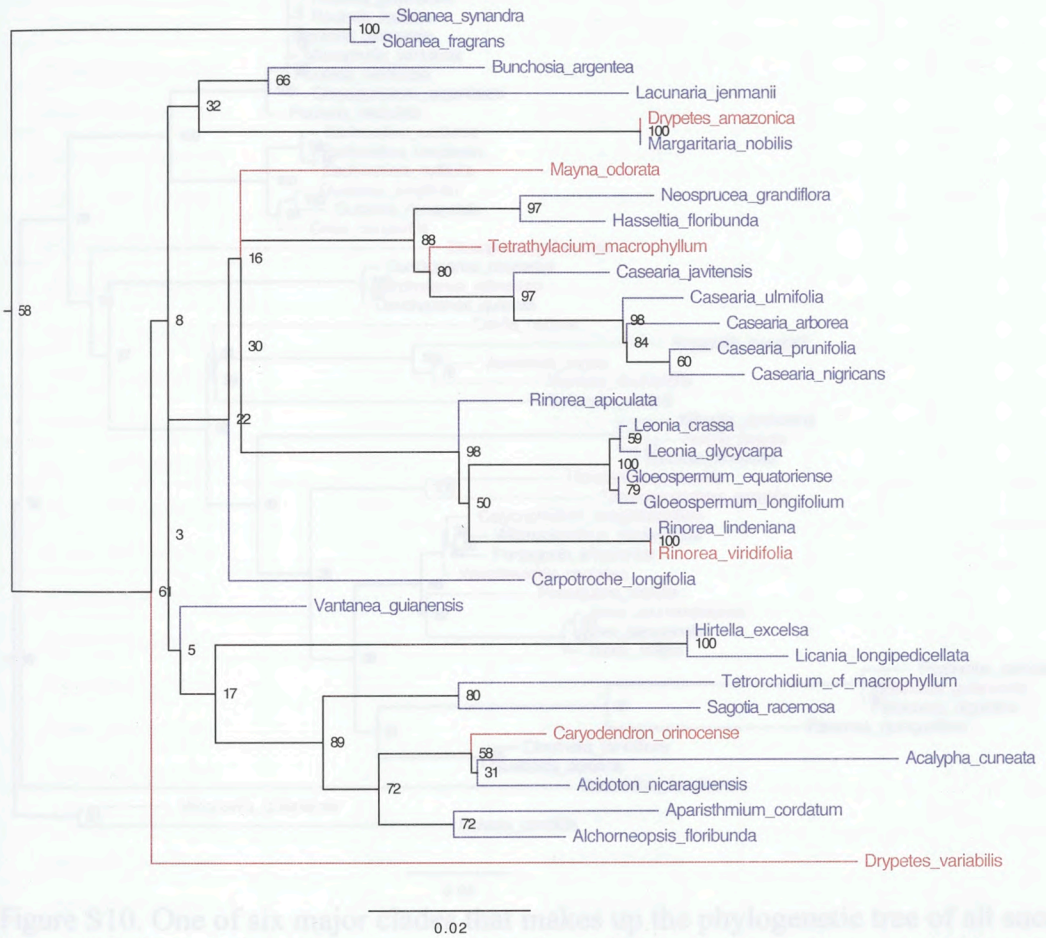


Figure S9. One of six major clades that makes up the phylogenetic tree of all successfully sequenced individuals from Yasuní (Fig. 8). This clade is comprised of Elaeocarpaceae, Malpighiaceae, Ochnaceae, Putranjivaceae, Phyllanthaceae, Achariaceae, Salicaceae, Violaceae, Humiriaceae, Chrysobalanaceae, and Euphorbiaceae families.

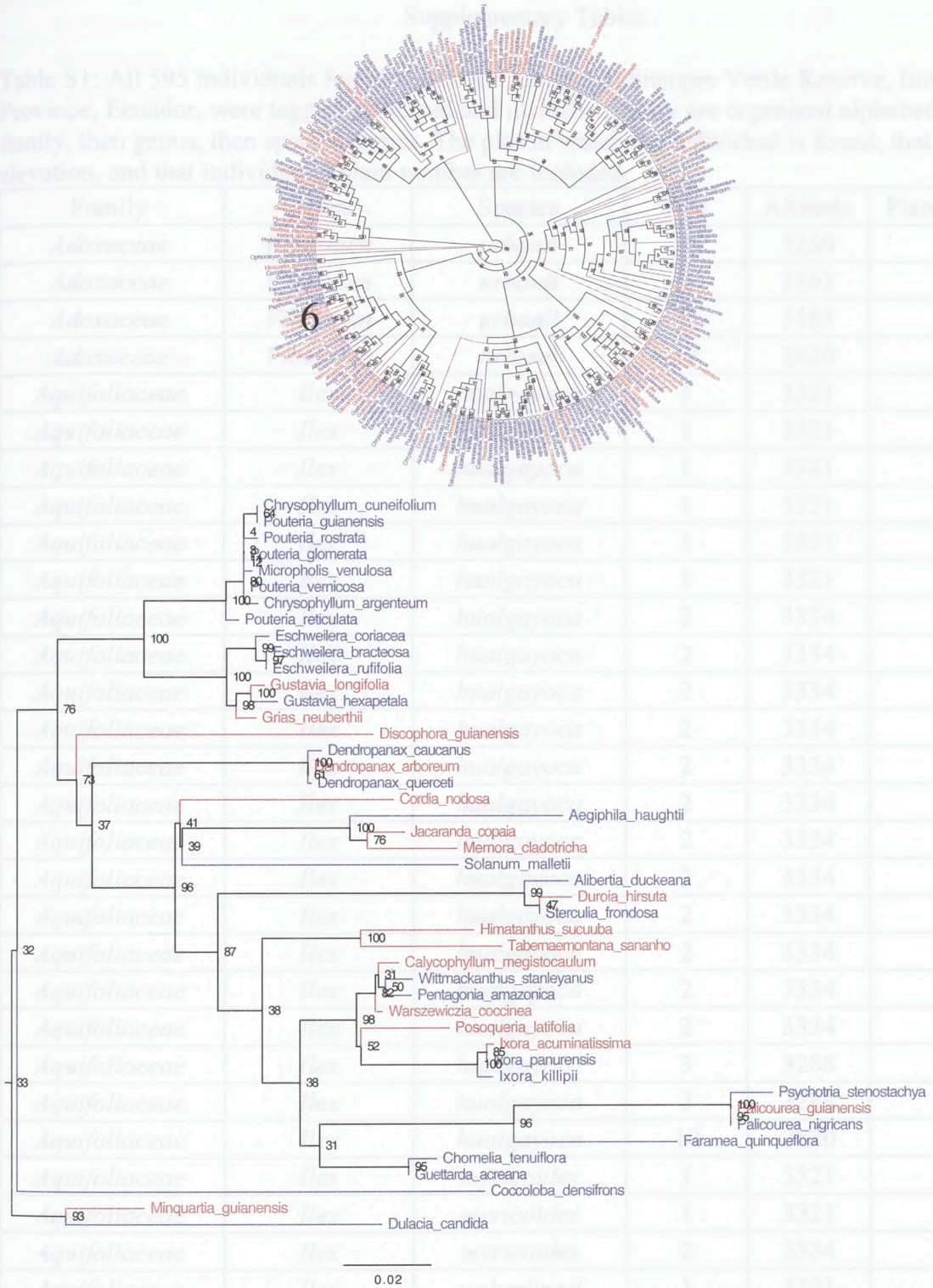


Figure S10. One of six major clades that makes up the phylogenetic tree of all successfully sequenced individuals from Yasuní (Fig. 8). This clade is comprised of Sapotaceae, Lecythidaceae, Stemonuraceae, Araliaceae, Boraginaceae, Lamiaceae, Bignoniaceae, Solanaceae, Rubiaceae, Apocynaceae, and Olacaceae.

Supplementary Tables

Table S1: All 595 individuals found within the transect at Siempre Verde Reserve, Imbabura Province, Ecuador, were tagged, collected and identified. They are organized alphabetically by family, then genus, then species names. The plot in which the individual is found, that plot's elevation, and that individual's plant number are included.

Family	Genus	Species	Plot	Altitude	Plant Number
Adoxaceae	*Viburnum*	*urbanii*	4	3250	375
Adoxaceae	*Viburnum*	*urbanii*	5	3163	357
Adoxaceae	*Viburnum*	*urbanii*	5	3163	361
Adoxaceae	*Viburnum*	*urbanii*	10	2820	226
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	499
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	534
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	553
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	567
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	585
Aquifoliaceae	*Ilex*	*hualgayoca*	1	3321	588
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	463
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	485
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	486
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	487
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	488
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	489
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	490
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	491
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	492
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	493
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	494
Aquifoliaceae	*Ilex*	*hualgayoca*	2	3334	495
Aquifoliaceae	*Ilex*	*hualgayoca*	3	3288	402
Aquifoliaceae	*Ilex*	*hualgayoca*	3	3288	439
Aquifoliaceae	*Ilex*	*hualgayoca*	10	2820	210
Aquifoliaceae	*Ilex*	*myricoides*	1	3321	503
Aquifoliaceae	*Ilex*	*myricoides*	1	3321	517
Aquifoliaceae	*Ilex*	*myricoides*	2	3334	474
Aquifoliaceae	*Ilex*	*weberlingii*	1	3321	587
Araliaceae	*Oreopanax*	*grandifolius*	5	3163	362
Araliaceae	*Oreopanax*	*grandifolius*	9	2860	253
Araliaceae	*Oreopanax*	*palamophyllus*	6	3093	326
Araliaceae	*Oreopanax*	*palamophyllus*	6	3093	330

Araliaceae	*Oreopanax*	*palamophyllus*	12	2700	139
Araliaceae	*Oreopanax*	*palamophyllus*	12	2700	140
Araliaceae	*Oreopanax*	*palamophyllus*	12	2700	145
Araliaceae	*Oreopanax*	*palamophyllus*	12	2700	148
Araliaceae	*Oreopanax*	*palamophyllus*	13	2669	120
Asteraceae	*Asteraceae*	*sp*	9	2860	242
Asteraceae	*Asteraceae*	*sp*	10	2820	202
Asteraceae	*Asteraceae*	*sp*	15	2437	5
Asteraceae	*Asteraceae*	*sp*	15	2437	40
Brunelliaceae	*Brunellia*	*acostae*	12	2700	154
Brunelliaceae	*Brunellia*	*tomentosa*	4	3250	374
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	10	2820	216
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	11	2773	159
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	11	2773	161
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	11	2773	172
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	11	2773	177
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	11	2773	194
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	12	2700	152
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	13	2669	96
Chloranthaceae	*Hedyosmum*	*cuatrecazanum*	13	2669	109
Clethraceae	*Clethra*	*ovalifolia*	1	3321	502
Clethraceae	*Clethra*	*ovalifolia*	1	3321	529
Clethraceae	*Clethra*	*ovalifolia*	1	3321	530
Clethraceae	*Clethra*	*ovalifolia*	1	3321	538
Clethraceae	*Clethra*	*ovalifolia*	1	3321	589
Clethraceae	*Clethra*	*ovalifolia*	1	3321	596
Clusiaceae	*Clusia*	*sp1*	7	3022	299
Clusiaceae	*Clusia*	*sp1*	8	2946	273
Clusiaceae	*Clusia*	*sp1*	9	2860	245
Clusiaceae	*Clusia*	*sp1*	10	2820	228
Clusiaceae	*Clusia*	*sp1*	10	2820	235
Clusiaceae	*Clusia*	*sp1*	10	2820	240
Clusiaceae	*Clusia*	*sp2*	14	2561	70
Clusiaceae	*Clusia*	*sp2*	14	2561	71
Clusiaceae	*Clusia*	*sp3*	9	2860	255
Clusiaceae	*Clusia*	*sp3*	10	2820	221
Clusiaceae	*Clusia*	*sp5*	13	2669	119
Clusiaceae	*Clusia*	*sp5*	13	2669	123
Clusiaceae	*Clusia*	*sp5*	14	2561	58

Clusiaceae	*Clusia*	*sp5*	14	2561	66
Clusiaceae	*Clusia*	*sp5*	14	2561	79
Clusiaceae	*Clusia*	*sp5*	15	2437	2
Clusiaceae	*Clusia*	*sp5*	15	2437	21
Clusiaceae	*Clusia*	*sp5*	15	2437	24
Clusiaceae	*Clusia*	*sp5*	15	2437	30
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	500
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	509
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	513
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	524
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	525
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	537
Cunoniaceae	*Weinmannia*	*auriculifera*	1	3321	546
Cunoniaceae	*Weinmannia*	*auriculifera*	3	3288	420
Cunoniaceae	*Weinmannia*	*auriculifera*	3	3288	428
Cunoniaceae	*Weinmannia*	*auriculifera*	3	3288	433
Cunoniaceae	*Weinmannia*	*auriculifera*	8	2946	284
Cunoniaceae	*Weinmannia*	*lentiscifolia*	4	3250	367
Cunoniaceae	*Weinmannia*	*lentiscifolia*	4	3250	370
Cunoniaceae	*Weinmannia*	*lentiscifolia*	4	3250	376
Cunoniaceae	*Weinmannia*	*lentiscifolia*	6	3093	331
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	288
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	289
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	294
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	303
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	311
Cunoniaceae	*Weinmannia*	*lentiscifolia*	7	3022	322
Cunoniaceae	*Weinmannia*	*lentiscifolia*	8	2946	264
Cunoniaceae	*Weinmannia*	*lentiscifolia*	8	2946	279
Cunoniaceae	*Weinmannia*	*lentiscifolia*	8	2946	285
Cunoniaceae	*Weinmannia*	*lentiscifolia*	10	2820	198
Cunoniaceae	*Weinmannia*	*lentiscifolia*	11	2773	163
Cunoniaceae	*Weinmannia*	*mariquitae*	1	3321	531
Cunoniaceae	*Weinmannia*	*mariquitae*	1	3321	573
Cunoniaceae	*Weinmannia*	*mariquitae*	1	3321	595
Cunoniaceae	*Weinmannia*	*pinnata*	1	3321	527
Cunoniaceae	*Weinmannia*	*pinnata*	1	3321	541
Cunoniaceae	*Weinmannia*	*pinnata*	1	3321	561
Cunoniaceae	*Weinmannia*	*pinnata*	1	3321	562

Cunoniaceae	*Weinmannia*	*pinnata*	1	3321	563
Cunoniaceae	*Weinmannia*	*pinnata*	5	3163	347
Cunoniaceae	*Weinmannia*	*pinnata*	5	3163	356
Cunoniaceae	*Weinmannia*	*pinnata*	5	3163	359
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	501
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	504
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	505
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	512
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	516
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	518
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	522
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	528
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	533
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	536
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	540
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	542
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	543
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	544
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	547
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	548
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	549
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	551
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	552
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	554
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	555
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	556
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	559
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	568
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	569
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	572
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	574
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	576
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	577
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	578
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	580
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	581
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	582
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	586
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	590

Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	591
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	592
Cunoniaceae	*Weinmannia*	*rollottii*	1	3321	594
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	447
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	449
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	453
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	454
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	457
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	459
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	461
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	462
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	464
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	468
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	469
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	470
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	471
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	472
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	473
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	475
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	476
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	477
Cunoniaceae	*Weinmannia*	*rollottii*	2	3334	482
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	403
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	406
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	407
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	410
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	412
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	413
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	419
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	427
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	429
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	430
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	435
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	440
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	441
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	442
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	443
Cunoniaceae	*Weinmannia*	*rollottii*	3	3288	446
Cunoniaceae	*Weinmannia*	*rollottii*	8	2946	269

Cunoniaceae	*Weinmannia*	*rollottii*	8	2946	270
Cyatheaceae	*Cyathea*	*cf frigida*	1	3321	558
Cyatheaceae	*Cyathea*	*cf frigida*	2	3334	466
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	387
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	389
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	392
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	393
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	397
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	398
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	405
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	409
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	426
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	436
Cyatheaceae	*Cyathea*	*cf frigida*	3	3288	444
Cyatheaceae	*Cyathea*	*cf frigida*	4	3250	366
Cyatheaceae	*Cyathea*	*cf frigida*	4	3250	369
Cyatheaceae	*Cyathea*	*cf frigida*	4	3250	373
Cyatheaceae	*Cyathea*	*cf frigida*	4	3250	377
Cyatheaceae	*Cyathea*	*cf frigida*	4	3250	378
Cyatheaceae	*Cyathea*	*cf frigida*	5	3163	351
Cyatheaceae	*Cyathea*	*cf frigida*	5	3163	353
Cyatheaceae	*Cyathea*	*cf frigida*	5	3163	358
Cyatheaceae	*Cyathea*	*cf frigida*	6	3093	325
Cyatheaceae	*Cyathea*	*cf frigida*	6	3093	328
Cyatheaceae	*Cyathea*	*cf frigida*	6	3093	334
Cyatheaceae	*Cyathea*	*cf frigida*	6	3093	338
Cyatheaceae	*Cyathea*	*cf frigida*	6	3093	343
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	295
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	297
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	298
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	300
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	308
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	314
Cyatheaceae	*Cyathea*	*cf frigida*	7	3022	320
Cyatheaceae	*Cyathea*	*cf frigida*	8	2946	275
Cyatheaceae	*Cyathea*	*cf frigida*	8	2946	280
Cyatheaceae	*Cyathea*	*cf frigida*	8	2946	286
Cyatheaceae	*Cyathea*	*cf frigida*	8	2946	287
Cyatheaceae	*Cyathea*	*cf frigida*	9	2860	258

Cyatheaceae	*Cyathea*	*cf frigida*	9	2860	261
Cyatheaceae	*Cyathea*	*cf frigida*	9	2860	262
Cyatheaceae	*Cyathea*	*cf frigida*	10	2820	199
Cyatheaceae	*Cyathea*	*cf frigida*	10	2820	201
Cyatheaceae	*Cyathea*	*cf frigida*	10	2820	212
Cyatheaceae	*Cyathea*	*cf frigida*	10	2820	220
Cyatheaceae	*Cyathea*	*cf frigida*	10	2820	234
Cyatheaceae	*Cyathea*	*cf frigida*	11	2773	164
Cyatheaceae	*Cyathea*	*cf frigida*	11	2773	184
Cyatheaceae	*Cyathea*	*cf frigida*	12	2700	149
Cyatheaceae	*Cyathea*	*cf frigida*	12	2700	150
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	98
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	100
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	103
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	110
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	112
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	117
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	122
Cyatheaceae	*Cyathea*	*cf frigida*	13	2669	128
Cyatheaceae	*Cyathea*	*cf frigida*	15	2437	22
Cyatheaceae	*Cyathea*	*cf frigida*	15	2437	47
Cyatheaceae	*Cyathea*	*cf frigida*	15	2437	52
Dicksoniaceae	*Dicksonia*	*sellowiana*	8	2946	266
Dicksoniaceae	*Dicksonia*	*sellowiana*	8	2946	268
Dicksoniaceae	*Dicksonia*	*sellowiana*	9	2860	252
Dicksoniaceae	*Dicksonia*	*sellowiana*	9	2860	259
Ericaceae	*Pernettya*	*prostrata*	2	3334	478
Escalloniaceae	*Escallonia*	*myrtilloides*	1	3321	514
Escalloniaceae	*Escallonia*	*myrtilloides*	2	3334	467
Escalloniaceae	*Escallonia*	*myrtilloides*	2	3334	484
Escalloniaceae	*Escallonia*	*myrtilloides*	3	3288	432
Escalloniaceae	*Escallonia*	*myrtilloides*	3	3288	434
Euphorbiaceae	*Hyeronima*	*macrocarpa*	13	2669	102
Euphorbiaceae	*Hyeronima*	*scabrida*	7	3022	313
Euphorbiaceae	*Hyeronima*	*scabrida*	12	2700	153
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	1
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	6
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	10
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	12

Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	13
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	15
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	34
Euphorbiaceae	*Sapium*	*laurifolium*	15	2437	36
Euphorbiaceae	*Sapium*	*stylare*	12	2700	142
Euphorbiaceae	*Sapium*	*stylare*	13	2669	106
Euphorbiaceae	*Sapium*	*stylare*	14	2561	83
Fabaceae	*Inga*	*cf insignis*	13	2669	99
Fabaceae	*Inga*	*cf insignis*	14	2561	65
Lamiaceae	*Aegiphila*	*bogotensis*	6	3093	337
Lauraceae	*Endlicheria*	*sp1*	12	2700	134
Lauraceae	*Nectandra*	*cf laurel*	10	2820	214
Lauraceae	*Nectandra*	*cf laurel*	11	2773	193
Lauraceae	*Nectandra*	*cf laurel*	14	2561	67
Lauraceae	*Nectandra*	*cf obtusata*	8	2946	283
Lauraceae	*Nectandra*	*cf obtusata*	15	2437	16
Lauraceae	*Nectandra*	*sp1*	10	2820	205
Lauraceae	*Nectandra*	*sp1*	10	2820	207
Lauraceae	*Nectandra*	*sp1*	14	2561	90
Lauraceae	*Nectandra*	*sp1*	14	2561	93
Lauraceae	*Nectandra*	*sp1*	14	2561	95
Lauraceae	*Ocotea*	*sericea*	13	2669	125
Lauraceae	*Ocotea*	*sericea*	15	2437	27
Lauraceae	*Persea*	*cf bullata*	15	2437	26
Lauraceae	*Persea*	*cf bullata*	15	2437	41
Melastomataceae	*Axinaea*	*cf sclerophylla*	7	3022	292
Melastomataceae	*Axinaea*	*cf sclerophylla*	7	3022	302
Melastomataceae	*Axinaea*	*cf sclerophylla*	8	2946	267
Melastomataceae	*Axinaea*	*macrophylla*	1	3321	565
Melastomataceae	*Axinaea*	*macrophylla*	2	3334	455
Melastomataceae	*Axinaea*	*macrophylla*	2	3334	480
Melastomataceae	*Axinaea*	*macrophylla*	2	3334	483
Melastomataceae	*Axinaea*	*macrophylla*	3	3288	431
Melastomataceae	*Meriania*	*maxima*	7	3022	315
Melastomataceae	*Meriania*	*tomentosa*	12	2700	132
Melastomataceae	*Meriania*	*tomentosa*	12	2700	133
Melastomataceae	*Meriania*	*tomentosa*	12	2700	136
Melastomataceae	*Miconia*	*cf sodiroi*	5	3163	344
Melastomataceae	*Miconia*	*cf sodiroi*	5	3163	345

Melastomataceae	*Miconia*	*cf sodiroi*	5	3163	350
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	323
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	332
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	333
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	336
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	341
Melastomataceae	*Miconia*	*cf sodiroi*	6	3093	342
Melastomataceae	*Miconia*	*cf sodiroi*	7	3022	319
Melastomataceae	*Miconia*	*cf sodiroi*	7	3022	321
Melastomataceae	*Miconia*	*corymbiformis*	2	3334	451
Melastomataceae	*Miconia*	*corymbiformis*	7	3022	305
Melastomataceae	*Miconia*	*corymbiformis*	7	3022	306
Melastomataceae	*Miconia*	*corymbiformis*	7	3022	307
Melastomataceae	*Miconia*	*corymbiformis*	7	3022	309
Melastomataceae	*Miconia*	*corymbiformis*	7	3022	310
Melastomataceae	*Miconia*	*corymbiformis*	8	2946	281
Melastomataceae	*Miconia*	*corymbiformis*	8	2946	282
Melastomataceae	*Miconia*	*lasiocalyx*	11	2773	186
Melastomataceae	*Miconia*	*lasiocalyx*	11	2773	187
Melastomataceae	*Miconia*	*lasiocalyx*	11	2773	191
Melastomataceae	*Miconia*	*lasiocalyx*	12	2700	151
Melastomataceae	*Miconia*	*lasiocalyx*	13	2669	97
Melastomataceae	*Miconia*	*lasiocalyx*	14	2561	59
Melastomataceae	*Miconia*	*sp1*	1	3321	519
Melastomataceae	*Miconia*	*sp1*	1	3321	523
Melastomataceae	*Miconia*	*theaezans*	7	3022	290
Melastomataceae	*Miconia*	*theaezans*	7	3022	291
Melastomataceae	*Miconia*	*theaezans*	7	3022	317
Melastomataceae	*Miconia*	*theaezans*	8	2946	271
Melastomataceae	*Miconia*	*theaezans*	9	2860	251
Melastomataceae	*Miconia*	*theaezans*	9	2860	254
Melastomataceae	*Miconia*	*theaezans*	10	2820	213
Melastomataceae	*Miconia*	*theaezans*	10	2820	215
Melastomataceae	*Miconia*	*theaezans*	10	2820	217
Melastomataceae	*Miconia*	*theaezans*	10	2820	219
Melastomataceae	*Miconia*	*theaezans*	10	2820	237
Melastomataceae	*Topobea*	*cf acuminata*	8	2946	272
Melastomataceae	*Topobea*	*cf acuminata*	8	2946	274
Melastomataceae	*Topobea*	*cf acuminata*	10	2820	197

Melastomataceae	*Topobea*	*cf acuminata*	10	2820	200
Melastomataceae	*Topobea*	*cf acuminata*	10	2820	203
Melastomataceae	*Topobea*	*cf acuminata*	10	2820	204
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	160
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	162
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	165
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	167
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	169
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	170
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	173
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	182
Melastomataceae	*Topobea*	*cf acuminata*	11	2773	192
Melastomataceae	*Topobea*	*cf acuminata*	12	2700	131
Melastomataceae	*Topobea*	*cf acuminata*	12	2700	137
Melastomataceae	*Topobea*	*cf acuminata*	12	2700	147
Melastomataceae	*Topobea*	*cf acuminata*	12	2700	155
Melastomataceae	*Topobea*	*cf acuminata*	13	2669	107
Melastomataceae	*Topobea*	*cf acuminata*	13	2669	113
Melastomataceae	*Topobea*	*cf acuminata*	14	2561	55
Melastomataceae	*Topobea*	*cf acuminata*	14	2561	85
Melastomataceae	*Topobea*	*cf acuminata*	15	2437	20
Meliaceae	*Guarea*	*kunthiana*	12	2700	138
Meliaceae	*Guarea*	*kunthiana*	13	2669	118
Meliaceae	*Guarea*	*kunthiana*	14	2561	73
Meliaceae	*Ruagea*	*membranacea*	13	2669	114
Meliaceae	*Ruagea*	*pubescens*	3	3288	399
Meliaceae	*Ruagea*	*pubescens*	6	3093	327
Meliaceae	*Ruagea*	*pubescens*	6	3093	335
Meliaceae	*Ruagea*	*pubescens*	8	2946	265
Meliaceae	*Ruagea*	*pubescens*	9	2860	248
Moraceae	*Ficus*	*dulciaria*	11	2773	178
Moraceae	*Ficus*	*dulciaria*	12	2700	156
Moraceae	*Ficus*	*dulciaria*	12	2700	157
Moraceae	*Ficus*	*dulciaria*	15	2437	11
Moraceae	*Ficus*	*dulciaria*	15	2437	19
Moraceae	*Ficus*	*dulciaria*	15	2437	28
Moraceae	*Ficus*	*dulciaria*	15	2437	51
Myrtaceae	*Myrcianthes*	*orthostemon*	9	2860	246
Myrtaceae	*Myrcianthes*	*orthostemon*	9	2860	257

Myrtaceae	*Myrcianthes*	*orthostemon*	10	2820	222
Myrtaceae	*Myrcianthes*	*orthostemon*	11	2773	196
Myrtaceae	*Myrcianthes*	*rhopaloides*	10	2820	227
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	57
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	60
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	69
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	75
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	77
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	80
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	81
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	84
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	2561	89
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	8
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	17
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	23
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	29
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	32
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	33
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	35
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	38
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	39
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	42
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	43
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	48
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	2437	49
Piperaceae	*Piper*	*puraceanum*	7	3022	312
Pentaphylacaceae	*Freziera*	*reticulata*	3	3288	437
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	510
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	511
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	515
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	520
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	526
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	532
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	539
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	557
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	570
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	575
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	579
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	583

Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	593
Pentaphylacaceae	*Freziera*	*verrucosa*	1	3321	597
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	452
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	456
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	458
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	460
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	465
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	479
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	481
Pentaphylacaceae	*Freziera*	*verrucosa*	2	3334	496
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	411
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	414
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	415
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	416
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	418
Pentaphylacaceae	*Freziera*	*verrucosa*	3	3288	445
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	9	2860	249
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	10	2820	230
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	11	2773	185
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	13	2669	111
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	13	2669	126
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	14	2561	76
Piperaceae	*Piper*	*sodiroi*	12	2700	129
Piperaceae	*Piper*	*sodiroi*	14	2561	78
Primulaceae	*Ardisia*	*sp1*	10	2820	211
Primulaceae	*Ardisia*	*sp1*	10	2820	223
Primulaceae	*Cybianthus*	*sp*	3	3288	438
Primulaceae	*Geissanthus*	*andinus*	1	3321	498
Primulaceae	*Geissanthus*	*andinus*	1	3321	508
Primulaceae	*Geissanthus*	*andinus*	1	3321	521
Primulaceae	*Geissanthus*	*andinus*	1	3321	535
Primulaceae	*Geissanthus*	*andinus*	1	3321	545
Primulaceae	*Geissanthus*	*andinus*	1	3321	550
Primulaceae	*Geissanthus*	*andinus*	1	3321	564
Primulaceae	*Geissanthus*	*andinus*	1	3321	566
Primulaceae	*Geissanthus*	*andinus*	1	3321	571
Primulaceae	*Geissanthus*	*andinus*	1	3321	584
Primulaceae	*Geissanthus*	*andinus*	3	3288	385
Primulaceae	*Geissanthus*	*andinus*	3	3288	386

Primulaceae	*Geissanthus*	*andinus*	3	3288	395
Primulaceae	*Geissanthus*	*andinus*	3	3288	417
Primulaceae	*Geissanthus*	*andinus*	3	3288	421
Primulaceae	*Geissanthus*	*ecuadorensis*	1	3321	560
Primulaceae	*Geissanthus*	*ecuadorensis*	5	3163	348
Primulaceae	*Geissanthus*	*ecuadorensis*	11	2773	176
Primulaceae	*Geissanthus*	*ecuadorensis*	12	2700	135
Primulaceae	*Geissanthus*	*ecuadorensis*	12	2700	144
Primulaceae	*Geissanthus*	*vanderwerffii*	2	3334	448
Primulaceae	*Geissanthus*	*vanderwerffii*	2	3334	450
Primulaceae	*Geissanthus*	*vanderwerffii*	3	3288	423
Primulaceae	*Myrsine*	*coriacea*	15	2437	18
Rosaceae	*Hesperomeles*	*obtusifolia*	1	3321	506
Rosaceae	*Hesperomeles*	*obtusifolia*	1	3321	507
Rosaceae	*Prunus*	*huantensis*	3	3288	384
Rosaceae	*Prunus*	*huantensis*	3	3288	394
Rosaceae	*Prunus*	*huantensis*	3	3288	422
Rosaceae	*Prunus*	*huantensis*	4	3250	371
Rosaceae	*Prunus*	*huantensis*	4	3250	372
Rosaceae	*Prunus*	*huantensis*	5	3163	349
Rosaceae	*Prunus*	*huantensis*	5	3163	360
Rosaceae	*Prunus*	*huantensis*	7	3022	293
Rubiaceae	*Cinchona*	*pitayensis*	5	3163	354
Rubiaceae	*Cinchona*	*pitayensis*	5	3163	363
Rubiaceae	*Cinchona*	*pitayensis*	13	2669	127
Rubiaceae	*Faramea*	*calyptrata*	14	2561	54
Rubiaceae	*Faramea*	*calyptrata*	14	2561	56
Rubiaceae	*Faramea*	*calyptrata*	14	2561	62
Rubiaceae	*Faramea*	*calyptrata*	14	2561	63
Rubiaceae	*Faramea*	*calyptrata*	14	2561	64
Rubiaceae	*Faramea*	*calyptrata*	14	2561	68
Rubiaceae	*Faramea*	*calyptrata*	14	2561	72
Rubiaceae	*Faramea*	*calyptrata*	14	2561	74
Rubiaceae	*Faramea*	*calyptrata*	14	2561	82
Rubiaceae	*Faramea*	*calyptrata*	14	2561	86
Rubiaceae	*Faramea*	*calyptrata*	14	2561	87
Rubiaceae	*Faramea*	*calyptrata*	14	2561	88
Rubiaceae	*Faramea*	*calyptrata*	14	2561	92
Rubiaceae	*Faramea*	*calyptrata*	14	2561	94

Rubiaceae	*Faramea*	*calyptrata*	15	2437	7
Rubiaceae	*Faramea*	*calyptrata*	15	2437	14
Rubiaceae	*Faramea*	*calyptrata*	15	2437	37
Rubiaceae	*Faramea*	*calyptrata*	15	2437	45
Rubiaceae	*Faramea*	*calyptrata*	15	2437	53
Rubiaceae	*Faramea*	*cf ovalis*	10	2820	209
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	171
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	174
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	179
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	181
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	188
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	189
Rubiaceae	*Faramea*	*cf ovalis*	11	2773	195
Rubiaceae	*Faramea*	*flavicans*	8	2946	277
Rubiaceae	*Faramea*	*flavicans*	9	2860	244
Rubiaceae	*Faramea*	*flavicans*	10	2820	232
Rubiaceae	*Faramea*	*flavicans*	10	2820	239
Rubiaceae	*Faramea*	*flavicans*	11	2773	166
Rubiaceae	*Faramea*	*flavicans*	11	2773	168
Rubiaceae	*Guettarda*	*dependens*	15	2437	50
Rubiaceae	*Guettarda*	*hirsuta*	12	2700	141
Rubiaceae	*Palicourea*	*amethystina*	4	3250	380
Rubiaceae	*Palicourea*	*amethystina*	4	3250	382
Rubiaceae	*Palicourea*	*amethystina*	5	3163	365
Rubiaceae	*Palicourea*	*amethystina*	7	3022	301
Rubiaceae	*Palicourea*	*amethystina*	12	2700	143
Rubiaceae	*Palicourea*	*amethystina*	13	2669	101
Rubiaceae	*Palicourea*	*amethystina*	13	2669	105
Rubiaceae	*Palicourea*	*amethystina*	13	2669	115
Rubiaceae	*Palicourea*	*amethystina*	13	2669	116
Rubiaceae	*Palicourea*	*amethystina*	13	2669	121
Rubiaceae	*Palicourea*	*amethystina*	13	2669	124
Rubiaceae	*Palicourea*	*cf stipularis*	7	3022	296
Rubiaceae	*Palicourea*	*cf stipularis*	14	2561	61
Rubiaceae	*Palicourea*	*cf stipularis*	14	2561	91
Rubiaceae	*Palicourea*	*stenosepala*	15	2437	9
Rubiaceae	*Palicourea*	*stenosepala*	15	2437	25
Rutaceae	*Zanthoxylum*	*andinum*	8	2946	278
Sabiaceae	*Meliosma*	*arenosa*	7	3022	304

Sabiaceae	*Meliosma*	*arenosa*	10	2820	231
Sabiaceae	*Meliosma*	*arenosa*	10	2820	233
Sabiaceae	*Meliosma*	*arenosa*	12	2700	146
Sabiaceae	*Meliosma*	*frondosa*	5	3163	355
Sabiaceae	*Meliosma*	*frondosa*	5	3163	364
Sabiaceae	*Meliosma*	*frondosa*	9	2860	263
Sabiaceae	*Meliosma*	*frondosa*	10	2820	238
Salicaceae	*Casearia*	*sylvestris*	15	2437	3
Salicaceae	*Casearia*	*sylvestris*	15	2437	4
Salicaceae	*Casearia*	*sylvestris*	15	2437	31
Sapindaceae	*Allophylus*	*excelsus*	9	2860	243
Siparunaceae	*Siparuna*	*piloso-lepidota*	5	3163	346
Siparunaceae	*Siparuna*	*piloso-lepidota*	6	3093	329
Solanaceae	*Cestrum*	*humboldtii*	4	3250	368
Solanaceae	*Solanum*	*sp2*	6	3093	340
Solanaceae	*Solanum*	*sp2*	15	2437	46
Solanaceae	*Solanum*	*sp2*	10	2820	218
Solanaceae	*Solanum*	*sp3*	6	3093	339
Styracaceae	*Styrax*	*heterotrichus*	10	2820	206
Styracaceae	*Styrax*	*heterotrichus*	10	2820	208
Styracaceae	*Styrax*	*heterotrichus*	10	2820	225
Symplocaceae	*Symplocos*	*quitensis*	11	2773	158
Symplocaceae	*Symplocos*	*quitensis*	13	2669	108
Symplocaceae	*Symplocos*	*subandina*	4	3250	379
Symplocaceae	*Symplocos*	*subandina*	4	3250	381
Theaceae	*Gordonia*	*fruticosa*	2	3334	497
Theaceae	*Gordonia*	*fruticosa*	3	3288	383
Theaceae	*Gordonia*	*fruticosa*	3	3288	388
Theaceae	*Gordonia*	*fruticosa*	3	3288	391
Theaceae	*Gordonia*	*fruticosa*	3	3288	396
Theaceae	*Gordonia*	*fruticosa*	3	3288	400
Theaceae	*Gordonia*	*fruticosa*	3	3288	401
Theaceae	*Gordonia*	*fruticosa*	3	3288	404
Theaceae	*Gordonia*	*fruticosa*	3	3288	408
Theaceae	*Gordonia*	*fruticosa*	3	3288	424
Theaceae	*Gordonia*	*fruticosa*	3	3288	425
Theaceae	*Gordonia*	*fruticosa*	6	3093	324
Theaceae	*Gordonia*	*fruticosa*	7	3022	316
Theaceae	*Gordonia*	*fruticosa*	7	3022	318

Theaceae	*Gordonia*	*fruticosa*	8	2946	276
Theaceae	*Gordonia*	*fruticosa*	9	2860	241
Theaceae	*Gordonia*	*fruticosa*	9	2860	247
Theaceae	*Gordonia*	*fruticosa*	9	2860	250
Theaceae	*Gordonia*	*fruticosa*	9	2860	256
Theaceae	*Gordonia*	*fruticosa*	9	2860	260
Theaceae	*Gordonia*	*fruticosa*	10	2820	224
Theaceae	*Gordonia*	*fruticosa*	10	2820	229
Theaceae	*Gordonia*	*fruticosa*	10	2820	236
Theaceae	*Gordonia*	*fruticosa*	11	2773	175
Theaceae	*Gordonia*	*fruticosa*	11	2773	180
Theaceae	*Gordonia*	*fruticosa*	11	2773	183
Theaceae	*Gordonia*	*fruticosa*	11	2773	190
Theaceae	*Gordonia*	*fruticosa*	12	2700	130
Theaceae	*Gordonia*	*fruticosa*	13	2669	104
Urticaceae	*Cecropia*	*andina*	15	2437	44

Table S2: These 152 individuals were sequenced for both the *rbc*L and *mat*K gene regions. They are organized alphabetically by family, then genus, then species names. The plot in which the individual is found along with that individual's plant number (Plant) are included. The *rbc*L and *mat*K columns signify whether that sequence was recovered (Yes), not recovered (No), or only one direction was successful (Uni). Individuals with an * by the family name were included in phylogenetic analyses. Individuals with an ** by the family name were added to the * data set to build a phylogeny for the entire transect (Fig. 3).

Family	Genus	Species	Plot	Plant	*rbc*L	*mat*K
Adoxaceae	*Viburnum*	*urbanii*	5	357	No	No
*Adoxaceae**	*Viburnum*	*urbanii*	10	226	Yes	Yes
*Adoxaceae***	*Viburnum*	*urbanii*	4	375	No	Yes
Aquifoliaceae	*Ilex*	*hualgayoca*	3	402	No	No
*Aquifoliaceae**	*Ilex*	*hualgayoca*	1	553	Yes	Yes
*Aquifoliaceae***	*Ilex*	*hualgayoca*	10	210	Yes	Yes
*Aquifoliaceae**	*Ilex*	*myricoides*	2	474	Yes	Yes
*Aquifoliaceae**	*Ilex*	*weberlingii*	1	587	Yes	Uni
*Araliaceae**	*Oreopanax*	*grandifolius*	9	253	Uni	Uni
*Araliaceae**	*Oreopanax*	*palamophyllus*	12	140	Yes	Yes
*Asteraceae**	*Asteraceae*	*sp*	10	202	Uni	Yes
*Asteraceae***	*Asteraceae*	*sp*	9	242	Uni	Yes
*Asteraceae***	*Asteraceae*	*sp*	15	5	Yes	Yes
*Asteraceae***	*Asteraceae*	*sp*	15	40	Yes	No
Brunelliaceae	*Brunellia*	*acostae*	12	154	No	No
*Chloranthaceae**	*Hedyosmum*	*cuatrecazanum*	11	194	Yes	No
*Chloranthaceae***	*Hedyosmum*	*cuatrecazanum*	13	96	Yes	No
Clethraceae	*Clethra*	*ovalifolia*	1	589	No	No
Clethraceae	*Clethra*	*ovalifolia*	1	502	No	No
Clethraceae	*Clethra*	*ovalifolia*	1	530	No	No
Clethraceae	*Clethra*	*ovalifolia*	1	596	No	No
Clusiaceae	*Clusia*	*sp1*	10	228	No	No
Clusiaceae	*Clusia*	*sp2*	14	71	No	No
*Clusiaceae**	*Clusia*	*sp2*	14	70	Yes	No
Clusiaceae	*Clusia*	*sp3*	10	221	No	No
Clusiaceae	*Clusia*	*sp3*	9	255	No	No
Clusiaceae	*Clusia*	*sp5*	15	2	No	No
Clusiaceae	*Clusia*	*sp5*	13	119	No	No
Clusiaceae	*Clusia*	*sp5*	14	58	No	No
Clusiaceae	*Clusia*	*sp5*	14	66	No	No
*Cunoniaceae**	*Weinmannia*	*auriculifera*	1	500	Yes	No
Cunoniaceae	*Weinmannia*	*lentiscifolia*	4	370	No	No

Cunoniaceae	*Weinmannia*	*lentiscifolia*	4	376	No	No
Cunoniaceae	*Weinmannia*	*lentiscifolia*	10	198	No	No
Cunoniaceae	*Weinmannia*	*mariquitae*	1	531	No	No
*Cunoniaceae**	*Weinmannia*	*mariquitae*	1	573	Uni	No
Cunoniaceae	*Weinmannia*	*mariquitae*	1	595	No	No
Cunoniaceae	*Weinmannia*	*pinnata*	1	541	No	No
Cunoniaceae	*Weinmannia*	*pinnata*	5	356	No	No
*Cunoniaceae**	*Weinmannia*	*pinnata*	1	527	Yes	No
*Cunoniaceae***	*Weinmannia*	*pinnata*	1	561	Yes	No
Ericaceae	*Pernettya*	*prostrata*	2	478	No	No
Escalloniaceae	*Escallonia*	*myrtilloides*	2	467	No	No
Escalloniaceae	*Escallonia*	*myrtilloides*	3	432	No	No
*Escalloniaceae**	*Escallonia*	*myrtilloides*	1	514	Uni	Yes
Euphorbiaceae	*Hyeronima*	*scabrida*	7	313	No	No
Euphorbiaceae	*Sapium*	*laurifolium*	15	1	No	No
*Euphorbiaceae**	*Sapium*	*stylare*	12	142	Yes	No
*Euphorbiaceae***	*Sapium*	*stylare*	14	83	Yes	No
*Fabaceae**	*Inga*	*cf insignis*	13	99	Yes	Yes
*Lamiaceae**	*Aegiphila*	*bogotensis*	6	337	Yes	No
*Lauraceae**	*Endlicheria*	*sp1*	12	134	Yes	No
Lauraceae	*Nectandra*	*cf laurel*	11	193	No	No
*Lauraceae**	*Nectandra*	*cf obtusata*	15	16	Yes	Yes
*Lauraceae***	*Nectandra*	*cf obtusata*	8	283	No	Uni
*Lauraceae**	*Nectandra*	*sp1*	14	93	Yes	Yes
Lauraceae	*Nectandra*	*sp1*	10	207	No	No
*Lauraceae***	*Ocotea*	*sericea*	15	27	Yes	No
*Lauraceae**	*Ocotea*	*sericea*	13	125	Yes	Uni
Lauraceae	*Persea*	*cf bullata*	15	26	No	No
Melastomataceae	*Axinaea*	*cf sclerophylla*	8	267	No	No
Melastomataceae	*Axinaea*	*macrophylla*	1	565	No	No
Melastomataceae	*Axinaea*	*macrophylla*	3	431	No	No
Melastomataceae	*Miconia*	*cf sodiroi*	5	345	No	No
Melastomataceae	*Miconia*	*cf sodiroi*	6	333	No	No
Melastomataceae	*Miconia*	*cf sodiroi*	7	319	No	No
Melastomataceae	*Miconia*	*corymbiformis*	2	451	No	No
Melastomataceae	*Miconia*	*lasiocalyx*	14	59	No	No
Melastomataceae	*Miconia*	*sp1*	1	523	No	No
Melastomataceae	*Miconia*	*theaezans*	10	213	No	No
Melastomataceae	*Topobea*	*cf acuminata*	8	272	No	No

Melastomataceae	*Topobea*	*cf acuminata*	11	167	No	No
Melastomataceae	*Topobea*	*cf acuminata*	12	131	No	No
Melastomataceae	*Topobea*	*cf acuminata*	13	107	No	No
Melastomataceae	*Topobea*	*cf acuminata*	15	20	No	No
*Melastomataceae**	*Topobea*	*cf acuminata*	14	55	Yes	No
*Melastomataceae**	*Meriania*	*maxima*	7	315	Yes	No
*Melastomataceae**	*Meriania*	*tomentosa*	12	132	Yes	No
Meliaceae	*Guarea*	*kunthiana*	13	118	No	No
*Meliaceae**	*Guarea*	*kunthiana*	12	138	Yes	Yes
*Meliaceae***	*Guarea*	*kunthiana*	14	73	Yes	Uni
*Meliaceae**	*Ruagea*	*membranacea*	13	114	Yes	Yes
*Meliaceae**	*Ruagea*	*pubescens*	3	399	Yes	Yes
*Meliaceae***	*Ruagea*	*pubescens*	6	327	Yes	Uni
*Meliaceae***	*Ruagea*	*pubescens*	6	335	Yes	Uni
*Meliaceae***	*Ruagea*	*pubescens*	9	248	Yes	Yes
*Meliaceae***	*Ruagea*	*pubescens*	8	265	Yes	Yes
Moraceae	*Ficus*	*dulciaria*	11	178	No	No
Moraceae	*Ficus*	*dulciaria*	15	19	No	No
*Moraceae**	*Ficus*	*dulciaria*	12	157	Yes	No
Myrtaceae	*Myrcianthes*	*orthostemon*	10	222	No	No
Myrtaceae	*Myrcianthes*	*orthostemon*	11	196	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	10	227	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	57	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	14	80	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	32	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	39	No	No
Myrtaceae	*Myrcianthes*	*rhopaloides*	15	42	No	No
Pentaphylacaceae	*Freziera*	*reticulata*	3	437	No	No
Pentaphylacaceae	*Freziera*	*verrucosa*	1	515	No	No
Pentaphylacaceae	*Freziera*	*verrucosa*	2	479	No	No
Pentaphylacaceae	*Freziera*	*verrucosa*	3	445	No	No
Pentaphylacaceae	*Ternstroemia*	*lehmannii*	13	111	No	No
*Pentaphylacaceae**	*Ternstroemia*	*lehmannii*	11	185	Yes	Yes
*Pentaphylacaceae***	*Ternstroemia*	*lehmannii*	9	249	Uni	Yes
*Pentaphylacaceae***	*Ternstroemia*	*lehmannii*	14	76	Yes	Yes
Phyllanthaceae	*Hieronyma*	*macrocarpa*	13	102	No	No
Phyllanthaceae	*Hieronyma*	*scabrida*	12	153	No	No
*Piperaceae**	*Piper*	*puraceanum*	7	312	Uni	No
Piperaceae	*Piper*	*sodiroi*	14	78	No	No

Piperaceae	*Piper*	*sodiroi*	12	129	No	No
Primulaceae	*Ardisia*	*sp1*	10	223	No	No
*Primulaceae**	*Ardisia*	*sp1*	10	211	Yes	Yes
Primulaceae	*Geissanthus*	*andinus*	1	550	No	No
*Primulaceae**	*Geissanthus*	*andinus*	1	498	Yes	Yes
*Primulaceae***	*Geissanthus*	*andinus*	1	545	Yes	Yes
*Primulaceae***	*Geissanthus*	*andinus*	3	386	Uni	No
*Primulaceae***	*Geissanthus*	*andinus*	3	421	Yes	Yes
*Primulaceae***	*Geissanthus*	*andinus*	1	584	Yes	Yes
*Primulaceae***	*Geissanthus*	*andinus*	3	395	Uni	Yes
Primulaceae	*Geissanthus*	*ecuadorensis*	11	176	No	No
Primulaceae	*Geissanthus*	*ecuadorensis*	12	135	No	No
*Primulaceae **	*Geissanthus*	*ecuadorensis*	12	135_A	Yes	Yes
*Primulaceae***	*Geissanthus*	*ecuadorensis*	5	348	Uni	Uni
Primulaceae	*Geissanthus*	*ecuadorensis*	12	144	No	No
Primulaceae	*Myrsine*	*coriacea*	15	18	No	No
Rubiaceae	*Guettarda*	*dependens*	15	50	No	No
Rutaceae	*Zanthoxylum*	*andinum*	8	278	No	No
Sabiaceae	*Meliosma*	*arenosa*	7	304	No	No
*Sabiaceae**	*Meliosma*	*arenosa*	12	146	Yes	No
Sabiaceae	*Meliosma*	*arenosa*	10	233	No	No
Sabiaceae	*Meliosma*	*frondosa*	5	364	No	No
Sabiaceae	*Meliosma*	*frondosa*	9	263	No	No
Salicaceae	*Casearia*	*sylvestris*	15	3	No	No
Salicaceae	*Casearia*	*sylvestris*	15	4	No	No
Salicaceae	*Casearia*	*sylvestris*	15	31	No	No
Sapindaceae	*Allophylus*	*excelsus*	9	243	No	No
Siparunaceae	*Siparuna*	*piloso-lepidota*	6	329	No	No
*Siparunaceae**	*Siparuna*	*piloso-lepidota*	5	346	No	Yes
*Solanaceae**	*Cestrum*	*humboldtii*	4	368	Yes	Yes
Solanaceae	*Solanum*	*sp2*	15	46	No	No
*Solanaceae**	*Solanum*	*sp2*	10	218	Yes	Yes
*Styracaceae**	*Styrax*	*heterotrichus*	10	206	Yes	Yes
*Styracaceae***	*Styrax*	*heterotrichus*	10	208	Yes	No
*Styracaceae***	*Styrax*	*heterotrichus*	10	225	Yes	No
Theaceae	*Gordonia*	*fruticosa*	3	396	No	No
Theaceae	*Gordonia*	*fruticosa*	11	175	No	No
Theaceae	*Gordonia*	*fruticosa*	9	256	No	No
*Theaceae**	*Gordonia*	*fruticosa*	7	316	Yes	Yes

Theaceae	*Gordonia*	*fruticosa*	13	104	No	No
*Theaceae***	*Gordonia*	*fruticosa*	12	130	Yes	No
Urticaceae	*Cecropia*	*andina*	15	44	No	No

Table S3. Standard effects sizes for phylogenetic diversity (PD) randomization for each plot (1-15) and when plots were grouped by elevation (low, medium, high). All randomizations were run 999 times.

Plot	ntaxa	pd.obs	rand.mean	rand.sd	obs.rank	obs.z	obs.p
1	9	0.441	0.655	0.086	5	-2.482	0.005
2	4	0.219	0.350	0.073	39	-1.789	0.039
3	5	0.306	0.416	0.078	92	-1.402	0.092
4	2	0.209	0.183	0.061	633	0.420	0.633
5	5	0.422	0.422	0.075	490	-0.009	0.49
6	6	0.551	0.483	0.080	796	0.839	0.796
7	4	0.404	0.352	0.071	765	0.727	0.765
8	5	0.392	0.415	0.073	375	-0.315	0.375
9	5	0.364	0.417	0.075	243	-0.715	0.243
10	12	0.555	0.804	0.092	6	-2.718	0.006
11	6	0.397	0.481	0.081	142	-1.030	0.142
12	11	0.790	0.758	0.092	626	0.351	0.626
13	10	0.570	0.708	0.089	69	-1.548	0.069
14	7	0.529	0.543	0.082	433	-0.165	0.433
15	6	0.506	0.481	0.079	615	0.312	0.615
Elevation Groups							
Low	20	0.994	1.112	0.093	113	-1.271	0.113
Medium	23	1.283	1.211	0.091	785	0.797	0.785
High	14	0.727	0.884	0.095	55	-1.651	0.055

Table S4. Standard effects sizes for mean pairwise distance (MPD) randomization for each plot (1-15) and when plots are grouped by elevation (low, medium, high). All randomizations were run 999 times.

Plot	ntaxa	mpd.obs	rand.mean	rand.sd	obs.rank	obs.z	obs.p
1	9	0.096	0.151	0.021	6	-2.632	0.006
2	4	0.031	0.074	0.018	6	-2.416	0.006
3	5	0.083	0.128	0.024	37	-1.877	0.037
4	2	0.060	0.092	0.031	143	-1.038	0.143
5	5	0.144	0.137	0.025	621	0.293	0.621
6	6	0.156	0.146	0.022	660	0.424	0.66
7	4	0.141	0.129	0.026	673	0.478	0.673
8	5	0.123	0.140	0.024	243	-0.705	0.243
9	5	0.082	0.114	0.021	73	-1.532	0.073
10	12	0.102	0.161	0.017	1	-3.547	0.001
11	6	0.094	0.129	0.024	85	-1.407	0.085
12	11	0.164	0.157	0.019	619	0.338	0.619
13	10	0.139	0.160	0.017	113	-1.237	0.113
14	7	0.154	0.150	0.021	573	0.195	0.573
15	6	0.170	0.138	0.023	929	1.434	0.929
Elevation Groups							
Low	20	0.169	0.168	0.013	536	0.092	0.536
Medium	23	0.120	0.160	0.016	4	-2.503	0.004
High	14	0.112	0.156	0.018	8	-2.509	0.008

Table S5. Standard effects sizes for mean nearest taxon distance (MNTD) randomization for each plot (1-15) and when plots are grouped by elevation (low, medium, high). All randomizations were run 999 times.

Plot	ntaxa	mntd.obs	rand.mean	rand.sd	obs.rank	obs.z	obs.p
1	9	0.022	0.097	0.027	6	-2.768	0.006
2	4	0.020	0.137	0.051	20	-2.283	0.02
3	5	0.065	0.126	0.037	72	-1.662	0.072
4	2	0.120	0.186	0.062	145	-1.057	0.145
5	5	0.115	0.124	0.036	388	-0.258	0.388
6	6	0.144	0.115	0.032	813	0.906	0.813
7	4	0.135	0.135	0.041	496	0.003	0.496
8	5	0.084	0.125	0.034	131	-1.193	0.131
9	5	0.071	0.125	0.039	108	-1.346	0.108
10	12	0.047	0.085	0.021	36	-1.799	0.036
11	6	0.040	0.116	0.035	26	-2.198	0.026
12	11	0.096	0.087	0.022	635	0.368	0.635
13	10	0.059	0.092	0.022	76	-1.511	0.076
14	7	0.114	0.109	0.028	548	0.191	0.548
15	6	0.146	0.115	0.035	811	0.885	0.811
Elevation Groups							
Low	20	0.054	0.062	0.011	262	-0.636	0.262
Medium	23	0.069	0.056	0.010	900	1.338	0.9
High	14	0.061	0.077	0.017	194	-0.927	0.194

Table S6. All 337 individuals in the data set. They are organized alphabetically by family, then genus, then species names (Pérez et al. 2014). In the *rbc*L and *mat*K columns, an herbarium number (indicated with a -) is given if the sequence was obtained from the sequencing facility. If the sequence was obtained from BOLD or GenBank, the accession number is given. The medicinal column clarifies whether the species was designated as medicinal or not. If the species is medicinal, symbols show which sources confirm this designation. ∗: Schultes and Raffauf 1990; +: Rios et al. 2007; ♦:Davis and Yost 1983; •:Waorani, ∞:Cerón and Montalvo 1998.

Family	Genus	Species	*rbc*L	*mat*K	Medicinal
Achariaceae	*Carpotroche*	*longifolia*	No	EF135514	No
Achariaceae	*Lindackeria*	*paludosa*	No	No	No
Achariaceae	*Mayna*	*odorata*	AP-3980	No	Yes∗+
Anacardiaceae	*Spondias*	*mombin*	JQ590141	AY594480	Yes∗+
Anacardiaceae	*Tapirira*	*guianensis*	JQ626278	No	Yes∗
Annonaceae	*Duguetia*	*hadrantha*	AY738161	AP-3705	No
Annonaceae	*Duguetia*	*quitarensis*	AY738173	AY740553	No
Annonaceae	*Duguetia*	*spixiana*	AP-2153	No	Yes∗
Annonaceae	*Guatteria*	*glaberrima*	No	AP-4478	No
Annonaceae	*Guatteria*	*multivenia*	AP-4381	AP-4381	No
Annonaceae	*Guatteria*	*recurvisepala*	AP-4677	AP-4677	No
Annonaceae	*Guatteria*	*scalarinervia*	AP-3033	AP-3033	No
Annonaceae	*Oxandra*	*mediocris*	AP-3709	AP-3709	No
Annonaceae	*Pseudomalmea*	*diclina*	AP-6308	AY841398	No
Annonaceae	*Trigynaea*	*triplinervis*	No	No	No
Annonaceae	*Unonopsis*	*floribunda*	No	No	Yes∗
Annonaceae	*Unonopsis*	*veneficiorum*	AP-4382	AP-4382	Yes∗
Annonaceae	*Xylopia*	*cuspidata*	AP-3251	AP-3251	No
Apocynaceae	*Himatanthus*	*sucuuba*	AP-4378	AP-4378	Yes∗
Apocynaceae	*Tabernaemontana*	*sananho*	AP-3976	AP-3976	Yes∗+

Araliaceae	*Dendropanax*	*arboreus*	AP-4229	AP-4229	Yes+
Araliaceae	*Dendropanax*	*caucanus*	AP-193	No	No
Araliaceae	*Dendropanax*	*querceti*	GV-1677	GV-1677	No
Arecaceae	*Aiphanes*	*ulei*	No	AP-4981	Yes+
Arecaceae	*Astrocaryum*	*chambira*	TAG-430422	TAG-430422	Yes+♦●
Arecaceae	*Astrocaryum*	*urostachys*	TAG-440569	TAG-440569	No
Arecaceae	*Attalea*	*maripa*	TAG-430406	No	No
Arecaceae	*Bactris*	*corossilla*	No	No	Yes∞
Arecaceae	*Bactris*	*maraja*	AP-2418	AP-2418	No
Arecaceae	*Bactris*	*simplicifrons*	GV-3026	HQ265561	No
Arecaceae	*Chamaedorea*	*pinnatifrons*	No	DQ178685	No
Arecaceae	*Euterpe*	*precatoria*	TAG-270118	TAG-270118	Yes*+●
Arecaceae	*Geonoma*	*maxima*	AP-4007	No	No
Arecaceae	*Geonoma*	*stricta*	No	No	Yes+
Arecaceae	*Geonoma*	*triglochin*	No	No	No
Arecaceae	*Geonoma*	*cf. aspidiifolia*	AP-2411	No	No
Arecaceae	*Hyospathe*	*elegans*	AP-4009	No	Yes*+
Arecaceae	*Iriartea*	*deltoidea*	TAG-440602	TAG-440602	No
Arecaceae	*Mauritia*	*flexuosa*	TAG-340096	TAG-340096	Yes*
Arecaceae	*Oenocarpus*	*bataua*	TAG-430717	No	Yes+●
Arecaceae	*Oenocarpus*	*mapora*	TAG-332156	TAG-332156	No
Arecaceae	*Phytelephas*	*tenuicaulis*	No	EF128238	No
Arecaceae	*Prestoea*	*schultzeana*	TAG-330812	TAG-330812	No
Arecaceae	*Socratea*	*exorrhiza*	AM110205	TAG-250255	Yes*
Arecaceae	*Wettinia*	*maynensis*	TAG-460194	TAG-460194	No
Bignoniaceae	*Jacaranda*	*copaia*	AP-4239	JQ626519	Yes*
Bignoniaceae	*Memora*	*cladotricha*	AP-1812	AP-1812	Yes+
Boraginaceae	*Cordia*	*nodosa*	AP-3714	AP-3714	Yes*+

Burseraceae	*Crepidospermum*	*rhoifolium*	No	No	No
Burseraceae	*Dacryodes*	*peruviana*	No	No	No
Burseraceae	*Protium*	*amazonicum*	No	No	No
Burseraceae	*Protium*	*aracouchini*	No	No	No
Burseraceae	*Protium*	*glabrescens*	No	No	No
Burseraceae	*Protium*	*guianense*	JQ625777	No	Yes*
Burseraceae	*Protium*	*nodulosum*	AP-4346	No	Yes+
Burseraceae	*Protium*	*sagotianum*	AP-1744	AP-1744	No
Burseraceae	*Tetragastris*	*panamensis*	GQ428579	No	Yes*
Cannabaceae	*Celtis*	*schippii*	GV-3594	GV-3594	No
Capparaceae	*Capparidastrum*	*solum*	AP-3487	AP-3487	No
Capparaceae	*Preslianthus*	*detonsus*	AP-3365	AP-3365	No
Chrysobalanaceae	*Hirtella*	*excelsa*	GV-1329	No	No
Chrysobalanaceae	*Licania*	*harlingii*	No	No	No
Chrysobalanaceae	*Licania*	*longipedicellata*	AP-4000	No	No
Cyatheaceae	*Cyathea*	*lasiosora*	No	No	No
Dichapetalaceae	*Tapura*	*juruana*	No	No	No
Dichapetalaceae	*Tapura*	*peruviana*	No	No	Yes+♦
Elaeocarpaceae	*Sloanea*	*fragrans*	No	AP-2493	No
Elaeocarpaceae	*Sloanea*	*pubescens*	No	No	No
Elaeocarpaceae	*Sloanea*	*synandra*	No	AP-4910	No
Erythroxylaceae	*Erythroxylum*	*macrophyllum*	No	No	Yes*
Euphorbiaceae	*Acalypha*	*cuneata*	AP-2581	No	No
Euphorbiaceae	*Acidoton*	*nicaraguensis*	AP-3257	AP-3257	No
Euphorbiaceae	*Alchornea*	*triplinervia*	No	No	Yes*
Euphorbiaceae	*Alchorneopsis*	*floribunda*	AP-6333	HM446655	No
Euphorbiaceae	*Aparisthmium*	*cordatum*	AP-3047	No	No
Euphorbiaceae	*Caryodendron*	*orinocense*	AP-6329	AP-6329	Yes*

Euphorbiaceae	*Conceveiba*	*rhytidocarpa*	No	No	No
Euphorbiaceae	*Pausandra*	*trianae*	No	No	No
Euphorbiaceae	*Sagotia*	*racemosa*	AP-3923	No	No
Euphorbiaceae	*Tetrorchidium*	*cf. macrophyllum*	AP-2973	AP-2973	No
Fabaceae	*Abarema*	*laeta*	AP-3341	No	No
Fabaceae	*Bauhinia*	*brachycalyx*	AP-3422	AP-3422	No
Fabaceae	*Brownea*	*grandiceps*	GV-1890	EU361892	No
Fabaceae	*Calliandra*	*trinervia*	AP-3413	No	No
Fabaceae	*Cedrelinga*	*cateniformis*	AP-3631	AP-3631	No
Fabaceae	*Dialium*	*guianense*	JQ625793	EU361930	Yes*
Fabaceae	*Hymenaea*	*oblongifolia*	AP-3942	AP-3942	Yes*
Fabaceae	*Inga*	*acreana*	AP-6305	AP-6305	No
Fabaceae	*Inga*	*alba*	AP-6304	AP-6304	No
Fabaceae	*Inga*	*auristellae*	AP-3989	AM920210	No
Fabaceae	*Inga*	*bourgonii*	HGS-3861	AM920191	No
Fabaceae	*Inga*	*brachyrhachis*	No	No	No
Fabaceae	*Inga*	*capitata*	JQ625753	No	No
Fabaceae	*Inga*	*cayennensis*	AP-4006	AP-4006	No
Fabaceae	*Inga*	*ciliata*	AP-3512	No	No
Fabaceae	*Inga*	*cordatoalata*	AP-6309	No	No
Fabaceae	*Inga*	*heteropylla*	AP-5622	No	Yes*
Fabaceae	*Inga*	*leiocalycina*	AP-5580	AP-5580	No
Fabaceae	*Inga*	*marginata*	AP-4841	AM920215	No
Fabaceae	*Inga*	*nobilis*	AM920263	AM920193	Yes*
Fabaceae	*Inga*	*oerstediana*	AP-4878	No	No
Fabaceae	*Inga*	*ruiziana*	FJ173751	AM920202	No
Fabaceae	*Inga*	*tenuistipula*	AP-3509	AP-3509	No
Fabaceae	*Inga*	*thibaudiana*	AP-4304	AP-4304	No

Fabaceae	*Inga*	*umbratica*	AP-4387	AM920207	No
Fabaceae	*Inga*	*vismiifolia*	FJ173758	AM920220	No
Fabaceae	*Inga*	*yacoana*	AP-3482	No	No
Fabaceae	*Lonchocarpus*	*seorsus*	AP-5587	AP-5587	No
Fabaceae	*Macrolobium*	*angustifolium*	GV-2888	GV-2888	No
Fabaceae	*Macrolobium*	*stenocladum*	No	No	No
Fabaceae	*Marmaroxylon*	*basijugum*	GV-993	No	No
Fabaceae	*Parkia*	*multijuga*	AP-6318	AP-6318	No
Fabaceae	*Parkia*	*nitida*	JQ626144	No	No
Fabaceae	*Pseudopiptadenia*	*suaveolens*	AP-3431	FJ037918	No
Fabaceae	*Pterocarpus*	*rohrii*	No	JN083564	Yes*
Fabaceae	*Swartzia*	*calophylla*	AP-4094	AP-4094	No
Fabaceae	*Swartzia*	*rosea*	No	No	No
Fabaceae	*Tachigali*	*formicarum*	GV-2895	No	No
Fabaceae	*Zygia*	*heteroneura*	TAG-380030	TAG-380030	No
Humiriaceae	*Vantanea*	*guianensis*	Z75679	EF135600	No
Lacistemataceae	*Lacistema*	*cf. nena*	No	No	No
Lacistemataceae	*Lozania*	*klugii*	No	No	No
Lamiaceae	*Aegiphila*	*haughtii*	AP-3396	No	No
Lauraceae	*Aniba*	*guianensis*	JQ626307	No	No
Lauraceae	*Aniba*	*hostmanniana*	AP-4922	AP-4922	No
Lauraceae	*Aniba*	*riparia*	AP-3310	AP-3310	No
Lauraceae	*Cryptocarya*	*yasuniensis*	No	AP-5097	No
Lauraceae	*Endlicheria*	*directonervia*	AP-3961	AP-3961	No
Lauraceae	*Endlicheria*	*tschudyana*	AP-3293	AP-3293	No
Lauraceae	*Licaria*	*cannella*	AP-4768	AP-4768	No
Lauraceae	*Nectandra*	*lineata*	AP-4405	AP-4405	No
Lauraceae	*Nectandra*	*membranacea*	AP-4774	AP-4774	No

Lauraceae	*Nectandra*	*oppositifolia*	No	No	No
Lauraceae	*Nectandra*	*viburnoides*	No	AP-3962	No
Lauraceae	*Ocotea*	*argyrophylla*	JQ626098	JQ626566	No
Lauraceae	*Ocotea*	*cernua*	AP-4466	AP-4466	Yes∞
Lauraceae	*Ocotea*	*cf. bofo*	No	No	No
Lauraceae	*Ocotea*	*floribunda*	HM446841	EU153866	No
Lauraceae	*Ocotea*	*javitensis*	No	No	No
Lauraceae	*Pleurothyrium*	*cuneifolium*	No	No	No
Lauraceae	*Pleurothyrium*	*glabrifolium*	No	No	No
Lauraceae	*Pleurothyrium*	*insigne*	No	No	No
Lauraceae	*Rhodostemonodaphne*	*juruensis*	No	No	No
Lecythidaceae	*Eschweilera*	*bracteosa*	AP-4251	AP-4251	No
Lecythidaceae	*Eschweilera*	*coriacea*	JQ626161	JQ626454	No
Lecythidaceae	*Eschweilera*	*rufifolia*	AP-4653	No	No
Lecythidaceae	*Grias*	*neuberthii*	AP-4330	AP-4330	Yes*
Lecythidaceae	*Gustavia*	*hexapetala*	AP-5096	AP-5096	No
Lecythidaceae	*Gustavia*	*longifolia*	GV-3522	GV-3522	Yes*
Malpighiaceae	*Bunchosia*	*argentea*	AP-3548	No	No
Malpighiaceae	*Byrsonima*	*putumayensis*	No	No	Yes∞
Malvaceae	*Apeiba*	*membranacea*	No	No	No
Malvaceae	*Ceiba*	*pentandra*	JX987572	HQ696701	No
Malvaceae	*Herrania*	*cuatrecasana*	No	No	No
Malvaceae	*Herrania*	*nitida*	AP-3522	AP-3522	No
Malvaceae	*Matisia*	*bracteolosa*	No	No	No
Malvaceae	*Matisia*	*huallagensis*	GV-3578	GV-3578	No
Malvaceae	*Matisia*	*malacocalyx*	No	No	No
Malvaceae	*Matisia*	*obliquifolia*	AP-3690	No	No
Malvaceae	*Matisia*	*oblongifolia*	No	No	No

Malvaceae	*Pachira*	*insignis*	No	HQ696704	No
Malvaceae	*Pachira*	*punga-schunkei*	AP-3390	AP-3390	No
Malvaceae	*Patinoa*	*paraensis*	GB-3592	GB-3592	No
Malvaceae	*Quararibea*	*wittii*	No	No	No
Malvaceae	*Sterculia*	*colombiana*	No	No	No
Malvaceae	*Sterculia*	*frondosa*	JQ625865	JQ626365	No
Malvaceae	*Sterculia*	*tessmannii*	GV-427	No	No
Malvaceae	*Theobroma*	*speciosum*	AP-3501	AP-3501	No
Malvaceae	*Theobroma*	*subincanum*	JQ626171	FJ514766	Yes∞
Melastomataceae	*Miconia*	*bubalina*	No	No	Yes*+
Melastomataceae	*Miconia*	*decurrens*	No	No	No
Melastomataceae	*Miconia*	*elata*	No	No	No
Melastomataceae	*Miconia*	*fosteri*	No	No	No
Melastomataceae	*Miconia*	*grandifolia*	No	No	No
Melastomataceae	*Miconia*	*klugii*	AP-3266	No	Yes*
Melastomataceae	*Miconia*	*multispicata*	No	No	No
Melastomataceae	*Miconia*	*napoana*	No	No	No
Melastomataceae	*Miconia*	*pilgeriana*	No	No	No
Melastomataceae	*Miconia*	*tomentosa*	No	No	Yes*
Melastomataceae	*Mouriri*	*grandiflora*	AP-3470	No	Yes*
Melastomataceae	*Tessmannianthus*	*heterostemon*	AP-4023	No	No
Meliaceae	*Cabralea*	*canjerana*	AP-4894	No	No
Meliaceae	*Cedrela*	*odorata*	AP-5601	AP-5601	Yes+
Meliaceae	*Guarea*	*carinata*	No	No	No
Meliaceae	*Guarea*	*fistulosa*	AP-3559	AP-3559	No
Meliaceae	*Guarea*	*glabra*	AP-4938	AP-4938	No
Meliaceae	*Guarea*	*gomma*	AP-4966	AP-4966	No
Meliaceae	*Guarea*	*grandifolia*	AP-4141	AP-4141	No

Meliaceae	*Guarea*	*guentheri*	AP-3536	AP-3536	No
Meliaceae	*Guarea*	*kunthiana*	GV-3606	No	No
Meliaceae	*Guarea*	*macrophylla ssp. pachycarpa*	AP-3308	AP-3308	Yes*
Meliaceae	*Guarea*	*pterorhachis*	AP-1579	AP-1579	No
Meliaceae	*Guarea*	*pubescens*	GV-1603	GV-1603	No
Meliaceae	*Guarea*	*purusana*	No	No	No
Meliaceae	*Guarea*	*silvatica*	AP-2956	AP-2956	No
Meliaceae	*Trichilia*	*cf. maynasiana*	No	No	No
Meliaceae	*Trichilia*	*elsae*	AP-4805	No	No
Meliaceae	*Trichilia*	*micrantha*	JQ625887	No	Yes*
Meliaceae	*Trichilia*	*obovata*	No	AP-4720	No
Meliaceae	*Trichilia*	*pallida*	AP-4496	HM446750	No
Meliaceae	*Trichilia*	*poeppigii*	AP-3557	No	No
Meliaceae	*Trichilia*	*rubra*	AP-3386	AP-3386	No
Meliaceae	*Trichilia*	*septentrionalis*	AP-3645	No	Yes*
Meliaceae	*Trichilia*	*solitudinis*	AP-3079	No	No
Menispermaceae	*Abuta*	*grandifolia*	FJ026459	AP-1656	Yes*+•
Monimaceae	*Mollinedia*	*killipii*	AP-3438	No	No
Moraceae	*Batocarpus*	*orinocensis*	AP-3708	AP-3708	No
Moraceae	*Brosimum*	*guianense*	AP-4200	JQ626530	Yes*
Moraceae	*Brosimum*	*utile subsp. ovatifolium*	AP-4977	AP-4977	Yes*+
Moraceae	*Castilla*	*ulei*	AP-3043	No	Yes*
Moraceae	*Clarisia*	*biflora*	GV-805	No	No
Moraceae	*Ficus*	*gomelleira*	AP-6312	No	No
Moraceae	*Ficus*	*nymphaeifolia*	AP-4145	No	Yes*
Moraceae	*Helicostylis*	*tomentosa*	AP-4678	FJ514761	Yes*
Moraceae	*Maquira*	*calophylla*	AP-3540	FJ514665	Yes*
Moraceae	*Naucleopsis*	*glabra*	AP-4937	AP-4937	No

Moraceae	*Naucleopsis*	*krukovii*	AP-3687	No	No
Moraceae	*Naucleopsis*	*ulei*	AP-3919	No	Yes*
Moraceae	*Perebea*	*angustifolia*	No	No	No
Moraceae	*Perebea*	*guianensis*	AP-3526	No	No
Moraceae	*Perebea*	*xanthochyma*	AP-4176	No	Yes•
Moraceae	*Pseudolmedia*	*laevigata*	KX640875	No	Yes*
Moraceae	*Pseudolmedia*	*laevis*	No	No	No
Moraceae	*Pseudolmedia*	*macrophylla*	AP-4082	AP-4082	No
Moraceae	*Sorocea*	*cf. steinbachii*	AP-4972	No	No
Moraceae	*Sorocea*	*muriculata*	AP-4899	AP-4899	No
Moraceae	*Sorocea*	*pubivena subsp. hirtella*	AP-4949	AP-4949	No
Moraceae	*Trymatococcus*	*amazonicus*	AP-3988	AP-3988	No
Myristicaceae	*Compsoneura*	*capitellata*	EU090509	EU090470	Yes*
Myristicaceae	*Iryanthera*	*hostmannii*	AP-1366	JQ626536	Yes•
Myristicaceae	*Iryanthera*	*juruensis*	AP-3474	No	Yes*+♦
Myristicaceae	*Otoba*	*glycycarpa*	No	No	No
Myristicaceae	*Virola*	*duckei*	No	No	No
Myristicaceae	*Virola*	*elongata*	No	No	Yes*∞
Myristicaceae	*Virola*	*flexuosa*	AP-4072	No	Yes*
Myristicaceae	*Virola*	*obovata*	No	No	No
Myristicaceae	*Virola*	*pavonis*	No	No	Yes*+
Myrtaceae	*Calyptranthes*	*bippennis*	AP-4059	No	No
Myrtaceae	*Calyptranthes*	*glandulosa*	No	AP-3409	No
Myrtaceae	*Calyptranthes*	*ruiziana*	No	No	No
Myrtaceae	*Calyptranthes*	*speciosa*	JQ626314	No	No
Myrtaceae	*Eugenia*	*feijoi*	AP-5628	AP-5628	No
Myrtaceae	*Eugenia*	*florida*	GV-3510	GV-3510	Yes*
Myrtaceae	*Eugenia*	*multirimosa*	No	No	No

Myrtaceae	*Eugenia*	*pusilliflora*	No	No	No
Myrtaceae	*Eugenia*	*schunkei*	No	No	No
Myrtaceae	*Eugenia*	*yasuniana*	No	No	No
Myrtaceae	*Myrcia*	*splendens*	HM446838	HM446718	Yes*
Myrtaceae	*Myrcia*	*vertipub*	No	No	No
Myrtaceae	*Myrciaria*	*floribunda*	AP-4758	AP-4758	No
Ochnaceae	*Lacunaria*	*jenmanii*	AP-4924	No	No
Ochnaceae	*Quiina*	*florida*	No	No	No
Olacaceae	*Dulacia*	*candida*	AP-3366	AP-3366	No
Olacaceae	*Heisteria*	*acuminata*	No	No	Yes+
Olacaceae	*Minquartia*	*guianensis*	AP-2964	AP-2964	Yes*+
Passifloraceae	*Dilkea*	*parviflora*	AP-4086	No	No
Phyllanthaceae	*Margaritaria*	*nobilis*	HM446823	HM446709	No
Phyllanthaceae	*Richeria*	*racemosa*	No	No	No
Picramniaceae	*Picramnia*	*juniniana*	No	No	No
Piperaceae	*Piper*	*arboreum*	GENG1678-16	No	Yes*
Piperaceae	*Piper*	*augustum*	No	DQ882203	Yes*♦●
Polygonaceae	*Coccoloba*	*densifrons*	AP-2140	AP-2140	No
Primulaceae	*Stylogyne*	*longifolia*	No	No	No
Putranjivaceae	*Drypetes*	*amazonica*	AP-4948	No	Yes∞
Putranjivaceae	*Drypetes*	*variabilis*	JQ626067	No	Yes∞
Rubiaceae	*Alibertia*	*duckeana*	KR-2975	No	No
Rubiaceae	*Alseis*	*cf. lugonis*	No	No	No
Rubiaceae	*Calycophyllum*	*megistocaulum*	AP-4815	AP-4815	Yes+
Rubiaceae	*Chomelia*	*tenuiflora*	GQ852316	No	No
Rubiaceae	*Duroia*	*hirsuta*	AJ286696	No	Yes*+♦●∞
Rubiaceae	*Faramea*	*capillipes*	No	No	No
Rubiaceae	*Faramea*	*quinqueflora*	AP-3299	AP-3299	No

Rubiaceae	*Guettarda*	*acreana*	JQ626041	No	No
Rubiaceae	*Ixora*	*acuminatissima*	AP-3960	AP-3960	Yes*
Rubiaceae	*Ixora*	*killipii*	GV-323	No	No
Rubiaceae	*Ixora*	*panurensis*	AP-3979	AP-3979	No
Rubiaceae	*Palicourea*	*guianensis*	AP-4208	No	Yes*+
Rubiaceae	*Palicourea*	*nigricans*	AP-1669	No	No
Rubiaceae	*Pentagonia*	*amazonica*	No	FJ905373	No
Rubiaceae	*Pentagonia*	*spathicalyx*	No	No	Yes*+♦
Rubiaceae	*Posoqueria*	*latifolia*	JQ626258	JQ626556	Yes*
Rubiaceae	*Psychotria*	*caerulea*	No	No	Yes+
Rubiaceae	*Psychotria*	*huampaniensis*	No	No	No
Rubiaceae	*Psychotria*	*stenostachya*	AP-1961	No	No
Rubiaceae	*Warszewiczia*	*coccinea*	AP-1224	AP-1224	Yes*+∞
Rubiaceae	*Warszewiczia*	*cordata*	No	No	Yes*
Rubiaceae	*Wittmackanthus*	*stanleyanus*	AP-3393	AP-3393	No
Rutaceae	*Esenbeckia*	*amazonica*	AP-3584	No	No
Sabiaceae	*Ophiocaryon*	*heterophyllum*	AP-4802	No	No
Salicaceae	*Casearia*	*arborea*	AP-4389	HM446663	No
Salicaceae	*Casearia*	*javitensis*	JQ626018	JQ626446	No
Salicaceae	*Casearia*	*nigricans*	GV-1686	No	No
Salicaceae	*Casearia*	*prunifolia*	AP-3278	AP-3278	No
Salicaceae	*Casearia*	*ulmifolia*	AP-3647	AP-3647	No
Salicaceae	*Hasseltia*	*floribunda*	No	EF135546	No
Salicaceae	*Neosprucea*	*grandiflora*	GV-3524	GV-3524	No
Salicaceae	*Ryania*	*speciosa*	No	No	Yes*
Salicaceae	*Tetrathylacium*	*macrophyllum*	GV-2790	No	Yes*∞
Sapindaceae	*Cupania*	*livida*	AP-6331	AP-6331	No
Sapindaceae	*Melicoccus*	*novogranatensis*	No	No	No

Sapotaceae	*Chrysophyllum*	*argenteum*	AP-3628	AP-3628	No
Sapotaceae	*Chrysophyllum*	*cuneifolium*	AP-4689	No	No
Sapotaceae	*Micropholis*	*venulosa*	JQ626105	JQ626490	No
Sapotaceae	*Pouteria*	*glomerata*	AP-3576	No	No
Sapotaceae	*Pouteria*	*guianensis*	AP-6345	No	No
Sapotaceae	*Pouteria*	*reticulata*	JQ625962	No	No
Sapotaceae	*Pouteria*	*rostrata*	AP-5091	No	No
Sapotaceae	*Pouteria*	*torta ssp. glabra*	No	No	No
Sapotaceae	*Pouteria*	*trilocularis*	No	No	No
Sapotaceae	*Pouteria*	*vernicosa*	AP-4941	No	No
Sapotaceae	*Sarcaulus*	*brasiliensis*	No	No	No
Simaroubaceae	*Simaba*	*orinocensis*	EU043033	EU042895	No
Simaroubaceae	*Simaba*	*polyphylla*	GV-3532	GV-3532	No
Siparunaceae	*Siparuna*	*cervicornis*	GV-1906	GV-1906	No
Siparunaceae	*Siparuna*	*cuspidata*	No	No	No
Siparunaceae	*Siparuna*	*decipiens*	FJ038199	GV-2798	No
Siparunaceae	*Siparuna*	*thecaphora*	No	No	Yes+•
Solanaceae	*Solanum*	*altissimum*	No	No	Yes∞
Solanaceae	*Solanum*	*malletii*	AP-1663	AP-1663	No
Staphyleaceae	*Turpinia*	*occidentalis*	AP-4150	HM446751	No
Stemonuraceae	*Discophora*	*guianensis*	JQ625904	JQ626375	Yes+∞
Ulmaceae	*Ampelocera*	*edentula*	AP-5583	No	No
Urticaceae	*Cecropia*	*ficifolia*	No	No	Yes*
Urticaceae	*Cecropia*	*sciadophylla*	No	No	Yes*
Urticaceae	*Coussapoa*	*orthoneura*	No	No	Yes*
Urticaceae	*Pourouma*	*bicolor*	AP-4051	AP-4051	No
Urticaceae	*Pourouma*	*guianensis*	No	No	No
Urticaceae	*Pourouma*	*minor*	JQ625720	AP-4665	No

Urticaceae	*Pourouma*	*tomentosa*	JQ626115	JQ626513	No
Violaceae	*Gloeospermum*	*equatoriense*	AP-209	AP-209	No
Violaceae	*Gloeospermum*	*longifolium*	AP-4020	AB354485	No
Violaceae	*Leonia*	*crassa*	AP-3566	AB354494	No
Violaceae	*Leonia*	*glycycarpa*	JQ626288	JQ626572	No
Violaceae	*Rinorea*	*apiculata*	AP-6315	AP-6315	No
Violaceae	*Rinorea*	*lindeniana*	AP-3443	No	No
Violaceae	*Rinorea*	*viridifolia*	AP-3682	AP-3682	Yes*
Vochysiaceae	*Qualea*	*paraensis*	No	No	No

Table S7: Standard effects sizes for mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) randomization after 999 runs. Statistical output for randomization of the D statistic is included. Pval1 is the p value for whether D is significantly different than 1. Pval0 is the p value for whether D is significantly different than 0. Observed, Mean Random, and Mean Brownian are the sums of sister-clade differences. nPermut is the number of permutations performed.

SES-MPD						
Medicinal	mpd.obs	rand.mean	rand.sd	obs.rank	obs.z	obs.p
67	0.205	0.196	0.005	974	1.798	0.974
SES-MNTD						
Medicinal	mntd.obs	rand.mean	rand.sd	obs.rank	obs.z	obs.p
67	0.047	0.036	0.005	989	2.43	0.989
D Randomization						
DEstimate	Pval1	Pval0	Observed	Mean Random	Mean Brownian	nPermut
0.881	0.073	<0.001	85.1	92.4	31.2	1000

APPENDIX

Appendix: Protocols for DNA extraction, PCR amplification, and sequencing that were conducted at the Canadian Center for DNA Barcoding, Biodiversity Institute of Ontario, Canada (see CCDB Protocols DNA Extraction; CCDB Protocols, PCR Amplification; CCDB Protocols, Sequencing).

DNA Extraction

1. Prior to processing, centrifuge plant boxes at 1500 g for 2 min.
2. Add one stainless steel bead to each tube which contains dry tissue and cover with fresh strip caps. Insert boxes, lids removed, into TissueLyser (Qiagen) adapters and shake at 28 Hz for 30 sec, rotate plates and repeat. Centrifuge at 1500 g for 2 min.
3. Add 250-350 µl of 2×CTAB to each tube, cover with fresh strip caps. If working with herbarium material, mix 25 ml of Insect Lysis Buffer + Na2SO3 with 2.5 ml of Proteinase K, 20 mg/ml; add 250 µl of mix to each tube, cover with fresh strip caps.
4. Mix once by gentle inverting of fully covered box. Centrifuge at 1500 g for 1 min. Incubate at 65ºC (56ºC – for herbarium material) for 1.5 hours.
5. Transfer 50 µl of lysate into 96-well Eppendorf plate.
6. Add 100 µl of Plant Binding Buffer (PBB) to each sample. Incubate for 5 min at room temperature.
7. Mix lysate 5-10 times by pipetting, transfer the lysate (about 150 µl) from the wells of microplate into the wells of the glass fiber plate placed on top of a square-well block. Seal the plate with self-adhering foil. Centrifuge at 5000 g for 5 min to bind DNA to the glass fiber membrane.
8. First wash step: Add 180 µl of Protein Wash Buffer (PWB) to each well of glass fiber plate. Seal with a new cover and centrifuge at 5000 for 2 min
9. Second wash step: Add 750 µl of Wash Buffer (WB) to each well of the glass fiber plate. Seal with a new self-adhering foil and centrifuge at 5000 for 5 min.
10. Remove the self-adhering foil. Place glass fiber plate on the lid of a tip box. Incubate at 56 °C for 30 min to evaporate residual ethanol.
11. Position a PALL collar on the collection microplate and place the glass fiber plate on top. Dispense 50 – 60 µl of ddH20 (prewarmed to 56°C) directly onto the membrane in each well and incubate at room temperature for 1 min. Seal plate.
12. Place the assembled plates on a clean square-well block to prevent cracking of the collection plate and centrifuge at 5000 g for 5 min to collect the DNA eluate.

PCR Amplification

Basic recipe for PCR for *rbc*L: PCR reagents per 10 µL reaction

# of reactions	1	100
5X HF Buffer (with MgCl2)	2 µL	200 µL
DMSO	0.3 µL	30 µL
10 mM dNTPs	0.056 µL	5.6 µL
10 µM Primer Forward	0.1 µL	10 µL
10 µM Primer Reverse	0.1 µL	10 µL
ddH2O	6.32 µL	632 µL
Phusion High Fidelity F530 (5U/ µL)	0.125 µL	12.5 µL
Total	9 µL	900 µL
DNA template	1 µL per reaction	

*rbc*L PCR thermocycling program: 98°C for 45 sec; 35 cycles of 98°C for 10 sec, 55°C for 30 sec, 72°C for 40 sec; final extension 72°C for 10 min.

Basic recipe for PCR for *mat*K:PCR reagents per 10 µL reaction

# of reactions	1	100
5X HF Buffer (with MgCl2)	2 µL	200 µL
DMSO	0.3 µL	30 µL
10 mM dNTPs	0.2 µL	20 µL
10 µM Primer Forward	0.5 µL	50 µL
10 µM Primer Reverse	0.5 µL	50 µL
ddH2O	5.375 µL	537.5 µL
Phusion High Fidelity F530 (5U/ µL)	0.125 µL	12.5 µL
Total	9 µL	900 µL
DNA template	1 µL per reaction	

*mat*K PCR thermocycling program: 98°C for 45 sec; 35 cycles of 98°C for 10 sec, 54°C for 30 sec, 72°C for 40 sec; final extension 72°C for 10 min.

Sequencing

*rbc*L: Dilute PCR product adding 15 µL of water in each well. Spin the plate.

*mat*K: Dilute PCR product adding 40 µL of water in each well. Spin the plate.

Sequencing chemical recipe:

# of reactions	1	104
5X Sequencing Buffer	1.875 µL	195 µL
DMSO	0.355 µL	37 µL
10 µM primer	1 µL	104 µL
BigDye	0.250 µL	26 µL
ddH2O	5.520 µL	574 µL
Total	9 µL	936 µL
Diluted DNA	2 µL per reaction	

Sequencing thermocycling programs:

*mat*K Forward (*mat*K-KIM-1R-f)
94°C for 10 sec; 35 cycles of 94°C for 20 sec, 48°C for 20 sec, 60°C for 4 min; hold at 4°C.

*rbc*L (forward and reverse) & *mat*K Reverse (*mat*K-MALP-R)
94°C for 10 sec; 35 cycles of 94°C for 20 sec, 50°C for 20 sec, 60°C for 4 min; hold at 4°C.

Sequencing Cleanup:

1. Add Sephadex powder to the Acroprep 96 filter plate. The standard amount of powder is measured by a column loader.
2. Add 300 µL of dH2O. Let Sephadex hydrate for 2 hours at room temperature, or overnight at 4°C.
3. Assemble the Sephadex plate onto collection plate and spin at 2100 rpm for 5 min.
4. Immediately proceed to loading sequencing product onto the Sephadex columns, to avoid drying. Use fresh plate as a collecting plate.
5. Spin at 2100 rpm for 5 min.
6. Dry the cleanup product at 88°C for 20 min, then cover the plate with rubber lid, and place at the freezer at -20°C until it being placed in ABI capillary sequencer.

I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

12/05/2016
Date

Samantha Jo Worthy
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We approve the thesis of Samantha Jo Worthy as presented here.

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