THE COMPLEX BIOGEOGRAPHIC HISTORY OF A WIDESPREAD TROPICAL TREE SPECIES

Christopher W. Dick[1,2,3,4] and Myriam Heuertz[5,6]

[1]*Department of Ecology and Evolutionary Biology, University of Michigan, 830 North University Ave, Ann Arbor, Michigan 48109*

[2]*University of Michigan Herbarium, 3600 Varsity Drive, Ann Arbor, Michigan 48108*

[3]*Smithsonian Tropical Research Institute, P.O. Box 0843-03092, Balboa Ancón, Republic of Panama*

   [4]*E-mail: cwdick@umich.edu*

[5]*Université Libre de Bruxelles, Faculty of Science, Behavioural and Evolutionary Ecology cp160/12, av. F.D. u*

[6]*Centre of Forest Research CIFOR-INIA, Unit of Forest Genetics, Carretera de la Coruña km 7.5, 28040 Madrid, Spain*

Many tropical forest tree species have broad geographic ranges, and fossil records indicate that population disjunctions in some species were established millions of years ago. Here we relate biogeographic history to patterns of population differentiation, mutational and demographic processes in the widespread rainforest tree *Symphonia globulifera* using ribosomal (ITS) and chloroplast DNA sequences and nuclear microsatellite (nSSR) loci. Fossil records document sweepstakes dispersal origins of Neotropical *S. globulifera* populations from Africa during the Miocene. Despite historical long-distance gene flow, nSSR differentiation across 13 populations from Costa Rica, Panama, Ecuador (east and west of Andes) and French Guiana was pronounced ($F_{ST} = 0.14$, $R_{ST} = 0.39$, $P < 0.001$) and allele-size mutations contributed significantly ($R_{ST} > F_{ST}$) to the divergences between cis- and trans-Andean populations. Both DNA sequence and nSSR data reflect contrasting demographic histories in lower Mesoamerica and Amazonia. Amazon populations show weak phylogeographic structure and deviation from drift–mutation equilibrium indicating recent population expansion. In Mesoamerica, genetic drift was strong and contributed to marked differentiation among populations. The genetic structure of *S. globulifera* contains fingerprints of drift-dispersal processes and phylogeographic footprints of geological uplifts and sweepstakes dispersal.

KEY WORDS: Bayesian clustering, F_{ST}, population structure, phylogeography, *Symphonia globulifera*, tropical rainforests.

Despite a longstanding interest in the origin of tree species diversity in tropical moist forests (Dobzhansky 1950; Ashton 1969; Wallace 1878), research on evolutionary processes in tropical tree species is at an early stage of development. Although recent studies indicate that lowland tropical tree species often have broad geographic distributions (e.g., Pitman et al. 1999), most genetic research on tropical trees has focused on local processes such as mating system and fine-scale spatial genetic structure (reviewed in Ward et al. 2005; Hardy et al. 2006; Dick et al. 2008). High levels of genetic diversity (Hamrick et al. 1992) and evidence of long-distance gene dispersal (Hamrick and Murawski 1990; Stacy et al.

1996) initially suggested that tropical forest trees should display low levels of genetic differentiation (e.g., F_{ST}) among populations (Alvarez-Buylla et al. 1996).

There are both historical and ecological reasons, however, to expect high levels of population differentiation in tropical forest tree species. Tropical forests date to Cretaceous times (Morley 2000; Davis et al. 2005) and have remained relatively stable through climatic fluctuations of the Pleistocene and earlier (Colinvaux et al. 1996; Fine and Ree 2006), thus providing ample time for divergence through isolation-by-distance processes (Wright 1943). Because most tropical moist forest tree species

occur at low population densities (<1 ha^{-1}), they may be more susceptible to genetic drift than the relatively common tree species studied in temperate and boreal forests (Fedorov 1966). Moreover, lowland tropical plants are thought to be physiologically sensitive to mild stress of drought and cold, especially to cool temperatures associated with increasing elevations (Janzen 1967). This combination of factors could promote a latitudinal gradient with stronger phylogeographic structure in tropical than temperate plants, as has been found in a recent survey of extra-tropical terrestrial and marine taxa (Martin and McKay 2004). In support of this idea, several recent studies have documented phylogeographic structure for Neotropical trees sampled around geographic barriers (Aide and Rivera 1998; Cavers et al. 2003; Dick et al. 2003; Novick et al. 2003) and higher F_{ST} in tropical trees than in their temperate zone or boreal forest counterparts (Hamrick et al. 1992; Dick et al. 2008).

In this study, we tested patterns of population differentiation, mutational and demographic processes to gain information on the biogeographic history of the widespread tropical forest tree *Symphonia globulifera* L. (Clusiaceae) at a regional scale within Panama, and at the continental scale in Central and South America. *Symphonia globulifera* is unusual among tropical forest tree species in that it has an extensive fossil record, which reveals a demographic history of sweepstakes dispersal from Africa and the invasion of several Neotropical forest domains. We expand upon a single-locus phylogeographic analysis based on the ribosomal Internal Transcribed Spacer (ITS) (Dick et al. 2003) with data from the chloroplast *trn*H-*psb*A intergenic spacer and expanded ITS sampling. Furthermore, we analyzed five nuclear SSRs in 13 populations of this species from Costa Rica, Panama, Ecuador (east and west of Andes) and French Guiana to infer genetic clusters, to test vicariance patterns based on the depth of SSR divergence and to look for signals of bottlenecks or population expansions testing SSR mutation-drift equilibrium. The results are placed in a context of fossil and regional landscape history, and contrasted with studies of other tropical and temperate zone tree species. The rate of *Symphonia*'s geographic expansion through species-rich Neotropical forests is compared to expectations based on Hubbell's (2001) neutral theory of community ecology and biogeography.

Materials and Methods
STUDY SPECIES

Symphonia globulifera L. f. (Clusiaceae) is a shade-tolerant rainforest tree species that is broadly distributed across the Neotropics and equatorial Africa. It is the only recognized species in its genus found outside of Madagascar, which harbors 16 *Symphonia* species (Abdul-Salim 2002). Nectar-feeding birds, including hummingbirds (Bittrich and Amaral 1996; Gill et al. 1998) pollinate the small scarlet flowers (Fig. 1). The large (4–5 cm) drupes are consumed and dispersed by bats and monkeys (Aldrich and

Figure 1. *Symphonia globulifera* **has distinctive aerial roots, flowers and fruits. Photo credits: Rolando Pérez, CTFS; Tropicos database, Missouri Botanical Garden.**

Hamrick 1998). The species is usually > 90% outcrossed (Degen et al. 2004; da Silva Carneiro et al. 2007), although higher levels of self-fertilization (>10%) have been documented in disturbed habitats (Aldrich and Hamrick 1998). *Symphonia globulifera* occurs in low population densities (<1 adult tree ha^{-1}) across much of its Neotropical range (Center for Tropical Forest Sciences, forest inventory plot data). It can be identified in the field by the combination of opposite simple leaves, bright yellow latex, and aerial roots (Fig. 1). There is some morphological variation across the Neotropical range; although *S. globulifera* are typically large canopy trees, some populations in Costa Rica occur only as understory treelets with an adult reproductive size of less than 10 cm diameter at breast height (dbh). In French Guiana, trees with large leaves and large flowers are sympatric with the common form, and are treated as separate species by local workers (Baraloto et al. 2007). None of this morphological variation has yet been considered sufficient to merit splitting of *S. globulifera* into more than one Neotropical species (Abdul-Salim 2002).

The genus *Symphonia* has morphologically distinctive fossil pollen (fossil taxon *Pachydermites diederexi*) used by the oil industry for stratigraphic dating. Hence, there is an unusually well-sampled fossil record for *S. globulifera* in Africa and the Neotropics. The combined fossil and DNA evidence indicates that *S. globulifera* migrated from Africa to the Neotropics via sweepstakes dispersal (Dick et al. 2003). The earliest *P. diederexi* appears in mid-Eocene deposits (ca. 45 Ma) in Nigeria (Jan-du-Chene et al. 1978). After a 30 million year fossil record in equatorial Africa, *P. diederexi* appeared abruptly in Miocene sediments off the coast of Venezuela (Germeraad et al. 1968) and Brazil (Regali et al. 1974). It is reported from mid-Pliocene sediments in Mexico (Graham 1976, pers. comm.) and Southeast Costa Rica (Graham and Dilcher 1998).

POPULATION SAMPLING

For the nSSR dataset, approximately 30 individuals were sampled in each of eight populations from Panama, two from Ecuador from either side of the Andes, two from Costa Rica, and one from French Guiana (Table 1). The populations in Costa Rica, Panama, and West Ecuador were sampled along transects with sufficient spacing to avoid sampling closely related individuals. In Paracou (French Guiana), Barro Colorado Island (BCI, Panama), and Yasuní (Amazonian Ecuador), individuals were randomly sampled (using a random sorting of tree ID tags) from forest inventory plots. The Paracou site consists of 15 permanent inventory plots of 6.25 ha, and a 25-ha plot (plot 16) of mapped trees ≥10 cm dbh. The BCI and Yasuní forest inventory plots consist of all trees ≥1.0 cm dbh in 50 ha (Losos and Leigh 2004). The Costa Rican populations represent the distinct understory form of *S. globulifera*. The Paracou plot contains some trees with the large-leaf and large-flower morphotype. The samples for the nSSR analysis were taken from the small-leafed form, although individuals of both forms were included in the phylogeographic analysis. The

Table 1. *Symphonia globulifera* study populations. Site abbreviation is indicated in parentheses in Column 2. Column 3 (SSR) is population size for SSR-based analyses. Column 4 (phylo) indicates sample size for DNA sequence-based phylogeographic analyses.

No.	Site	SSR	Phylo	Latitude	Longitude	Alt. (m)
1	Pipeline Road (PR), Panama	26	12	9.13°N	79.68°W	30
2	Santa Rita ridge (SR), Panama	35	11	9.25°N	79.62°W	200
3	Cerro Campana (CC), Panama	33	12	8.67°N	79.92°W	800
4	Fort Sherman (FS), Panama	30	11	9.35°N	79.95°W	8
5	Cerro Jefe (CJ), Panama	30	12	9.25°N	79.38°W	634
6	Veraguas (VER), Panama	30	12	8.53°N	81.08°W	832
7	El Cope, Panama	29	13	8.57°N	80.58°W	220
8	Barro Colorado Island (BCI), Panama	30	9	9.17°N	79.83°W	26
9	Chilamate, Sarapiqui (FCH), Costa Rica	28	4	10.43°N	85.05°W	266
10	La Selva (LS), Costa Rica	25	11	10.42°N	84.00°W	54
11	Paracou (FG), French Guiana	30	11	5.30°N	52.88°W	18
12	Yasuní, Amazonas (YAS), Ecuador	30	8	0.68°S	76.40°W	194
13	Muisne, Esmeraldas (Esm), Ecuador	24	10	0.60°N	80.02°W	1
14	Puerto Viejo, Sarapiqui, Costa Rica	–	2	10.32°N	83.93°W	88
15	Blue Fields, Nicaragua	–	1	12.08°N	83.86°W	22
16	Oaclanza, Honduras	–	5	15.37°N	84.54°W	240
17	Manaus, Brazil	–	5	2.30°S	60.0°W	85
18	Madreselva, Peru	–	3	3.62°S	72.23°W	115
19	Los Amigos, Peru	–	6	12.53°S	70.08°W	248
20	Valle del Sajta, Bolivia	–	2	17.00°S	64.75°W	206

DNA sequence-based phylogeographic analyses included samples from the above-mentioned populations ($N \leq 10$ per site) and supplemental field collections or herbarium accessions from Honduras, Nicaragua, Bolivia, West Indies, and Peru for a total of 20 Neotropical locations (Table 1).

Leaf samples were dried in silica gel in the field or stored in liquid nitrogen prior to DNA extraction, which was performed using DNeasy kits (Qiagen Corporation, Valencia, CA). The DNA aliquot from each tree was assigned an identification number (Lab ID), which is linked to information on geographic location, herbarium label information, or tag numbers for trees sampled in permanent inventory plots (Appendix).

SSR ANALYSIS

Of the 15 published SSR primer pairs developed for *S. globulifera* populations in Costa Rica (Aldrich et al. 1998), French Guiana (Degen et al. 2004), and Brazil (Vinson et al. 2005), only five primer pairs (Table 2) consistently amplified across the geographic range represented by the study populations. The Sg92 primers used in this study (designed by Henri Caron, INRA, Bordeaux, France) amplify the same locus as the Sg18 primers published by Degen (2004): CCCACCAAACACAAATCAT (Sg92f) and GTTGAGGATTGTTTGCCCAG (Sg92r). The Polymerase Chain Reaction (PCR) cocktail contained $1\times$ QIAGEN buffer, 3.75 mM $MgCl_2$, 100 μM of each dNTP, 2.0 μM of each primer, 0.25 units of QIAGEN Taq polymerase, and 1.0 μl of undiluted template DNA. The thermal cycle was 5 min at 94°C; 25 cycles of 45 sec at 94°C, 1.0 min at 55°C, and 30 sec at 72°C; ending with 15 min at 72°C. The SSR primers were labeled with fluorescent dyes (TAMRA, NED, ROX, 6-FAM) and electrophoresed on an ABI 3700 automated DNA sequencing machine with Liz 500 size standard. Genotypes were scored using STRand version 2.2 (M. Locke, E. Baack and R. Toonen, University of California).

SSR DIVERSITY AND INBREEDING

For each locus, we recorded the number of alleles along with their sizes and computed Nei's (1978) gene diversity and Wright's inbreeding coefficient using SPAGeDI version 1.2g (Hardy and Vekemans 2002). Within populations we evaluated the same statistics as well as allelic richness, a measure of the number of alleles standardized for sample size, using Fstat version 2.9.3 (Goudet 1995). Departure from Hardy–Weinberg expectations was tested with exact tests in GENEPOP v. 3.3 (Raymond and Rousset 1995).

BAYESIAN CLUSTER ANALYSIS OF SSR GENOTYPES

The program STRUCTURE version 2.2 (Pritchard et al. 2000) was run 10 times on individual multilocus SSR genotypes for a number of clusters K ranging from 1 to 20, using a burn-in length of 50,000 and a run length of 500,000 iterations. We used the admixture model without prior information on sample population membership and allowed allele frequencies to be correlated among clusters (Falush et al. 2003). The likelihood for the data given each of K clusters was recorded. Because it increased steadily with increasing K without displaying a clearly highest value for any K, the most likely number of clusters was inferred with the ΔK statistic of Evanno et al. (2005). For the best inferred K, the proportion of ancestry Q in each cluster of each sampling location (sample population) was computed as the average proportion of ancestry over individuals and plotted on a map. To further explore the genetic structure, we ran a mixture analysis (Manel et al. 2005) on population samples in BAPS, a software for Bayesian Analysis of Population Structure (Corander et al. 2003), using either a uniform prior over populations or considering their spatial coordinates ("spatial clustering of groups" option) and testing 10 replicates of $1 \leq K \leq 13$ groups.

TESTS OF SSR DIFFERENTIATION, MUTATIONAL, AND DEMOGRAPHIC PROCESSES

After defining the global pattern of population structure with the above methods, differentiation statistics (F_{ST}, R_{ST}) were computed between (pairs of) sampling locations or inferred clusters using SPAGeDI version 1.2.g (Hardy and Vekemans 2002). The null hypothesis of no population differentiation was tested for overall F_{ST} and R_{ST} using 10,000 permutations of individuals

Table 2. Gene diversity of five nuclear SSR loci developed for *S. globulifera*. Column 2 refers to geographic origin of SSR isolates (Costa Rica, French Guiana, Brazil) and reference: (1) Aldrich et al. (1998), (2) Degen et al. (2004), (3) Vinson et al. (2005), (4) this article. *N*, number of genes sampled; *K*, total number of alleles; Size (var), mean allele size and variance (var); H_E, Nei's (1978) gene diversity; F_{IS}, Wright's inbreeding coefficient; $F_{IS} \neq 0$ refers to the number of populations (out of the number of populations genotyped at each locus) showing significant departure from Hardy–Weinberg genotypic proportions.

No.	Locus	Origin	N	*K*	Size (var)	H_E	F_{IS}	$F_{IS} \neq 0$
1	Sg19	CR (1)	724	24	173±44	0.885	−0.002	1 pop/13
2	SgC4	CR (1)	630	24	172±70	0.916	−0.010	1 pop/12
3	Sg92	FG (4)	732	19	179±35	0.892	0.049	4 pops/13
4	Sg03	FG (2)	602	24	318±69	0.822	0.318	6 pops/12
5	Sg06	Br (3)	644	41	198±285	0.921	0.106	5 pops/11

among locations or clusters in SPAGeDI, and for pairwise F_{ST} using exact tests in GENEPOP. The presence of phylogeographic structure, i.e., whether alleles within populations were more related than alleles in the overall sample, was tested by comparing R_{ST} with its value after permuting allele sizes within loci ("permuted R_{ST}") using SPAGeDI (10,000 permutations). A significant one-sided test establishes the alternative hypothesis of $R_{ST} >$ "permuted R_{ST}." This means that allele size mutations contributed to population differentiation and can be interpreted as phylogeographic structure (Hardy and Vekemans 2002). Sequential Bonferroni corrections (Rice 1989) were applied to significance levels of multiple tests.

The demographic history of a population leaves a signature in its allele frequency distributions: a reduction in effective population size (population bottleneck) leads to the preferential loss of rare alleles, whereas new alleles accumulate under population growth. When rare alleles are lost, the frequencies of the remaining alleles are more evenly distributed than under drift–mutation equilibrium, which results in a gene diversity value higher than expected under drift–mutation equilibrium with the same number of alleles. The program BOTTLENECK (Piry et al. 1999) permits testing for such "heterozygosity excess," and for "heterozygosity deficit" in the converse case of population expansion, by comparing gene diversity values H_E to their expected values based on the number of alleles, H_A, obtained from coalescent simulations using an infinite allele model (IAM) or a stepwise mutation model (SMM) adapted to microsatellites. To test for deviations from equilibrium demographic processes in sampling locations and genetic clusters of *S. globulifera*, we computed the $T2$ statistic of Cornuet and Luikart (1996), which represents an average over loci of standardized deviates for heterozygosity, and tested its significance with the Wilcoxon signed ranks test (Piry et al. 1999). We used $T2$ under the SMM model because alleles at our loci were separated by multiples of 2 bp, with the exception of a single allele at locus SG19.

DNA SEQUENCE ANALYSIS

The ITS region (ITS1, ITS2, and 5S ribosomal gene) was amplified using the ITS4 (White et al. 1990) and ITSi primers (Urbatsch et al. 2000). *Trn*H-*psb*A was amplified using primers developed for plant DNA barcoding (Kress et al. 2005). The PCR contained $1\times$ QIAGEN buffer, 3.75 mM MgCl$_2$, 100μM of each dNTP, 2μM of each primer, 0.25 units of Taq polymerase (Qiagen), and 1.0 μl of undiluted template DNA. The thermal cycle for ITS and *trn*H-*psb*A was 94°C for 4.0 min, followed by 30 cycles of 94°C for 45 sec, 55°C for 45 sec and 72°C for 3 min. PCR products were extracted from low melting point agarose using GELASE (Epicentre Biotechnologies, Madison, WI) prior to sequencing. Forward and reverse DNA strands were sequenced using BIG DYE chemistry (Applied Biosystems Incorporated [ABI], Foster City,

CA) on an ABI 3700 capillary sequencer. DNA chromatograms were aligned and edited using SEQUENCHER 4.1 (Gene Codes Corporation, Ann Arbor, MI), MacClade 4.0 (Maddison and Maddison 2000), and Se-Al version 2.0 (Rambaut 1996). Indels and multiple-nucleotide substitutions were coded as single changes. Haplotype networks were created in PAUP* 4.0b10 (Swofford 1998) using neighbor joining and in TCS (Clement et al. 2000) using statistical parsimony. Indices of haplotype diversity (h) and nucleotide (π) diversity were obtained using DNasp 4.0 (Rozas et al. 2003). Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to estimate the within and among population components of nucleotide diversity (Φ_{PT}) using GenAlEx (Peakall and Smouse 2006).

Results

SSR DIVERSITY AND INBREEDING

Multilocus SSR genotypes were obtained for 380 individuals from 13 *S. globulifera* populations, although some loci did not amplify consistently in all populations. Amplification problems occurred for SgC4 in Paracou, Sg03 in Esmeraldas, and Sg06 in both Chilamate and Esmeraldas and those locus/population combinations were coded as missing data. The mean number of alleles per locus was 26.4 (range: 19–41) for a total of 132 alleles (Table 2). Expected heterozygosity (H_E) per locus ranged from 0.822 (Sg03) to 0.921 (Sg06) (mean $H_E = 0.887$). Multilocus H_E ranged from 0.677 (Esmeraldas) to 0.881 (Paracou). The global inbreeding coefficient was significantly positive ($F_{IS} = 0.087$; $R_{IS} = 0.214$) and single population estimates ranged from -0.084 (Chilamate) to 0.253 (Paracou) (Table 3). All of the loci displayed deviation from Hardy–Weinberg expectations in at least one population (Table 2), but deviations were not consistent across loci within populations, which suggests that the homozygote excess was caused by null alleles rather than inbreeding. Exceptions were BCI and Paracou where mating system might have an effect on consanguinity because both had three loci with significantly positive F_{IS}.

BAYESIAN CLUSTER ANALYSIS OF SSR GENOTYPES

Run lengths of 500,000 iterations with the STRUCTURE algorithm were deemed sufficient because run parameters such as the log likelihood of data LnP(D) stabilized within 100,000 iterations for all tested values of the number of clusters K ($1 \leq K \leq 20$). The true number of clusters K in the data was difficult to determine empirically because LnP(D) steadily increased as K increased until a plateau was reached at approximately $K = 11$ (Fig. 2.A). The ΔK statistic of Evanno et al. (2005) however permitted detection of a rate change in LnP(D) corresponding to $K = 3$ (Fig. 2.B). This type of clustering made geographical sense as it essentially separated the Esmeraldas population on the Pacific side of the Andes (mostly Cluster 01, Fig. 3) from the cis-Andean populations

Table 3. SSR diversity values per population. *N*, average number of genes sampled/locus; *A*, mean number of alleles per locus; *AR*, allelic richness or number of alleles expected in a sample of 14 individuals; H_E, expected heterozygosity; F_{IS}, Wright's inbreeding coefficient and probability of departure from Hardy–Weinberg genotypic proportions, *T2*(SMM), *T2* statistic of Cornuet and Luikart (1996) under the stepwise mutation model and probability of heterozygosity excess/deficit; Nc, not computed, ns, not significant, ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$).

Population/cluster	Region	N	A	AR	H_E	F_{IS}	$T2$ (SMM)
Pipeline Road	Panama	24.8	6.4	5.81	0.779	0.044[ns]	0.928[ns/ns]
Santa Rita	Panama	32.6	11.4	7.99	0.771	0.027***	0.257[ns/ns]
Cerro Campana	Panama	32	7.6	5.89	0.737	0.125**	−0.006[ns/ns]
Fort Sherman	Panama	23.8	7.2	6.29	0.790	0.025*	0.910[*/ns]
Cerro Jefe	Panama	28.6	9.4	7.60	0.713	0.050**	1.410[*/ns]
Alto de la Piedra	Panama	28	9.4	7.44	0.722	0.045[ns]	0.543[ns/ns]
El Cope	Panama	28.6	10.2	8.13	0.828	0.100***	1.144[*/ns]
BCI	Panama	30	10.6	8.47	0.831	0.133***	−0.017[ns/ns]
Chilamate	Costa Rica	19.8[1]	8[1]	6.66[1]	0.767[1]	−0.084[ns/1]	−0.217[ns/ns]
La Selva	Costa Rica	23.8	10.2	8.57	0.782	0.128*	1.442[ns/ns]
Paracou	French Guiana	30[1]	14[1]	10.86[2]	0.881[1]	0.253[***/1]	−0.711[ns/ns]
Yasuní	East Ecuador	27.8	10.6	8.38	0.792	0.047*	−0.535[ns/ns]
Esmeraldas	West Ecuador	22.3[3]	4.7[3]	4.34[3]	0.677[3]	0.206[**/3]	0.352[ns/ns]
Cluster 01	Trans-Andes	101.6	11.8	7.58	0.833	nc	1.019[ns/ns]
Cluster 02	Panama	86.2	13	6.91	0.789	nc	0.551[ns/ns]
Cluster 03	Cis-Andes-Costa Rica	92.8	23	10.23	0.893	nc	−1.756[ns/*]

[1]data from loci 1–4;
[2]data from loci, 1, 3–5;
[3]data from loci 1–3.
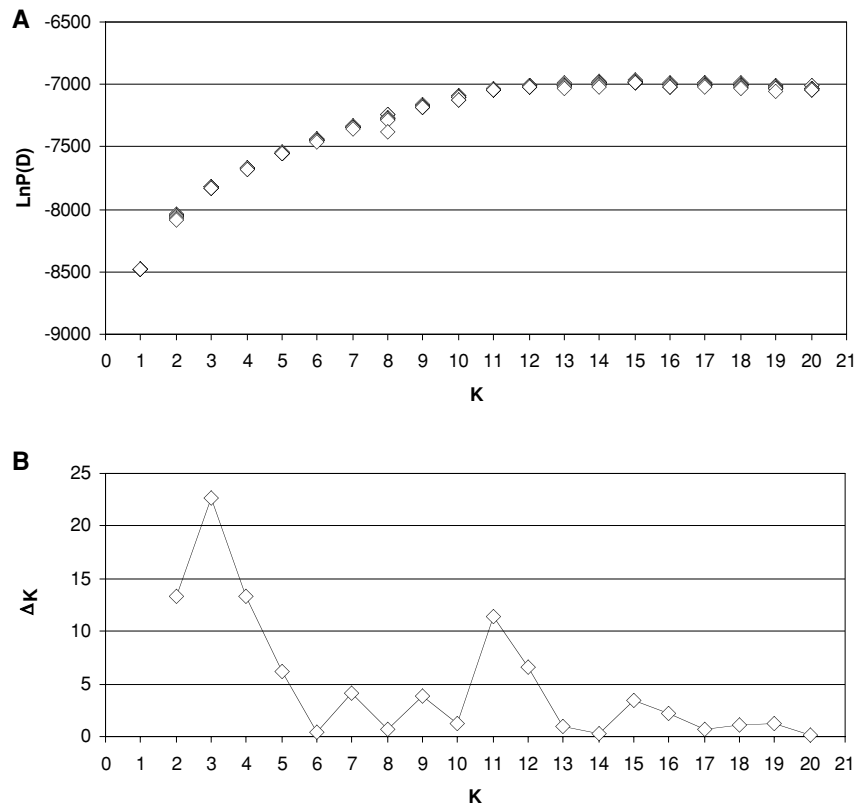
Figure 2. (A) Log-likelihood of the microsatellite data on 13 *S. globulifera* populations given *K* clusters, obtained through 10 runs of the STRUCTURE algorithm (Falush et al. 2003). (B) Corresponding ΔK statistic (Evanno et al. 2005) showing peaks at $K = 3$ and $K = 11$, indicating that those are the best solutions for *K* given the data.
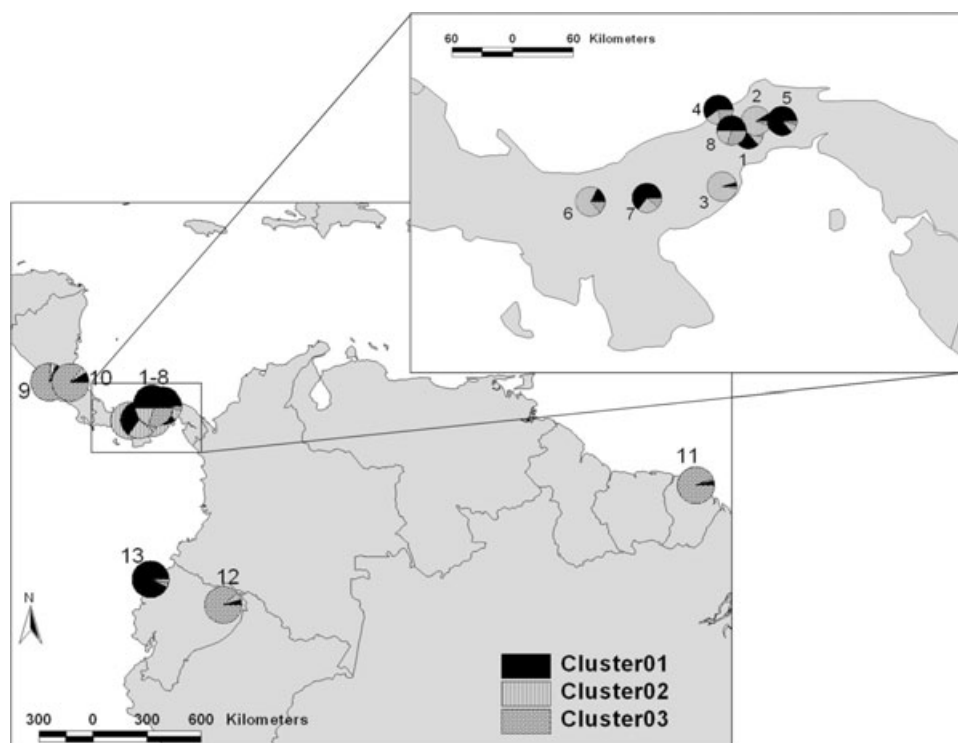
Figure 3. Structure results (Pritchard et al. 2000) for $K = 3$ clusters. The proportion of ancestry in each cluster of each sampling location (sample population) was computed as the average proportion of ancestry over individuals.

(mostly Cluster 03, Fig. 3). Both Costa Rican populations had the highest proportion of ancestry, over 76%, in Cluster 03 as well. In Panama, an additional gene pool, Cluster 02, was detected. It was predominant with over 84% ancestry in two populations (Cerro Campana and Santa Rita ridge), but most Panamanian populations were admixed between Cluster 01 and Cluster 02 with smaller shares of the cis-Andes-Costa Rica Cluster 03. Evanno et al.'s (2005) ΔK detected a further rate change in LnP(D) at $K = 11$ (Fig. 2.B). With $K = 11$ individuals essentially clustered into their population of origin, except that both Costa Rican populations shared one gene pool, and Panamanian populations Fort Sherman and BCI another (results not shown). The strong pattern of among-population structure was confirmed with BAPS, where both spatial and nonspatial mixture analysis resulted in a best partition of sampling locations into 13 clusters, one per population (results not shown).

TESTS OF SSR DIFFERENTIATION, MUTATIONAL, AND DEMOGRAPHIC PROCESSES

On the basis of these results, we computed differentiation statistics directly between sampling locations, which resulted in strong genetic differentiation, with $F_{ST} = 0.138$ ($P \leq 0.001$) and $R_{ST} = 0.391$ ($P \leq 0.001$). When individual multilocus genotypes were attributed to the STRUCTURE cluster ($K = 3$) in which they had $> 70\%$ ancestry, differentiation was slightly weaker with $F_{ST} = $

0.084 ($P \leq 0.001$) and $R_{ST} = 0.352$ ($P \leq 0.001$) among clusters. Phylogeographic structure was detected in both cases ($R_{ST} >$ permuted R_{ST}, $P \leq 0.001$). For STRUCTURE results with $K = 3$, this was due to a significant influence of allele size mutations between the cis-Andes-Costa Rica Cluster 03 and both other clusters (Cluster 03 vs. Cluster 01: $R_{ST} = 0.469$, Cluster 03 vs. Cluster 02: $R_{ST} = 0.442$, $R_{ST} >$ permuted R_{ST} with $P \leq 0.001$ in both cases), but not between the trans-Andes Cluster 01 and the Panamanian Cluster 02 ($R_{ST} = 0.026$). The same phylogeographic pattern is reflected between pairs of populations, where comparisons involving populations from cis-Andes or Costa Rica on the one hand and Panama on the other resulted in significant $R_{ST} >$ permuted R_{ST} (Table 4). All pairs of populations were strongly and significantly differentiated based on F_{ST} ($P < 0.01$ for all pairwise comparisons, Table 4). Strong differentiation was also found at short spatial scales within Panama, with overall $F_{ST} = 0.111$ ($P \leq 0.001$) and $R_{ST} = 0.149$ ($P \leq 0.001$), but no contribution of mutations to differentiation was detected (Table 4).

A total of 34 population-specific alleles were detected, of which 32 were private to populations from the cis-Andes-Costa Rica Cluster 03: La Selva had 19 private alleles, Chilamate 5, Paracou 5, and Yasuni 3. The Panamanian population El Cope had the two remaining private alleles. An accumulation of new mutations can be a sign of a recent population expansion. Results from the BOTTLENECK program (Table 3) support this with a

Table 4. Pairwise measures of differentiation between populations. Above diagonal: F_{ST}, all values are significant at $P<0.01$ after multiple test correction; below diagonal: R_{ST}, for shaded values $R_{ST}>$permuted R_{ST} at $P<0.05$ after multiple test correction, indicating that mutations contributed to population differentiation.

	$R_{ST}\backslash$ F_{ST}	Panama								Costa Rica		French Guiana	East Ecuador	West Ecuador
		PR	SR	CC	FS	CJ	VER	ElCope	BCI	FCH	LS	FG	YAS	Esm
Panama	PR		0.106	0.097	0.108	0.097	0.119	0.092	0.080	0.121	0.127	0.127	0.159	0.126
	SR	0.089		0.086	0.143	0.145	0.112	0.100	0.106	0.179	0.167	0.168	0.151	0.220
	CC	0.221	0.212		0.160	0.149	0.067	0.098	0.117	0.215	0.185	0.154	0.165	0.233
	FS	0.250	0.195	0.197		0.171	0.149	0.089	0.056	0.124	0.110	0.113	0.138	0.197
	CJ	0.125	0.098	0.320	0.374		0.140	0.118	0.138	0.219	0.197	0.159	0.203	0.182
	VER	0.081	0.124	0.061	0.074	0.244		0.108	0.112	0.204	0.168	0.164	0.127	0.193
	ElCope	−0.004	0.117	0.160	0.252	0.116	0.080		0.068	0.135	0.133	0.103	0.121	0.136
	BCI	0.087	0.039	0.199	0.097	0.156	0.063	0.125		0.124	0.093	0.083	0.082	0.189
Costa Rica	FCH	0.345	0.299	0.473	0.192	0.394	0.321	0.383	0.249		0.056	0.120	0.182	0.230
	LS	0.345	0.169	0.490	0.390	0.277	0.397	0.372	0.266	0.069		0.113	0.161	0.279
F. Guiana	FG	0.479	0.380	0.613	0.586	0.449	0.554	0.483	0.495	0.060	0.156		0.117	0.194
E. Ecuador	YAS	0.671	0.476	0.767	0.745	0.606	0.714	0.661	0.649	0.161	0.278	0.171		0.226
W. Ecuador	Esm	0.187	0.439	0.313	0.464	0.377	0.272	0.089	0.422	0.442	0.641	0.147	0.527	

negative average for the $T2$ statistic in cis-Andean and Costa Rican populations ($T2 = -0.623$) and a significant heterozygosity deficit (for individuals with $>70\%$ ancestry) in the cis-Andes-Costa Rica Cluster 03 ($T2 = -1.767$, $P = 0.016$). On the other hand, $T2$ was on average positive in trans-Andean and Panamanian populations ($T2 = 0.613$) and the heterozygosity excess was significant in Fort Sherman, Cerro Jefe, El Cope but also in the Costa Rican La Selva, which may reflect local bottlenecks.

DNA SEQUENCE VARIATION

The aligned ITS region sampled from 102 individuals of *S. globulifera* was 645 base pairs in length (range: 645–753 bp). The ITS dataset contained eight haplotypes and 42 polymorphic sites, 15 of which were parsimony informative. Indels were found in the cis-Andean haplotype (5-bp deletion), coastal Ecuador (1 bp insertion), and Belize-Nicaragua (1 bp insertion). The average number of nucleotide differences (k) among haplotypes was 4.05 bp. Nucleotide diversity (π) was 0.00815. Consistent with previous analyses (Dick et al. 2003) Mesoamerican ITS haplotypes clustered together in the parsimony network and were fairly differentiated from a haplotype found in Dominica and from the single cis-Andean haplotype, which occurred over large distances (> 2500 km) from French Guiana to Peru and Bolivia. There was no ITS haplotype diversity within populations in any Neotropical location, despite examination of relatively large samples of trees from locations such as BCI, Panama ($N = 18$) and Yasuní, Ecuador ($N = 15$). The same haplotype was found across moderate distances within some of the sampling areas shown as circles in Figure 4: Belize [20 km between samples]; Western

Ecuador [20 km]; Manaus, Brazil [60 km]. The 36 additional ITS sequences of this study increased the geographic breadth of the previous analysis to Peru, Honduras, and Nicaragua, but did not reveal new haplotypes in these countries. One novel ITS haplotype was encountered in northeastern Panama (Nusagandi; Fig. 4 haplotype 5; $N = 2$) and is nested within the Mesoamerican ITS clade.

The 574 bp of aligned *trn*H-*psb*A sequences from 153 individuals (Appendix) contained 20 haplotypes (excluding cpSSR variation; Fig. 5). *Trn*H-*psb*A variation consisted of single nucleotide substitutions ($N = 23$); a tri-nucleotide substitution; single nucleotide indels ($N = 5$); and indels of six bp ($N = 1$), seven bp ($N = 1$), 16 bp ($N = 1$), and 22 bp ($N = 1$). The *trn*H-*psb*A locus also contained a poly-T site (cpSSR) with eight length variants at position 355 to 375. Excluding indels, the average nucleotide difference among haplotypes was 6.02 bp and nucleotide diversity (π) was 0.01109. Haplotypes from the Amazonian basin and French Guiana clustered together in the parsimony network, as did haplotypes from Mesoamerica. One Paracou haplotype did, however, cluster with the Mesoamerican group. There were 11 *trn*H-*psb*A haplotypes in the 13 populations sampled for SSR analyses (133 individuals), with a total of 41 polymorphisms. In these 13 populations, 95% of the haplotype diversity was distributed among populations ($\Phi_{PT} = 0.953$, $P < 0.001$). BCI and Paracou each harbored two haplotypes, and in Paracou the two haplotypes were distributed among both large- and small-leaved trees. Only three *trn*H-*psb*A haplotypes were shared among proximal populations ([BCI, Sherman], [La Selva, Chilamate], [BCI, Santa Rita, Pipeline Road]).
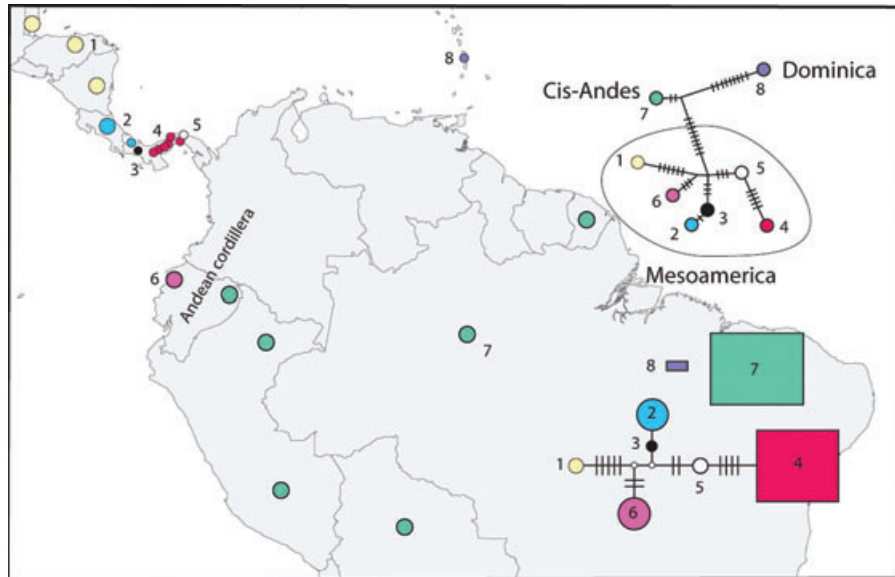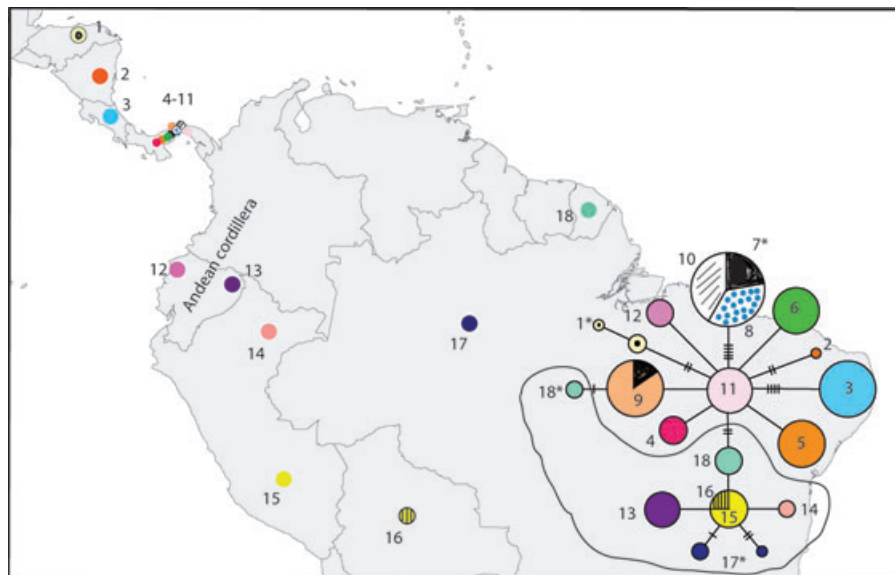
Figure 4. Statistical parsimony network of seven ITS haplotypes, represented by colors and numbers. The upper network was constructed using neighbor joining; the lower network was constructed using statistical parsimony in the program TCS (Clement et al. 2000) in which the size of the circle or square is proportional to the number of sequences for the haplotype. The Dominica and cis-Andean ITS haplotypes are disjunct due to the large number of changes.

Discussion

MUSEUMS AND CRADLES

The geographic differentiation of *S. globulifera* populations contrasts with patterns in temperate zone trees such as the European ash *Fraxinus excelsior* (Heuertz et al. 2004). Using a similar sampling design and five SSR loci (275 total alleles), the authors found no evidence of allele size variance and only very weak differentiation ($F_{ST} = 0.023$) among populations spanning 2000 km from the British Isles over central Europe to the Baltic States. In addition to life-history differences such as wind-mediated pollen and seed dispersal of *Fraxinus*, there is a major historical difference between the *Symphonia* and *Fraxinus* comparison: the

Figure 5. Statistical parsimony network of 18 *Trn*H-*psb*A haplotypes sampled from 18 Neotropical populations of *Symphonia globulifera*. The size of the circle is proportional to number of sequences for the haplotype. Multiple-nucleotide indels were considered as single changes and poly-T length polymorphisms were excluded. Cis-Andean haplotypes (Amazon basin + Guianas) are encircled. Colors and numbers represent geographic locations. The BCI (#8) and Paracou (#18) populations each contained two haplotypes.

common ash has occupied most of its present distribution only in the past 10,000 years, whereas *S. globulifera* has occupied parts of its Neotropical distribution for over 10 million years.

The high levels of genetic differentiation across the range of *S. globulifera* are due in some part to the age of the species, which permitted it to be subject to both successful sweepstakes dispersal and to subdivision by emerging geographic barriers. *Symphonia globulifera* falls into one end of a spectrum of tropical tree ages and geographic distributions. Based on a fossil-calibrated molecular phylogeny of the species-rich Neotropical tree genus *Inga* (Richardson et al. 2001), which comprises nearly 300 lowland forest species, Lavin (2006) estimated the age of the *Inga* crown clade at less than 2 million years. Many *Inga* species are widespread in the Neotropics, and their ancestral populations should be much younger than those of codistributed *Symphonia*. Thus, compared to nearly 300 species of *Inga*, *S. globulifera* is a living fossil. *Inga*, however, may be the youthful outlier in the spectrum of ages of tropical tree lineages. Like *Symphonia*, which is monotypic in the Neotropics, most Neotropical tree genera contain relatively few species (Bermingham and Dick 2001). The cross-Andean disjunction of many widespread rainforest plant species also suggests antiquity. In Ecuador, approximately 1432 lowland plant species (∼30% of Ecuador's flora) have population disjunctions east and west of the Andes (Jørgensen and León-Yánez 1999) and experience no contemporary gene flow. Raven (1999) has suggested that these disjunctions derive from vicariance by the Andean uplift several million years ago. Although *S. globulifera* populations are old enough to fit this model, recent groups such as *Inga* and some well-dispersed species, such as the kapok tree *Ceiba pentandra* (Dick et al. 2007), appear to have recently dispersed around the Andes. Further analyses focused on genetic divergences of lowland populations around the Andes may help determine if most widespread species are good dispersers with weak phylogeographic population structure, like *Inga* and *C. pentandra*, or old and dispersal limited within terrestrial rainforest biomes, like *S. globulifera*.

LONG-DISTANCE DISPERSAL

Although its seeds are not adapted for water dispersal, long-distance marine dispersal has played a primary role in the migration and establishment of *S. globulifera* in the Neotropics. *Symphonia globulifera* fossils in Central and South America pre-date the Pliocene emergence of the Panama landbridge, and the West Indian island of Dominique was never connected to mainland. The colonization of these three regions may derive from a single dispersal event from Africa followed by oceanic dispersals within the Neotropics, or by multiple oceanic dispersal events originating from Africa. The nSSR, ITS, and *trn*H-*psb*A data show association between trans-Andean Ecuador and Mesoamerica indicative of marine or terrestrial connections between the continents.

An unanticipated result of the nSSR Bayesian cluster analysis was the finding that Costa Rican populations share more recent ancestry with cis-Andean (Fig. 2) than with trans-Andean populations of west Ecuador and Panama. The relationship is corroborated by low pairwise F_{ST}-based estimates between Costa Rican and cis-Andean populations suggesting a marine connection between Costa Rica and South America that post-dates the establishment of *S. globulifera* elsewhere in Mesoamerica. Alternatively, a cis-Andes-Mesoamerican gene pool homogenized through marine or aerial gene flow could have become disjoint when a differentiated trans-Andes gene pool immigrated into Mesoamerica after closure of the Panama isthmus. The coancestry of Costa Rica and cis-Andean populations is not supported by ITS or *trn*H-*psb*A data, however, which indicate genetic divergence of the Costa Rican populations but no link to South American populations. Events of chloroplast capture and losses of ITS haplotypes through concerted evolution (Hughes et al. 2005) could account for discrepancies between the nSSR and sequence datasets. Another scenario is that alleles of equal length in Costa Rica and Amazonia attained similar frequencies by chance. If populations in either region established as small founder populations following oceanic dispersal, the latter hypothesis may in fact be most realistic.

POPULATION EXPANSION AND OTHER DEMOGRAPHIC PROCESSES

Following its abrupt fossil appearance in the Neotropics during the early to mid Miocene, *S. globulifera* invaded species-rich rainforests in all the major forest domains of Neotropics, including Mesoamerica, Chocó, Amazon basin, Guiana shield, and the Atlantic forests of Brazil; *S. globulifera* thus coexists with several thousand tree species across its Neotropical range. *Symphonia globulifera* expanded through Neotropical forests with great speed in light of expectations based on Hubbell's (2001) neutral theory, which was formulated in part to explain the generation and maintenance of tropical forest tree diversity. Under the neutral theory, propagules of coexisting tree species arrive at open germination sites with probabilities equal to their relative abundances in the local community. The appearance of a new species, following transoceanic dispersal, is equivalent to Hubbell's "point mutation mode" of speciation, in which a new species enters the metacommunity as a single individual. As pointed out by Leigh et al. (2004), the rate of spread of the new species under Hubbell's model is approximately equal to the rate of increase of a neutral allele in an ideal population, taking approximately N generations to reach a size of N individuals (Fisher 1930; Ewens 1972). Using conservative estimates of the generation time and population density of *S. globulifera* in Neotropical forests, Leigh (2007) calculated a probability of approximately 2×10^{-9} for the expansion of *S. globulifera* across Neotropical forests within 20 million

years by stochastic processes. Hence, although *S. globulifera* is a relatively old species, it required some competitive advantage to become established across vast expanses of species-rich forests.

Our genetic data support a population expansion event in modern *S. globulifera*, at least in the Amazon basin and probably beyond. A heterozygosity deficit and abundance of population-specific alleles in the cis-Andes-Costa Rica Cluster 03 (and in the cis-Andean populations analyzed jointly, results not shown) suggest that population expansion affected this gene pool. The absence of ITS variation across the Amazon basin documented by Dick et al. (2003) and extended to further sampling locations in northern and southern Peru, as well as lesser divergence in cis-Andean *trn*H-*psb*A haplotypes (Fig. 5) and star-shaped phylogenetic relationships between them (e.g., Bailey et al. 1996), were also in agreement with a hypothesis of recent population expansion.

Although the spread of *S. globulifera* left a trace of population expansion in its genomes, our data indicate that genetic drift can be strong at the local scale in this species. A significant heterozygosity excess consistent with bottlenecks was detected in several populations, mostly from Panama (Table 3), which presumably led to losses of alleles. Allelic richness is on average lower in populations belonging to the trans-Andes and Central American Clusters 01 and 02 than in the cis-Andes Costa Rica Cluster 03 affected by expansion (Table 3), although comparative tests were nonsignificant because of low power. Also, pronounced nSSR and cpDNA differentiation with limited haplotype sharing between proximal populations in Panama provides evidence of very restricted gene flow. Historical climatic variations in a particular topographic setting might be at the origin of these genetic patterns (see below).

CIS- AND TRANS-ANDEAN CONTRASTS

Both ITS and *trn*H-*psb*A support a Mesoamerican (including Western Ecuador) and a cis-Andean clade and the deepest nSSR divergence was found between populations separated by the Andes. This pattern of ancient divergence is consistent with *Symphonia* fossil records of a broad South American and Mesoamerican distribution prior to the uplift of the northern Andes, which reached altitudes above ca. 1800 m in the late Miocene (about 10 Ma, Gregory-Wodzicki 2000), and the Pliocene formation of the Panama land bridge (Coates and Obando 1996). There are presently no continuous habitat connections between lowland rainforests over or around the northern tips of the Andes. Although DNA sequence and nSSR divergence is consistent with Andean vicariance, the uplift of the cordilleras may alternatively be a barrier to secondary contact of populations that had differentiated in Central and South America before the two continents were connected (Dick et al. 2003). The Talamanca cordilleras of Central America are smaller in stature than the northern An-

des and punctuated by lowland passes, but these mountains also delimit high F_{ST} and/or reciprocal monophyly between Atlantic and Pacific slope populations for several tropical tree species studied (Chase et al. 1995; Cavers et al. 2003, 2005; Novick et al. 2003).

An unusual result is the regional asymmetry in phylogeographic structure between cis- and trans-Andean populations of *S. globulifera*, with high haplotype diversity, strong phylogeographic structure, and high F_{ST} in Mesoamerica, and in Amazonia an absence of ITS variation and relatively low F_{ST} between populations separated by over 2500 km. The signature of strong genetic drift in populations from Mesoamerica, including divergence among populations within Panama, may be partly explained by topographic heterogeneity that is not found in lowland Amazonia. The spine of mountains that run through peninsular Mesoamerica create rainfall gradients that define habitats available to rainforest trees (Pyke et al. 2001; Engelbrecht et al. 2007) and thus place constraints on patterns of gene flow (McRae and Beier 2007). In the watershed of the Panama Canal, for example, annual rainfall ranges from 1600 mm yr^{-1} on the Pacific coast to 4000 mm yr^{-1} along the Caribbean coast less than 50 km to the north. The topographic variation may have further constricted gene flow during the last glacial maximum (LGM), when average rainfall was reduced by ~30% in Panama (Piperno and Jones 2003). Forest cover persisted in sites that presently receive ≥3000 mm rainfall per year (Bush and Colinvaux 1990) whereas Pacific slope sites that presently receive ~1800 mm rainfall per year were dominated by grass pollen (Bush et al. 1992). *Symphonia globulifera* is uncommon on the dryer Pacific coast of Mesoamerica, but it is common in the upper elevation forests that were probably isolated during the LGM (Whitmore and Prance 1987; Gentry 1992). This historical contraction of Mesoamerican rainforests, which has not been demonstrated in core Amazon basin, could have produced population bottlenecks resulting in the observed levels of genetic differentiation.

SUMMARY

Some tropical tree species have had cross-continental or intercontinental geographic ranges dating long before Pleistocene climate changes. The relative stability of the tropical forest biome suggests that sweepstakes dispersal, vicariance histories, and strong phylogeographic structure may be found more frequently in tropical species than in their temperate zone counterparts. Tertiary geological history was reflected in the genetic structure of *S. globulifera*, and our analysis indicates that regional populations have been genetically isolated through the Pleistocene and earlier. We expect that studies of other widespread species, especially those with cross-Andean disjunctions, will reveal strong phylogeographic structure and distinct regional patterns of population differentiation. In particular, the study of *S. globulifera* suggests

that tropical forest tree populations will be more highly differentiated in Mesoamerica than across similar spatial scales in the Amazon basin.

ACKNOWLEDGMENTS

LITERATURE CITED

Abdul-Salim, K. 2002. Systematics and biology of *Symphonia* L. f. (Clusiaceae). Department of Organismic and Evolutionary Biology. Harvard Univ., Cambridge.

Aide, T. M., and E. Rivera. 1998. Geographic patterns of genetic diversity in *Poulsenia armata* (Moraceae): implications for the theory of Pleistocene refugia and the importance of riparian forest. J. Biogeogr. 25:695–705.

Aldrich, P. R., and J. L. Hamrick. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science 281:103–105.

Aldrich, P. R., J. L. Hamrick, P. Chavarriaga, and G. Kochert. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. Mol. Ecol. 7:933–944.

Alvarez-Buylla, E. R., R. Garcia-Barrios, C. Lara-Moreno, and M. Martinez-Ramos. 1996. Demographic and genetic models in conservation biology: applications and perspectives for tropical rain forest tree species. Ann. Rev. Ecol. Syst. 27:387–421.

Ashton, P. S. 1969. Speciation among tropical forest trees: some deductions in the light of recent evidence. Biol. J. Linn. Soc. 1:155–196.

Bailey, C. D., M. B. Richards, V. A. Macaulay, I. B. Colson, D. G. Bradley, R. E. M. Hedges, and B. C. Sykes. 1996. Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. Proceedings: Biol. Sci. 263:1467–1473.

Baraloto, C., F. Morneau, D. Bonal, L. Blanc, and B. Ferry. 2007. Seasonal water stress tolerance and habitat associations within four neotropical tree genera. Ecology 88:478–489.

Bermingham, E., and C. Dick. 2001. The *Inga:* newcomer or museum antiquity. Science 293:2214–2216.

Bittrich, V., and M. C. E. Amaral. 1996. Pollination biology of *Symphonia globulifera* (Clusiaceae). Plant Syst. Evol. 200:101–110.

Bush, M. B., and P. A. Colinvaux. 1990. A pollen record of a complete glacial cycle from lowland Panama. J. Veg. Sci. 1:105–118.

Bush, M. B., D. R. Piperno, P. A. Colinvaux, P. E. Deoliveira, L. A. Krissek, M. C. Miller, and W. E. Rowe. 1992. A 14,300-yr paleoecological profile of a lowland tropical lake in Panama. Ecol. Monogr. 62:251–275.

Cavers, S., C. Navarro, and A. J. Lowe. 2003. Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica. Mol. Ecol. 12:1451–1460.

Cavers, S., C. Navarro, P. Hopkins, R. A. Ennos, and A. J. Lowe. 2005. Regional and population-scale influences on genetic diversity partitioning within Costa Rican populations of the pioneer tree *Vochysia ferruginea* Mart. Silvae Genet. 54:258–264.

Chase, M. R., D. H. Boshier, and K. S. Bawa. 1995. Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree.1. Genetic variation in natural populations. Am. J. Bot. 82:468–475.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1659.

Coates, A. G., and J. A. Obando. 1996. The geologic evolution of the Central American isthmus. Pp. 21–56 *in* J. B. C. Jackson, A. F. Budd, and A. G. Coates, eds. Evolution and environment in tropical America. Univ. of Chicago Press, Chicago.

Colinvaux, P. A., P. E. de Oliveira, J. E. Moreno, M. C. Miller, and M. B. Bush. 1996. A long pollen record from lowland Amazonia: forest and cooling in glacial times. Science 274:85–88.

Corander, J. M., P. Waldmann, and M. J. Sillanpää. 2003. Bayesian analysis of genetic differentiation between populations. Genetics 163:367–374.

Cornuet, J. M., and G. Liuikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

da Silva Carneiro, F., A. M. Sebbenn, M. Kanashiro, and B. Degen. 2007. Low interannual variation of mating system and gene flow of *Symphonia globulifera* in the Brazilian Amazon. Biotropica 39:628–636.

Davis, C. C., C. O. Webb, K. J. Wurdack, C. A. Jaramillo, and M. J. Donoghue. 2005. Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests. Am. Nat. 165:E36–E65.

Degen, B., E. Bandou, and H. Caron. 2004. Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. Heredity 93:585–591.

Dick, C. W., K. Abdul-Salim, and E. Bermingham. 2003. Molecular systematics reveals cryptic Tertiary diversification of a widespread tropical rainforest tree. Am. Nat. 162:691–703.

Dick, C. W., E. Bermingham, M. R. Lemes, and R. Gribel. 2007. Extreme long distance dispersal of the lowland rainforest tree *Ceiba pentandra* L. (Malvaceae) in Africa and the Neotropics. Mol. Ecol. 16:3039–3049.

Dick, C. W., O. J. Hardy, F. A. Jones, and R. J. Petit. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Trop. Plant Biol. 1:20–33.

Dobzhansky, T. 1950. Evolution in the tropics. Am. Sci. 38:208–221.

Engelbrecht, B. M. J., L. S. Comita, R. Condit, T. A. Kursar, M. T. Tyree, B. L. Turner, and S. P. Hubbell. 2007. Drought sensitivity shapes species distribution patterns in tropical forests. Nature 447:80–83.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611–2620.

Ewens, W. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3:87–112.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial restriction sites. Genetics 131:479–491.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Fedorov, A. A. 1966. The structure of the tropical rain forest and speciation in the humid tropics. J. Ecol. 54:1–11.

Fine, P. V. A., and R. H. Ree. 2006. Evidence for a time-integrated species-area effect on the latitudinal gradient in tree diversity. Am. Nat. 168:796–804.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Gentry, A. H. 1992. Tropical forest biodiversity—distribution patterns and their conservation significance. Oikos 63:19–28.

Germeraad, J. H., C. A. Hopping, and J. Muller. 1968. Palynology of tertiary sediments from tropical areas. Rev. Palaeobot. Palynol. 6:189–348.

Gill, G. E., R. T. Fowler, and S. A. Mori. 1998. Pollination biology of *Symphonia globulifera* (Clusiaceae) in central French Guiana. Biotropica 30:139–144.

Goudet, J. 1995. Fstat version 1.2: a program to calculation F statistics. J. Hered. 86:485–486.

Graham, A. 1976. Studies in neotropical paleobotany. II. The Miocene communities of Veracruz, Mexico. Ann. Mo. Bot. Gard. 63:787–842.

Graham, A., and D. L. Dilcher. 1998. Studies in Neotropical paleobotany. XII. A palynoflora from the Pliocene Rio Banano Formation of Costa Rica and the Neogene vegetation of Mesoamerica. Am. J. Bot. 85:1426–1438.

Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: a review. Geo. Soc. Am. Bull. 112:1091–1105.

Hamrick, J. L., and D. A. Murawski. 1990. The breeding structure of tropical tree populations. Plant Species Biol. 5:157–165.

Hamrick, J. L., M. J. W. Godt, and S. L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6:95–124.

Hardy, O. J., and X. Vekemans. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2:618–620.

Hardy, O. J., L. Maggia, E. Bandou, P. Breyne, H. Caron, M. H. Chevallier, A. Doligez, C. Dutech, A. Kremer, C. Latouche-Halle, et al. 2006. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Mol. Ecol. 15:559–571.

Heuertz, M., J.-F. Hausman, O. J. Hardy, G. G. Vendramin, N. Frascaria-Lacoste, and X. Vekemans. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). Evolution 58:976–988.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton Univ. Press, Princeton, NJ.

Hughes, C. E., R. J. Eastwood, and C. D. Bailey. 2005. From famine to feast? Selecting nuclear DNA sequence loci for plant species-level phylogeny reconstruction. Philos. Trans. R. Soc. Lond. B 361:211–225.

Jan-du-Chene, R. E., M. S. Onyike, and M. A. Sowunmi. 1978. Some new Eocene pollen of the Ogwashi-Asaba Formation, southeastern Nigeria. Revista Española de Micropaleontologia 10:285–322.

Janzen, D. H. 1967. Why mountain passes are higher in the tropics. Am. Nat. 101:233–249.

Jørgensen, P. M., and S. León-Yánez. 1999. Catalogue of the vascular plants of Ecuador. Missouri Botanical Garden, St. Louis.

Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weigt, and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. USA 102:8369–8374.

Lavin, M. 2006. Floristic and geographic stability of discontinuous seasonally dry tropical forests explains patterns of plant phylogeny and endemism. Pp. 433–448 *in* R. T. Pennington, J. A. Ratter, and G. P. Lewis, eds. Neotropical savannas and seasonally dry forests: plant biodiversity, biogeographic patterns and conservation. CRC Press, Boca Raton, Florida.

Leigh, E. G. J. 2007. Neutral theory: a historical perspective. J. Evol. Biol. 20:2075–2091.

Leigh, E. G., P. Davidar, C. W. Dick, J.-P. Puyravaud, J. Terborgh, H. Ter Steege, and S. J. Wright. 2004. Why do some tropical forests have so many kinds of trees? Biotropica 36:447–473.

Losos, E. C., and E. G. J. Leigh, eds. 2004. Tropical forest diversity and dynamism. Univ. of Chicago Press, Chicago.

Maddison, D. R., and W. P. Maddison. 2000. MacClade 4: analysis of phylogeny and character evolution. Version 4.0. Sinauer Associates, Sunderland, MA.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions techniques with appropriate. Trends Ecol. Evol. 20:136–142.

Martin, P. R., and J. K. McKay. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58:938–945.

McRae, B. H., and P. Beier. 2007. Circuit theory predicts gene flow in plant and animal populations. Proc. Natl. Acad. Sci. USA 104:19885–19890.

Morley, R. J. 2000. Origin and evolution of tropical rain forests. John Wiley & Sons Ltd, West Sussex.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Novick, R. R., C. Dick, M. R. Lemes, C. Navarro, A. Caccone, and E. Bermingham. 2003. Genetic structure of Mesoamerican populations of Big-leaf mahogany (*Swietenia macrophylla*) inferred from microsatellite analysis. Mol. Ecol. 12:2885–2893.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295.

Piperno, D. R., and J. G. Jones. 2003. Paleoecological and archaeological implications of a Late Pleistocene/Early Holocene record of vegetation and climate from the Pacific coastal plain of Panama. Q. Res. 59:79–87.

Piry, S. G., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting reductions in the effective population size using allele frequency data. J. Hered. 86:502–503.

Pitman, N. C. A., J. Terborgh, M. R. Silman, and P. Nuez. 1999. Tree species distributions in an upper Amazonian forest. Ecology 80:2651–2661.

Pritchard, J. K., J. C. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pyke, C. R., R. Condit, S. Aguilar, and S. Lao. 2001. Floristic composition across a climatic gradient in a neotropical lowland forest. J. Veg. Sci. 12:553–556.

Rambaut, A. 1996. Se-Al; Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk.

Raven, P. H. 1999. Foreward. Pp. vi–viii *in* P. M. Jørgensen, and S. León-Yánez, eds. Catalogue of the vascular plants of Ecuador. Missouri Botanical Garden, Saint Louis, USA.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Regali, M. S. P., N. Uesugui, and A. S. Santos. 1974. Palynology of the Mesozoic-Cenozoic sediments of Brazil. Boletim Técnico da Petrobras (Rio de Janeiro) 17:177–301.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Richardson, J. E., R. T. Pennington, T. D. Pennington, and P. M. Hollingsworth. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293:2242–2245.

Rozas, J., J. C. Sanchez-Delbarrio, Z. Messequer, and R. Rozas. 2003. DNASP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

Stacy, E. A., J. L. Hamrick, J. D. Nason, S. P. Hubbell, R. B. Foster, and R. Condit. 1996. Pollen dispersal in low-density populations of three neotropical tree species. Am. Nat. 148:275–298.

Swofford, D. S. 1998. Phylogenetic analysis using parsimony, version 4.04b8. Sinauer, Sunderland, MA.

Urbatsch, L. E., B. G. Baldwin, and M. J. Donoghue. 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based in nuclear

rDNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction site data. Syst. Bot. 25:539–565.

Vinson, C. C., A. C. Amaral, I. Sampaio, and A. Y. Ciampi. 2005. Characterization and isolation of DNA microsatellite primers for the tropical tree, *Symphonia globulifera* Linn. f. Mol. Ecol. Notes 5:202–204.

Wallace, A. R. 1878. Tropical nature and other essays. Macmillan, New York and London.

Ward, M., C. W. Dick, R. Gribel, and A. J. Lowe. 2005. To self, or not to self... A review of outcrossing and pollen-mediated gene flow in neotropical trees. Heredity 95:246–254.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. Innis, D. Gelfand, J. Sninsky, and T. White, eds. PCR protocols: a guide to methods and application. Academic Press, San Diego.

Whitmore, T. C., and G. Prance, eds. 1987. Biogeography and Quaternary History in Tropical America. Oxford Science Publications, Clarendon Press, Oxford, UK.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Associate Editor: W. O. McMillan

Appendix. Voucher information for sequenced individuals of *Symphonia globulifera*. The laboratory ID refers to the voucher number for the leaf tissue and DNA maintained at the University of Michigan. GenBank accession numbers are provided for all *trn*H-*psb*A sequences, and for previously unpublished ITS sequences. GenBank accessions for published ITS sequences are available in Dick et al. (2003).

Population	Lab ID	*trn*H-*psb*A	ITS	Population	Lab ID	*trn*H-*psb*A	ITS	Population	Lab ID	*trn*H-*psb*A	ITS
Campana	1385	EU841731	EU841877		1438	EU841812		BCI	253	EU841724	
	1386	EU841732	EU841878		1439	EU841813			497	EU841725	
	1387	EU841733			1440	EU841814			508	EU841726	
	1388	EU841734			1441	EU841815			560	EU841727	
	1389	EU841735		Chilimate	899	EU841755			570	EU841728	
	1390	EU841736			897	EU841756			1470	EU841720	
	1391	EU841737		Pipeline	1241	EU841832			1471	EU841721	
	1392	EU841738			1242	EU841833	EU841901		1472	EU841722	
	1393	EU841739			1243	EU841834	EU841902		1473	EU841723	
	1394	EU841740			1244	EU841835		Bolivia	940	EU841729	
	1395	EU841741			1245	EU841836			941	EU841730	
	1396	EU841742			1246	EU841837		Nicaragua	1474	EU841821	EU841890
Paracou	1397	EU841766			1247	EU841838		Peru	1154	EU841822	
	1398	EU841767			1248	EU841839			1155	EU841823	
	1399	EU841768			1249	EU841840			1156	EU841824	
	1400	EU841769			1250	EU841841			1157	EU841825	
	1401	EU841770			1251	EU841842			1158	EU841826	
	1402	EU841771			1252	EU841843			1159	EU841827	
	1403	EU841772		Santa Rita	1442	EU841844	EU841903		1162	EU841828	EU841894
	1404	EU841773			1443	EU841845	EU841904		1163	EU841829	EU841895
	1405	EU841774			1444	EU841846			1164	EU841830	EU841896
	406	EU841775			1445	EU841847			1165	EU841831	EU841897
	407	EU841776			1446	EU841848			1166		EU841898
C. Jefe	1406	EU841792	EU841886		1447	EU841849			1167		EU841899
	1407	EU841793	EU841887		1448	EU841850			1168		EU841900
	1408	EU841794			1449	EU841851		Yasuní	257	EU841869	
	1409	EU841795			1450	EU841852			263	EU841870	
	1410	EU841796			1451	EU841853			647	EU841871	
	1411	EU841797			1452	EU841854			651	EU841872	
	1412	EU841798		Sherman	1453	EU841777	EU841905		652	EU841873	
	1413	EU841799			1454	EU841778	EU841906		653	EU841874	
	1414	EU841800			1455	EU841779			654	EU841875	
	1415	EU841801			1456	EU841780			655	EU841876	
	1416	EU841802			1457	EU841781			1492	EU841867	
	1417	EU841803			1458	EU841782			1493	EU841868	

Appendix. Continued.

Population	Lab ID	trnH-psbA	ITS	Population	Lab ID	trnH-psbA	ITS	Population	Lab ID	trnH-psbA	ITS
El Cope	1418	EU841743	EU841879		1459	EU841783		Veraguas	1475	EU841855	EU841907
	1419	EU841744	EU841880		1460	EU841784			1476	EU841856	EU841908
	1420	EU841745			1461	EU841785			1477	EU841857	
	1421	EU841746			1462	EU841786			1478	EU841858	
	1422	EU841747			1463	EU841787			1479	EU841859	
	1423	EU841748		Esmeraldas	1464	EU841757			1480	EU841860	
	1424	EU841749			1465	EU841758			1481	EU841861	
	1425	EU841750			1466	EU841759			1482	EU841862	
	1426	EU841751			1477	EU841760			1483	EU841863	
	1427	EU841752			1478	EU841761			1484	EU841864	
	1428	EU841753			1479	EU841762			1485	EU841865	
	1429	EU841754			260	EU841763			1486	EU841866	
La Selva	1430	EU841804	EU841888		261	EU841764		Honduras	1487	EU841788	EU841881
	1431	EU841805	EU841889		657	EU841765			1488	EU841789	EU841882
	1432	EU841806			658	EU841757			1489	EU841790	EU841883
	1433	EU841807		Manaus	259	EU841816			1490		EU841884
	1434	EU841808			640	EU841817			1491	EU841791	EU841885
	1435	EU841809			641	EU841818		Nusagandi	1090		EU841891
	1436	EU841810			642	EU841819			1494		EU841892
	1437	EU841811			643	EU841820			1495		EU841893