



Diversity and floristic enrichment with montane cloud forest species, in an **urban forest** in Chiapas, Mexico

Diversidad y enriquecimiento florístico con especies nativas de bosque mesófilo de montaña, en un bosque urbano de Chiapas, México

Bárbara Cruz-Salazar¹*, Lorena Ruiz-Montoya², María Teresa Pérez-Gómez³,
Maricela García-Bautista³, & Neptalí Ramírez-Marcial²

¹ Consejo Nacional de Ciencia y Tecnología-Universidad Autónoma de Tlaxcala. Centro Tlaxcala de Biología de la Conducta, Tlaxcala, Tlaxcala, México.

² El Colegio de la Frontera Sur. Departamento de Conservación de la Biodiversidad. San Cristóbal de Las Casas, Chiapas, México.

³ El Colegio de la Frontera Sur. Laboratorio de Genética. San Cristóbal de Las Casas, Chiapas, México.

* Corresponding author. barbaracruzsalazar@gmail.com

ABSTRACT

The mountain mesophilic forests (MCF) occupy only between 0.5% and 1% of the Mexican territory and are seriously threatened due to the high rate of deforestation. To contribute to the conservation of tree species of MCF, the present study carried out the objectives of (1) determining the diversity of trees in the urban forest El Cerrito de San Cristóbal, Chiapas, Mexico, (2) floristically enriching the urban forest El Cerrito de San Cristóbal with the plantation of native species of MCF of Chiapas, and (3) describe the genetic diversity introduced through floristic enrichment. The structure and composition of the tree community was recorded through 1000 m² circular sampling plots, while herbaceous species were recorded in walks along tracks. Fourteen MCF native species were first planted in El Cerrito; then, the introduced genetic diversity was determined using chloroplast DNA PCR-RFLPs (tRNL). A composition dominated by species previously introduced 40 years ago (*Cupressus lusitanica*, *Eucalyptus camaldulensis*, and *Pinus pseudostrobus*) was detected. No recruitment of seedlings and juveniles of canopy trees was observed, and some naturally established individuals of native species were spotted. The genetic diversity introduced was low by species, but for the whole forest it was moderate. The low genetic diversity by species is likely associated with the use of a conservative marker. Urban forests constitute an approach for the conservation of native tree species and their genetic diversity, while promoting the regeneration and diversification of urban forest. This study contributes to the conservation and genetic knowledge of tree species characteristic of montane forests in Mexico.

KEYWORDS: forestation, conservation genetics, floristic enrichment, RFLP.

RESUMEN

Los bosques mesófilos de montaña (BMM) se presentan entre 0.5% y 1% del territorio mexicano y están seriamente amenazados debido a la alta tasa de deforestación. Para contribuir a la conservación de especies arbóreas de BMM, se realizó el presente estudio con los objetivos de (1) determinar la diversidad de árboles en el bosque urbano El Cerrito de San Cristóbal, Chiapas, México, (2) enriquecer florísticamente el bosque urbano El Cerrito de San Cristóbal con la plantación de especies nativas del BMM de Chiapas y (3) describir la diversidad genética introducida mediante el enriquecimiento florístico. A través de un muestreo en parcelas circulares de 1000 m², se registró la estructura y composición de la comunidad de árboles y, por recorridos, se registraron las especies de herbáceas. Se plantaron 14 especies nativas del BMM para enriquecimiento de El Cerrito y, mediante la técnica PCR-RFLPs de ADN de cloroplasto (tRNL), se determinó la diversidad genética introducida. Se detectó una composición de árboles dominada por especies introducidas hace 40 años (*Cupressus lusitanica*, *Eucalyptus camaldulensis* y *Pinus pseudostrobus*). No se observó reclutamiento de plántulas y juveniles de árboles del dosel y se identificaron algunos individuos de especies nativas establecidos naturalmente. La diversidad genética introducida fue baja por especie, pero en conjunto fue moderada. La baja diversidad por especie se atribuye a que el marcador utilizado es conservador. Los bosques urbanos pueden constituir un sistema de conservación de especies de árboles nativos y de su diversidad genética, al mismo tiempo que se promueve la regeneración y diversificación de bosque urbanos. Este estudio contribuye con la conservación y conocimiento genético de especies arbóreas de bosques de montaña de México.

PALABRAS CLAVE: forestación, genética de la conservación, enriquecimiento florístico, RFLP.

INTRODUCTION

Changes in land use as a result of human activities are the leading cause of the loss of forests and biodiversity associated with them (Sebbenn, 2011; Toledo-Aceves, Meave, González-Espínosa, & Ramírez-Marcial, 2011; Agramont, Maas & Nava, 2012; Food and Agriculture Organization [FAO], 2016). The montane cloud forest is one of the natural systems recording the highest loss rate in Mexico (Toledo-Aceves *et al.*, 2011). This forest system generates unique environmental conditions for the coexistence of temperate and Neotropical plant species, where endemic species grow (Challenger, 1998; Williams-Linera, 2007; Toledo-Aceves *et al.*, 2011).

The estimated cover of these forests in Mexico ranges between 0.5% and 1% (Rzedowski, 1996; Jiménez, 2012). Chiapas, and particularly Los Altos region, has recorded an annual deforestation rate of 18.9%, caused mainly by illegal logging, grazing, and agriculture (González-Espínosa, Quintana-Ascencio, Ramírez-Marcial, & Gaytan-Guzman, 1991; González-Espínosa, Ochoa-Gaona, Ramírez-Marcial, & Quintana-Ascencio, 1995; Cayuela, 2006; González-Espínosa *et al.*, 2007).

A conservation strategy for forested areas is the restoration and floristic enrichment of forest remnants, as well as the introduction of new genetic variants to increase genetic diversity (Frankham, Ballou, & Briscoe, 2002). Unfortunately, urban forests are reforested primarily with exotic species; however, based on the ecological knowledge of local forests, native species could also be incorporated. The introduction of new organisms to previously settled communities brings ecological consequences (e.g., competition, diseases) (Begon, Townsend, & Harper, 2006). Also, genetic factors may compromise the long-term viability of introduced populations, e.g., through inbreeding, deleterious alleles, and founder effect, all of which should be factored in during the introduction of organisms (Frankham *et al.*, 2002; Freeland, 2005).

El Cerrito de San Cristóbal de Las Casas, Chiapas (hereafter called El Cerrito) is an urban forest developed by the afforestation of a volcanic mound some 60 years ago,

by planting three species: *Cupressus lusitanica*, *Pinus pseudostrobus*, and *Eucalyptus camaldulensis*, the latter two being exotic species. Although *C. lusitanica* is considered to be native to Mexico, its biological properties restrain the growth of other plant species (Fernández-Pérez, Ramírez-Marcial, & González-Espínosa, 2013). This study determines the current diversity and propose floristic enrichment, and the genetic richness associated with it, taking this urban forest as an example of a conservation strategy for native cloud forest species.

OBJECTIVES

The specific objectives are: (1) describing the composition and floristic richness of El Cerrito de San Cristóbal urban forest, (2) increasing tree diversity in El Cerrito with MCF native species from Los Altos de Chiapas (Chiapas Highlands) as a conservation strategy for these species, and (3) estimating the introduced genetic diversity in the cluster of species used for floristic enrichment.

MATERIALS AND METHODS

Study area

El Cerrito is a promontory located in the central part of the valley of San Cristóbal de las Casas, Chiapas, seeming like an "island" of tree vegetation surrounded by human settlements and roads (Ruiz-Montoya, 2014). Its altitude ranges from 2128 m to 2190 m above sea level, stretching across 80 hectares approximately. It is located between 16°43'54.72"–16°44'08.38" N and 92°38'52.59"–92°38'24.52" E (Fig. 1).

The first afforestation effort took place in the late 1960s to embellish the chapel dedicated to the patron saint San Cristóbal. These efforts hardly prospered, given the scarcity of soil and water. A new afforestation attempt was conducted in 1973 as an initiative of municipal and state government authorities, through the then Socio-Economic Development Program of the Chiapas Highlands (Prodech), with the participation of the local society. In that year, El Cerrito became public property; it was then that some 5000 plants of cypress (*Cupressus lusitanica*), eucalyptus

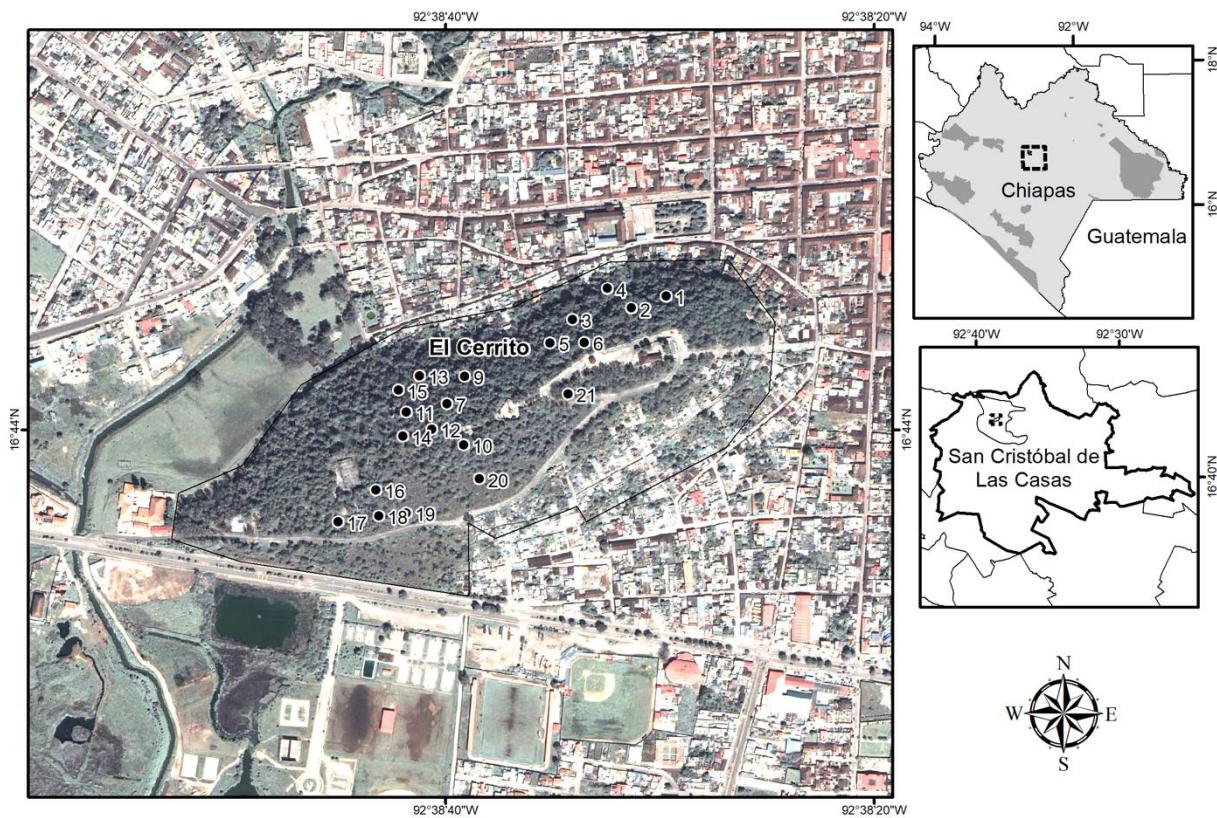


FIGURE 1. Study area in El Cerrito, San Cristóbal de Las Casas, Chiapas, for the analysis of tree composition, floristic enrichment, and genetic diversity of 14 introduced tree species.

(*Eucalyptus camaldulensis*), and Japanese privet (*Ligustrum japonicum*) were planted. Planting and maintenance activities were carried out mostly by citizens.

Tree Diversity.

Twenty 1000 m² circular plots were established across the entire polygon of El Cerrito (Ramírez-Marcial *et al.*, 2010). Within each plot, the diameter at breast height (DBH) was measured for all individual trees, both planted and grown naturally, which had a trunk ≥ 5 cm in diameter. The presence of shrub and herbaceous species was recorded in the entire area in July and August 2012 and 2013 through walks along tracks. Absolute and relative data on density, frequency, and basal area of each species were recorded.

Floristic enrichment

All introduced plants were grown in nurseries after thorough seed tree selection and seed collection processes,

along with feasibility and planting analyses (Guillén, Luna & Ruiz-Montoya, 2014). Introduced plants were between two and four years old. Planting was conducted in 20 plots of 400 m², distributed across El Cerrito slopes. Individuals of each species were assigned to plots according to local site conditions (microrelief, accumulated soil, radiation) and shade tolerance (Ramírez-Marcial, Camacho-Cruz & González-Espínosa, 2005; Ramírez-Marcial, Camacho-Cruz, González-Espínosa, & López-Barrera, 2006; Ramírez-Marcial *et al.*, 2008). Trees were planted at two time points during the rainy season: late June 2012 and early June 2013. Given the high ecological relevance of oaks (*Quercus*) in forests of Chiapas Highlands (González-Espínosa *et al.*, 2012), we used a larger number of individuals of oak species.

The survival of the plantation was assessed in January 2014 by quantifying the number of live plants versus the total number of plants introduced in the two consecutive

years. In addition, growth rate was determined by comparing the height and diameter at two time points — September 2012, one month after the end of the plantation, and January 2013 — in a random sample of individuals of the 2012 plantation. Plant height was measured from the plant base up to the highest part of the main stem; stem diameter was measured at 10 cm from the ground. The relative growth rate was calculated for the two variables (height and diameter) using the formula $TCR = \ln(M2 - M1)/4$, where TCR is the relative growth rate, $M1$ and $M2$ are the measurements recorded at times 1 and 2, respectively, and the denominator refers to the time elapsed (4 months) between times 1 and 2 (Wilson & Tilman, 1991).

Introduced Genetic Diversity

The genetic diversity introduced into the cluster of species managed was estimated by assessing genetic variation through Restriction Fragment Length Polymorphism (RFLP) of a universal chloroplast region; this allowed reducing time and costs associated with obtaining specific markers. We used the spacer region of the chloroplast genome (cpDNA) corresponding to exon 5' of tRNA (UAA) and to exon 3' of tRNA (UAA; Taberlet, Gielly, Pautou, & Bouvier, 1991), with genbank access number: B49317 (5' CGAAATCGGTAGACGCTAGGG 3') and A49855 (5' GGGGATAGAGGGACTTGAAC 3').

Two to three leaves were collected from 29 to 30 individuals, according to availability, of each of the 14 introduced species prior to planting, and were stored at -70 °C in the Genetics Laboratory at El Colegio de la Frontera Sur (Ecosur). DNA was extracted with a modification of the CTAB 2X method described by Doyle and Doyle (1987). DNA amplification was performed using the Polymerase Chain Reaction (PCR). The amplification conditions used were initial denaturation at 94 °C for 2 min, followed by 30 cycles at 94 °C for 1 min (denaturation), 52 °C for 1 min (alignment), 72 °C for 1 min (extension), and 72 °C for 7 min (final extension). The amplification

products were visualized by electrophoresis in 2% agarose gels.

Once the DNA region of the target chloroplast was obtained, it was digested with eight restriction enzymes (Fermentas): *HinfI*, *HaeIII*, *MspI*, *HhaI*, *TaqI*, *MboI*, *AluI*, and *DraI*. The restriction reaction was conducted with 10 µl of nuclease-free water, 2 µl of buffer (Green Buffer), 0.7 µl of 10X enzyme, and 5 µl of the amplified product. Each reaction was incubated in a water bath at 37 °C for 90 min. The restriction fragments were separated by electrophoresis in 8% acrylamide gels. The patterns of bands obtained by electrophoresis were interpreted as suggested by Dumolin-Lapegue *et al.* (1997), where each polymorphic restriction fragment is considered a character and each state is encoded according to fragment size.

The following parameters were used as genetic diversity estimators: haplotype diversity (h), percent polymorphism (%P), expected heterozygosity (uh), and number of polymorphic sites (restriction fragments) (s); all were obtained using the program GenAlEx v. 6.51 (Peakall & Smouse, 2006). The genetic differentiation between species was estimated through an Analysis of Molecular Variance (AMOVA) and the fixation index (F_{st}), determined using the software Arlequin v. 3.5.1.2 (Excoffier, 2010). In addition, we obtained the Shannon's genetic diversity index (SI) using the program MVSP v. 3.1 (Kovach, 2007).

The genetic similarity between species was examined through the analysis of Nei's genetic distances (Nei, 1978) with the program GenAlEx v. 6.51 (Peakall & Smouse, 2006), depicted graphically applying the Neighbor-Joining method including 1000 replicates, with the program PAUP v. 4.0a147 (Swofford, 2002). In addition to the genetic diversity parameters, clade width (defined by genetic distances) was used as an indirect estimator of genetic diversity: a broader width is related to a lower genetic relationship, i.e., a greater genetic diversity due to phylogenetic remoteness (distance).



RESULTS

Species Diversity

We identified six shrub, 11 tree, and 107 herb species. The most abundant species was *C. lusitanica*, representing nearly one-third of all trees (Appendix 1). Herbaceous species showed a high species richness (107), 68 being dicotyledons (63.55%), 24 monocotyledons (22.43%), and 15 ferns and lycophytes (14.02%). The most species-rich family was Asteraceae with 16 species, followed by Poaceae and Lamiaceae with eight species, and Polypodiaceae with six species. The genus *Polypodium* (ferns) showed the largest number of species (Appendix 1). Species richness, density, and basal area were highly variable in all parcels evaluated, indicating a high heterogeneity in the composition and structure of the tree vegetation.

Floristic enrichment

The number of individuals planted in years 2012 and 2013 amounted to 2762 and 590, respectively, for a total of 3352 plantlets (186 ind/ha) across 8000 m², with a mean survival of 45%. The species showing the highest percent survival one year after planting were *Olmediella betscheriana* (88%) and *Myrsine juergensenii* (84%). The species with the lowest survival were *Quercus sebifera* (0%), *Oreopanax xalapensis* (10%), and *Symplocos breedlovei* (20%).

A low average growth was recorded both in height (0.001 cm/month) and in stem diameter (0.018 cm/month). *Quercus rugosa* displayed the highest growth rate (0.22 cm/month in height and 0.030 cm/month in stem diameter), while other species showed even a decrease in size (e.g., *Styrax magnus*, -0.079 cm/month in height; *Q. laurina*, -0.013 cm/month in stem diameter; Table 1).

TABLE 1. Growth rate (cm/month) and percent survival (%) of 14 native tree species introduced to El Cerrito de San Cristóbal de Las Casas, Chiapas, Mexico. - = no data obtained, N = Sample, S = survival.

Species	Growth			Survival	
	N	Height	Diameter	No. of Individuals Planted	S
<i>Alnus acuminata</i>	-	-	-	61	0.33
<i>Arbutus xalapensis</i>	-	-	-	62	0.48
<i>Myrsine juergensenii</i>	5	0.010	0.007	101	0.84
<i>Olmediella betscheriana</i>	8	-0.029	0.010	102	0.88
<i>Oreopanax xalapensis</i>	-	-	-	50	0.10
<i>Prunus barbata</i>	5	-0.040	0.009	85	0.47
<i>Prunus rhamnoides</i>	7	0.008	0.010	85	0.53
<i>Symplocos breedlovei</i>	-	-	-	50	0.20
<i>Styrax magnus</i>	4	-0.079	0.011	102	0.54
<i>Quercus crassifolia</i>	17	0.006	0.039	412	0.48
<i>Quercus crispipilis</i>	18	-0.001	0.022	907	0.44
<i>Quercus laurina</i>	18	-0.005	-0.013	733	0.48
<i>Quercus rugosa</i>	25	0.22	0.030	571	0.54
<i>Quercus sebifera</i>	5	0.005	0.013	31	0.00
Average/total*		0.001	0.018	3352*	0.45

Introduced Genetic Diversity

The amplification fragment of the chloroplast spacer region included 600 base pairs (bp). After the digestion of fragments with restriction enzymes, we identified 20 restriction sites (loci) with *HinfI*, 23 with *HaeIII*, 11 with *MspI*, 20 with *HhaI*, 27 with *TaqI*, 21 with *MboI*, 14 with *AluI*, and 13 with *DraI*, for a total of 149 loci.

Styrax magnus showed the highest genetic diversity ($s = 57$, $h = 0.13$, %P = 46.31), followed by *Prunus rhamnoides* ($s = 33$, $h = 0.08$, %P = 30.87), *M. juergensenii* ($s = 41$, $h = 0.08$, %P = 27.52), and *Olmediella betscheiana* ($s = 38$, $h = 0.09$, %P = 25.50). *Symplocos breedlovei* was the species with the

lowest genetic diversity ($s = 17$, $h = 0.03$, %P = 9.72) (Table 2). In general, mean genetic diversity was low ($s = 30.5$, $h = 0.06$, %P = 22.67; Table 2).

The analysis of molecular variance (AMOVA) showed that 67.21% of variation was found between species and 32.79% within species, with a fixation index $F_{st} = 0.67$, $P = 0.001$. The tree of genetic distances showed a clear grouping only for *Quercus* species, with *Q. crassifolia* and *Q. crispipilis* as the closest species. A genetic relationship was also found between *Oreopanax xalapensis* and *Olmediella betscheiana*, as well as between *Symplocos breedlovei* and *Alnus acuminata*. *Prunus barbata* was identified as the basal species (Fig. 2).

TABLE 2. Genetic diversity of 14 native tree species introduced to El Cerrito de San Cristóbal, Chiapas, Mexico, obtained with eight restriction enzymes of the tRNRL region of chloroplast DNA (cpDNA).

Species	N	s	h	%P	SI	uh
<i>Alnus acuminata</i>	27	30	0.04	20.13	0.06	0.04
<i>Arbutus xalapensis</i>	30	17	0.04	18.12	0.06	0.04
<i>Myrsine juergensenii</i>	30	41	0.08	27.52	0.13	0.09
<i>Olmediella betscheiana</i>	28	38	0.09	25.50	0.13	0.09
<i>Oreopanax xalapensis</i>	30	23	0.04	22.82	0.07	0.04
<i>Prunus barbata</i>	30	23	0.05	15.44	0.08	0.05
<i>Prunus rhamnoides</i>	30	33	0.08	30.87	0.12	0.08
<i>Symplocos breedlovei</i>	29	17	0.03	11.41	0.04	0.03
<i>Styrax magnus</i>	30	57	0.13	46.31	0.20	0.13
<i>Quercus crassifolia</i>	30	35	0.06	23.49	0.10	0.07
<i>Quercus crispipilis</i>	30	18	0.03	12.08	0.05	0.03
<i>Quercus laurina</i>	30	37	0.07	24.83	0.10	0.07
<i>Quercus rugosa</i>	30	30	0.08	20.13	0.11	0.08
<i>Quercus sebifera</i>	30	28	0.08	18.79	0.11	0.08
Average	29.57	30.5	0.06	22.67	0.10	0.07

N = number of sequences analyzed, s = number of polymorphic sites, h = haplotype diversity, %P = percentage of polymorphism, SI = Shannon's genetic diversity index, uh = expected heterozygosity [$uh = 1 - (p^2 + q^2)$].

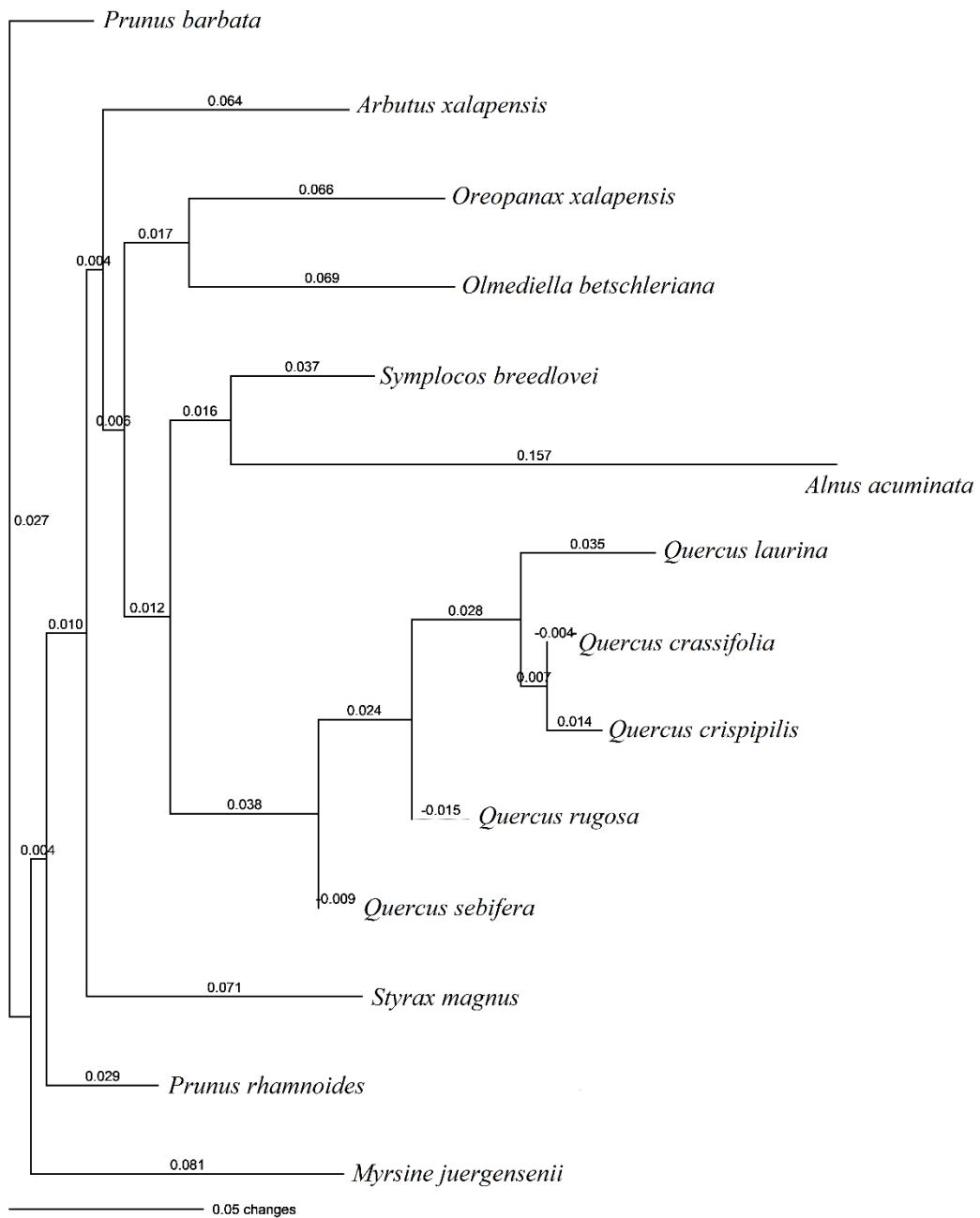


FIGURE 2. Tree of Nei's genetic distances (Nei 1978) with 1 000 permutations for 14 tree species introduced to El Cerrito de San Cristóbal de Las Casas, Chiapas, based on eight restriction enzymes of the tRNAL region of chloroplast DNA.

DISCUSSION

Tree Diversity

As expected, the floristic composition analysis revealed that the forest is dominated by those species that were initially selected to be planted in a mound devoid of tree vegetation. This choice was likely based on the availability of plants produced at that time to supply the timber industry. The area was forested aiming to embellish a site with religious significance and, unintentionally, it is currently a landscape element that certainly serves to regulate temperature and probably captures atmospheric pollutants; at the same time, it is used as a leisure area for the local population and it also contributes to the conservation of native plant and animal species, especially birds (Mena & Silva, 2014), and insects (León-Cortés, Caballero, Miss-Barrera, & Giron-Intzin, 2019).

Despite the small number of tree species selected for the afforestation of El Cerrito 60 years ago, the current forest is characterized by heterogeneous vegetation composition and structure, albeit with a low diversity of native tree species. A null recruitment of seedlings and juveniles of the species that dominate the canopy was also observed. The low diversity of native species may be due to the fact that *C. lusitanica* prevents the establishment of other tree species (Fernández-Pérez, Ramírez-Marcial, & González-Espínosa, 2013). In addition, it is known that monospecific plantations lead to a low diversity of plants and animals, as well as to alterations of the properties of soil (e.g., acidification) (Cavelier & Tobler, 1998). In El Cerrito, soil pH is near-neutral (6.7-7.9) (Ruiz-Montoya, 2014), with a high cation-exchange capacity, suggesting suitable conditions for the establishment of plantlets of the 14 species introduced.

Floristic Enrichment

The introduction of native species is widely recommended for reforestation, ecological restoration, or forest enrichment, as this preserves the functionality and resilience of communities, which become better adapted to

the current changing conditions (Thomas *et al.*, 2014). All the species introduced to the El Cerrito urban forest are native to montane cloud forests of Los Altos de Chiapas, and, given their tolerance to high irradiation, are easily established in disturbed areas (Alcantara, Luna, & Velázquez, 2002; Ramírez-Marcial *et al.*, 2012).

Enrichment increased the diversity of tree species in the early development stages (between two- and four-years post-planting) (Guarigata, Rheingans, & Montagnini, 1995; Jiménez 2012); an effective establishment is expected, so that subsequent growth should maintain the forest cover in the area in decades to come. However, a decline may occur because the local coexistence of introduced species depends on their specific tolerance to the prevailing environmental conditions in the site, as well as on those that may emerge as the forest develops (e.g., shade) (Quintana-Ascencio & González-Espínosa, 1993; Ramírez-Marcial, 2003; Quintana-Ascencio, Ramírez-Marcial, González-Espínosa, & Martínez-Icó, 2004).

In the short follow-up of the plantation, *Q. rugosa* showed the highest growth rate, likely because of its tolerance to moderate shade (Quintana-Ascencio, González-Espínosa, & Ramírez-Marcial, 1992; Bonfil & Soberón, 1999). On the other hand, five species (*Olmendiella betschleriana*, *Prunus barbata*, *Styrax magnus*, *Q. crispipilis*, & *Q. laurina*) showed a decrease in height and stem diameter, maybe associated with water stress and damage caused by passers-by that cut off growing tips, thus negatively affecting their development and, ultimately, their establishment.

According to Galindo-Jaimes *et al.* (2002), *Pinus*-dominated forests in Los Altos de Chiapas are less fertile because of low organic matter and nitrogen in the soil; in addition, according to these authors, the establishment of many species and forest regeneration depend on a canopy dominated by *Quercus*. In this sense, our study recorded a 39% survival of *Quercus* spp. two years after planting, which supports a good prognosis for short- and long-term survival. However, it is suggested to continue planting native trees aiming to increase population density and



species richness, as well as to monitor their establishment and growth.

Introduced Genetic Diversity

Planting native species for enrichment purposes increases species richness and, from a genetics perspective, enhances genetic flow and the founder effect (Glaubitz, Wu, & Moran, 2003; Li *et al.*, 2005). The enrichment planting conducted at El Cerrito introduced previously absent genotypes and alleles, with populations that may show a low genetic diversity due to the founder effect, depending on the degree of diversity managed for the cluster of introduced species, and on the number of individuals with genetic variants that reached the reproductive stage.

To achieve a long-term development of introduced populations, it is recommended to manage high genetic diversity levels (Frankham *et al.*, 2002; Glaubitz, Wu, & Moran, 2003; Stockwell, Hendry, & Kinnison, 2003). This study estimated genetic diversity with a molecular marker selected based on its universality, but the genetic variation recorded was low ($\%P = < 46.31$, $b = 0.13$, $s = < 41$). These low genetic diversity levels are probably related to the collection of seeds from just a few parent trees, and to the chloroplast being a region of DNA of exclusively maternal inheritance with no recombination and a low evolutionary rate (Palmer and Stein, 1986), particularly as regards the tRNA region used in our study (7% variation in *Symplocos*; Fritsch, Cruz, Almeda, Wang, & Shi 2006).

The PCR-RFLP technique detects specific differences in DNA based on the presence or absence of fragments recognized by restriction enzymes (endonucleases); when the enzyme detects the specific unprotected (methylated) sequence, it cleaves the 4-6 nucleotide fragment. This produces fragments of variable length modified by DNA mutations, thus facilitating the comparison between genomes (Valadez & Kahl, 2000). Although this technique reveals the genetic variation based on the frequency of alleles (fragments), it involves only a relatively small DNA fraction; additionally, it does not identify heterozygous conditions, hence limiting the scope of the information

obtained (Rentería-Alcántara, 2007). Therefore, future studies should include nuclear markers to obtain a complete estimate of genetic diversity (Rentería-Alcantára, 2007); also, a larger number of seed trees should be considered in future reforestation works.

Up to now, studies addressing the genetic diversity of wild populations of *Quercus* species are available for central and northern Mexico, yielding diversity estimates of 0.289 for *Q. havardii* based on the expected heterozygosity (He) with RFLP (Mayes, McGinley, & Werth, 1998). For the species covered in this study, it is known that genetic diversity levels of *Q. crassifolia* based on cpDNA ranges from 0.639 to 0.76 (Gorgonio-Ramírez, Clark, Campos, Monsalvo, & Alfonso, 2017), while the use of microsatellites yielded a He of 0.764; these studies suggest a high genetic diversity in oak species. We recommend exploring the genetic diversity of wild *Quercus* populations in montane cloud forests and contemplate such diversity in propagation and restoration programs of oak forests in Chiapas.

The genetic diversity of *Oreopanax xalapensis* in Los Altos de Chiapas has also been studied through enzymes, showing high genetic diversity and low genetic structuring at relatively small spatial scales (Ruiz-Montoya, Correa-Vera, Alfaro-González, Ramírez-Marcial, & Vallejo, 2011; Vera-Maloof, Ruiz-Montoya, & Ramírez-Marcial, 2019). Our results showed lower levels of genetic diversity than previously reported in *Oreopanax xalapensis*; possibly, the difference is due to the bias by sample size (low number of parent trees), so it is suggested to increase population genetics studies in native species of montane cloud forest trees.

As expected, the AMOVA showed the greatest percent variation between species, with a high and significant fixation index ($F_{st} = 0.67$), a finding that highlights the importance of each species in terms of the contribution to genetic variation introduced in El Cerrito. In addition, genetic distances identified *Prunus barbata* as the most evolutionarily conserved and differentiated species from all others (Weir, 1996; Week, Daly & Simpson, 2005). Since no previous information is available about the

phylogenetic relationships of the species studied, the genetic distances between species reported in this document are valuable information for future phylogenetic analyses of tree species in montane cloud forests.

CONCLUSIONS

The results of this study support our proposal that urban forests are suitable for the conservation of native tree species. Planting native species improves forest richness and, in the case of El Cerrito, promotes the spatial continuity of montane cloud forests. The floristic enrichment of El Cerrito is a feasible strategy to boost the recovery of the forest community, i.e., improve habitat and microclimate conditions, thereby leading to the regeneration of a high diversity of tree species and local wildlife. The creation of forested areas including native species should be further promoted, in both rural and urban spaces, since it is one of the urgent actions needed to mitigate the effects of climate change.

The genetic diversity introduced by each individual species was relatively low, but nonetheless it was high at the community level, as assessed in terms of the distant phylogenetic relationships among species. If the plantation in El Cerrito urban forest progresses, it will likely translate into favorable environmental characteristics for the colonization of other species (Sebbenn, 2011).

This work shows that urban forests are a suitable approach for the conservation of native tree species, which in advanced stages may protect wild animal species and boost natural forest regeneration (De la Mora-Estrada *et al.*, 2017; León-Cortés *et al.*, 2019; Lorenzo, Bolaños, García-Méndez, & Ruiz, 2014; Mena & Silva, 2014; Rao & Hodgkin, 2002; Ruiz-Montoya *et al.*, 2014). Our results are relevant for ecological restoration and conservation of tree species of the montane cloud forest in urban forest systems, as well as for the knowledge of genetic diversity of tree species in Mexico.

ACKNOWLEDGMENTS

To Trinidad Alejandro Guillen Diaz for his support in sample collection. To Carlos Molina, Maria Hernandez,

Fernando Kante, Antelmo G. Estrada, Paola A. Covarrubias, and Nancy A. Reyes for their assistance in field and laboratory work; to Diego Díaz for his help in preparing the location map. This work was partially funded by the Comisión Nacional Forestal (Special Project 2012-Ecological Restoration of Cerrito de San Cristóbal granted to LRM). María Elena Sánchez-Salazar translated the manuscript into English.

REFERENCES

Agramont, A. R. E., Maass, S. F., & Nava, B. G. (2012). Effect of human disturbance on the structure and regeneration of forest in the Nevado de Toluca National Park, Mexico. *Journal of Forestry Research*, 23(1), 39-44. doi: 10.1007/s11676-012-0226-8

Alcántara, O., Luna, I., & Velázquez, A. (2002). Altitudinal distribution patterns of Mexican cloud forest based upon preferential characteristic genera. *Plant Ecology*, 161(2), 167-174. doi: 10.1093/jpe/rty053.

Begon, M., Townsend, C. R., & Harper, J. L. (2006). *Ecology, from individuals to ecosystems*. United Kingdom: Blackwell Publishing.

Bonfil, C., & Soberón, J. (1999). *Quercus rugosa* seedling dynamics in relation to its re-introduction in a disturbed Mexican landscape. *Applied Vegetation Science*, 2(2), 189-200. doi: 10.1111/avsc.12438

Cavelier, J., & Tobler, A. (1998). The effect of abandoned plantations of *Pinus patula* and *Cupressus lusitanica* on soils and regeneration of a tropical montane rain forest in Colombia. *Biodiversity and Conservation*, 7(3), 335-347. doi: 10.1007/s10531-019-01800-0

Cayuela, L. (2006). Deforestación y fragmentación de bosques tropicales montanos en los Altos de Chiapas, México. Efectos sobre la diversidad de árboles. *Ecosistemas*, 15(3), 191-196.

Challenger, A. (1998). *Utilización y conservación de los ecosistemas terrestres de México: pasado, presente y futuro. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad*. United State: Cornell University.

Cole, R. J. (2009). Postdispersal seed fate of tropical montane trees in an agricultural landscape, southern Costa Rica. *Biotropica*, 41(3), 319-327. doi: 10.1111/btp.12409

De la Mora-Estrada, L. F., Ruiz-Montoya, L., Ramírez-Marcial, N., Morón-Ríos, A., & Mayorga-Martínez, M. C. (2017). Diversidad de chinches (Hemiptera: Heteroptera) en bosques secundarios de pino-encino de San Cristóbal de Las Casas, Chiapas, México. *Revista Mexicana de Biodiversidad*, 88(1), 86-105. doi: 10.1016/j.rmb.2017.01.016



Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, 19, 11-15.

Dumolin-Lapergue, S., Demesure, B., Le Corre, V., Fineschi, S., & Petit, R. J. (1997). Phylogeographic structure of white oaks throughout the European continent. *Genetics*, 146(4), 1475-1487. Retrieved from <https://www.genetics.org/content/genetics/146/4/1475.full.pdf>

Excoffier, L. (1998-2010). *ARLEQUIN v. 3.5.1.2. An Integrated Software Package for Population Genetics Data Analysis*. Institute of Ecology and Evolution, University of Berne. Swiss Institute of Bioinformatics. Retrieved from <http://cmpg.unibe.ch/software/arlequin3>

Organización de las Naciones Unidas para la Alimentación [FAO] (2016). *El estado de los bosques del mundo*. Retrieved from <http://www.fao.org/3/a-i5850s.pdf>

Fernández-Pérez, L., Ramírez-Marcial, N., & González-Espinosa, M. (2013). Reforestación con *Cupressus lusitanica* y su influencia en la diversidad del bosque de pino-encino en Los Altos de Chiapas. *Botanical Sciences*, 91(2), 207-216. Retrieved from <http://www.scielo.org.mx/pdf/bs/v91n2/v91n2a8.pdf>

Frankham, R. J., Ballou, D., & Briscoe, D. A. (2002). *Introduction to conservation genetics*. England: Cambridge University Press, Cambridge.

Freeland, J. R. (2005). *Molecular Ecology*. England: John Wiley & Sons, Ltd.

Fritsch, P. W., Cruz, B. C., Almeda, F., Wang, Y., & Shi, S. (2006). Phylogeny of *Symplocos* based on DNA sequences of the Chloroplast *trnC-trnD* intergenic region. *The American Society of Plant Taxonomist*, 31(1), 181-192. doi: 10.1600/036364406775971877

Galindo-Jaimes, L., González-Espinosa, M., Quintana-Ascencio, P., & García-Barrios, L. (2002). Tree composition and structure in disturbed stands with varying dominance by *Pinus* spp. in The Highlands of Chiapas, México. *Plant Ecology*, 162(2), 259-272. doi: 10.1093/jpe/rty053

Glaubitz, J. C., Wu, H. X., & Moran, G. F. (2003). Impacts of silviculture on genetic diversity in the native forest species *Eucalyptus sieberi*. *Conservation Genetics*, 4(3), 275-287. doi: 10.1007/s10592-019-01165-5.

González-Espinosa, M., Quintana-Ascencio, P. F., Ramírez-Marcial, N., & Gaytán-Guzmán, P. (1991). Secondary succession in disturbed *Pinus-Quercus* forest in the highlands of Chiapas, México. *Journal of Vegetation Science*, 2(3), 351-360. doi: 10.1111/jvs.12796

González-Espinosa, M., Ochoa-Gaona, S., Ramírez-Marcial, N., & Quintana-Ascencio, P. F. (1995). Current land-use trends and conservation of old-growth forest habitats in The Highlands of Chiapas, Mexico. In M. Wilson, & S. Sader (Eds.), *Neotropical Migratory Birds in Mexico* (pp. 190-198). United States: Maine Agricultural and Forest Experimental Station.

González-Espinosa, M., Ramírez-Marcial, N. R., Camacho-Cruz, A., Holz, S. C., Rey-Benayas, J. M., & Parra-Vázquez, M. R. P. (2007). Restauración de bosques en territorios indígenas de Chiapas: modelos ecológicos y estrategias de acción. *Boletín de la Sociedad Botánica de México*, 80, 11-23. doi: 10.17129/botsci.1045

González-Espinosa, M., Meave, J. A., Ramírez-Marcial, N., Toledo-Aceves, T., Lorea-Hernández, F. G., & Ibarra-Manríquez, G. (2012). Los bosques de niebla de México: Conservación y restauración de su componente arbóreo. *Ecosistemas*, 21(1-2), 36-54. Retrieved from https://www.redalyc.org/pdf/540/Resumenes/Resumen_54026849004_1.pdf

Gorgonio-Ramírez, M., Clark, T. R., Campos, J. E., Monsalvo, R. A., & Alfonso, C. C. (2017). Diversidad y estructura genética de *Quercus crassifolia* en sitios de manejo forestal y uso local en Sierra Juárez, Oaxaca. *Madera y bosques*, 23(2), 85-98. doi: 10.21829/myb.2019.2511553

Guariguata, M. R., Rheingans, R., & Montagnini, F. (1995). Early woody invasion under tree plantations in Costa Rica: implications for forest restoration. *Restoration Ecology*, 3(4), 252-260. doi: 10.1111/rec.13015

Guillén, D. T. A., Luna, G. A., & Ruiz-Montoya, L. (2014). Colecta, siembra y germinación de semillas de árboles de Los Altos de Chiapas. En L. Ruiz-Montoya (Coord.), *Diversidad biológica y enriquecimiento florístico del Cerrito de San Cristóbal* (pp. 95-108). Mexico: Comisión Nacional Forestal/ El Colegio de la Frontera Sur.

Jiménez, V. R. E. (2012). *Assessing success of forest restoration efforts in degraded montane cloud forests in southern Mexico*. Master thesis. Michigan Technological University.

Kovach, W. L. (2007). MVSP - A multivariate statistical package for Windows, ver. 3.1. Retrieved from <https://mvsp-a-multivariate-statistical-package.apponic.com/>

León-Cortés J. L., Caballero, U., Miss-Barrera, I. D., & Girón-Intzin, M. (2019). Preserving butterfly diversity in an ever-expanding urban landscape? A case study in the highlands of Chiapas, México. *Journal of Insect Conservation*, 23(2), 405-418. doi: 10.1007/s10841-019-00149-7

Li, Y-Y., Chen, X-Y., Zhang, X., Wu, T-Y., Lu, H-P., & Cai, Y.W. (2005). Genetic Differences between wild and artificial populations of *Metasequoia glyptostroboides*: Implications for species recovery.

Conservation Biology, 19(1), 224-231. doi: 10.1111/j.1523-1739.2005.00025.x

Lorenzo, C., Bolaños, J., García-Méndez, A., & Ruiz, C. M. (2014). Mamíferos pequeños en el Cerrito de San Cristóbal. In L. Ruiz-Montoya, & S. Sader (Coord.), *Diversidad biológica y enriquecimiento florístico del Cerrito de San Cristóbal* (pp. 39-56). Mexico: El Colegio de la Frontera Sur.

Mayes, S. G., McGinley, M. A., & Werth, C. R. (1998). Clonal population structure and genetic variation in sand-shinnery oak, *Quercus havardii* (Fagaceae). *American Journal of Botany*, 85(11), 1609-1617. doi: 10.3732/ajb.92.1.13

Mena, D. L. G., & Silva, R. J. M. (2014). Aves del Cerrito de San Cristóbal. In L. Ruiz-Montoya (Ed.), *Diversidad biológica y enriquecimiento florístico del Cerrito de San Cristóbal* (pp. 57-78). Mexico: Comisión Nacional Forestal/ El Colegio de la Frontera Sur.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89 (3), 586-590. Retrieved from: <https://www.genetics.org/content/146/4/1475>

Palmer, J. D., & Stein, D. B. (1986). Conservation of chloroplast genome structure among vascular plants. *Current Genetics*, 10(11), 823-833. Retrieved from: doi: 10.1007/BF00418529

Peakall, R. P., & Smouse, E. (2006). Genetic Analysis in Excel (GENALEX 6.5.1). Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), 288-295. doi: 10.1111/mec.15199

Quintana-Ascencio, P. F., & González-Espinosa, M. (1993). Afinidad fitogeográfica y papel sucesional de la flora leñosa de los bosques de pino-encino de Los Altos de Chiapas, México. *Acta Botánica Mexicana*, 21, 43-57. doi: 10.21829/abm118.2017.1207

Quintana-Ascencio, P., González-Espinoza, M., & Ramírez-Marcial, N. (1992). Acorn removal, seedling survivorship, and seedling growth of *Quercus crispipilis* in sucesional forests of the highlands of Chiapas, México. *Bulletin of the Torrey Botanical Club*, 119, 6-18.

Quintana-Ascencio, P. F., Ramírez-Marcial, N., González-Espinosa, M., & Martínez-Ico, M. (2004). Sapling survival and growth of coniferous and broad-leaved trees in successional highland habitats in Mexico. *Applied Vegetation Science*, 7(1), 81-88. doi:10.1111/j.1654-109X.2004.tb00598.x

Ramírez-Marcial, N. (2003). Survival and growth of tree seedlings in anthropogenically disturbed mexican montane rain forests. *Journal of Vegetation Science*, 14(6), 881-890. doi: 10.1111/jvs.12796

Ramírez-Marcial, N., Camacho-Cruz, A., & González-Espinosa, M. (2005). Potencial florístico para la restauración de bosques en Los Altos y montañas del norte de Chiapas. In M. González-Espinosa, N. Ramírez-Marcial, & L. Ruiz-Montoya (Eds.), *Diversidad biológica en Chiapas* (pp. 325-369). México: Plaza y Valdés.

Ramírez-Marcial, N., Camacho-Cruz, A., González-Espinosa, M., & López-Barrera, F. (2006). Establishment, survival and growth of tree seedlings under successional montane oak forests in Chiapas, Mexico. En M. Kappelle (Ed.), *Ecology and Conservation of Neotropical Oak Forests* (pp. 177-189). Heidelberg: Ecological Studies, Springer.

Ramírez-Marcial, N., Camacho-Cruz, A., González-Espinosa, M., Sánchez-Velázquez, L. R., Galindo-González, J., & Díaz-Fleischer, F. (2008). Clasificación de grupos funcionales vegetales para la restauración del Bosque Mesófilo de Montaña. *Ecología, Manejo y Conservación de los Ecosistemas de montaña en México*, 1, 51-72.

Ramírez-Marcial, N., Camacho-Cruz, A., Martínez-Icó, M., Luna-Gómez, A., Golicher, D., & González Espinosa, M. (2010). *Árboles y arbustos de los bosques de montaña en Chiapas*. San Cristóbal de Las Casas, Chiapas. El Colegio de la Frontera Sur, Chiapas, Mexico.

Ramírez-Marcial, N., Luna-Gómez, A., Castañeda, O. H. E., Martínez-Icó, M., Holz, S. C., Camacho, C. A., & González-Espinosa, M. (2012). *Guía de propagación de árboles nativos de para la recuperación de bosques*. El Colegio de la Frontera Sur, Chiapas, México.

Rao, V. R., & Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell Tissue and Organ Culture*, 68 (1), 1-19. doi: 10.1023/A:1013359015812

Rentería-Alcántara, M. (2007). Breve revisión de los marcadores moleculares. In L. Eguíarte, V. Souza, & X. Aguirre (Eds.), *Ecología Molecular* (pp. 541-566). México: Semarnat, Instituto Nacional de Ecología, UNAM, Conabio.

Ruiz-Montoya, L., Correa-Vera, V., Alfaro-González, F. C., Ramírez-Marcial, N., & Vallejo R. V. (2011). Diversidad genética de *Oreopanax xalapensis* (Araliaceae) en Los Altos de Chiapas. *Boletín de la Sociedad Botánica de México*, 88, 15-25. doi: 10.17129/botsci.1045

Ruiz-Montoya, L. (2014). *Diversidad biológica y enriquecimiento florístico del Cerrito de San Cristóbal*. Mexico: Comisión Nacional Forestal/ El Colegio de la Frontera Sur.

Ruiz-Montoya, L., Alias, V. L. A., Alias, V. M. de J., Guillén, D. T. A., & de la Mora, E. L. F. (2014). Diversidad y distribución de familias de insectos en el Cerrito de San Cristóbal. In L. Ruiz-Montoya (Ed.), *Diversidad biológica y enriquecimiento florístico del Cerrito de San Cristóbal* (pp. 39-56). México: El Colegio de la Frontera Sur, Chiapas.



Rzedowski, J. (1996). Análisis preliminar de la flora vascular de los bosques mesófilos de montaña de México. *Acta Botánica Mexicana*, 35, 25-44. doi: 10.21829/abm118.2017.1207

Sebbenn, A. (2011). Effects of forest fragmentation on the effective and realized gene flow of Neotropical tree species: implications for genetic conservation. *BMC Proceedings*, 5(7), O6. doi: 10.1186/1753-6561-5-S7-O6

Stockwell, C. A, Hendry, A. P., & Kinnison, M. T. (2003). Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution*, 18(2), 94-101. doi: 10.1016/S0169-5347(02)00044-7

Swofford, D. L. (2002). PAUP: phylogenetic analysis using parsimony, Version 4.0. Sinauer Associates, Sunderland, Massachusetts. Retrieved from <https://paup.phylosolutions.com/get-paup/>

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17(5), 1105-1109. Retrieved from doi: 10.1007/BF00037152

Thomas, E., Jalon, R., Loo, J., Boshier, D., Gallo, L., Cavers, S., Bordács, S., Smith, P., & Bozzano, M. (2017). Genetic considerations in ecosystem restoration using native tree species. *Forest Ecology and Management*, 333, 66-75. doi: 10.1016/j.foreco.2014.07.015

Toledo-Aceves, T., Meave, J. A., González-Espinosa, M., & Ramírez-Marcial, N. (2011). Tropical montane cloud forests: Current threats and opportunities for their conservation and sustainable management in Mexico. *Journal of Environmental Management*, 92(3), 974-981. doi: 10.1016/j.jenvman.2010.11.007

Valadez, E., & Kahl, G. (2000). *Huellas de ADN en genomas de plantas*. Universidad Autónoma Chapingo. Texcoco, México.

Vera-Maloof, F. Z., Ruiz-Montoya, L., & Ramírez-Marcial, N. (2019). Does the genetic diversity of macuillillo *Oreopanax xalapensis* (Araliaceae), change along successional gradients of the montane cloud forest? *Tropical Conservation Science*, 12, 1-11. doi: 10.1177/1940082919872923

Week, A., Daly, D. C., & Simpson, B. B. (2005). The phylogenetic history and biogeography of the frankicense and myrrh familia (urseraceae) based on nuclear and chloroplast sequence data. *Molecular Phylogenetics and Evolution*, 35(1), 85-101. doi:10.1016/j.ympev.2004.12.021

Weir, B. S. (1996). *Genetic data analysis II: methods for discrete population genetic data*. Sunderland: Sinauer Assoc.

Williams-Linera, G. (2007). *El bosque de niebla del centro de Veracruz: Ecología, historia y destino en tiempos de fragmentación y cambio climático*. Xalapa, Veracruz, Mexico: Instituto de Ecología, A.C.-Conabio.

Wilson, S. D., & Tilman, D. (1991). Components of plant competition along an experimental gradient of nitrogen availability. *Ecology*, 72(3), 1050-1065. Retrieved from https://www.jstor.org/stable/1940605?seq=1#page_scan_tab_contents

Received: 14-January-2020

Accepted: 06-May-2020

Published: 17-December-2020

This paper must be cited as:

Cruz-Salazar, B., Ruiz-Montoya, L., Pérez-Gómez, M. T., García-Bautista, M., & Ramírez-Marcial, N. (2020). Diversity and floristic enrichment with montane cloud forest species, in an urban forest in Chiapas, Mexico. *Madera y Bosques*, 26(3), e2632100. doi: 10.21829/myb.2020.2632100



Madera y Bosques by Instituto de Ecología, A.C. is distributed under a Creative Commons Licence Attribution-NonCommercial-ShareAlike 4.0 International.