Journal of Biogeography (J. Biogeogr.) (2013)

ORIGINAL ARTICLE

Influence of the geological history of the Trans-Mexican Volcanic Belt on the diversification of *Nolina parviflora* (Asparagaceae: Nolinoideae)

Eduardo Ruiz-Sanchez[1,2*] and Chelsea D. Specht[1]

[1]*University of California, Berkeley, Department of Plant and Microbial Biology and the University and Jepson Herbaria, Berkeley, CA, 94270, USA,* [2]*Instituto de Ecología A.C., Centro Regional de Bajío, Red de Biodiversidad y Sistemática, 61600, Pátzcuaro, Michoacán, México*

Correspondence: Eduardo Ruiz-Sanchez, Instituto de Ecología A.C., Centro Regional de Bajío, Red de Biodiversidad y Sistemática, Av. Lázaro Cárdenas 253, 61600 Pátzcuaro, Michoacán, México.
E-mail: eduardo.ruiz@inecol.edu.mx

ABSTRACT

Aim Our aims were to determine the pattern of genetic variation in the endemic shrub *Nolina parviflora*, and to evaluate the influence of the geological history of the Trans-Mexican Volcanic Belt (TMVB) and nearby mountainous regions on plant population divergence.

Location Trans-Mexican Volcanic Belt, Sierra Madre Occidental, Sierra Madre Oriental and Sierra Madre del Sur mountain ranges in Mexico.

Methods Twenty-eight populations (210 individuals) were sequenced for one nuclear (*rpb*2) and two chloroplast (*trn*L–F and *psb*A–*trn*H) DNA markers. Intraspecific phylogenetic relationships among haplotypes were reconstructed, and molecular dating, population genetic analyses and group testing were performed on the data. Isolation-by-distance analysis was conducted for the populations spanning the distribution of the species.

Results Twenty-four chloroplast marker haplotypes and 36 *rpb*2 haplotypes were recovered from the populations sampled. The combined marker phylogeny indicates the presence of two well-supported clades within the *N. parviflora* populations. Clade 1 includes populations from Jalisco and Zacatecas and Clade 2 comprises the remaining populations. We found an east–west geographical pattern of chloroplast DNA (cpDNA) haplotype distribution, indicating a lack of gene flow between these two regions. Divergence time estimates indicate an Oligocene to mid-Miocene divergence between *Nolina* and *Dasylirion*. Divergence estimates for Clade 1 are from the mid-Miocene to early Pleistocene, and for Clade 2 from the early Miocene to mid-Pliocene. Values of cpDNA G_{ST} (0.702) indicate a strong population structure and differentiation. A spatial analysis of molecular variance indicates 11 groups among the sampled populations and detects various well-supported geographical barriers.

Main conclusions Divergence time estimates suggest a correlation between the time of divergence between distinct *N. parviflora* populations and periods of uplift in the TMVB. We infer that the orogeny of this mountain range played an important role in driving the diversification of plant populations in central Mexico by creating topographical barriers that limited gene flow.

Keywords
Divergence time, geology, Mexico, nuclear marker, phylogeography, plant speciation, *psb*A–*trn*H, *rpb*2, Trans-Mexican Volcanic Belt, *trn*L–F.

INTRODUCTION

The Trans-Mexican Volcanic Belt (TMVB) is a complex geological region (Gómez-Tuena *et al.*, 2007). This mountain range is formed by a continental magmatic arc of nearly 8000 volcanic structures that extends east–west from the Pacific coast in San Blás, Nayarit and Banderas Bay, Jalisco to the Gulf of Mexico coast at Palma Sola, Veracruz. The TMVB originated during the mid-Miocene around the modern-day cities of Morelia and Querétaro in central Mexico,

Journal of Biogeography

eventually extending eastward to Veracruz and westward to Nayarit. Geologists have divided the TMVB into three sectors (west, east and central) based on age, orogeny and tectonic features (Gómez-Tuena *et al.*, 2007). The volcanic activity of the TMVB may be divided into four main episodes that occurred during: (1) the middle to late Miocene, (2) late Miocene, (3) latest Miocene to the Pliocene, and (4) the Pliocene to the Holocene (Gómez-Tuena *et al.*, 2007). Volcanic activity in this geological province continues today.

The TMVB connects three major mountain ranges: the Sierra Madre Occidental (SMOc) to the north-west; the Sierra Madre Oriental (SMOr) to the north-east; and the Sierra Madre del Sur (SMS) to the south. Together with the mountain ranges of Chiapas, these ranges constitute the biogeographical area known as the Mexican Transition Zone (Morrone, 2010), which extends from south-western USA through Mexico down to the Nicaraguan lowlands. The Mexican Transition Zone is a complex geological area where Neotropical and Nearctic biota overlap (Halffter, 1987; Morrone, 2010), creating a unique and diverse set of ecosystems. Located centrally within this ecological transition zone, the TMVB extends east–west for *c.* 1000 km and its width is 80–230 km (Metcalfe, 2006; Gómez-Tuena *et al.*, 2007). Because much of the TMVB lies above 2000 m a.s.l., its highlands have a remarkably temperate climate with pine–oak forests the dominant natural vegetation type (Metcalfe, 2006). The highest peaks exceed 5000 m a.s.l. and have permanent ice cover. Studies show that the western part of the region was wetter and the eastern part drier in the late Pleistocene than they are at present (Metcalfe, 2006).

While it connects the mountain chains that make up the Mexican Transition Zone, the TMVB is quite different from the mountain ranges that surround it in Mexico. It is geologically recent, tectonically and volcanically active, and has long been a focus for human settlement.

Bryson *et al.* (2011a) recently reviewed the extent of phylogenetic and phylogeographical studies that regard the TMVB as a geographical barrier to gene flow for animals, including studies on insects, fishes, amphibians, reptiles, birds and mammals. For some of these groups, the TMVB acted as a shallow barrier, whereas for others it acted as a hard geographical barrier, separating populations to the north of the TMVB from those south of it. Two phylogeographical studies on plants (Sosa *et al.*, 2009; Ruiz-Sanchez *et al.*, 2012) also showed that the TMVB was a geographical barrier to gene flow between northern and southern populations.

In addition to acting as a barrier to north–south gene flow, the TMVB itself – with its diverse subtropical ecosystems separated by high montane habitat – may play a role in restricting gene flow among the lineages distributed along and within the TMVB. Recent phylogenetic (Bryson *et al.*, 2011b,c; Bryson & Riddle, 2012) and phylogeographical (Bryson *et al.*, 2012; Parra-Olea *et al.*, 2012) studies show that the formation of the TMVB is correlated with the diversification of bunchgrass lizards (*Sceloporus scalaris*; Bryson *et al.*, 2011b), alligator lizards (*Barisia*; Bryson & Riddle, 2012) and salamanders (*Pseudoeurycea leprosa*; Parra-Olea *et al.*, 2012), indicating that habitat diversity within the TMVB may drive speciation in these lineages. However, to date no phylogeographical studies of plant species distributed along the TMVB have been performed on the influence of the volcanic belt ecosystem on population diversification. Understanding the gene flow patterns of a plant lineage in a comparative framework may shed some light on how geological patterns impact divergence and speciation in populations of plants on the TMVB.

Here we evaluate the historical events that have influenced patterns of genetic variation among populations of the endemic Mexican shrub *Nolina parviflora* Hemsl. (Asparagaceae: Nolinoideae). The main goal of this phylogeographical study was to reveal the evolutionary and ecological history of *N. parviflora* populations located along the TMVB, in addition to those north and south of the belt, and to test the idea that the belt is a barrier to gene flow and driver of speciation. The genus *Nolina* has *c.* 30 described species distributed in pine–oak–juniper forests and xerophytic areas in Mexico and the United States. The genus is closely related to *Beaucarnea*, *Calibanus* and *Dasylirion* according to molecular and morphological data (Kim *et al.*, 2010; Seberg *et al.*, 2012).

Nolina parviflora is a pachycaulous, rosette-leaved, polycarpic shrub, flowering every year but with mass flowering cycles that last up to 3 years (E. Ruiz-Sanchez, pers. obs.). It has a wide geographical distribution in the pine forest, pine–oak–juniper forests, xerophytic scrub and tropical dry forest localities of the TMVB, Sierra Madre Occidental, Sierra Madre Oriental and Sierra Madre del Sur mountain ranges (Fig. 1). Bees, wasps and flies pollinate the dioecious flowers. Fruit are one-seeded and capsular with inflated carpels, giving them an intermediate character between winged and balloon fruit. The fruit falls from the plant by disarticulation of the pedicel; if there is wind, the seeds are wind-dispersed in the fruit. If there is no wind, the fruit falls to the ground and the seeds are dispersed as the fruits roll along the ground and break apart (Trelease, 1911).

By combining a multi-locus phylogeographical study and phylogenetic-based estimates of divergence times, we can place genetic divergences within *N. parviflora* in a temporal context and can relate genetic divergences to abiotic factors that influence habitat composition and affect evolutionary processes (e.g. Carstens & Richards, 2007; Bryson *et al.*, 2011c,d, 2012; Cavender-Bares *et al.*, 2011). Here we use molecular data sampled from all known populations of *N. parviflora*, combined with divergence time estimates, to help identify the historical ecological and geographical processes that may have influenced the observed pattern of genetic variation in this species.

MATERIALS AND METHODS

Sampling

A total of 210 individuals from 28 populations were sampled, covering the entire geographical range of *N. parviflora*

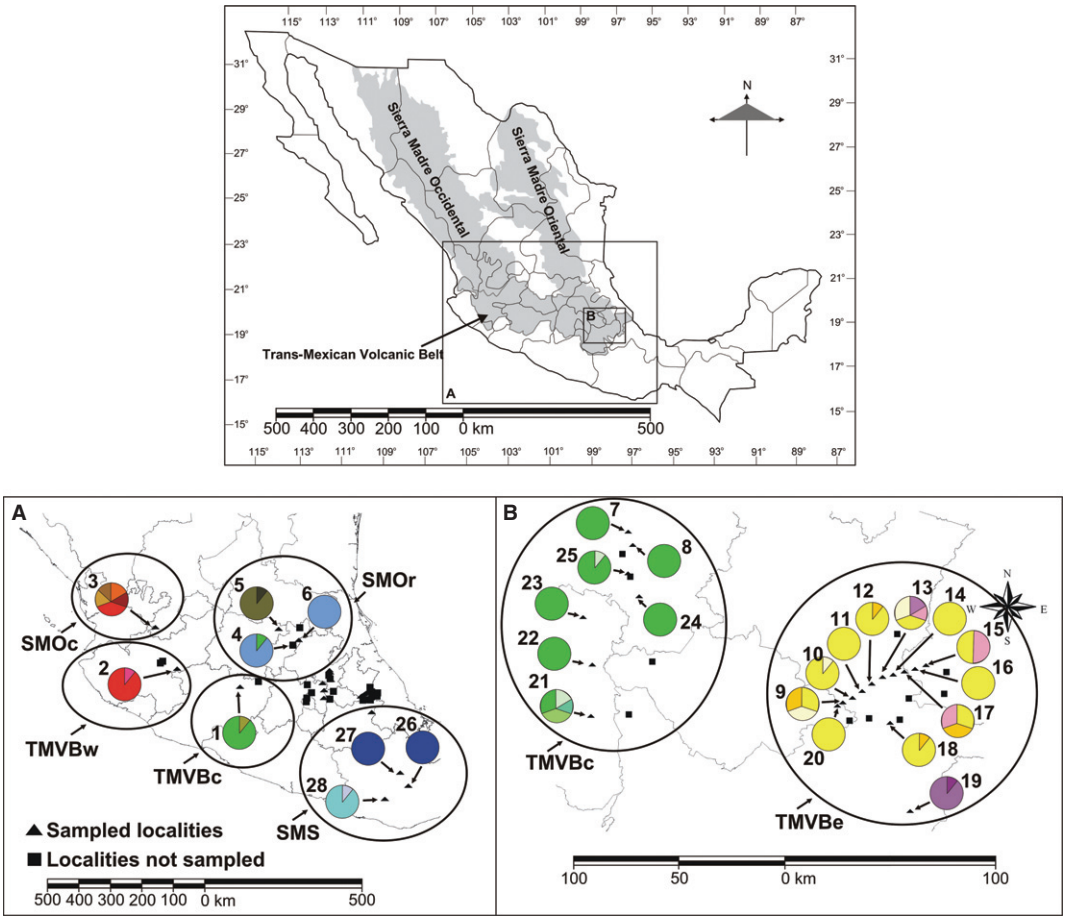

Figure 1 Geographical distribution of *Nolina parviflora* in Mexico. Population numbers correspond to those in Table 1. The lower panels are enlargements of the area within the squares labelled A and B in the top panel. Pie charts represent chloroplast haplotypes for each sampling locality. The colour coding of haplotypes is the same as in Fig. 4. Circles indicate six regions: SMOc, Sierra Madre Occidental; SMOr, Sierra Madre Oriental; SMS, Sierra Madre del Sur; TMVBw, the western part of the Trans-Mexican Volcanic Belt; TMVBc, the central Trans-Mexican Volcanic Belt; TMVBe, the eastern part of the Trans-Mexican Volcanic Belt.

(Table 1, Fig. 1). Leaf material was obtained from a maximum of 11 individuals at each locality, dried in silica gel (Chase & Hills, 1991), and stored at −80 °C. We used *Dasylirion leiophyllum* and *Dasylirion wheeleri* as outgroups for this analysis, based on the proposed position of *Dasylirion* as sister to *Nolina* (Kim *et al.*, 2010; Seberg *et al.*, 2012).

DNA extraction, molecular marker selection, amplification and sequencing

Whole genomic DNA was extracted with a modified sodium dodecyl sulfate (SDS) and sodium chloride protocol, followed by washing with ethanol (Edwards *et al.*, 1991; Konieczny & Ausubel, 1993). DNA quality and quantity were verified by measuring DNA absorbance at 260 and 280 nm with a NanoDrop® ND1000 (NanoDrop Technologies, Wilmington, DE, USA). We selected two plastid markers (intragenic spacer regions *trn*L–F and *psb*A–*trn*H) based on previously published phylogeographical analyses (Sosa *et al.*, 2009; Ornelas *et al.*, 2010) and one nuclear gene (the 23rd intron of RNA polymerase beta subunit II or '*rpb2*') previously shown to provide species and potentially population-level resolution (Sass & Specht, 2010). Polymerase chain reaction (PCR) amplification for both markers was performed from genomic DNA with Phire® HotStart II DNA polymerase (Finnzymes, Keilaranta, Finland).

Previously published primers 'e' and 'f' (Taberlet *et al.*, 1991) were used to amplify the *trn*L–F spacer in 10-μL aliquots with 10–100 ng of genomic DNA and the following reagents: 0.02 U Phire® HotStart II DNA polymerase (Finnzymes), 1× Phire® Plant PCR Buffer (Finnzymes), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 mM of each primer. PCRs were run on a MyCycler thermal cycler (Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation at 95 °C for 3 min; followed by 35 cycles of 98 °C for 5 s, 62 °C for 5 s, 72 °C for 20 s; with a final extension at 72 °C for 1 min. Primers trnH2 (Tate & Simpson, 2003) and psbA (Sang *et al.*, 1997) were used to amplify *psb*A–*trn*H following the same protocols as described for *trn*L–F but with an annealing temperature of 64 °C. For *rpb2*, previously

Table 1 Geographical location and population code for the 28 Mexican populations of *Nolina parviflora* used in the study. SMOc, Sierra Madre Occidental; SMOr, Sierra Madre Oriental; SMS, Sierra Madre del Sur; TMVB, Trans-Mexican Volcanic Belt.

Population	Locality	Code	Region	Latitude N	Longitude W	Elevation (m a.s.l.)	*n* nrDNA	*n* cpDNA	Haplotype (n_a)
1	México, Michoacán, Morelia	MICH	TMVB	19°39′	101°08′	2050	6	5	A(4) G(1)
2	México, Jalisco, Tizapan el Alto	JAL	TMVB	20°09′	102°56′	1714	8	10	C(9) D(1)
3	México, Zacatecas, Teul de González	ZAC	SMOc	21°21′	103°34′	1708	7	10	C(5) S(2) T(1) U(1) V(1)
4	México, Querétaro, Caderyta	QUE1	SMOr	20°53′	99°39′	2213	6	9	R(8) A(1)
5	México, Guanajuato, Xichú	GTO	SMOr	21°18′	100°05′	1880	1	6	W(5) X(1)
6	México, Querétaro, San Joaquín	QUE2	SMOr	20°58′	99°29′	1607	3	7	R(7)
7	México, Hidalgo, Epazoyucán	HID1	TMVB	20°07′	98°36′	2639	4	7	A(7)
8	México, Hidalgo, Epazoyucán	HID2	TMVB	20°03′	98°35′	2667	4	7	A(7)
9	México, Puebla, Libres	PUE8	TMVB	19°23′	97°41′	2465	2	7	K(5) L(1) O(1)
10	México, Puebla, Libres	PUE7	TMVB	19°24′	97°39′	2307	2	7	K(6) O(1)
11	México, Puebla, Libres	PUE6	TMVB	19°26′	97°36′	2251	4	7	K(7)
12	México, Puebla, Tepeyahualco	PUE5	TMVB	19°28′	97°34′	2261	1	7	K(6) L(1)
13	México, Puebla, Tepeyahualco	PUE4	TMVB	19°29′	97°31′	2294	4	6	K(2) M(2) N(1) O(1)
14	México, Puebla, Tepeyahualco	PUE3	TMVB	19°30′	97°28′	2337	3	7	K(7)
15	México, Veracruz, Perote	VER	TMVB	19°32′	97°20′	2292	4	7	K(3) M(4)
16	México, Veracruz, Perote	VER1	TMVB	19°32′	97°28′	2334	5	7	K(7)
17	México, Puebla, Tepeyahualco	PUE2	TMVB	19°31′	97°25′	2357	4	7	K(5) L(1) M(1)
18	México, Puebla, Guadalupe Victoria	PUE10	TMVB	19°31′	97°25′	2357	8	10	K(9) L(1)
19	México, Puebla, Ciudad Serdán	PUE9	TMVB	18°55′	97°24′	2357	3	7	P(6) Q(1)
20	México, Tlaxcala, Cuapiaxtla	TLAX	TMVB	19°17′	97°30′	2384	3	7	K(7)
21	México, Edo. México, Ixtapaluca	MEX1	TMVB	19°20′	98°46′	2800	4	6	A(1) B(3) E(1) F(1)
22	México, Edo. México, Tepetlaoxtoc	MEX2	TMVB	19°33′	98°45′	2660	2	6	A(6)
23	México, Edo. México, Pirámides	MEX3	TMVB	19°45′	98°48′	2668	3	7	A(7)
24	México, Hidalgo, Tlanalapa	HID4	TMVB	19°50′	98°33′	2521	6	7	A(7)
25	México, Hidalgo, Zempoala	HID3	TMVB	19°56′	98°36′	2460	4	7	A(7)
26	México, Oaxaca, Matatlán	OAX2	SMS	16°49′	96°21′	2006	5	9	J(9)
27	México, Oaxaca, Ixtepeji	OAX3	SMS	17°12′	96°35′	2400	10	11	J(11)
28	México, Oaxaca, Sola	OAX1	SMS	16°27′	97°01′	1493	8	10	H(9) I(1)

n cpDNA, sample size of chloroplast markers; *n* nrDNA, sample size of the nuclear marker; n_a, number of distinct haplotypes found in individuals sampled, with the number of individuals per haplotype in parentheses.

published forward and reverse primers (Sass & Specht, 2010) were used to amplify multiple fragments; these fragments were gel extracted and sequenced and from these sequences a set of specific primers was designed to amplify a single *rpb*2 region for *Nolina*. The primers used were forward 'rpb2-Np.J.F' (CCA TTG GTC AGC TCA TTG AA) and reverse 'rpb2-Np.Q.R' (CTC TGA AAA GCA ACT GAA GGT T) for PCR amplification, and 'rpb2-877.F' (TGC TGA TTT GCC ATA CTT TAG C) and 'rpb2-878.R' (AAC ACC ATA ATC AAG GCA AAA A) for cycle sequencing. PCR conditions were the same as for *trn*L–F with an annealing temperature of 58 °C.

Prior to sequencing, PCR products were purified with exonuclease I and thermosensitive alkaline phosphatase to remove single-stranded primers and any remaining dNTPs (Fermentas International, Burlington, Ontario, Canada). PCR products were cycle-sequenced using PCR (chloroplast DNA) or sequencing (*rpb*2) primers and the ABI Prism BigDye Terminator Cycle Sequence Ready Reaction kit (ver. 3.1; Perkin-Elmer/Applied Biosystems, Foster City, CA, USA). Products of cycle-sequencing were visualized on an ABI Prism 3100 automated sequencer (Applied Biosystems).

Phylogenetic analysis

Bayesian inference (BI) analyses were used to infer phylogenetic relationships among the populations of *N. parviflora*, with the chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) analysed both separately and as a combined matrix. BI analyses were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). jModelTest 0.1.1 (Posada, 2008) was used to detect the model of molecular evolution that best fit the cpDNA (TrN+I) and nrDNA (F81+G) matrices under the Akaike information criterion (AIC). BI analyses were performed using two independent runs for each molecular dataset and the combined matrix to assess the repeatability of estimating stationarity between runs. For each run, we employed one cold and three heated chains for 30,000,000 generations, sampling one tree every 1000 generations. Sample points collected prior to stationarity (convergence of likelihood scores) were eliminated as the burn-in (10%). Posterior probabilities for supported clades were determined by a 50% majority-rule consensus of the trees retained after burn-in.

Divergence time estimates

Divergence times were estimated using the combined matrix with a mixed model Bayesian approach as implemented in BEAST 1.6.1 (Drummond & Rambaut, 2007). We used the HKY + I model of sequence evolution for the two chloroplast spacers (*psb*A–*trn*H and *trn*L–F) and the HKY + G model for the nuclear *rpb*2 dataset under an uncorrelated lognormal relaxed clock model. To model the tree, the Yule speciation process was used as a prior. To calibrate the root we used the dates obtained by Bell *et al.* (2010), and the estimated 24 Ma (standard deviation 12–30 Ma) for the divergence of *Nolina* from the remaining Asparagaceae genera. We used a normal distribution for the root calibration prior, following the recommendation of Ho & Phillips (2009). One independent 50^7 generation run was performed with random starting trees, sampling every 1000 generations. TRACER 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) was used to assess convergence and estimate effective sample sizes (ESS) for all parameters. Results were summarized in a single tree using TREEANNOTATOR 1.6.1 (Drummond & Rambaut, 2007) and visualized with FIGTREE 1.5.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Genetic structure and population differentiation

For the cpDNA data, parameters of population diversity, i.e. average within-population gene diversity (h_S), total gene diversity (h_T) and genetic differentiation over all populations (G_{ST}), as well as equivalent parameters (v_S, v_T, N_{ST}) were estimated using PERMUT (Pons & Petit, 1996), taking into account the evolutionary distances of recovered haplotypes. For the 28 populations analysed for the *rpb*2 nuclear maker, the level of differentiation (G_{ST}) was estimated for these samples using DNASP 5 (Librado & Rozas, 2009).

Statistical parsimony haplotype networks for the cpDNA data were generated with TCS 1.2.1 (Clement *et al.*, 2000) using the 95% connection probability limit, with gaps coded using the modified complex indel coding (MCIC) in SEQSTATE (Müller, 2005). Some ambiguities were detected in the networks (loops); these loops were deleted from the analysis according to three criteria (frequency, topology and geography) as proposed by Pfenninger & Posada (2002). The haplotype genealogy was reconstructed based only on the chloroplast sequence data, which is non-recombining and maternally inherited (Salzburger *et al.*, 2011). We did not use the nrDNA sequences to reconstruct the haplotype genealogy because recombination processes could influence our biogeographical interpretations of the nuclear marker (Zhang & Hewitt, 2003).

A spatial analysis of molecular variance (SAMOVA), implemented in SAMOVA 1.0 (Dupanloup *et al.*, 2002), was conducted in order to reconstruct groups of locations that are both geographically homogeneous and genetically differentiated from each other, maximizing the proportion of total genetic variance that is due to differences among groups of locations (F_{CT}). The most likely number of groups (K) was determined by repeatedly running the SAMOVA analysis with two to 12 groups and choosing those partitions with a maximum F_{CT} value, as suggested by Dupanloup *et al.* (2002). We explored K values with 100 simulated annealing simulations for each K.

Additionally, BARRIER 2.2 (Manni *et al.*, 2004) was used following Monmonier's algorithm (Monmonier, 1973) to predict geographical barriers between the *N. parviflora* localities. Zones of abrupt changes in the pattern of genetic variation among sample populations in the presence of isolating factors (geography) can be detected (Manni *et al.*, 2004) and are thought to indicate the presence of current or historical barriers to gene flow. The combined cpDNA matrix was transformed into a distance matrix using the F84 model of nucleotide substitution implemented in DNADIST, and SEQBOOT was used to generate 100 bootstrapped distance matrices from DNA sequences to evaluate support for the observed barriers; both programs are part of the PHYLIP 3.69 package (Felsenstein, 1989). Isolation by distance was tested by regressing pairwise estimates of $F_{ST}/(1-F_{ST})$ against the log-transformed geographical distance between localities (Rousset, 1997) with 10,000 permutations using TFPG 1.3 (Miller, 1997).

RESULTS

Sampling

We sequenced 210 individuals from 28 populations for the plastid markers. The total length of the aligned combined chloroplast DNA matrix (*trn*L–F and *psb*A–*trn*H) was 979 bp. Nineteen substitutions were detected and four indels of 1–56 bp were coded using MCIC as described above. An 8 bp inversion near the 3′ of the *psb*A–*trn*H was treated as a single evolutionary event by manually reversing the region for the alignment and coding the inversion as a separate character. For the nuclear *rpb*2, we obtained 124 sequences from 28 populations sampled, the aligned length was 776 bp and only 18 substitutions were detected. The GenBank accession numbers of the sequences generated from *N. parviflora* and outgroups are: *trn*H–*psb*A (JX178963–JX178986), *trn*L–F (JX178987–JX179010), and *rpb*2 (JX179011–JX179046).

Phylogenetic relationships

The Bayesian concatenated (cpDNA + nrDNA) 50% majority consensus tree recovered 69 unique terminals from the 28 *N. parviflora* populations forming two well-supported clades within *N. parviflora* (Fig. 2). Clade 1 (PP = 0.91) includes individuals from Jalisco (JAL) and Zacatecas (ZAC) populations and is sister to Clade 2 (PP = 1.0). Clade 2 includes the remaining populations and is largely unresolved with only a few well-supported subclades (Fig. 2). The individual cpDNA and *rpb*2 phylogenies (not shown) resulted in similar topology to the concatenated tree, recovering the same two main supported clades.

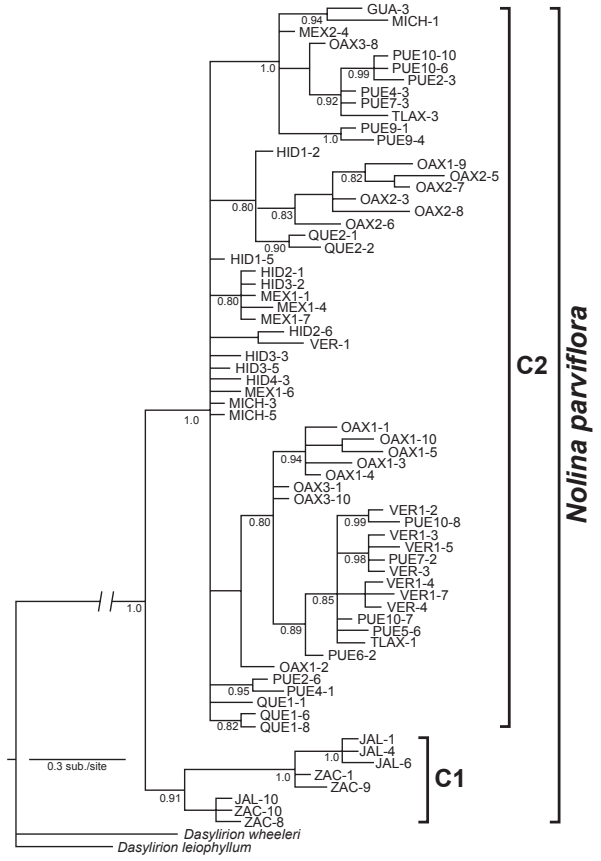

Figure 2 Bayesian 50% majority consensus tree based on concatenated chloroplast (*psb*A–*trn*H and *trn*L–F) and nuclear *rpb*2 gene markers, showing the relationships among populations of *Nolina parviflora* in Mexico. Two main clades are identified in brackets: C1 and C2. Numbers below the branches are Bayesian posterior probabilities (PP). Code names are the same as those used in Table 1.

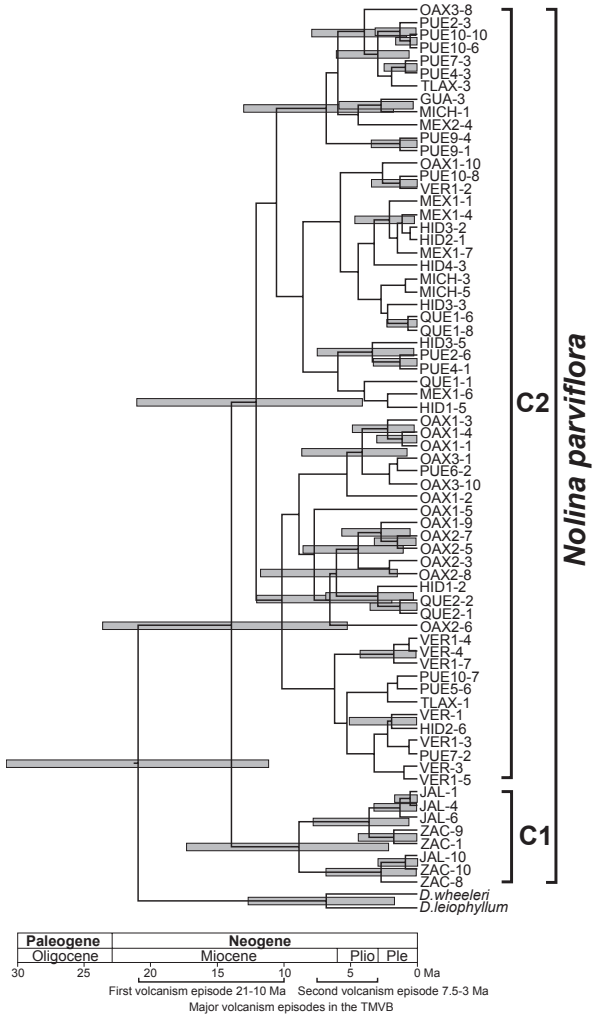

Figure 3 Chronogram based on a Bayesian approach to the *Nolina parviflora* phylogeography from concatenated chloroplast (*psb*A–*trn*H and *trn*L–F) and nuclear *rpb*2 gene markers in Mexico. Grey bars indicate 95% highest posterior density (HPD) intervals for node age estimates. Brackets identify Clades 1 and 2. Plio, Pliocene; Ple, Pleistocene; Ma, million years ago.

Divergence time estimates

Divergence time estimates for *N. parviflora* are shown in Fig. 3. The divergence of *Nolina* and *Dasylirion* occurred during the transition from the Oligocene to the mid-Miocene (mean = 20.9 Ma; 95% highest posterior density, HPD = 30.8–11.1), with subsequent divergences between *N. parviflora* populations occurring from the early Miocene to the Pleistocene (Fig. 3). Clade 1 and Clade 2 diverged from one another in the late Oligocene to late Pliocene (mean = 13.9 Ma; 95% HPD = 23.6–5.2). Divergence estimates for Clade 1 are from the mid-Miocene to the early Pleistocene (mean = 8.8 Ma; 95% HPD = 17.3–2.1). For Clade 2 divergence estimates are from the early Miocene to the early Pliocene (mean = 12.0 Ma; 95% HPD = 21.0–4.0) (Fig. 3). For major subclades within Clade 1 and Clade 2, estimates for population-level divergence range from the mid- or late Miocene to the Pleistocene, while for others divergence occurred during the Pliocene and into the Pleistocene (Fig. 3).

cpDNA haplotype relationships and geographical distribution

For the cpDNA data, we recovered 24 haplotypes within *N. parviflora*. The most common haplotypes, K and A, were recovered in 63 and 46 individuals, respectively (Fig. 4), representing almost 50% of all individuals sampled. The western Trans-Mexican Volcanic Belt (TMVBw) population has two haplotypes and the Sierra Madre Occidental (SMOc) contains five different haplotypes. These two regions share only a single haplotype between them, and they do not share haplotypes with populations sampled in any other regions. The central Trans-Mexican Volcanic Belt (TMVBc) has a total of five haplotypes and is the only region that shares haplotype A with the Sierra Madre Oriental (SMOr). The eastern Trans-Mexican Volcanic Belt (TMVBe) has the highest haplotype diversity, with a total of seven haplotypes recovered from its populations.

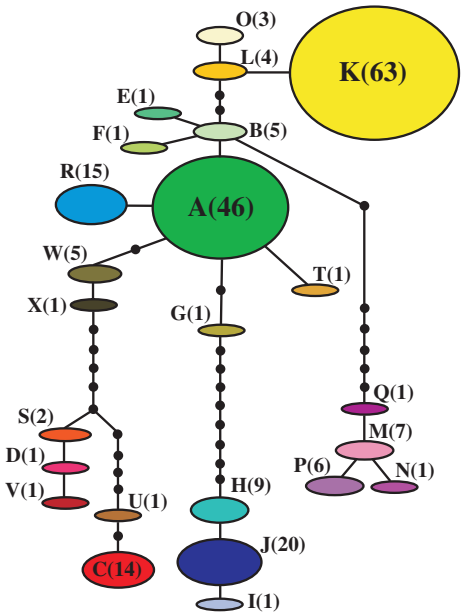

Figure 4 Statistical parsimony network of 24 cpDNA haplotypes found in *Nolina parviflora* in Mexico. A–X: sampled haplotypes. Solid black circles: hypothetical haplotypes. Numbers in parentheses indicate the number of individuals. See also Fig. 1.

SMOr populations have four haplotypes, and the Sierra Madre del Sur (SMS) populations have the least diversity with only three haplotypes recovered (Table 1, Fig. 1).

The haplotypes from SMOr and the central and eastern regions of the TMVB occupy the central part of the statistical parsimony network (Fig. 4). The haplotypes that occur in the western TMVB and in the western SMOc are separated by five predicted non-sampled haplotypes from the recovered SMOr and other eastern haplotypes. Eight non-sampled haplotypes separate the three haplotypes found in the southern populations of the SMS from Oaxaca, and surprisingly the haplotypes from the Puebla (PUE2, 4, 9) and Veracruz (VER (N, M, P and Q) populations are separated from their closest relatives by six non-sampled haplotypes. These populations are more closely related to haplotype B in the central region of the TMVB than to haplotypes K, L or O from the more proximal eastern region of the TMVB (Figs 1 & 4).

Genetic structure, population differentiation and spatial analyses

Differentiation among populations based on cpDNA variation (G_{ST} = 0.702, SE 0.0671) indicates a large degree of population genetic structure within *N. parviflora*. Within-population gene diversity (h_S) is 0.253 (SE 0.0505) and total genetic diversity (h_T) is 0.847 (SE 0.0396). The parameters v_S (0.181, SE 0.0476) and v_T (0.849, SE 0.749) are almost similar to h_S and h_T values. Allelic differentiation (N_{ST}) (0.787 SE 0.0553) is greater than and significantly different from G_{ST}, indicating that populations are strongly differentiated genetically and with some phylogeographical structure. Differentiation among populations based on nrDNA variation is G_{ST} = 0.295 with few haplotypes recovered.

SAMOVA results revealed a significant genetic and geographical structure among 11 groups of locations with a F_{CT} value of 0.838 ($P < 0.00001$) (Fig. 5). Barrier 2.2 estimated nine geographical boundaries (Fig. 5). The boundaries with the greatest support separate the Jalisco population (JAL) from the Zacatecas population (ZAC) (Barriers 1–2; Fig. 5). There is a barrier that separates the Guanajuato (GTO) from

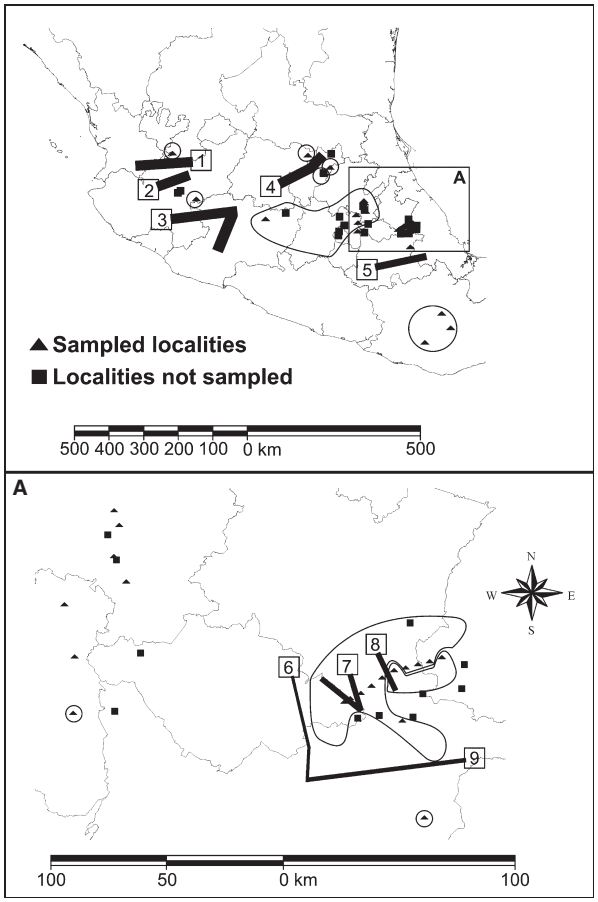

Figure 5 Geographical breaks identified in the distribution of *Nolina parviflora* in Mexico using Monmonier's algorithm. The lower panel is an enlargement of the area within the square labelled A in the top panel. The populations of *N. parviflora* sampled are indicated by filled triangles, with breaks as recovered by the software Barrier 2.2 (Manni *et al.*, 2004) indicated with black bars. The confidence level of the barrier is indicated by the weight of the line, with heavy lines indicating the best-supported breaks as determined by analyses run on boot-strapped distance matrices. Biogeographical barriers are those with numbers: (1) Santiago River, (2) Lerma-Santiago Basin, (3) Balsas Basin, (4) Rio Pánuco Basin, (5) Trans-Mexican Volcanic Belt and (6–9) unnamed barriers. The populations inside unfilled circles or irregular polygons are the 11 groups found by the SAMOVA analysis.

the Querétaro (QUE1 and 2) populations in the Sierra Madre Oriental (Barrier 4); the central region from the TMVBe; and there are several barriers that separate the individual populations that occur in the TMVBe (Barriers 6–9; Fig. 5). Finally, there is a barrier that separates the southernmost populations of the TMVBe from the Oaxaca populations in the Sierra Madre del Sur (Barrier 5; Fig. 5). The Mantel test revealed a significant correlation between the pairwise distances $F_{ST}/(1-F_{ST})$ and the geographical distances among populations ($r = 0.615$, $P = 0.0001$), suggesting a pattern of isolation by distance.

DISCUSSION

Haplotype diversity and geographical distribution

We found a high amount of genetic structure within *N. parviflora* populations inhabiting the TMVB, indicating that gene flow among the populations of *N. parviflora* is largely restricted geographically. Gene flow even appears to be limited within the montane region of the TMVB itself, indicating that habitat fragmentation inherent to this volcanic chain may act as a barrier to gene flow and ultimately increase the potential for TMVB populations to undergo diversification and speciation. We found only one individual from the Querétaro (QUE1) population in the Sierra Madre Oriental that shares haplotype A with the populations from the TMVBc (Fig. 1), and one haplotype (C) that is shared between the Jalisco (TMVBw) and Zacatecas (Sierra Madre Occidental) populations (Fig. 1). Otherwise, the populations distributed along the TMVB are grouped into three distinct regions (eastern, central and western) with no haplotypes shared among them (Fig. 1). Our findings in chloroplast haplotype distribution could support the hypothesis of Corona *et al.* (2007) that the TMVB may not be a single biogeographical entity, owing to its historically complex connections with other biogeographical regions of Mexico. Gómez-Tuena *et al.* (2007) divided the TMVB into three sectors or regions (east, central and west) with different geological ages and tectonic features. We found an almost perfect concordance between these three regions and our haplotype data (Fig. 1).

It is difficult to directly compare our results with those of previous plant phylogenetic or phylogeographical studies (Jaramillo-Correa *et al.*, 2008; Moreno-Letelier & Piñero, 2009; Aguirre-Planter *et al.*, 2012) of species (*Abies* and *Pinus*) that have some overlap between their distributions and those of *N. parviflora* populations because of the different markers used (mitochondrial and single sequence repeats, respectively). However, there is some concordance with the pattern we recovered and that found in previous studies, suggesting a strong east–west distribution of haplotypes and a clear separation of haplotypes between east and west indicating a lack of gene flow between these two regions (Jaramillo-Correa *et al.*, 2008; Moreno-Letelier & Piñero, 2009; Aguirre-Planter *et al.*, 2012). This same pattern is present in some phylogenetic and phylogeographical studies of animals (McCormack *et al.*, 2008, 2011; Bryson *et al.*, 2011a,b,c,d, 2012; Bryson & Riddle, 2012) and in the central–east pattern found by Parra-Olea *et al.* (2012). This east–west pattern of haplotype distribution is also corroborated by our combined cp and nrDNA phylogenetic analysis.

Additionally, we found significant genetic differentiation between *N. parviflora* to the north and south of the TMVB, indicating that the TMVB itself acts as a barrier to gene flow. This is consistent with the findings of previous studies whose authors proposed that the TMVB is a barrier to species distribution (Sosa *et al.*, 2009; Bryson *et al.*, 2011a,c, 2012; Ruiz-Sanchez *et al.*, 2012).

Temporal congruence with Trans-Mexican Volcanic Belt volcanism

Our divergence time estimates for populations of *N. parviflora* coincide with two major volcanic episodes along the TMVB, the first of which occurred 21–10 Ma and the second 7.5–3 Ma (García-Palomo *et al.*, 2002; Gómez-Tuena *et al.*, 2007) (Fig. 3). The estimated divergence of the western clade (Clade 1) from the eastern clade around the mid-Miocene to the beginning of the Pliocene coincides with these two episodes of volcanic activity in the TMVB (García-Palomo *et al.*, 2002; Gómez-Tuena *et al.*, 2007) (Fig. 3). Associated error must be kept in mind when using a secondary calibration point, such as the one we used in this study, along with the error bars around each node, as stressed by Graur & Martin (2004).

The eastern clade (Clade 2) diversified from the early Miocene to the mid-Pliocene, and some subclades diversified from the Pliocene to Pleistocene. These periods coincide with the fourth period of volcanism along the TMVB, which occurred *c.* 21–1.8 Ma (García-Palomo *et al.*, 2002; Rosas-Elguera *et al.*, 2003; Gómez-Tuena *et al.*, 2007) (see Fig. 2 in Gómez-Tuena *et al.*, 2007). Previous studies of lizards and salamanders are also consistent both spatially and temporally with the two volcanic episodes (Bryson *et al.*, 2011b) or with the last volcanic episode in the eastern TMVB (Parra-Olea *et al.*, 2012). The Neogene formation of the TMVB and the different volcanic episodes that resulted in the uplift of this mountain range are thought to have resulted in the diversification of several of the animal lineages (small mammals, birds, lizards, rattlesnakes, Mexican horned lizard) that are distributed in this region of central Mexico (Sullivan *et al.*, 1997; Demastes *et al.*, 2002; Edwards & Bradley, 2002; León-Paniagua *et al.*, 2007; McCormack *et al.*, 2008; Navarro-Sigüenza *et al.*, 2008; Bryson *et al.*, 2011c, 2012; Bryson & Riddle, 2012). Our study demonstrates that the volcanic episodes mentioned by Gómez-Tuena *et al.* (2007) are temporally concordant with the divergence of our two main clades and the diversification of several other subclades, and therefore could be important in the population-level diversification of *N. parviflora*.

Geographical barriers and isolation

The SAMOVA analysis indicated the presence of 11 distinct geographical groups (Fig. 5). According to the SAMOVA results, the Sierra Madre Oriental is divided into three distinct groups, the TMVB into five different groups, and the population of the Sierra Madre Occidental (ZAC) and the three populations from the Sierra Madre del Sur (OAX 1, 2 and 3) are recognized as individual groups.

Monmonier's algorithm as implemented in Barrier (Manni *et al.*, 2004) is a powerful tool for discovering geographical barriers that are likely to have played a role in preventing gene flow among extant populations. We found nine geographical barriers separating the 28 populations sampled. Barriers 1 and 2 (Fig. 5) are at the current locations of the Santiago River and Lerma-Santiago Basin that separate the population of Jalisco (JAL) from that of Zacatecas (ZAC) in the Sierra Madre Occidental. These same geographical barriers have been identified as defining the population structure of other taxa, including *Dendroctonus mexicanus*, *Pinus strobiformis*, *Aphelocoma ultramarina*, the *Crotalus triseriatus* species group and *Phrynosoma orbiculare* (reviewed in Bryson *et al.*, 2011c, 2012) (Fig. 5). Barrier 3 separates the populations of Jalisco (JAL) and Zacatecas (ZAC) from the southernmost Oaxaca populations (Fig. 5); this barrier coincides with the Balsas Basin, also identified as a barrier to gene flow for the *Crotalus triseriatus* species group, the *Peromyscus aztecus* species group, *Dendroctonus mexicanus, Buarremon*, and the *Neotoma mexicana* species group (reviewed in Bryson *et al.*, 2011c).

Geographical Barrier No. 4 was found to separate the Guanajuato (GTO) population from the Querétaro (QUE) population within the Sierra Madre Oriental (Fig. 5). These populations are separated by the Rio Pánuco Basin, a geological feature found to be important in preventing gene flow among the *Crotalus triseriatus* species group, and the *Dendroctonus mexicanus* and *Phrynosoma orbiculare* populations (reviewed in Bryson *et al.*, 2011c, 2012). Populations from the central and eastern TMVB are separated from the Oaxaca populations in the Sierra Madre del Sur (Fig. 5) by Barrier 5. A similarly located barrier was found to separate the northern populations of *Hunnemannia fumariifolia* from the southern populations in Oaxaca (Ruiz-Sanchez *et al.*, 2012), indicating a lack of gene flow between these regions.

Barriers 6–9, which separate populations of *Nolina parviflora* that span the TMVB, have been identified in a few other biogeographical studies (Bryson *et al.*, 2011a,c). These are likely to result from the effect of the varied types of habitat that occur along the TMVB acting as barriers to gene flow among populations located within the TVMB ecosystem; it is notable that this pattern was also recovered in studies that sampled extensively within naturally fragmented ecosystems (e.g. Sosa *et al.*, 2009; Bryson *et al.*, 2011a,c, 2012; Ruiz-Sanchez *et al.*, 2012). The main boundaries defined by the SAMOVA and Barrier analyses seem to fit the west–east and north–south scenarios of genetic discontinuities among populations between regions.

Tests of genetic differentiation (G_{ST} = 0.702) indicate a high degree of geographical structure among *N. parviflora* populations. These data, when combined with the SAMOVA results and the genetic barrier analyses, indicate that the complex topography of the TMVB results in a high degree of habitat heterogeneity and environmental diversity, promoting isolation and the opportunity for speciation and diversification (Jetz *et al.*, 2004). Our distribution-wide study of *N. parviflora* revealed remarkable variation in genetic structure among populations for both chloroplast (G_{ST} = 0.702) and nuclear (G_{ST} = 0.295) markers, the G_{ST} values of which are quite high, especially when compared with the genetic variation observed for populations of other monocotyledonous species (e.g. see Appendices S1 and S2 in Petit *et al.*, 2005). The differences between the cpG_{ST} and nrG_{ST} values could reflect differences in pollen-mediated versus seed-mediated gene flow (Ennos, 1994). The inflated fruits of *N. parviflora* are thought to be wind-dispersed while the pollen grains are thought to be animal-dispersed, by Hymenoptera and Diptera (Trelease, 1911), indicating the potential for more efficient animal-mediated pollination (i.e. pollen-mediated gene flow) than wind-mediated fruit dispersal (i.e. seed-mediated gene flow).

Our phylogenetic, SAMOVA and geographical barriers do not refute the idea of different lineages being defined from within the cluster currently described as *N. parviflora*. Closer observation yields discrete morphological differences between the populations of the TMVBw and SMOc and the other populations (E. Ruiz-Sanchez, pers. obs.); however, a detailed morphological analysis is required to support the recognition of additional species.

CONCLUSIONS

Our study of the phylogeography of *Nolina parviflora* suggests that the Trans-Mexican Volcanic Belt played a major role in driving diversification and limiting gene flow among the populations of this species throughout this mountain range and between populations distributed in the Sierra Madre Occidental, Oriental and del Sur. There is a correlation between the different periods of uplift in the TMVB and the diversification times of *N. parviflora*, indicating that geological changes could have influenced genetic differentiation in this species, potentially causing allopatric fragmentation between the populations in the west and the central–eastern and southern populations, as corroborated by the isolation-by-distance results. Combined, our results indicate that there is the potential for the description of new species within the *N. parviflora* lineage, although to confirm this, a detailed morphological analysis of the populations is required.

ACKNOWLEDGEMENTS

We thank Ismael Valdivieso, Arturo de Nova, Etelvina Gándara, Pablo Carrillo, Raymundo Ramírez, Diego Angulo and Flor Rodríguez for field assistance; Heather Driscoll,

Roxana Yockteng, Ana Almeida and Tanya Renner for laboratory assistance; and Juan Francisco Ornelas for helpful comments on previous versions of the manuscript. We also thank Pauline Ladiges and three anonymous referees whose suggestions greatly improved the manuscript. Special thanks to Pablo Carrillo for providing samples for the Michoacán and Guanajuato localities, and to Francisco and Maria Rodríguez for their hospitality and Taqueria Las Vegas for providing burritos to E.R-S. during his stay in CA. E.R-S. was supported by a UC-MEXUS and Consejo Nacional de Ciencia y Tecnología (CONACyT) post-doctoral fellowship, and research funding and support was provided by UC Berkeley College of Natural Resources and the University and Jepson Herbaria.

REFERENCES

Aguirre-Planter, E., Jaramillo-Correa, J.P., Gómez-Acevedo, S., Khasa, D.P., Bousquet, J. & Eguiarte, L.E. (2012) Phylogeny, diversification rates and species boundaries of Mesoamerican firs (*Abies*, Pinaceae) in a genus-wide context. *Molecular Phylogenetics and Evolution*, **62**, 263–274.

Bell, C.D., Soltis, D.E. & Soltis, P.S. (2010) The age and diversification of the angiosperms re-revisited. *American Journal of Botany*, **97**, 1296–1303.

Bryson, R.W., Jr & Riddle, B.R. (2012) Tracing the origins of widespread highland species: a case of Neogene diversification across the Mexican sierras in an endemic lizard. *Biological Journal of the Linnean Society*, **105**, 382–394.

Bryson, R.W., Jr, García-Vázquez, U.O. & Riddle, B.R. (2011a) Phylogeography of Middle American gopher-snakes: mixed responses to biogeographical barriers across the Mexican Transition Zone. *Journal of Biogeography*, **38**, 1570–1584.

Bryson, R.W., Jr, García-Vázquez, U.O. & Riddle, B.R. (2011b) Relative roles of Neogene vicariance and Quaternary climate change on the historical diversification of bunchgrass lizards (*Sceloporus scalaris* group) in Mexico. *Molecular Phylogenetics and Evolution*, **62**, 447–457.

Bryson, R.W., Jr, Murphy, R.W., Lathrop, A. & Lazcano-Villareal, D. (2011c) Evolutionary drivers of phylogeographical diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of montane rattlesnakes. *Journal of Biogeography*, **38**, 697–710.

Bryson, R.W., Jr, Murphy, R.W., Graham, M.R., Lathrop, A. & Lazcano, D. (2011d) Ephemeral Pleistocene woodlands connect the dots for highland rattlesnakes of the *Crotalus intermedius* group. *Journal of Biogeography*, **38**, 2299–2310.

Bryson, R.W., Jr, García-Vázquez, U.O. & Riddle, B.R. (2012) Diversification in the Mexican horned lizard *Phrynosoma orbiculare* across a dynamic landscape. *Molecular Phylogenetics and Evolution*, **62**, 87–96.

Carstens, B.C. & Richards, C.L. (2007) Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution*, **61**, 1439–1454.

Cavender-Bares, J., Gonzalez-Rodriguez, A., Pahlich, A., Koehler, K. & Deacon, N. (2011) Phylogeography and climatic niche evolution in live oaks (*Quercus* series *Virentes*) from the tropics to the temperate zone. *Journal of Biogeography*, **38**, 962–981.

Chase, M.W. & Hills, H.G. (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon*, **40**, 215–220.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Corona, A.M., Toledo, V.H. & Morrone, J.J. (2007) Does the Trans-Mexican Volcanic Belt represent a natural biogeographic unit? An analysis of the distributional patterns of Coleoptera. *Journal of Biogeography*, **34**, 1008–1015.

Demastes, J.W., Spradling, T.A., Hafner, M.S., Hafner, D.J. & Reed, D.L. (2002) Systematics and phylogeography of pocket gophers in the genera *Cratogeomy*s and *Pappogeomys*. *Molecular Phylogenetics and Evolution*, **22**, 144–154.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Dupanloup, I., Schneider, S. & Excoffier, L. (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Edwards, C.W. & Bradley, R.D. (2002) Molecular systematics and historical phylobiogeography of the *Neotoma mexicana* species group. *Journal of Mammalogy*, **83**, 20–30.

Edwards, K., Johnstone, C. & Thompson, C. (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research*, **19**, 1349.

Ennos, R.A. (1994) Estimating the relative rates of pollen and seed migration among plant-populations. *Heredity*, **72**, 250–259.

Felsenstein, J. (1989) PHYLIP – Phylogeny Inference Package (Version 3.2). *Cladistics*, **5**, 164–166.

García-Palomo, A., Macías, J., Tolson, G., Valdez, R. & Mora, J.C. (2002) Volcanic stratigraphy and geological evolution of the Apan region, east-central sector of the Trans-Mexican Volcanic Belt. *Geofísica Internacional*, **41**, 133–150.

Gómez-Tuena, A., Orozco-Esquivel, M.T. & Ferrari, L. (2007) Igneous petrogenesis of the Trans-Mexican Volcanic Belt. *Geological Society of America Special Paper*, **422**, 129–181.

Graur, D. & Martin, W. (2004) Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics*, **20**, 80–86.

Halffter, G. (1987) Biogeography of the montane entomofauna of Mexico and Central America. *Annual Review of Entomology*, **32**, 95–114.

Ho, S.Y.W. & Phillips, M.J. (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology*, **58**, 367–380.

Jaramillo-Correa, J.P., Aguirre-Planter, E., Khasa, D.P., Eguiarte, L.E., Piñero, D., Furnier, G.R. & Bousquet, J. (2008) Ancestry and divergence of subtropical montane forest isolates: molecular biogeography of the genus *Abies*

(Pinaceae) in southern Mexico and Guatemala. *Molecular Ecology*, **17**, 2476–2490.

Jetz, W., Rahbek, C. & Colwell, R.K. (2004) The coincidence of rarity and richness and the potential signature of history in centres of endemism. *Ecology Letters*, **7**, 1180–1191.

Kim, J.-H., Kim, D.-K., Forest, F., Fay, M.F. & Chase, M.W. (2010) Molecular phylogenetics of Ruscaceae *sensu lato* and related families (Asparagales) based on plastid and nuclear DNA sequences. *Annals of Botany*, **106**, 775–790.

Konieczny, A. & Ausubel, F.M. (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. *The Plant Journal*, **4**, 403–410.

León-Paniagua, L., Navarro-Sigüenza, A.G., Hernández-Baños, B.E. & Morales, J.C. (2007) Diversification of the arboreal mice of the genus *Habromys* (Rodentia: Cricetidae: Neotominae) in the Mesoamerican highlands. *Molecular Phylogenetics and Evolution*, **42**, 653–664.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Manni, F., Guérard, E. & Heyer, E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology*, **76**, 173–190.

McCormack, J.E., Peterson, A.T., Bonaccorso, E. & Smith, T.B. (2008) Speciation in the highlands of Mexico: genetic and phenotypic divergence in the Mexican jay (*Aphelocoma ultramarina*). *Molecular Ecology*, **17**, 2505–2521.

McCormack, J.E., Heled, J., Delaney, K.S., Peterson, A.T. & Knowles, L.L. (2011) Calibrating divergence times on species trees versus gene trees: implications for speciation history of *Aphelocoma* jays. *Evolution*, **65**, 184–202.

Metcalfe, S.E. (2006) Late Quaternary environments of the northern deserts and central Transvolcanic Belt of Mexico. *Annals of the Missouri Botanical Garden*, **93**, 258–273.

Miller, M. (1997) *Tools for Population Genetics Analysis (TFPGA), version 1.3. A Windows® program for the analysis of allozyme and molecular population genetic data*. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

Monmonier, M.S. (1973) Maximum-difference barriers: an alternative numerical regionalization method. *Geographical Analysis*, **5**, 245–261.

Moreno-Letelier, A. & Piñero, D. (2009) Phylogeographic structure of *Pinus strobiformis* Engelm. across the Chihuahuan Desert filter-barrier. *Journal of Biogeography*, **36**, 121–131.

Morrone, J.J. (2010) Fundamental biogeographic patterns across the Mexican Transition Zone: an evolutionary approach. *Ecography*, **33**, 355–361.

Müller, K. (2005) SeqState: primer design and sequence statistics for phylogenetic DNA data sets. *Applied Bioinformatics*, **4**, 65–69.

Navarro-Sigüenza, A.G., Peterson, A.T., Nyari, A., García-Deras, G. & García-Moreno, J. (2008) Phylogeography of the *Buarremon* brush-finch complex (Aves, Emberizidae) in Mesoamerica. *Molecular Phylogenetics and Evolution*, **47**, 21–35.

Ornelas, J.F., Ruiz-Sánchez, E. & Sosa, V. (2010) Phylogeography of *Podocarpus matudae* (Podocarpaceae): pre-Quaternary relicts in northern Mesoamerican cloud forests. *Journal of Biogeography*, **37**, 2384–2396.

Parra-Olea, G., Windfield, J.C., Velo-Antón, G. & Zamudio, K.R. (2012) Isolation in habitat refugia promotes rapid diversification in a montane tropical salamander. *Journal of Biogeography*, **39**, 353–370.

Petit, R.J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D. & Vendramin, G.G. (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, **14**, 689–701.

Pfenninger, M. & Posada, D. (2002) Phylogeographic history of the land snail *Candidula unifasciata* (Helicellinae, Stylommatophora): fragmentation, corridor migration, and secondary contact. *Evolution*, **56**, 1776–1788.

Pons, O. & Petit, R.J. (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144**, 1237–1245.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Rosas-Elguera, J., Alva-Valdivia, L.M., Goguitchaichvili, A., Urrutia-Fucugauchi, J., Ortega-Rivera, M.A., Salinas Prieto, J.C. & Lee, J.K.W. (2003) Counterclockwise rotation of the Michoacan Block: implications for the tectonics of western Mexico. *International Geology Review*, **45**, 814–826.

Rousset, F. (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation-by-distance. *Genetics*, **145**, 1219–1228.

Ruiz-Sanchez, E., Rodriguez-Gomez, F. & Sosa, V. (2012) Refugia and geographic barriers of populations of the desert poppy, *Hunnemannia fumariifolia* (Papaveraceae). *Organisms Diversity and Evolution*, **26**, 991–1010.

Salzburger, W., Ewing, G.B. & von Haeseler, A. (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, **20**, 1952–1963.

Sang, T., Crawford, D.J. & Stuessy, T.F. (1997) Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany*, **84**, 1120–1136.

Sass, C. & Specht, C.D. (2010) Phylogenetic estimation of the core bromelioids with an emphasis on the genus *Aechmea*. *Molecular Phylogenetics and Evolution*, **55**, 559–571.

Seberg, O., Petersen, G., Davis, J.I., Pires, C.P., Stevenson, D.W., Chase, M.W., Fay, M.F., Devey, D.S., Jørgensen, T., Sytsma, K.J. & Pillon, Y. (2012) Phylogeny of the Asparagales based on three plastid and two mitochondrial genes. *American Journal of Botany*, **99**, 875–889.

Sosa, V., Ruiz-Sanchez, E. & Rodriguez-Gomez, F.C. (2009) Hidden phylogeographic complexity in the Sierra Madre

Oriental: the case of the Mexican tulip poppy *Hunnemannia fumariifolia* (Papaveraceae). *Journal of Biogeography*, **36**, 18–27.

Sullivan, J., Markert, J.A. & Kilpatrick, C.W. (1997) Phylogeography and molecular systematics of the *Peromyscus aztecus* group (Rodentia: Muridae) inferred using parsimony and likelihood. *Systematic Biology*, **46**, 426–440.

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**, 1105–1109.

Tate, J.A. & Simpson, B.B. (2003) Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany*, **28**, 723–737.

Trelease, W. (1911) The desert group Nolineae. *Proceedings of the American Philosophical Society*, **50**, 404–443.

Zhang, D.-X. & Hewitt, G.M. (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, **12**, 563–584.

BIOSKETCHES

Eduardo Ruiz-Sanchez is a full-time researcher and professor at the Instituto de Ecología A.C., Centro Regional del Bajío, Pátzcuaro, Michoacán. His research interests include the systematics and evolution of Neotropical bamboos and the phylogeography of Mexican endemic plants.

Chelsea D. Specht is an associate professor in the Department of Plant and Microbial Biology and Curator of the monocot collections in the University and Jepson Herbaria at the University of California, Berkeley. Her research interests include the systematics and evolution of monocots, particularly Zingiberales, in addition to Mexican and California native plants.

Editor: Pauline Ladiges