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BRIEF COMMUNICATION

High phylogeographic and genetic diversity of *Tidestromia lanuginosa* supports full-glacial refugia for arid-adapted plants in southern and central Coahuila, Mexico

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PREMISE: Recent phylogeographic work suggests the existence of latitudinal gradients in genetic diversity in northern Mexican plants, but very few studies have examined plants of the Chihuahuan Desert. *Tidestromia lanuginosa* is a morphologically variable annual species whose distribution includes the Chihuahuan Desert Region. Here we undertook phylogeographic analyses of chloroplast loci in this species to test whether genetic diversity and differentiation of Mexican populations of *T. lanuginosa* change along a latitudinal gradient and whether diversity is higher in Coahuila, consistent with ideas of lower plant community turnover during the Pleistocene.

METHODS: Haplotype network, maximum likelihood tree, and Bayesian phylogenetic haplotype were reconstructed, and genetic diversity was assessed among 26 populations. Barrier analysis was used to explore barriers to gene flow.

RESULTS: Four major population groups were identified, corresponding with physiographic provinces in Mexico. Each population group displayed high levels of genetic structure, haplotype, and nucleotide diversity. Diversity was highest in southern populations across the species as a whole and among the Chihuahuan Desert populations.

CONCLUSIONS: *Tidestromia lanuginosa* provides an important example of high phylogeographic and genetic diversity in plants of northern Mexico. Barriers to gene flow among the major population groups have most likely been due to a combination of orographic, climatic, and edaphic variables. The high genetic diversity of *T. lanuginosa* in southern and central Coahuila is consistent with the hypothesis of full-glacial refugia for arid-adapted plants in this area, and highlights the importance of this region as a center of diversity for the Chihuahuan Desert flora.

KEY WORDS Amaranthaceae; Chihuahuan Desert; Cuatro Ciénegas; gene flow; genetic diversity; genetic structure; Gomphrenoideae; Mexican physiographic provinces; Pleistocene refugia; *Tidestromia lanuginosa*.

With approximately 10% of the world's plant species, Mexico represents one of the richest countries botanically (Rzedowski, 1991; Rodríguez and López-Toledo, 2016). While overall plant species richness is highest in the wetter ecosystems of Mexico, endemism is much higher in the drier regions (Rzedowski, 1991; Meiners and Hernández-López, 2007). For example, the arid and semiarid regions of Mexico and adjacent parts of the United States are home to numerous endemic genera and even

a few endemic families (e.g., Fouquieriaceae, Koeberliniaceae; Rzedowski, 1991).

Despite this wealth of botanical diversity, our understanding of Mexican plant phylogeographic diversity has only begun to develop over the last decade. These studies have demonstrated relatively high levels of genetic diversity and structure (e.g., Aguilar-Meléndez et al., 2009; Gutiérrez-Rodríguez et al., 2011; Colin and Eguiarte, 2016). Very few studies, however, have examined plant phylogeography in the Chihuahuan Desert region of north central Mexico (e.g., Aguirre-Liguori et al., 2014; Angulo et al., 2014; Loera et al., 2017). The Chihuahuan Desert is geographically the largest and possesses the highest species richness of the warm deserts of North America (Morafka, 1977; Hoyt, 2002; Granados-Sánchez et al., 2011). The greater Chihuahuan Desert region hosts 826 endemic taxa, with only 116 from non-arid habitats (Villarreal-Quintanilla et al., 2017). Geologically, the Chihuahuan Desert region is characterized by a basin-and-range topography with typically calcareous mountain ranges surrounding basins that often contain salt playas, although igneous intrusions and gypsum exposures are also present throughout the region (Hoyt, 2002). In fact, many Chihuahuan Desert endemic taxa are adapted to grow in these unusual edaphic conditions such as gypsum and saline soils, but of these, only the gypsum endemic species *Fouquieria shrevei* I.M.Johnst. has been explored phylogeographically (Aguirre-Liguori et al., 2014).

Paleobotanical evidence, especially from packrat middens, indicates that significant shifts in vegetation occurred in the Chihuahuan Desert region during glacial/interglacial periods of the Pleistocene, leading earlier researchers to suggest that many arid-adapted taxa may have gone locally extinct or become restricted to one or more refugia during glacial maxima (Van Devender, 1986; Hernández and Bárcenas, 1995). Some studies (Sosa et al., 2009; Loera et al., 2017; Scheinvar et al., 2017) have suggested the existence of latitudinal gradients in the diversity of arid-adapted plants of northern Mexico, explained in part by higher diversity in southern regions due to the existence of climatic refugia during full-glacial periods, from which these desertscrub species expanded northward during interglacial periods (Van Devender, 1986). Similar elevational shifts in species distribution during glacial–interglacial cycles of the Pleistocene have been reported for *Agave lechuguilla* Torr. (Scheinvar et al., 2017), *Hunnemannia fumariifolia* Sweet (Sosa et al., 2009), and *Pinus strobiformis* Engelm. (Moreno-Letelier and Piñero, 2009) from the Chihuahuan Desert. The climatic fluctuations of the Pleistocene also provided opportunities for reproductive isolation and even speciaton (Van Devender, 1986). The specific effects of these climate shifts on the genetic diversity of individual species depends on the ecological affinities and distribution ranges of each, with arid-adapted taxa contracting into more arid microclimates during full-glacial episodes and more mesic-adapted taxa becoming isolated on montane sky islands during interglacial periods (Moreno-Letelier et al., 2009). In any case, levels and patterns of genetic diversity were likely significanctly affected by the repeated glacial–interglacial cycles of the Pleistocene (Gámez and Castellanos-Morales, 2019).

The few phylogeographic studies that have been done in plants from the Chihuahuan Desert suggest that significant geographic variation and structure exists, even over relatively small distances, and particularly in the Mexican portions of the desert (Aguirre-Liguori et al., 2014). Many widespread taxa in the Chihuahuan Desert region are morphologically variable (e.g., Yang, 1967; Echelle and Echelle, 1986; Macdonald et al., 2011), and it largely remains to be tested whether such variation is correlated with phylogeographic structure.

The broad geographic distribution and morphological variation of the herbaceous, arid-adapted *Tidestromia lanuginosa* (Nutt.) Standl. (Gomprhenoideae, Amaranthaceae) make it an excellent system for exploring patterns of plant phylogeography in the arid and semiarid regions of northern Mexico. *Tidestromia* Standl. comprises a clade of eight herbaceous to suffruticose, C_4 photosynthetic species distributed in the arid regions of northern Mexico and the southwestern United States, with a center of diversity in the Chihuahuan Desert, and especially in Coahuila, where a majority of the species can be found (Henrickson, 1993; Sánchez-del Pino and Motley, 2010; Bena et al., 2017). *Tidestromia lanuginosa* is the most widely distributed species in the genus, occurring in arid and semiarid habitats throughout northern Mexico and the southwestern United States, with disjunct populations in the Dominican Republic (Robertson, 1981; Sánchez-del Pino and Flores-Olvera, 2006). This species is found in association with various types of substrates including calcareous soils, gypsum, granite, sand, and saline soils, among others (Sánchez-del Pino, 2001) and is common in disturbed areas such as roadsides (Sánchez-del Pino, 2007). *Tidestromia lanuginosa* also contains significant anatomical variation in leaves, stems, and roots, and in the concentrations of tannins and mucilage (Bucio, 2008), even over relatively small geographic distances. For example, the number of leaf trichomes and stomata differed between samples from Lampazos, Nuevo León and those from Saltillo, Coahuila (Valencia, 2009).

We used chloroplast loci and phylogeographic methods to test whether genetic diversity in the widespread, variable species *Tidestromia lanuginosa* exhibits a latitudinal gradient in northern Mexico, with higher diversity farther south. Within the Chihuahuan Desert proper, we further hypothesized that diversity would be highest in southern Coahuila because this region is known to have experienced lower rates of plant community turnover during the peak of the last full-glacial period because of its relatively southern position within the Chihuahuan Desert (Van Devender and Burgess, 1985; Van Devender, 1990). Chloroplast DNA may be highly valuable in phylogeographic studies because it is generally uniparentally inherited, has relatively low mutation rates, and is haploid (Avise, 2000; Greiner et al., 2014; Hu et al., 2019). Because of its maternal inheritance through seeds, cpDNA often shows a more highly geographical structure than the nuclear genome (Yuan, 2008; Bai et al., 2014), and cpDNA markers have been successfully used to document phylogeographic patterns in many plant species (e.g., Petit et al., 2005; Garrick et al., 2015).

MATERIALS AND METHODS

Population sampling

We sampled 122 individuals from 26 populations of *Tidestromia lanuginosa* in the Mexican states of Chihuahua, Coahuila, Nuevo León, Tamaulipas, and San Luis Potosí to represent the geographic distribution and morphological variation documented for the species in Mexico (Fig. 1A). Leaves of 10–20 individuals per population

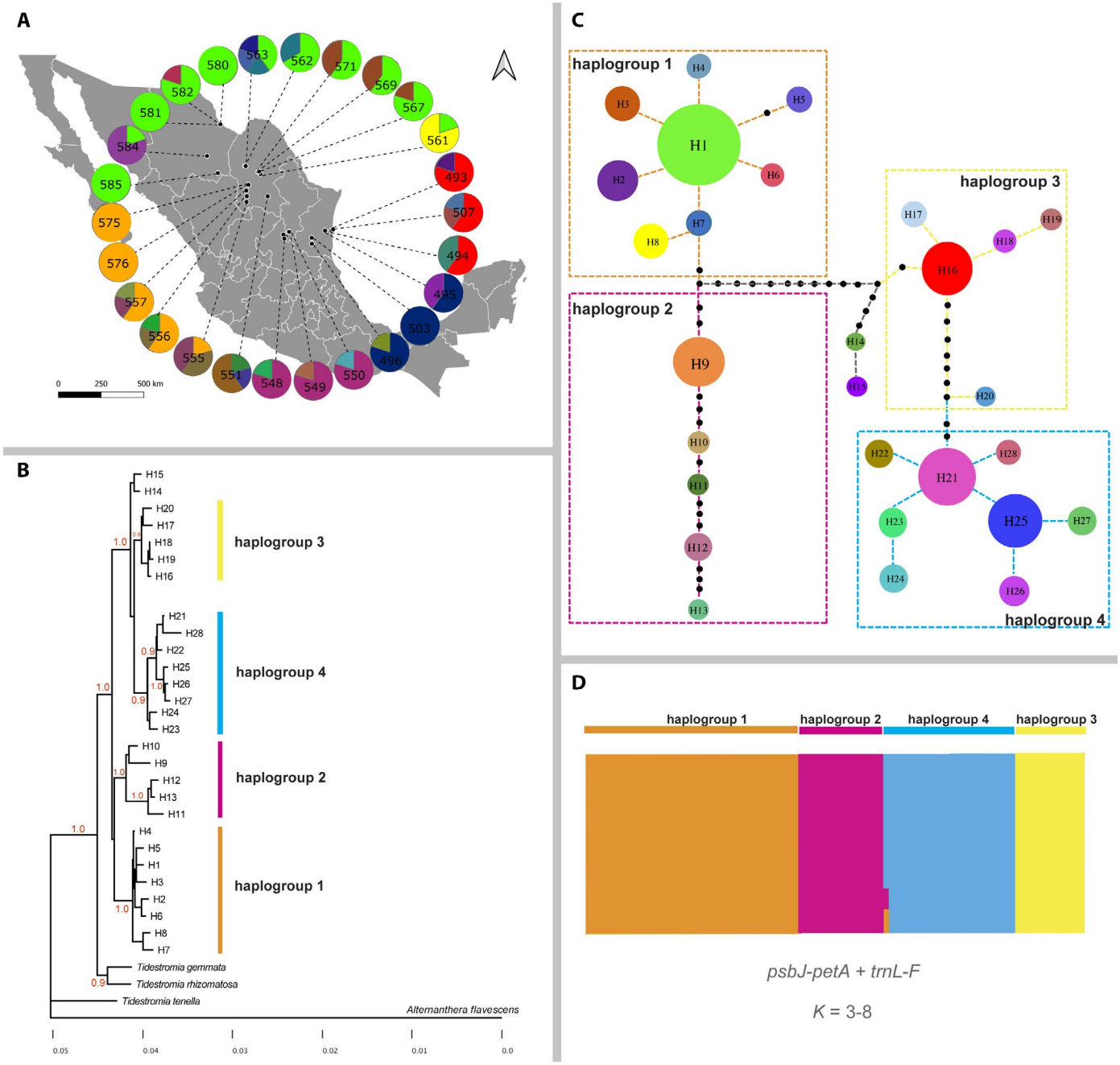


FIGURE 1. (A) Map of the collection localities for *Tidestromia lanuginosa* in Mexico (taken and edited using Google Earth, 2017), showing distribution of the 28 haplotypes found for 26 populations of *Tidestromia lanuginosa*. (B) Bayesian-derived tree for chloroplast haplotypes of *Tidestromia lanuginosa*, indicating the four main haplogroups. Posterior probability values ≥ 0.9 in the consensus tree are in red. (C) Haplotype network showing relationships among the 28 haplotypes and four haplogroups inferred for *Tidestromia lanuginosa* in Mexico. The sizes of circles are approximately proportional to sample size. (D) Results of Bayesian analysis of population genetic structure (BAPS) based on *psbJ-petA* and *trnL-F* sequences ($K = 3–8$).

were collected in silica gel (Appendix S1), but we included only up to five individuals per population because González (2015) showed that such sampling was sufficient to represent population variation. Four closely related taxa (*T. gemmata* I.M.Johnst., *T. rhizomatosa* I.M.Johnst., *T. tenella* I.M.Johnst., and *Alternanthera flavescens* Kunth) were selected as outgroups based on recent phylogenetic studies (e.g., Sánchez-del Pino et al., 2009; Sánchez-del Pino and Motley, 2010). Collecting and voucher information is provided in Appendix 1.

DNA extraction, amplification, and sequencing

Total DNA was extracted using the Qiagen Plant DNeasy kit (Qiagen, Valencia, CA, USA) and the manufacturer's protocol.

The plastid *trnL-F* intron and spacer region and the *psbJ-petA* spacer region were sequenced based on previous studies that demonstrated their informativeness in addressing phylogenetic relationships, including in *Tidestromia* (Shaw et al., 2007; Sánchez-del Pino and Motley, 2010; Aguirre-Liguori et al., 2014). The *trnL-F* region was amplified and sequenced using primers "c" and "f" of Taberlet et al. (1991), whereas the *psbJ-petA* spacer region was amplified using primers "*psbJ*" and "*petA*" from Shaw et al. (2007). The PCR conditions of Taberlet et al. (1991) and Shaw et al. (2007) were utilized with the following modifications: a total volume of 20 µl of reaction mixture contained 1× reaction buffer, 12.5 pmol dNTPs, 10 pmol of each primer, 1 U *Taq* DNA polymerase (Qiagen) and 10 ng DNA template. All PCRs were run on a flexid Mastercycler nexus gradient (Eppendorf AG, Germany). The thermocycler conditions for *trnL-F* were based on Sánchez-del Pino et al. (2009) and for *psbJ-petA* were based on those for *rpl16* from Shaw and Small (2005), with a slight modification to remove a ramp step: initial denaturation at 80°C for 5 min; 30 cycles at 95°C for 1 min, 50°C for 1 min, and 65°C for 1 min; 65°C for 5 min, hold at 4°C.

PCR products were purified by Macrogen (Seoul, Korea) using ExoSAP-IT (USB Corp., Cleveland, OH, USA), and forward and reverse strands were sequenced by Macrogen using an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were assembled and edited using Sequencher v. 4.1.4. (Gene Codes Corp., Ann Arbor, MI, USA). Edited sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in Geneious version 8.1.6 (Kearse et al., 2012), with default parameters, and by visual examination using PhyDE (Phylogenetic Data Editor) v. 0.9971 (Müller et al., 2010). The sequences reported in this study were deposited in GenBank (Appendix 1).

Genetic diversity and haplotype analyses

A haplotype network was built using the program TCS v. 1.21 (Templeton et al., 1987; Clement et al., 2000), considering gaps as a fifth state. We corroborated the network with a maximum likelihood (ML) tree that was estimated using the program MEGA 7 (Kumar et al., 2015), with 1000 bootstrap replicates. The program Arlequin v. 3.5 (Excoffier and Lischer, 2010) was used to quantify nucleotide (π; Nei, 1987; Nei and Miller, 1990) and haplotype diversity (Hd; Nei, 1987) among the population groups identified in the ML tree and haplotype network and across all groups and per population. Rarefaction analyses were used to determine the genetic variation with respect to the number of individuals sampled and genotyped between phylogeographic areas (*Tidestromia lanuginosa* from the northern and the southern parts of the Chihuahuan Desert) and species (*T. lanuginosa* and *Agave lechuguilla*). We used the software online "iNEXT" (Chao et al., 2016) to obtain the rarefaction curves, with 100 randomizations and extrapolating to 146 individuals. The produced rarefaction curve is a result of repeatedly re-sampling (generally without replacement) *N* individuals or samples at random plotting the average number of species represented by *N* individuals or samples (Gotelli and Colwell, 2001). The differences between two samples are clearly not significant when the 95% confidence interval of the haplotype richness estimates overlap (Colwell et al., 2004; Gotelli and Colwell, 2011).

Phylogeographic structure

Genetic structure was evaluated using three approaches. First, SAMOVA (Dupanloup et al., 2002) was used to infer the spatial structure of genetic variation. This program identifies groups of locations that are geographically homogeneous and genetically differentiated from each other, maximizing the proportion of total genetic variance due to differences between groups of populations (F_{CT}). We used 100 simulated annealing processes for each value of *K* from 2 and 10.

The second method employed a Bayesian clustering approach as implemented in BAPS (Bayesian Analysis of Population Structure; Corander et al., 2013) to group the individuals into *K* populations. In the population mixture analysis, 10 independent simulations were run for each value of *K* ($K = 3$, $K = 4$, $K = 5$, $K = 6$, $K = 7$, K = 8), and the *P* value was fixed to 0.05. These mixture results were then run using the admixture model, with the following settings: a minimum population size of one, 100,000 iterations, 100 iterations to estimate the admixture coefficient for reference individuals, and 300 iterations for reference individuals from each location. The third method employed N_{ST} and G_{ST}, as calculated in DnaSP version 6 (Rozas et al., 2017). The estimator N_{ST} is analogous to F_{ST} and is appropriate for estimating genetic structure from sequence data (Lynch and Crease, 1990). N_{ST} is a measure of genetic differentiation that incorporates phylogenetic distance, and G_{ST} is an unordered measure of genetic differentiation that does not incorporate phylogenetic distance (Jian et al., 2016). In this study, the G_{ST} values were then compared with N_{ST} with 1000 permutations to evaluate any significant differences between the two estimators that test for the existence of phylogeographic structure.

To assess the geographical location of possible genetic discontinuities among sampling sites, we used the program BARRIER v.2.2 (Manni et al., 2004). BARRIER creates a map of the sampling locations from geographical coordinates and then identifies possible barriers in the edges of polygons where the maximum distances occur between sampling sites from a matrix of pairwise genetic distances (F_{ST}). The pairwise F_{ST} matrix was obtained using ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010).

Phylogenetic analyses

To estimate the phylogeny of the haplotypes within *T. lanuginosa,* optimal substitution models were determined using AICc values as implemented in jModelTest 0.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012). For *T. lanuginosa,* the optimal models were TVM and HKY, respectively, for *petA-psbJ* and the *trnL-F* intron/spacer. A Bayesian tree was estimated from the same data using MrBayes 3.2.7a (Ronquist et al., 2012). Two independent runs were conducted to assess the repeatability of stationarity between runs for each analysis. For each run, four chains were run with temperature of 0.2, as defined by default in the program, set for 10,000,000 runs, and sampling one tree every 1000 generations. Stationarity was determined based on the likelihood scores for time to converge, and sampling points collected prior to stationarity were eliminated (12.5%). Posterior probabilities for supported clades were determined. Although a 50% majority-rule consensus of the trees was retained after burn-in, one of the 10,000 trees that approached the ML tree topology was selected.

RESULTS

Sequence variation

The length of the concatenated two-locus alignment used in this study, after excluding poly-A/T repeats, was 1774 bp. There were 22 parsimony-informative sites, five parsimony-uninformative substitutions and nine indels. The *trnL-F* region was more phylogenetically informative than the *psbJ-petA* region (Appendix S2).

Phylogeographic structure and haplotype network

Across all individuals of *Tidestromia lanuginosa,* 28 haplotypes were identified from 26 populations (Fig. 1C; Appendix S1). Total haplotype diversity was higher (Hd = 0.886 [0.020 SD]; Table 1) than the average intrapopulation diversity (Hd = 0.356) with a range of variation of 0.000 to 0.800, indicating strong genetic differentiation among populations.

Haplotypes were divided into four haplogroups based on sequence divergence, the haplotype network, the ML tree, and phylogeographic structure analysis (Tables 1, 2; Fig. 1C, D; Appendices S3, S4). Haplotypes were interconnected by 1–13 mutational steps (Fig. 1C). Haplotypes H14 and H15 were resolved into haplogroup 4 based on BAPS analysis (Fig. 1D). Haplogroup 1 (H1–H8) had eight haplotypes, haplogroups 2 (H9–H13) and 3 (H16–H20) each had five haplotypes, and haplogroup 4 (H14, H15, H21–H28) had 10 haplotypes. Haplogroup 1 had the most individuals followed by haplogroup 4, haplogroup 2, and haplogroup 3 (Table 1). Two main lineages were evident in the haplotype network: the North cluster comprised haplogroups 1 and 2, and the South cluster comprised haplogroups 3 and 4. The South cluster had the highest haplotype (Hd) and nucleotide diversity (π) despite including fewer individuals (49 vs 73; Table 1). Each of the four haplogroups was dominated by 1 or 2 haplotypes and was relatively diverse genetically (Table 1; Fig. 1C). Haplotype H1 was the most frequent in haplogroup 1, with 34 individuals widely distributed in Chihuahua and Coahuila. Haplotype H9 was most frequent in haplogroup 2, and included 13 individuals, all in Coahuila. Haplotype H16 was most frequent in haplogroup 3, including 10 individuals limited to the southern coast of Tamaulipas. Haplotypes H21 and H25 were most frequent in haplogroup 4, occurring west and east of the Sierra Madre Oriental, respectively. At the group level, haplogroup 4 had the highest Hd (Table 1).

The SAMOVA and BAPS results also supported the subdivision of *T. lanuginosa* into four haplogroups. SAMOVA analysis found the greatest increment in F_{CT} value for $K = 2$ ($F_{CT} = 0.615$). BAPS analysis revealed high genetic differentiation in the four haplogroups (Fig. 1D), although one population (550-05) shared alleles with three haplogroups (1, 2, and 4).

N_{ST} and G_{ST} values indicated high levels of genetic differentiation among the populations sampled and among the four groups recognized for *T. lanuginosa* (Table 2). N_{ST} was significantly higher than G_{ST} (Table 2), which is considered evidence of phylogeographic structure (Pons and Petit, 1996).

BARRIER analysis identified three genetic discontinuities among the four haplogroups of *T. lanuginosa*. One discontinuity separated haplogroup 1 from haplogroup 2, the second barrier separated haplogroup 3 from haplogroup 4, and the third separated haplogroup 2 from haplogroup 4 (Fig. 2A).

TABLE 1. Indices of genetic diversity for clusters, haplogroups and populations of *Tidestromia lanuginosa* in Mexico obtained from cpDNA sequences, including population size (*N*), number of haplotypes (*h*), haplotype diversity (Hd ± SD), nucleotide diversity (π ± SD), standard deviations (SD), and number of segregating sites (*S*).

Cluster	*N*	*h*	*S*	Hd (±SD)	π (±SD)
North	73	13	22	0.702 (0.046)	0.00208 (0.00118)
South	49	15	24	0.827 (0.028)	0.00331 (0.00179)
Chihuahuan Desert	93	20	48	0.828 (0.031)	0.00252 (0.00016)
Coahuila state	54	14	45	0.854 (0.030)	0.00228 (0.00021)
Haplogroups					
1	52	8	8	0.508 (0.0778)	0.00041 (0.00008)
2	21	5	10	0.381 (0.1093)	0.00044 (0.00011)
3	15	5	11	0.362 (0.1434)	0.00037 (0.00020)
4	34	10	19	0.758 (0.494)	0.00080 (0.00015)
Populations					
493	5	2	1	0.400 (0.237)	0.00023 (0.00014)
494	5	2	1	0.000 (0.000)	0.0000 (0.00000)
495	5	2	1	0.600 (0.175)	0.00034 (0.00037)
496	5	2	1	0.000 (0.000)	0.00000 (0.00000)
503	4	1	0	0.000 (0.000)	0.00000 (0.00000)
507	5	3	10	0.700 (0.218)	0.00091 (0.00040)
548	5	2	1	0.400 (0.237)	0.00023 (0.00014)
549	5	2	1	0.400 (0.237)	0.00023 (0.00029)
550	5	2	2	0.400 (0.237)	0.00045 (0.00027)
551	5	3	14	0.700 (0.218)	0.00160 (0.00047)
555	5	3	6	0.800 (0.175)	0.00068 (0.00020)
556	5	3	6	0.400 (0.237)	0.00045 (0.00027)
557	5	3	7	0.600 (0.175)	0.00102 (0.00030)
561	5	2	2	0.400 (0.237)	0.00045 (0.00027)
562	3	2	1	0.000 (0.000)	0.00000 (0.0000)
563	5	4	4	0.700 (0.218)	0.00045 (0.00017)
567	5	2	1	0.400 (0.237)	0.00023 (0.00014)
569	5	2	1	0.600 (0.175)	0.00034 (0.00010)
571	5	2	1	0.600 (0.175)	0.00034 (0.00010)
575	5	1	0	0.000 (0.000)	0.00000 (0.00000)
576	1	—	—	—	—
580	5	1	0	0.000 (0.000)	0.00000 (0.00000)
581	4	1	0	0.000 (0.000)	0.00000 (0.00000)
582	5	2	1	0.400 (0.237)	0.00023 (0.00029)
584	5	2	1	0.400 (0.237)	0.00023 (0.00029)
585	5	1	0	0.000 (0.000)	0.00000 (0.00000)
Total	122	28	56	0.886 (0.020)	0.00308 (0.00009)

Haplogroups 1 and 2 were located entirely within the Chihuahuan Desert (Fig. 2B; Appendix S1), whereas the distribution of haplogroups 3 and 4 can be better explained through the physiographic provinces classification. For instance, haplogroup 3 was limited to the Tamaulipas Coastal Plain (Fig. 2C; Appendix S1), and haplogroup 4 was recovered with two clusters divided by the Sierra Madre Oriental. One cluster was dominated by haplotype 21 and contained three populations (548, 549, and 550) found only in the Occidental Mountains and Plains, whereas haplotype H25 predominated in the other cluster, which contained three populations (495, 496, and 503) restricted to the North Gulf Coastal Hills (Fig. 2C; Appendix S1). Three of the four haplogroups (1, 2, and 4) were present in Coahuila (Fig. 2A).

Rarefaction curves based on individuals suggest there are no significant differences in haplotype richness between phylogeographic areas (confidence intervals overlap between the North and South groups of *Tidestromia lanuginosa*; Appendix S5A), whereas rarefaction curves between *T. lanuginosa* and *Agave lechuguilla* suggest

TABLE 2. Comparison of indices of genetic differentiation (G_{ST} vs. N_{ST}) between population groups of *Tidestromia lanuginosa* in Mexico (**P < 0.0001, obtained with a permutation test in DnaSP, using 1000 permutations).

Source of variation	G_{ST}	N_{ST}	P
North vs. South clusters			
Among groups	0.13079	0.63352**	0.0001
Haplogroup 1 vs. Haplogroup 2			
Among groups	0.32687	0.86940**	0.0001
Haplogroup 3 vs. Haplogroup 4			
Among groups	0.22927	0.81437**	0.0001

significant differences in haplotype richness (confidence intervals do not overlap; Appendix S5B).

Phylogenetic analyses

The MrBayes phylogeny of haplotypes revealed that all *T. lanuginosa* haplotypes formed a well-supported clade (PP = 1.0) and identified four well-suported subclades (PP = 0.9, 1.0; Fig. 1B). The positions of H14 and H15 were unresolved in the analyses.

DISCUSSION

Genetic diversity and structure in *Tidestromia lanuginosa*

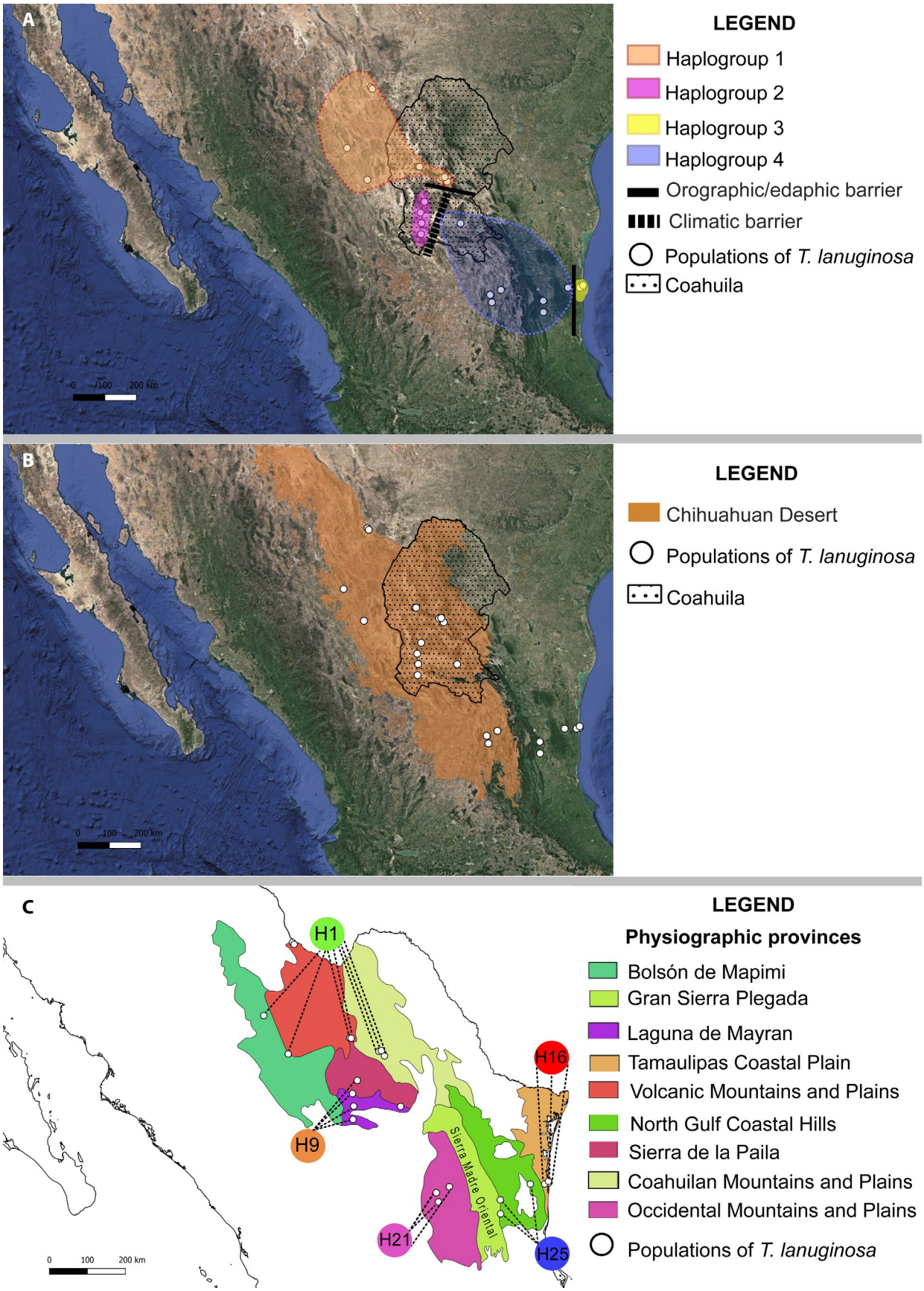
Our work provides evidence of the relatively high levels of phylogeographic structure in northern Mexican plants and importantly reveals relatively high genetic diversity of *Tidestromia* in the Chihuahuan Desert. The presence of five abundant haplotypes (H1, H9, H16, H21, and H25) within *Tidestromia lanuginosa*, some of which were completely restricted to non-overlapping physiographic provinces, highlights the level of diversity in this species (Fig. 2). Other studies of phylogeography in northern Mexican plants, although relatively few in number, demonstrate similarly elevated levels of genetic differentiation within species. For example, strong phylogeographic signal is evident in *Agave lechuguilla,* one of the dominant taxa of Chihuahuan Desert scrub, with a broad distribution from central Mexico to the southwestern United States (Scheinvar et al., 2017). Likewise, strong phylogeographic patterns are also evident in *Fouquieria shrevei*, a gypsum endemic with a much more limited distribution in central and southern Coahuila (Aguirre-Liguori et al., 2014). *Ephedra compacta* Rose has also been found to exhibit high genetic diversity on the Mexican Plateau (Loera et al., 2017).

A comparison of haplotype diversity of *Tidestromia lanuginosa* with that reported in the literature for other species in the aridlands of Mexico is informative despite the fact that different genomic regions have been used. Population-level diversity of *Tidestromia lanuginosa* in Mexico is high (Hd = 0.886, SD ± 0.020 for *trnL-F* and *psbJ-petA*; Table 1). This diversity includes the Chihuahuan Desert, where diversity in *T. lanuginosa* is higher (Hd = 0.828, SD ± 0.031; Table 1) than values reported for southern Chihuahuan Desert populations of *Ephedra* (Hd = 0.716, SD ± 0.029) using three cpDNA regions (*clpP-psbB*, *matK* and *trnS-trnFm*), but lower than in Chihuahuan Desert *Agave lechugilla* (Hd = 0.931, SD ± 0.000) based on four noncoding chloroplast fragments (*psbJ-petA*, *3′rps16-5′trnK*, *rpl32-trnL,* and *trnL-trnF*). Interestingly, Coahuilan populations of *Tidestromia lanuginosa* show higher haplotypic diversity (Hd = 0.854, SD ± 0.030; Table 1) than that of Coahuilan populations of *Fouquieria shrevei* (Hd = 0.743) based on three pairs of polymorphic intergenic chloroplast sequences (*psbJ-petA*, *ndhF-rpl32*, and *rpl32-trnL*).

Although effective population size is a relevant factor in determining the amount of genetic variability that can be maintained in a population (Lande and Barrowclough, 1987), it may also be influenced by life history traits, ecological traits, and historical events (Zhang et al., 2017). It is possible that the short life cycle of *T. lanuginosa* may have played an important role in promoting genetic diversity in this species, as might the dispersal ability of pollen and seeds. However, we must emphasize that little is known of the reproductive biology of *T. lanuginosa*. For example, seed dispersal mechanisms are poorly known for *Tidestromia*. A few observational reports have mentioned that *T. lanuginosa* seeds are eaten by ants (Davidson et al., 1980; Whitford, 1978) and birds (Campbell et al., 1973; Davis et al., 1975). Sánchez-del Pino and Flores-Olvera (2006) postulated that the floral involucre might be involved in seed dispersal or protection, but further studies are necessary to understand and postulate better hypotheses on reproduction and migration. Regarding pollination, it has been reported that *Tidestromia* flowers are pollinated by insects, with bees mainly reported to pollinate *T. lanuginosa* (Hurd and Linsley, 1963; Lytle-Webb 1978, Timberlake, 1980; Rozen, 1989; Discover Life, 2019).

An important factor that might also contribute to the high genetic differentiation found in *Tidestromia lanuginosa* is its potential adaptation to different environments, including different climatic and edaphic conditions such as gypsum, saline, calcareous, limestone, sandy, granite, and clay soils (Sánchez-del Pino and Motley, 2010). As currently circumscribed, *Tidestromia lanuginosa* has an unusually broad ecogeographic distribution, with populations across northern Mexico, ranging from relatively wetter environments on the Gulf of Mexico coast to very dry environments in the center of the Chihuahuan Desert. The largest divergence in *T. lanuginosa*—that of the four haplogroups—separates the core Chihuahuan Desert populations of Coahuila and Chihuahua, which occupy very dry areas, from the populations further south, which occupy areas with higher annual rainfall. Chloroplast genetic diversity is relatively high in both of these groups, with almost no sharing of haplotypes, suggesting that these groups have inhabited their respective regions for a relatively long period.

FIGURE 2. (A) Map of the collection localities for *Tidestromia lanuginosa* in Mexico (from and edited using Google Earth, 2017) showing the barriers obtained using BARRIER for chloroplast regions and the major haplogroups found for *Tidestromia lanuginosa* within Coahuila (black dotted area). Solid black lines represent the location of the most probable barriers concordant with mountainous and edaphic areas that divide major groups found for *Tidestromia lanuginosa*; the dotted line represents an inferred barrier that does not correlate with an obvious abiotic barrier. (B) Map showing the Chihuahuan Desert in Mexico where sampled populations of *Tidestromia lanuginosa* were collected. (C) Map showing the physiographic provinces in Mexico where sampled populations of *Tidestromia lanuginosa* were collected and distinctive haplotypes of each haplogroup following a latitudinal gradient (North: H1 and H9; South: H16, H25, and H21), with emphasis on those located east and west of the Sierra Madre Oriental in haplogroup 4.


A
0 100 200 km
LEGEND
Haplogroup 1
Haplogroup 2
Haplogroup 3
Haplogroup 4
Orographic/edaphic barrier
Climatic barrier
Populations of T. lanuginosa
Coahuila
B
0 100 200 km
LEGEND
Chihuahuan Desert
Populations of T. lanuginosa
Coahuila
C
H1
H9
H16
H21
H25
Sierra Madre Oriental
0 100 200 km
LEGEND
Physiographic provinces
Bolsón de Mapimi
Gran Sierra Plegada
Laguna de Mayran
Tamaulipas Coastal Plain
Volcanic Mountains and Plains
North Gulf Coastal Hills
Sierra de la Paila
Coahuilan Mountains and Plains
Occidental Mountains and Plains
Populations of T. lanuginosa

By including only chloroplast genetic information, our data are limited to maternal influences and are most likely best aligned with seed dispersal patterns. Likewise, we cannot exclude the possibility of chloroplast capture, i.e., introgression of chloroplasts from related species (Rieseberg and Soltis, 1991), especially since other species of *Tidestromia* (e.g., *T. suffruticosa*, *T. gemmata*) co-occur in their distribution range and occasionally grow at the same site. Also, hybridization has been hypothesized to occur within the genus based on molecular data, as well as pollen and chromosome observations (Sánchez-del Pino and Motley, 2010).

Barriers to gene flow

Differentiation among and within haplogroups generally correlates with known orographic, edaphic, and/or climatic barriers (Fig. 2A). An orographic barrier separates haplogroup 1 from haplogroup 2 by the Sierra de la Paila, located in Coahuila, which is an element of the northern discontinuous part of the Sierra Madre Oriental (Villarreal, 1994). The Sierra Madre Oriental formed earlier (between 80 to 50 million years ago [Ma] according to English and Johnston, 2004) than the postulated origin of *Tidestromia* (8.1 ± 4.9 Ma according to Sage, 2016), and it was believed to play an important role in the speciation of *T. lanuginosa*. We hypothesize a relatively recent dispersal event that resulted in the differentiation of two haplogroups (1 and 2). Differentiation is also evident within haplogroup 4, although the haplotypes within this group are separated by one mutational step (Figs. 1C, 2C). Specifically, most of the haplogroup 4 populations fall within two groups, located east and west of the Sierra Madre Oriental, respectively. Each of these sets of populations is dominated by a single, distinct haplotype, with no shared haplotypes, suggesting that gene flow via seed dispersal has been minimal between them. Scarcity of maternal gene flow is not surprising given the great altitude of the Sierra Madre Oriental, which reaches approximately 3500 m at its peak at this latitude. The eastern side of this mountain range receives a much higher annual rainfall than the western side, leading to sometimes dramatic differences in biomes at the same latitude (e.g., wet forest vs. desert scrub), and hence it is possible that the populations of haplogroup 4 may be adapted to the differing rainfall regimes (Fig. 2C). The barrier that separates haplogroups 2 and 4 may also correspond to differences in climate. Populations of haplogroup 2 are found in very dry regions, whereas haplogroup 4 populations occur in less dry climate regions (Instituto Nacional de Estadística y Geografía, INEGI). Thus, significant differences in rainfall may serve as another strong ecological pressure for natural selection.

In contrast, an edaphic barrier was found between the coastally distributed haplogroup 3 and the inland haplogroup 4 (Figs. 1C, 2A). This barrier is located at the limit of the Tamaulipas Coastal Plain (Appendix S1; Fig. 2A). We found evidence of strong genetic differentiation based on cpDNA regions within these groups. Populations in haplogroup 3 were all collected in Tamaulipas on coastal dunes that occur on extremely saline and flooded Solonchak soils, while those of haplogroup 4 grow on soils that are much less saline and sandy. Hence, edaphic factors likely play an important role in controlling the distribution of these populations. Such extreme edaphic conditions can act as strong selective forces (Rajakaruna, 2004), possibly leading to differentiation between some haplogroups (3 and 4). Sorrie and Weakley (2001) have noted that edaphic endemicity can be high on the diverse soil types within the North American Coastal Plain, which includes the Tamaulipas Coastal Plain. Given the phylogeographic and ecological distintiveness of the *T. lanuginosa* populations in this area, it is possible that they are in the process of speciation. However, additional population sampling, molecular and morphological data, and greenhouse and field experiments will be necessary to test for reproductive barriers and possible speciation for these and other haplogroups.

Pleistocene refugia in the southern Chihuahuan Desert

Although uniparental molecular markers have some limitations, our results are consistent with the hypothesis that *Tidestromia lanuginosa* persisted in more southerly regions, particularly in Coahuila, during full-glacial periods of the Pleistocene. Ample packrat midden evidence indicates that during the last glacial maximum (approximately 21,000 yr ago), most areas that currently harbor Chihuahuan Desert scrub instead harbored grasslands and savannas (Van Devender, 1990). However, rates of community turnover appear to have been lower in regions of southern and central Coahuila, and some typical desert succulents have been recovered from packrat middens during the later stages of the Wisconsin glacial period in the region of the Bolsón de Mapimí (Van Devender and Burgess, 1985; Van Devender 1990). Hence, these parts of Coahuila may have served as an important refugium for the Chihuahuan Desert biota during the cooler and wetter periods of the Pleistocene (Moore and Jansen, 2007; Gámez and Castellanos-Morales, 2019).

Geographic patterns of haplotype diversity in *Tidestromia lanuginosa* are consistent with the hypothesis of southern refugia for arid-adapted taxa. Both across the species as a whole and within the Chihuahuan Desert, we detected a latitudinal gradient of decreasing diversity farther north in *T. lanuginosa* (Fig. 2A, C; Table 1). Across the species as a whole, the South cluster of populations (haplogroups 3 and 4) had a higher haplotype (Hd) and nucleotide diversity (π) than the North cluster (haplogroups 1 and 2), despite having fewer individuals (Table 1). The rarefaction curves show that the South and the North clusters have nonsignificant differences in amount of genetic variation (Appendix S5A).

Of *T. lanuginosa* populations within the Chihuahuan Desert, southern and central Coahuila possessed the highest haplotype diversity. For example, haplotypes from three of the four haplogroups exist in Coahuila, whereas only one haplogroup has been detected so far in Chihuahua. Moreover, haplogroups 1 and 2 occupy largely non-overlapping central vs. southern parts of Coahuila (Fig. 2A). Relatively high within-population haplotype diversity also characterizes many populations in Coahuila, as for example, populations 551, 555, 556, and 557 in southern Coahuila, and population 563 in central Coahuila (Fig. 2; Table 1; Appendix S1). Lastly, unique haplotypes were documented from some parts of Coahuila (e.g., H3 and H8 unique haplotypes from Cuatro Ciénegas; H4, H5, and H7 from Ocampo; and H11, H13, H14, H15, and H22 from Saltillo).

These results are consistent with those from the few other Chihuahuan Desert plants that have been investigated. For example, haplotype diversity was found to be higher in *A. lechuguilla* in the southern Chihuahuan Desert (in Coahuila and Hidalgo) than in more northern areas (Scheinvar et al., 2017). Rarefaction curves provide evidence that *Tidesromia lanuginosa* has more haplotype richness than *Agave lechuguilla*. Therefore, *Tidestromia lanuginosa* may be more genetically diverse in Coahuila than *Agave lechuguilla*, especially considering that only two plastid loci were

included for *T. lanuginosa* (*trnL-F* and *petA-psbJ*) vs. the four included for *A. lechuguilla* (*psbJ-petA*, *3′rps16-5′trnK*, *rpl32-trnL* and *trnL-trnF*; Appendix S5B). Aside from *A. lechuguilla*, the gypsum endemic species *Fouquieria shrevei*, which is restricted to gypsum in central and southern Coahuila, also possesses populations with relatively high haplotype diversity (Aguirre-Liguori et al., 2014).

Above the species level, Coahuila is also an important center of endemism for several plant groups, including Cactaceae (Hernández and Bárcenas, 1995; Gómez-Hinostrosa and Hernández, 2000), as well as for *Tidestromia* itself. Six of the eight species of *Tidestromia* occur in Coahuila, some in sympatry, and three species (*T. rhizomatosa* I.M.Johnst., *T. tenella, T. valdesiana* Sánch.Pino & FloresOlv.) are endemic to the southern and central parts of the state (Sánchez-del Pino, 2001; Sánchez-del Pino and Flores Olvera, 2006; Sánchez-del Pino and Motley, 2010). Collectively, these results suggest that arid-adapted plant species likely were able to persist through climatically unfavorable full-glacial periods of the Pleistocene in at least southern and central Coahuila, and reinforce the importance of Coahuila as a center of diversity for the Chihuahuan Desert flora.

CONCLUSIONS

Our findings reveal that *Tidestromia lanuginosa* in Mexico contains high phylogeographic diversity that correlates relatively well with major geographic barriers and abiotic factors. The molecular and geographical differentiation within *T. lanuginosa* may be related to previously reported morphological differences (Sánchez-del Pino, 2001; Bucio, 2008; Valencia, 2009), which highlights the need for further molecular, morphological, and anatomical investigations in this complex. More broadly, our work demonstrates that the flora of the arid and semi-arid regions of northern Mexico is not only rich in endemic species, but also rich in genetic diversity. This is particularly true for the state of Coahuila, which may represent an important core of genetic diversity for the Chihuahuan Desert flora, and seems to have served as an important refugium for the arid-adapted flora of northern Mexico. Additional work is needed in other groups with broad distributions in Coahuila and the Chihuahuan Desert to explore whether phylogeographic diversity has been underestimated more broadly.

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AUTHOR CONTRIBUTIONS

I.S.P. conceived the ideas, collected plant material, analyzed data, and wrote the manuscript. A.A. conducted experiments and collected and analyzed data. R.H.A.N collected plant material, analyzed data, and participated in manuscript preparation. A.M.O. collected plant material and reviewed the manuscript. A.I.M. analyzed data and participated in manuscript preparation. M.C.P. conducted analysis and reviewed the manuscript. M.J.M. and H.F.O. participated in manuscript preparation and assisted with ideas. All authors contributed to the drafts and approved the final publication.

DATA AVAILABILITY

All sequences generated in this study are deposited in GenBank (Appendix 1). The alignments used in this study are available from the treebase repository: http://purl.org/phylo/treebase/phylows/study/TB2:S26441.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Details of sampling locations and haplotypes of *Tidestromia lanuginosa*.

APPENDIX S2. Sequence variation in *psbJ-petA*, *trnL* intron, and *trnL-F* spacer among individuals of *Tidestromia lanuginosa* from Mexico.

APPENDIX S3. Maximum likelihood tree of the populations of *Tidestromia lanuginosa* in Mexico indicating major groups.

APPENDIX S4. Consensus tree obtained from the Bayesian analysis.

APPENDIX S5. Results of rarefaction analyses based on individuals.

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APPENDIX 1. Collection information and GenBank accessions for cpDNA sequences. Specimens were deposited in CICY and ANSM (abbreviations following Thiers, 2020).

Taxon	Voucher	Collection site	GenBank accessions		
			psbJ-petA	***trnL* intron**	***trnL-F* spacer**
Outgroups					
Alternanthera flavescens Kunth.	*Sánchez-del Pino, 590* (MEXU)	Mexico.Yucatán. Mpio. Progreso.	MT146869	MT146873	MT146877
Tidestromia gemmata I.M.Johnst.	*Sánchez-del Pino, González & Simá 565* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146871	MT146875	MT146879
Tidestromia rhizomatosa I.M.Johnst.	*Sánchez-del Pino & Simá 574* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146870	MT146874	MT146878
Tidestromia tenella I.M.Johnst.	*Sánchez-del Pino, Andueza & Mora 508* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146872		
Tidestromia tenella I.M.Johnst.	*Sánchez-del Pino, Andueza & Mora 577* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.		MT146876	MT146880
Ingroups					
Tidestromia lanuginosa (Nutt.) Standl.					
Population 493	*Sánchez-del Pino, Andueza & Mora 493* (MEXU)	Mexico. Tamaulipas. Mpio. La Pesca.	MT146503– MT146507	MT146625– MT146629	MT146747– MT146751
Population 494	*Sánchez-del Pino, Andueza & Mora 494* (MEXU)	Mexico. Tamaulipas. Mpio. Soto la Marina.	MT146508– MT146512	MT146630– MT146634	MT146752–MT146756
Population 495	*Sánchez-del Pino, Andueza & Mora 495* (MEXU)	Mexico. Tamaulipas. Mpio. Soto la Marina.	MT146513– MT146517	MT146635– MT146639	MT146757– MT146761
Population 496	*Sánchez-del Pino, Andueza & Mora 496* (MEXU)	Mexico. Tamaulipas. Mpio. Cd. Victoria.	MT146518– MT146522	MT146640– MT146644	MT146762–MT146766
Population 503	*Sánchez-del Pino, Andueza & Mora 503* (MEXU)	Mexico. Tamaulipas. Mpio. Lleras.	MT146523– MT146526	MT146645– MT146648	MT146767– MT146770
Population 507	*Sánchez-del Pino, Andueza & Mora 507* (MEXU)	Mexico. Tamaulipas. Mpio. Cruillas.	MT146527– MT146531	MT146649– MT146653	MT146771– MT146775
Population 548	*Sánchez-del Pino & Simá 548* (MEXU)	Mexico. San Luis Potosí. Mpio. Matehuala.	MT146532– MT146536	MT146654– MT146658	MT146776– MT146780
Population 549	*Sánchez-del Pino & Simá 549* (MEXU)	Mexico. San Luis Potosí. Mpio. Matehuala.	MT146537– MT146541	MT146659– MT146663	MT146781–MT146785
Population 550	*Sánchez-del Pino & Simá 550* (MEXU)	Mexico. Nuevo León. Mpio. Dr. Arroyo.	MT146542– MT146546	MT146664– MT146668	MT146786– MT146790
Population 551	*Sánchez-del Pino & Simá 551* (MEXU)	Mexico. Coahuila. Mpio. Saltillo.	MT146547– MT146551	MT146669– MT146673	MT146791– MT146795
Population 555	*Sánchez-del Pino & Simá 555* (MEXU)	Mexico. Coahuila. Mpio. Viesca.	MT146552– MT146556	MT146674– MT146678	MT146796– MT146800
Population 556	*Sánchez-del Pino & Simá 556* (MEXU)	Mexico. Coahuila. Mpio. Saltillo.	MT146557– MT146561	MT146679– MT146683	MT146801– MT146805
Population 557	*Sánchez-del Pino & Simá 557* (MEXU)	Mexico. Coahuila. Mpio. San Pedro de las Colonias.	MT146562– MT146566	MT146684– MT146688	MT146806– MT146810
Population 561	*Sánchez-del Pino & Simá 561* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146567– MT146571	MT146689– MT146693	MT146811– MT146815
Population 562	*Sánchez-del Pino & Simá 562* (MEXU)	Mexico. Coahuila. Mpio. Ocampo.	MT146572– MT146574	MT146694– MT146696	MT146816– MT146818
Population 563	*Sánchez-del Pino & Simá 563* (MEXU)	Mexico. Coahuila. Mpio. Ocampo.	MT146575– MT146579	MT146697– MT146701	MT146819– MT146823
Population 567	*Sánchez-del Pino, González & Simá 567* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146580– MT146584	MT146702– MT146706	MT146824– MT146828
Population 569	*Sánchez-del Pino, González & Simá 569* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146585– MT146589	MT146707– MT146711	MT146829– MT146833
Population 571	*Sánchez-del Pino, González & Simá 571* (MEXU)	Mexico. Coahuila. Mpio. Cuatro Ciénegas.	MT146590– MT146594	MT146712– MT146716	MT146834– MT146838
Population 575	*Sánchez-del Pino, González & Simá 575* (MEXU)	Mexico. Coahuila. Mpio. San Pedro de las Colonias.	MT146595– MT146599	MT146717– MT146721	MT146839– MT146843
Population 576	*Sánchez-del Pino, González & Simá 576* (MEXU)	Mexico. Coahuila. Mpio. San Pedro de las Colonias.	MT146600	MT146722	MT146844
Population 580	*Sánchez-del Pino & Simá 580* (MEXU)	Mexico. Chihuahua. Mpio. Ojinaga.	MT146601– MT146605	MT146723– MT146727	MT146845– MT146849

(Continues)

APPENDIX 1. Continued

Taxon	Voucher	Collection site	GenBank accessions		
			psbJ-petA	*trnL* intron	*trnL-F* spacer
Population 581	*Sánchez-del Pino & Simá 581* (MEXU)	Mexico. Chihuahua. Mpio. Ojinaga.	MT146606– MT146609	MT146728– MT146731	MT146850– MT146853
Population 582	*Sánchez-del Pino & Simá 582* (MEXU)	Mexico. Chihuahua. Mpio. Ojinaga.	MT146610– MT146614	MT146732– MT146736	MT146854– MT146858
Population 584	*Sánchez-del Pino & Simá 584* (MEXU)	Mexico. Chihuahua. Mpio. Las Delicias.	MT146615– MT146619	MT146737– MT146741	MT146859– MT146863
Population 585	*Sánchez-del Pino & Simá 585* (MEXU)	Mexico. Chihuahua. Mpio. Jiménez.	MT146620– MT146624	MT146742– MT146746	MT146864– MT146868