Genética de la conservación y filogenia de la musaraña de Arizona en las "islas del cielo" del Suroeste de los Estados Unidos

Conservation genetics and phylogeny of the Arizona shrew in the "Sky Islands" of the Southwestern United States

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Introduction: Little is known about the phylogenetic relationships and population genetic structure of the Arizona shrew (*Sorex arizonae*), a small mammal restricted to a few mountainous areas in the southwestern United States and northern Mexico. Information on genetic variability is needed, as the species is restricted to sky islands, which are small and often locally fragmented habitats.

Methods: Using mitochondrial cytochrome-*b* sequences and 12 microsatellite loci, we assessed genetic differentiation among 64 individuals from four mountain ranges in Arizona and New Mexico: Animas, Chiricahua, Huachuca, and Santa Rita ranges.

Results: We found a unique haplotype for the Animas population, while the other mountain ranges shared a common haplotype but they also each had a unique haplotype at lower frequencies. F-statistics indicate that significant population differentiation has occurred among the Chiricahua and Animas populations compared with the other two populations. The F_{ST} statistics revealed high levels of genetic differentiation between the Chiricahua and Animas populations, compared to the other two populations that show lower but significant F_{ST} values. Nevertheless, the STRUCTURE analysis using microsatellite markers did not show significant differentiation between Chiricahua and Animas but it did detect strong signatures of differentiation between the cluster formed by these two populations and Huachuca and Santa Rita. Phylogenetic analysis using cytochrome-*b* sequences confirmed that the Arizona shrew is most closely related to *S. trowbridgii* among North American shrews that have been sequenced for the *mt*DNA cytochrome-*b* region to date. Both species are placed in a monophyletic group sister to a clade consisting of Nearctic shrews.

Discussion and conclusions: Our results suggest that the *S. arizonae* populations have high levels of differentiation in comparison to other North American shrews. We also detected high levels of population genetic structure with both *mt*DNA and microsatellite although the pattern between them is different. Given the inherent vulnerability of these naturally isolated populations with their locally fragmented distributions, the genetic data support a need for conservation focused on protection of suitable habitats. As our fieldwork indicates, suitable habitats include mid- to high-elevation riparian zones and upland areas, including ridgelines and mountaintop saddles with having sufficient moisture, vegetation, and soil development to provide cover and a food base for shrews.

Key words: Arizona shrew; haplotype diversity; mitochondrial cytochrome-*b*; sky islands.

Introduction

The Arizona shrew (*Sorex arizonae*) is one of the most geographically restricted shrews in the world and is currently listed as "least concern" by the IUCN (IUCN 2014). Due to their small size, semifossorial habits, and habitat specialization (Simons and Hoffmeister 2003), the vagility of the Arizona shrew is likely to be low relative to other small mammals and their populations are expected to be highly isolated. In addition, shrews must feed often to maintain their high basal metabolic rates, further restricting their movements between and within habitats (Hanski 1994). Like many other shrews, *S. arizonae* rarely lives much longer than a year. Spring populations are composed entirely of adults, which die before the next spring and are replaced by young born the previous summer and fall. These ecological and physiological constraints severely limit the ability of *S. arizonae* to move between suitable habitats and especially to occupy the drier lower elevation habitats that separate areas known to support the species. As a result, we hypothesize that the genetic variation of *S. arizonae* will be highly structured between mountain ranges harboring the shrew.

The Arizona shrew was described in 1977 (Diersing and Hoffmeister 1977) from ten specimens found in the mountain ranges of southern Arizona. The Arizona shrew is distinguished from other shrews by a well-defined post-mandibular foramen among other differences in dental morphology. *S. arizonae* inhabits the montane conifer forests and oak-pine woodlands of certain mountain ranges in Arizona, New Mexico, and Mexico. Its range is known to include the higher elevations (above about 1,500 m) of the Chiricahua, Huachuca, and Santa Rita Mountains of southern Arizona, the Animas Mountains of southwestern New Mexico, and the Sierra Madre Occidental of Chihuahua, Mexico. *S. arizonae* is listed as a species of special concern in Arizona and as endangered in the state of New Mexico and in Mexico (Simons and Hoffmeister 2003).

Montane forest habitats occupied by *S. arizonae* present discrete ecosystems in which to study populations that, since the end of the Pleistocene, have been isolated by the surrounding lower elevation desert scrub and grassland habitats. These lower elevation habitats are not occupied by *S. arizonae*. Known as "sky islands", described above, the mountain ranges support a large number of endemic species with limited genetic exchange, including many of the threatened and endangered species in the Southwest (Nishida *et al*. 1992). Each sky island has a unique topography with horizontal layers of life zones ranging from arid (at the bottom) to boreal (at the top). In Arizona, for example, the Pinalenos Mountains contain the most life zones in the shortest vertical distance of any mountain in North America (Warshall 1986). Several studies of species present on sky islands report varying degrees of genetic differentiation among populations (DeChaine and Martin 2005). These results typically reveal an unusual degree of *mt*DNA genetic structure as a result of isolation between the different mountain ranges (Masta 2000; Knowles 2001). In addition, a model based on mixing of populations during interglacial expansions of "sky island" ecosystems suggested that glaciations did not inhibit divergence among populations of species having low vagility (Knowles 2001).

In this paper, we measure the genetic variation of *S. arizonae* populations inhabiting the four mountain ranges known to support the species in the United States using mitochondrial DNA (*mt*DNA) cytochrome-*b* sequences. We quantify variation using cytochrome-*b* because it has been previously shown to be useful in examining phylogeographic patterns in mammals (Avise *et al*. 1987) and is known to have moderate rates of evolution in a wide range of shrew species with recent divergence times (Fumagalli *et al*. 1999; Demboski and Cook 2001; 2003; Maldonado *et al*. 2001; Brant and Orti 2002; Mascheretti *et al*. 2003; McAliley *et al*. 2007). Additionally, we used nuclear microsatellite loci to determine the level of genetic differentiation between populations of *S. arizonae* from the four mountain ranges. Through this effort we seek to elucidate patterns

of genetic variation and the phylogenetic status of *S. arizonae*. Based on this information we provide management recommendations for the species, including directions for further research.

Material and methods

Tissue samples from 64 individuals were obtained from four different mountain ranges in Arizona and New Mexico. Shrews were collected in 1992, 1999, and 2001, using pitfall traps generally set among vegetation, logs, rocks, and/or debris piles (Simons and Van Pelt 1999; Simons and Maldonado 2007). Traps were set at 20 sites in a variety of situations including within and near riparian zones, in canyons, along a ridgeline with seeps, and within a mountaintop saddle. Sixteen sites (80 %) yielded at least one *S. arizonae* specimen (Animas: 2 of 4 sites; Chiricahua: 4 of 5 sites; Huachuca: 7 of 8 sites; Santa Rita: all of 3 sites). Trap locations varied between 1,745 and 2,675 meters in elevation; captures of *S. arizonae* occurred from 1,745 to 2,510 meters in elevation. Samples were collected following guidelines approved by the American Society of Mammalogists (Sikes *et al*. 2011). Tissue samples were obtained from each specimen and stored in ethanol (95 %) at -70 °C. The balance of each of 43 specimens was preserved in ethanol and accessioned at the National Museum of Natural History (USNM) and DNA samples extracted from all 64 individuals used in this study were deposited at the Center for Conservation and Evolutionary Genetics (CCEG) at the Smithsonian Institution (Table 1).

DNA Extraction and mtDNA Sequencing. Whole genomic DNA was extracted using the phenol-chloroform method with a final concentration of 1 μg/μL (Sambrook *et al*. 1989). Initially we utilized two universal primers, LGL765 and LGL766 (Bickham *et al*. 1995), to amplify the complete cytochrome-*b* gene of *mt*DNA of a representative sample from each locality. However, our amplification rates with the larger universal primers were low and worked only in samples with high quality and quantity of DNA. Therefore, we used the larger fragment sequence from a representative sample of *Sorex arizonae* from each of the four localities to design two internal primers, cytbLshrw (3'-TGG CTC CCT ATG TAT CTG T-5') and cytbHshrw (3'-ATA GCA ACT GCC TTT ATG GGG-5'), that amplify a shorter 249 base pair fragment of the mitochondrial cytochrome-*b* region. This shorter fragment was capable of capturing sufficient variation to distinguish different haplotypes as it includes the variable region of the left domain of the cytochrome-*b* that has been previously used for the intraspecific and population level analysis of other North American shrews (Maldonado *et al.* 2001).

For both primer sets, polymerase chain reactions (PCRs) were performed in a volume of 25 μL with 1.0 unit of AmpliTaq Gold (Applied Biosystems), 1x PCR Buffer, 0.2 mM each dNTP, 0.6 μM each primer, 3 mM $MgCl_2$ and 20 μg BSA. A 10 minute denaturation at 95 °C was used followed by 35 cycles of 94°C for 1 min, 53°C for 1 min, and 72 °C for 1 minute and finally a 10 minute elongation step at 72 °C. PCR products were cleaned using Qiaquick PCR purification columns (Qiagen Inc.) and sequenced using BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems) according to manufacturer's recommendations. Reactions were purified via centrifugation through Sephadex columns (Amersham Biosciences). Sequences were run on the ABI Prism 3100 Genetic Analyzer and edited in Sequencher 4.1 (Gene Codes Corporation, Inc.).

Microsatellite genotyping. Twelve dinucleotide microsatellite loci were amplified for the species. We used six polymorphic microsatellite loci designed specifically for *S. arizonae* (Ortega *et al*. 2005). PCR conditions consisted of an initial denaturation at 95 °C for 10 min, followed by 30 cycles at 94 °C for 30s, original annealing temperature for each primer (Ortega *et al*. 2005) for 45s and 72 °C for 45s, and a final extension of 72 °C for 10 min. All reactions were performed in a Perkin Elmer 9700 Thermalcycler. Amplifications of microsatellites were carried out in a 15 μL volume containing 30 ng of DNA, 0.2 μM of each primer, 0.2 μM of dNTPs, 1x *Taq* buffer (1.5 μM

Table 1. *Sorex arizonae* specimen accession numbers for DNA samples deposited at the Center For Conservation and Evolutionary Genetics (CCEG) and for museum voucher specimens deposited at the National Museum of Natura History (USNM) at the Smithsonian Institution.

Accession Numbers		Locality		
CCEG Tissue #	USNM #	Mountains	Site	Sex
CCEG-JEM001	602126	Huachuca	Miller Canyon	M
CCEG-JEM006	602128	Huachuca	Miller Canyon	M
CCEG-JEM008	602101	Animas	Saddle @ 8050'	F
CCEG-JEM010	602113	Chiricahua	Pine Canyon	M
CCEG-JEM011	602123	Huachuca	Miller Canyon	M
CCEG-JEM012	602132	Santa Rita	Cottonwood Can.	M
CCEG-JEM013	602140	Santa Rita	Florida Canyon	unk
CCEG-JEM016	602134	Santa Rita	Cottonwood Can.	M
CCEG-JEM018	602130	Huachuca	Miller Canyon	unk
CCEG-JEM019	NA	Santa Rita	Cottonwood Can.	unk
CCEG-JEM020	602138	Santa Rita	Black Bill Canyon	M
CCEG-JEM024	602100	Animas	Pinery Canyon	unk
CCEG-JEM025	602114	Chiricahua	Florida Canyon	unk
CCEG-JEM026	602141	Santa Rita	Madera Canyon	M
CCEG-JEM027	602144	Santa Rita	Cottonwood Can.	F
CCEG-JEM028	602135	Santa Rita	McClure Canyon	M
CCEG-JEM031	602122	Huachuca	Pinery Canyon	unk
CCEG-JEM039	602116	Chiricahua	Pine Canyon	unk
CCEG-JEM040	602111	Chiricahua	Pinery Canyon	M.
CCEG-JEM041	602118	Chiricahua	Pine Canyon	unk
CCEG-JEM043	602133	Santa Rita	Cottonwood Can.	unk
CCEG-JEM047	602099	Animas	Black Bill Canyon	unk
CCEG-JEM052	602145	Santa Rita	Madera Canyon	unk
CCEG-JEM053	602110	Chiricahua	Pine Canyon	unk
CCEG-JEM055	602139	Santa Rita	Florida Canyon	F
CCEG-JEM056	602127	Huachuca	Miller Canyon	unk
CCEG-JEM057	602109	Chiricahua	Pine Canyon	unk
CCEG-JEM059	602124	Huachuca	Miller Canyon	unk
CCEG-JEM061	602142	Santa Rita	Florida Canyon	unk
CCEG-JEM062	602119	Huachuca	Garden Canyon	unk
CCEG-JEM063	602143	Santa Rita	Madera Canyon	unk
CCEG-JEM066	602146	Santa Rita	Madera Canyon	unk
CCEG-JEM068	602136	Santa Rita	Cottonwood Can.	unk
CCEG-JEM075	602120	Huachuca	Garden Canyon	M
CCEG-JEM076	602107	Chiricahua	Pine Canyon	unk
CCEG-JEM078	602121	Huachuca	Garden Canyon	F
CCEG-JEM081	602117	Chiricahua	Pinery Canyon	unk
CCEG-JEM082	602131	Santa Rita	Cottonwood Can.	unk

Continuation table 1

| Accession Numbers | | Locality | | |
CCEG Tissue #	USNM #	Mountains	Site	Sex
CCEG-JEM085	602108	Chiricahua	Pine Canyon	unk
CCEG-JEM086	602125	Huachuca	Miller Canyon	F
CCEG-JEM088	602129	Huachuca	Miller Canyon	unk
CCEG-JEM092	602106	Chiricahua	Pine Canyon	unk
CCEG-JEM096	602147	Santa Rita	Madera Canyon	M
CCEG-JEM100	602137	Santa Rita	Cottonwood Can.	unk
CCEG-LHS476	NA	Chiricahua	E.Turkey Creek	unk
CCEG-LHS477	NA	Huachuca	McClure Spring	unk
CCEG-LHS478	NA	Huachuca	McClure Spring	unk
CCEG-LHS479	NA	Huachuca	McClure Spring	unk
CCEG-LHS480	NA	Huachuca	McClure Spring	unk
CCEG-LHS481	NA	Huachuca	McClure Spring	unk
CCEG-LHS482	NA	Huachuca	McClure Spring	unk
CCEG-LHS483	NA	Huachuca	McClure Spring	unk
CCEG-LHS484	NA	Huachuca	McClure Spring	unk
CCEG-LHS489	NA	Huachuca	Ramsey Canyon	unk
CCEG-LHS490	NA	Huachuca	Ramsey Canyon	unk
CCEG-LHS491	NA	Huachuca	Ramsey Canyon	unk
CCEG-LHS492	NA	Huachuca	Ramsey Canyon	unk
CCEG-LHS493	NA	Huachuca	Ramsey Canyon	unk
CCEG-LHS494	NA	Huachuca	L.Blacktail Can.	unk
CCEG-LHS496	NA	Huachuca	L.Blacktail Can.	unk
CCEG-LHS498	NA	Huachuca	Huachuca	unk
CCEG-LHS500	NA	Huachuca	Blacktail Canyon	unk
CCEG-LHS502	NA	Huachuca	Blacktail Canyon	unk
CCEG-LHS503	NA	Huachuca	Blacktail Canyon	unk

Key: NA-Not accessioned at NMNH

MgCl$_2$, 10 mM Tris-HCl, 50 mM KCl), and 0.75 U of *Taq* polymerase. Analysis was performed on an ABI Prism 3100 Genetic Analyzer. Analysis of computer-generated results was executed using GeneScan (version 2.1) software, and final allele-sizing was done with the ABI Genotyper package (version 2.1). Additionally, we used six loci originally isolated in *S. ornatus* (Maldonado *et al.* 2006). PCR reactions were performed in a volume of 25 μL with 1.0 unit of AmpliTaq (Applied Biosystems), 1x PCR Buffer, 0.2 mM each dNTP, 0.6 μM each primer, and 3 mM MgCl$_2$. A 5 minute denaturation at 95 °C was used followed by 30 cycles of 94 °C for 1 min, 54-56 °C for 1 min, and 72 °C for 1 min and finally a 10 min elongation step at 72 °C.

Analyses of mtDNA sequence data. For alignment of all *mt*DNA sequences, the computer program SEQUENCHER 4.1 (Gene Codes Corp.) was utilized and then each sequence was inspected by eye and checked for the presence of stop codons to detect pseudogenes. The resulting multiple sequence alignment was imported into the computer program MacClade (Madison and Madison 2000) to

identify variable nucleotide positions. Genetic divergence among haplotypes was estimated using the Tamura and Nei (1993) distance with the computer program PAUP* 4.0 (Swofford 2001).

Cytochrome-*b* sequences generated using the 249 bp fragment were aligned and compared among the 64 *S. arizonae* samples. The resulting haplotypes were utilized to generate a statistical parsimony haplotype network using TCS 1.21 (Clement *et al.* 2000) to visualize potential regional mtDNA structure. TCS calculates the number of mutational steps among all pairs of haplotypes and then joins the most similar haplotypes together into a network where their combined probability is .95%. Genetic variability within populations was estimated in terms of haplotypic (*h*) and nucleotide (π) diversity, as implemented in the program ARLEQUIN 2.000 (Schneider *et al.* 2000). We used the analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) to investigate the proportion of total genetic variation within and among described populations. We calculated F_{ST} statistics based on corrected sequence distances among haplotypes. We generated pairwise genetic distances to calculate fixation indices (F_{ST}) between all population pairs. Statistical significance was ascertained by conducting 10,000 permutations in the software ARLEQUIN 2.000.

To examine the phylogenetic status of *S. arizonae*, we obtained a 1,118 bp fragment of the cytochrome-*b* from each representative haplotype from each of the four localities. The *S. arizonae* sequences were aligned and compared with sequences from 38 other *Sorex* species (Ohdachi *et al.* 1997, 2001, 2006; Fumagalli *et al.* 1999; Demboski and Cook 2001, 2003; Maldonado *et al.* 2001; Brant and Orti 2002; Mascheretti *et al.* 2003; Sipe and Browne 2004; Stewart *et al.* in litt.). We aligned our *Sorex* sequences with two short-tailed shrews (*Blarina* and *Cryptotis*; Brant and Orti 2002) and a desert shrew (*Notiosorex crawfordi)* that were used as outgroups (Ohdachi *et al.* 2006). Genbank accession numbers for all of the sequences used in the phylogenetic analysis comparing *S. arizonae* to other shrew species are listed in Table 2.

In order to evaluate the appropriate model of molecular evolution for our cytochrome-*b* sequences we used the program JModeltest v2.1.4 (Darriba *et al.* 2012). The model selected for all codon positions was GTR + I + G based on the corrected Akaike information criterion. Bayesian posterior probabilities were determined using MrBayes 3.2.1 and were used to place confident limits on tree branches (Ronquist and Huelsenbeck 2003). The Bayesian analysis was run using 5,000,000 generations along four chains with two replicates at a temperature of 0.05. The convergence between the two replicates run in MrBayes was assessed by the average standard deviation of split frequencies (ASDSF) between runs. After 5,000,000 generations, the ASDSF was 0.003. Sample frequency was set to 1000 with a burn-in of 1,250.

Analyses of Microsatellite data. For microsatellite data, allelic frequencies, F statistics (Weir and Cockerham 1984), R_{ST} (Rousset 1996), Hardy-Weinberg equilibrium, and genotypic disequilibrium among all loci pairs, as well as expected and observed heterozygosity were estimated using the software program GENEPOP 3.1b (Raymond and Rousset 1995). Sequential Bonferroni corrections (Rice 1989) were made to avoid type I errors.

To examine levels of genetic divergence using microsatellite data, POPULATIONS Version 1.2.28 (O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Génétique et Evolution, Gif sur Yvette; HYPERLINK "http://www.cnrs-gif.fr/pge/bioinfo/populations") was used to generate a Nei's standard genetic distance matrix (Saitou and Nei 1987).

To determine the degree of population genetic structure, the program STRUCTURE Version 2 (Pritchard *et al.* 2000) was used. This program uses Bayesian analysis to cluster individuals into subpopulations (K) with no prior information as to known populations. In this case, 100,000 MCMC repetitions following a 100,000 burn-in period were used. We allowed the value of K to vary between 1 and 10 and performed 10 independent runs for each value of K. An admixture

Table 2. Genbank accession numbers for Cytochrome -*b* sequences used in phylogenetic analysis.

Species	Locality	Ref.	Genbank Accession #
Sorex alpinus	Valais Canton, Switzerland	1	AB175119
Sorex antinorii	Piacenca, Italy	1	AB175125
Sorex araneus	Sweden	7	AJ245893
Sorex arizonae	Arizona, Chricahua, Pine Cyn. Hap-A	8	FJ657536.1
Sorex arizonae	Arizona, Santa Rita, Madera Cyn.Hap-B	8	FJ657537.1
Sorex arizonae	Arizona, Animas, Saddle Cyn. Hap-C	8	FJ657538.1
Sorex arizonae	Arizona, Huachuca, Miller Cyn. Hap-D	8	FJ657539.1
Sorex bendirii	Oregon, Lane County	2	AF238032
Sorex caecutiens	Magadan State, Russia	5	AB028551
Sorex camtschatica	Magadan State, Russia	2	AY014920
Sorex cinereus	Minnesota State, USA	2	AY014952
Sorex coronatus	Vercors, France	6	AJ000419
Sorex cylindricauda	Kyangjin Gompa, Nepal	1	AB175121
Sorex dapaenodon	Magadan State, Russia	1	AB175126
Sorex excelsus	Yushu Aut. Pref., Quinghai, China	1	AB175123
Sorex fumeus	Pennsylvania State, USA	1	AB175116
Sorex gracillimus	Hokkaido Pref., Japan	1	AB175131
Sorex granarius	Piedrahita, Avila, Spain	6	AJ000417
Sorex haydeni	South Dakota State,USA	2	AY014940
Sorex hoyi	Alaska State, USA	2	AF238040
Sorex isodon	Gangwon Pref., R. Korea	4	AB062736
Sorex jacksoni	Alaska State, USA	2	AY014926
Sorex longirostris	Tennessee State, USA	2	AY014954
Sorex minutissimus	Primorskii Territory, Russia	1	AB175129
Sorex minutus	Laghi di Ceretto, Italy	1	AB175133
Sorex mirabilis	Gangwon Pref., R. Korea	4	AB062737
Sorex monticolus	Washington State, USA	3	AB100273
Sorex ornatus	California, San Diego County	2	AF238035
Sorex palustris	Alaska State, USA	2	AF238033
Sorex portenkoi	Provideniya, Russia	2	AY014921
Sorex preblei	Oregon State, USA	2	AY014937
Sorex pribilofensis	Alaska State, USA	2	AY014933
Sorex roboratus	Magadan State, Russia	1	AB175128
Sorex samniticus	Opi, Abruzzi, Italy	6	AJ000429
Sorex saussurei	Guerrero State, Mexico	1	AB175117
Sorex shinto	Iwate Pref., Japan	5	AB028544
Sorex tenellus	California State, USA	2	AY014955
Sorex trowbridgii	Washington State, USA	2	AY014956
Sorex tundrensis	Magadan State, Russia	1	AB175127

Continuation table 2

Species	Locality	Ref.	Genbank Accession #
Sorex ugyunak	Alaska State, USA	2	AY014930
Sorex unguiculatus	Hokkaido Pref., Japan	5	AB028530
Sorex vagrans	Montana State, USA	2	AF154551
Blarina brevicauda	Michigan State, USA	1	AB175134
Cryptotis parva	Texas State, USA	1	AB175135
Notiosorex crawfordi	Texas State, USA	1	AB175145

References

1 Ohdachi *et al.* 2006.
2 Demboski and Cook 2003.
3 Shinohara and Suzuki 2003 (published in database only).
4 Ohdachi *et al.*2004.
5 Ohdachi *et al.* 2001.
6 Brünner *et al.* 2002.
7 Mouchaty *et al.* 2000.
8 This study.

model was used and correlated allele frequencies were assumed. The mean posterior probability was calculated for each set of ten runs of K and used to determine an optimal K. We also used the ΔK method (Evanno *et al.* 2005) based on the second-order rate of change in the log Pr(X|K), implemented in the program Structure Harvester (Earl and von Holdt 2012). Two monomorphic loci (A3-26 and 1S) were removed from the dataset for all of the analyses.

Results

Mitochondrial DNA analysis. A 249 bp fragment of the variable region of the left domain of the cytochrome-*b* gene was compared between 64 Arizona shrews. Four different haplotypes were identified differing by one to three base pairs (Table 3). Haplotype A was the most common, and it was found in a total of 57 individuals from three populations (Santa Rita, Huachuca, Chiricahua). Haplotype B was found in only two individuals from the Chiricahua population. Haplotype C was restricted to all three individuals from Animas population and, finally, haplotype D was found in two individuals restricted to the Huachuca population (Figure 1a).

Haplotype diversity values (*h*) ranged from zero in the Santa Rita Mountains, where only one haplotype was recorded, to 0.32 in the Huachuca Mountains. Nucleotide diversity values (π) ranged from 0.03 to 0.15, indicating close relationship among haplotypes (Table 4). Hierarchical analysis of *mt*DNA variation revealed that 54.3 % of the genetic variation was attributable to differences among individuals within regions, whereas 45.7 % of the variation was partitioned among regions. When analyzed in a pairwise fashion, comparisons revealed some statistically significant F_{ST} values ($P < 0.05$; Table 5). Santa Rita and Huachuca populations showed a non-significant difference, but both were significantly different from the Animas and Chiricahua populations.

A hierarchical AMOVA analysis was performed to detect population genetic structure. For the four defined sites, 21.7 % of the variation was detected among groups, 37.5 % of the variation was partitioned among populations within groups, and 40.8 % of the variation was partitioned within populations.

*mt*DNA haplotypes separated by several mutational steps have a > 0.95 probability of

being connected in a parsimonious way. Within this network, we found no ambiguous haplotype connections and haplotypes only differed by one or two mutations for a total of four substitutions (Table 3). All groups showed no ambiguities, were well resolved, and showed a star-shaped pattern (Figure 1b). Although the analysis did not incorporate geographic distance between sites, it revealed important associations among haplotype A with Santa Rita, Huachuca, and Chiricahua sites, while haplotype C in Animas is a unique haplotype in an exclusive site.

The Bayesian tree based on the 1,118 bp fragment of the cytochrome-*b* gene demonstrated support values ranging from 64 to 100 % for the nodes and the position of *S. arizonae* was well resolved (Figure 2). The four haplotypes of *S. arizonae* formed a monophyletic group that is closely related to *S. trowbridgii* (BI = 0.99) and *S. sausseri* (BI = 0.83). The overall tree topology supports the hypothesis originally generated from allozyme data (George 1988) that three major groupings can be detected in shrews of the genus *Sorex* and does not support classification based primarily on dental characters by Carraway (2007) that *S. arizonae* should be classified within the subgenus *Sorex*. Members of the Paleartic subgenus *Sorex* form a monophyletic group, and members of the Nearctic subgenus *Otisorex* form two monophyletic clades. One clade (clade B)includes the Nearctic species *S. trowbridgii* and *S. arizonae,* which are sister to *S. saussure*i. In general, the long-branch lengths and high Bayesian posterior probabilities for the node that clusters *S. arizonae* haplotypes as a monophyletic group in the tree are consistent with the hypothesis that this is a well defined species with a long evolutionary trajectory.

Microsatellite analysis. Tests for Hardy-Weinberg equilibrium (HWE) showed that all populations

Table 3. The four mitochondrial cytochrome-*b* haplotypes detected in 64 *S. arizonae* including nucleotide changes in 249 bp fragment. GenBank accession numbers are provided.

Sequence position					GenBank Accession numbers
Haplotype	70	100	173	194	
A	T	C	A	A	EF626663.1
B	C	C	A	A	EF626664.1
C	T	C	T	G	EF626665.1
D	T	T	A	A	EF626666.1

were in equilibrium after Bonferroni correction. In addition, none of the tests for linkage disequilibrium were significant at the 0.05 level with a sequential Bonferroni correction.

Population pairwise R_{ST} values exhibited low to high levels of genetic differentiation (0.03 - 0.6). Four population pairwise R_{ST} comparisons indicated significant genetic differentiation between Santa Rita and Huachuca shrews compared to Animas and also to Chiricahua populations. These data also suggest that moderate levels of differentiation, although not significant, exist between the Santa Rita and Huachuca and between Animas and Huachuca populations (Table 5). Heterozygosity levels ranged from 0.9 in locus A3 - 35 and locus 42S, to 0.0 in two monomorphic loci A3 - 36 and 1S (Table 6). Genetic diversity levels are highly variable across loci; expected heterozygosity is about equal for the four populations. We did not observed any microsatellite loci that were out of Hardy-Weinberg equilibrium, nor did we report linkage disequilibrium in any population. We detected that a high levels of population admixture has occurred between the Animas and the Chiricahua populations, but not among the rest of the populations. This was reflected in different genotypic frequencies and high levels of private alleles. An analysis of allele frequency distribution reveals that a large number of alleles are not shared among populations: 5, 14, 20, and 6 private (unique) alleles are found in the Animas, Chiricahua, Huachuca, and Santa Rita populations, respectively.

Clear population genetic structure was detected using the Bayesian cluster approach; the

Figure 1. a) Map of the sampling locations of each population in Arizona and New Mexico (Marshall 1957), including the mtDNA haplotype frequencies for each site. b) Cytochrome-*b* minimum-spanning network based on 249 base pairs (bp) for the four representative haplotypes. Each node represents a 1-bp change.
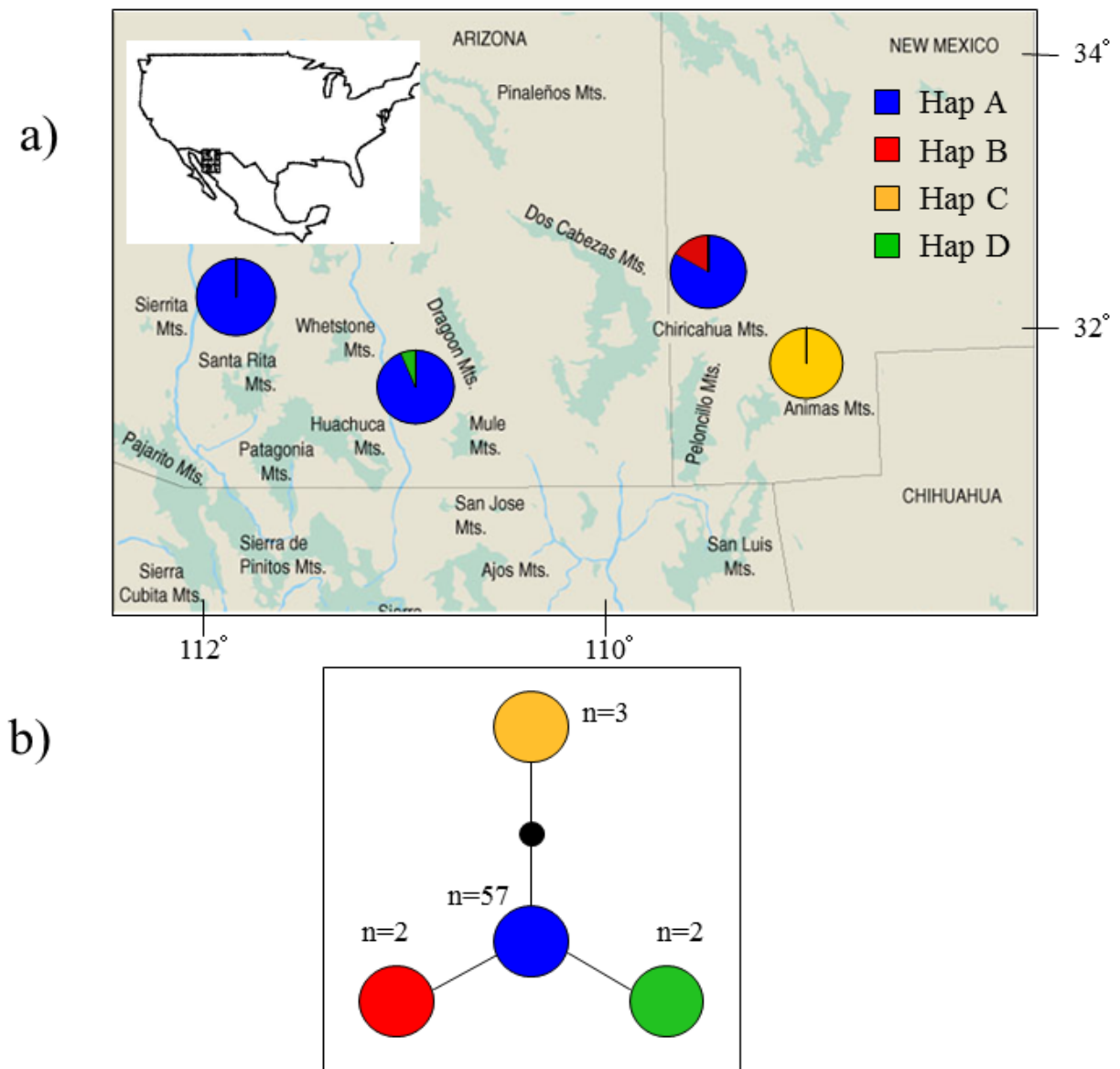
STRUCTURE program suggested that the sampled *S. arizonae* most likely represent three well-defined groups. The probability of the data was highest for K = 3; these population clusters were identified without any population locality prior information. All of the individuals sampled from the Animas and Chiricahua populations fell within a single cluster, while individuals from Huachuca and Santa Rita were assigned to two distinct clusters (Figure 3). There was very little evidence of admixture among the three defined clusters.

Discussion

The Arizona shrew is restricted to mountain ranges of southeastern Arizona, southwestern New Mexico, and northern Mexico. Only a few specimens of Arizona shrew have been collected due to their restricted difficult to access range, specialized habitat preferences, and elusive behavior. Until recently, information about the species' biology was scarce and based on only a few

specimens (Diersing and Hoffmeister 1977). In recent decades however, additional field surveys have improved our understanding of this poorly known shrew (Simons *et al*. 1990; Simons and Van Pelt 1999). The present study represents the largest sampling of *S. arizonae* to date (see also Simons and Maldonado 2007). Our work suggests that *S. arizonae* remains well distributed and relatively abundant within its restricted and fragmented range in Arizona and New Mexico, particularly in many riparian zones and upland areas, including some ridgelines and mountaintop saddles with sufficient moisture, vegetation, and soil development to provide cover and a food base for shrews.

Phylogenetic status of Sorex arizonae. In terms of the phylogenetic position of these shrews with

Table 4. Haplotype (*h*) and nucleotide diversity (π) along with their respective standard error (SE) for the four geographic populations.

Population	*h*	SE	π	SE
Animas (n = 3)	0.0	0.00	0.00	0.0000
Chiricahua (n = 12)	0.21	0.046	0.09	0.0026
Huachuca (n = 31)	0.32	0.024	0.15	0.0041
Santa Rita (n = 18)	0.0	0.00	0.05	0.0028

respect to other Nearctic and Eurasian shrews, our data supports the close affinity of *S. arizonae* to *S. trowbridgii* from Western North America and *S. saussurei* from Central Mexico.

Sorex trowbridgii shows some similarities with *S. arizonae* (George 1989), and it should be noted that the distribution of these species does not overlap; together they form part of a unique radiation of *Sorex* in North America. Our results suggest that *S. arizonae* may represent an earlier radiation with disjunct refugial populations now persisting in the mountains of southeastern Arizona, southwestern New Mexico, and northern Mexico.

Before 1977, all dark shrews from higher elevations in southern Arizona were thought to be *S. monticola* or *S. vagrans*. *S. arizonae* was unrecognized because it shares some external features with the other widely distributed shrews. Relationships among these species have been difficult to assess, with several different classifications proposed based on morphology (Diersing and Hoffmeister 1977). However, *S. arizonae* is distinguished based on a unique combination of cranial and dental features (Junge and Hoffmann 1981; Simons and Hoffmeister 2003). Moreover, our phylogenetic analysis of *mt*DNA data shows that *S. arizonae* is a well-defined species with a clear genetic from both *S. monticola* and *S. vagrans*. Our analysis placed *S. arizonae* in a well supported clade with *S. trowbridgii* and *S. saussurei* in a clade, separate from other North America shrews that are also considered to be members of the subgenus *Otisorex* (Figure 2). This enigmatic clade was previously suggested by allozyme analysis by George (1988) to be part of a distinct yet unnamed subgenus, however, these conclusions were discordant with the conclusions of other more recent molecular studies (Fumagalli *et al*. 1999; Ohdachi et al. 2006) that divided the genus *Sorex* into two monophyletic groups: the subgenus *Sorex*, with a primarily Palearctic distribution, and the subgenus *Otisorex*, distributed chiefly throughout the New World. This clade was considered as a sister group to *Otisorex* and did not support the validity of a third subgenus. Furthermore, a more recent study that examined the phylogenetic relationships of *Sorex* from Mexico and Guatemala (Esteva *et al*. 2010) also reported that several middle American species of shrews (*S. saussurei, S. veracrucis, S. macrodon* and *S. veraepacis*) formed a second major clade in the subgenus *Otisorex* (Clade B in Esteva *et al*. 2010) and were also included with *S. trowbridgii* and appeared to represent an early split of North American soricine shrews. This study dated the diversification of this clade to the late Miocene and only had members of Clade B extending beyond the Isthmus of Tehuantepec. Our study suggests that as member of the *S. trowbridgii-saussuri* clade, *S. arizonae* was also part of this older wave of diversification into southern Mexico.

Population structure of Sorex arizonae. Our molecular genetic data provides insight into the population structure of *S. arizonae*. Animas and Chiricahua populations form a single cluster which suggests that there has been gene flow in the recent past. However, with the exception of these two populations, all other individuals from the Santa Rita and Huachuca populations, were assigned with highest likelihood to inferred populations in the STRUCTURE analysis. This analysis coupled with F_{ST} and R_{ST} statistics indicate a highly significant nuclear differentiation between shrews from the Santa Rita and Huachuca mountains with those in the Chiricahua and Animas Mountains. The difference in patterns between *mt*DNA and microsatellite markers may be due to the fact that *mt*DNA shows a historical pattern with perhaps more gene flow between the Arizona populations in the past while microsatellites reveal events which could be correlated with changes in recent habitat connectivity.

Genetic differentiation between populations of *S. arizonae* is explained by the existence of isolated mountain habitats separated by lowland arid areas inhospitable for these shrews. Although *S. arizonae* is restricted to the higher parts of these mountains (*e. g.* pine-oak woodland;

Figure 2. Phylogenetic tree based on Bayesian inference, depicting cytochrome-*b* relationships of the four S. arizonae haplotypes compared with GenBank deposited sequences from 38 other Sorex species. We aligned Sorex sequences with a short-tailed shrew (Blarina), small eared-shrew (Cryptotis), and a desert shrew (Notiosorex crawfordi) that were used as outgroups. Numbers designate nodes supported by Bayesian posterior probabilities. The three major clades are denoted on the right-hand side of the tree.
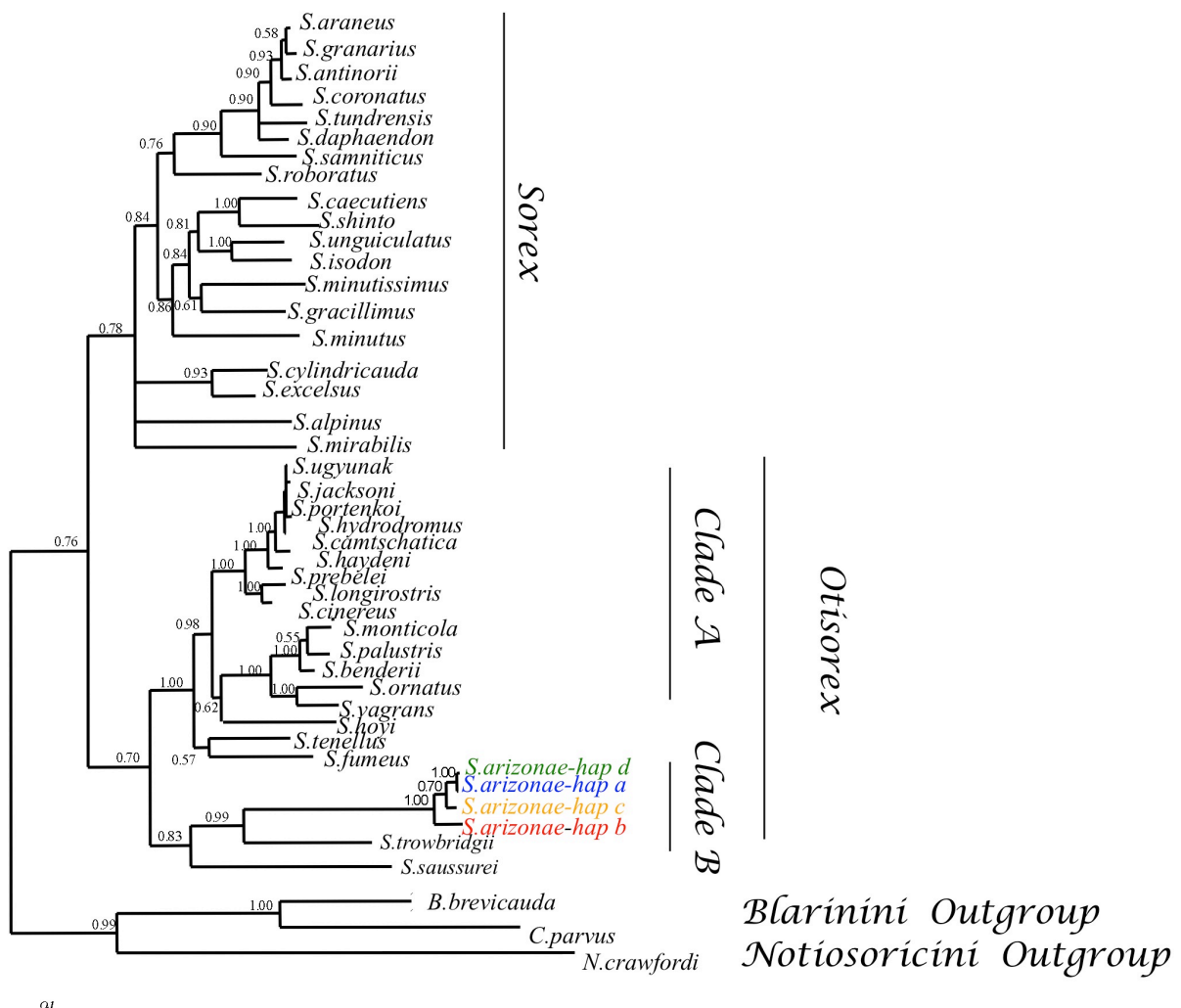
Marshall 1957), our extensive field surveys suggest that *S. arizonae* remains well distributed within its restricted and fragmented range, and reasonably abundant within quality habitat. A restricted range and low vagility presumably facilitate population isolation between localities, promoting the development of significant genetic structure among populations. Environmental and geographical factors presumably accelerate genetic differentiation among the three subpopulations. However, details of this isolation, such as the stability of isolating mechanisms through time, remains to be understood.

The microsatellite data suggest that recent gene flow among *S. arizonae* populations is quite limited amongst most of the populations, however, cytochrome-*b* sequences show that there is a widely distributed haplotype (A) among three populations, and therefore, warrant further research based on larger sample sizes and knowledge of dispersal rates that have been inferred in other shrew species (Mascheretti *et al*. 2003).

Table 5. Pairwise comparison of genetic differentiation among the four geographic populations. Values above diagonal are *Fst*-values based on DNA sequence variation of *mt*DNA cytochrome-*b*. Values below the diagonal are Rst-values based on microsatellite data. Numbers denoted by an asterisk (*) are significantly different from 0 ($P < 0.05$).

|	Animas	Chiricahua	Huachuca	Santa Rita
Animas	---	0.1617	0.2047*	0.3175*
Chiricahua	0.0397	---	0.2096*	0.3079*
Huachuca	0.4298*	0.307*	---	0.1909
Santa Rita	0.6102*	0.358*	0.1303	---

Sorex arizonae's distribution should be assessed more widely in the United States and particularly in northern Mexico, where only a single individual has been reported and the distribution of the species remains to be established (Simons and Val Pelt 1999). Based on the species' known habitat requirements and *mt*DNA differences indicated in this paper, genetic differentiation can be detected between Arizona and New Mexico. However, nuclear microsatellite differentiation among *S. arizonae* is also marked between the three Arizona subpopulations.

Overall, haplotype A was the most widely dispersed as it was found in three of four mountain ranges (Chiricahua, Huachuca, and Santa Rita). From these localities, the Huachuca population has only two haplotypes, the most private alleles, and greater nucleotide diversity and was generally the most variable with respect to measures of microsatellite variation. The Chiricahua and Santa Rita populations demonstrated intermediate levels of variation. In contrast, the Animas population presented one unique haplotype, the least number of private alleles, and moderate nucleotide diversity. Note, however, that the Animas population also had the smallest sample size in this study by far.

Finally, Arizona shrews demonstrated less genetic diversity at *mt*DNA and/or microsatellite loci than ornate shrews (*S. ornatus*; Maldonado *et al*. 2001, 2004, 2006), masked shrew (*S. cinereus*; Sipe and Browne 2004), smoky shrew (*S. fumeus*; Sipe and Browne 2004), and dusky shrew (*S. monticola*; Demboski and Cook 2001).

Conservation and management implications. Our results have implications for the conservation and management of the Arizona shrew. *S. arizonae* is arguably one of the most threatened small mammals in southern Arizona, southern New Mexico, and northern Mexico (Warshall 1986). It is thought to be threatened primarily by destruction of its habitat. In Mexico, the shrew is listed as an endangered species and; however, throughout its distributional range is considered least concern (Woodman *et al*. 2008). The one location known to support the species in Chihuahua Mexico was reportedly logged and its habitat possibly destroyed sometime after the single specimen was

Figure 3. a) Individual population assignment from STRUCTURE. b) Barplot from STRUCTURE depicting population assignment for individuals mapped and sorted by sampling location.
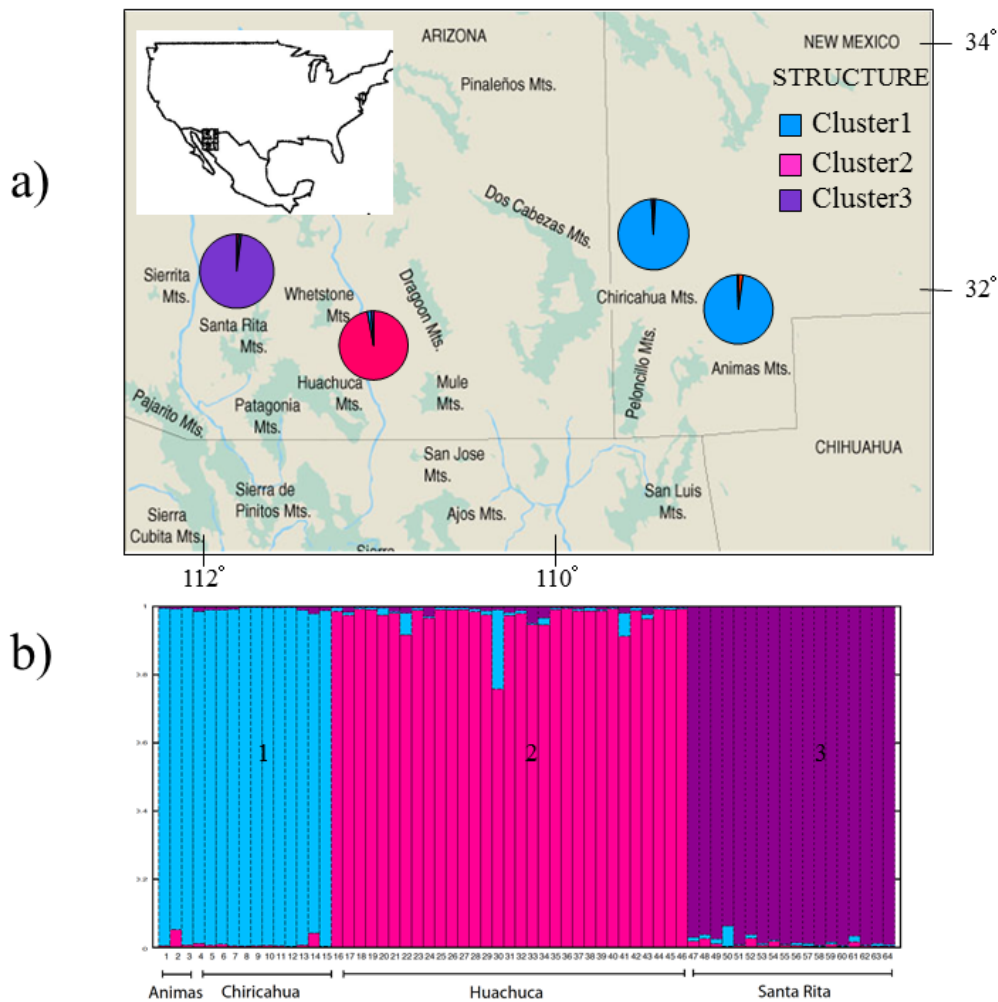
discovered (Hoffmeister 1986). In the United States, field assessments suggest that the shrew is restricted to patchy habitats along riparian zones and other well-vegetated areas. This situation suggests that populations may have a high probability of local extinction, particularly if movement corridors are limited.

Sorex arizonae can be reasonably common in high-quality habitat, but these areas are restricted within the desert sky islands where the species occurs (Simons and Maldonado 2007). Furthermore, signatures of recent gene flow were revealed with microsatellite markers between the Animas and Chiricahua populations and, therefore, additional research is needed to assess the extent to which shrews move between the relatively discrete patches of high-quality habitat.

Our data indicate that Arizona and New Mexico populations of *S. arizonae* form three distinct clusters representing independent evolutionary trajectory. We consider each cluster a management unit (MU) because they differ in allele frequencies and haplotype diversity (Moritz 1994). These MU's may respond differently to environmental changes and should be managed as separate units for conservation.

Levels of genetic variability observed in *S. arizonae* populations were low (*mt*DNA) to moderate (microsatellite). A single *mt*DNA haplotype was fixed in the New Mexico population and this population also displayed a lower mean heterozygosity at microsatellite loci compared with

Table 6. Descriptive statistics for each locus for each population including number of alleles (A_N), expected heterozygosity (H_E), and observed heterozygosity (H_O).

Locus	Population	Animas (*n* = 3)	Chiricahua (*n* = 12)	Huachuca (*n* = 31)	Santa Rita (*n* = 18)
A3-35	A_N	4	6	5	4
	H_E	0.87	0.84	0.35	0.66
	H_O	1	0.58	0.32	0.69
A4-20	A_N	3	6	2	2
	H_E	0.73	0.63	0.03	0.11
	H_O	0.67	0.58	0.03	0.11
A4-5	A_N	4	7	10	6
	H_E	0.8	0.77	0.79	0.7
	H_O	1	0.58	0.8	0.56
SH-25	A_N	2	1	3	4
	H_E	0.6	0	0.5	0.63
	H_O	1	0	0.5	0.41
A3-26	A_N	1	1	1	1
	H_E	0	0	0	0
	H_O	0	0	0	0
1S	A_N	1	1	1	1
	H_E	0	0	0	0
	H_O	0	0	0	0
42S	A_N	5	10	8	7
	H_E	0.93	0.91	0.73	0.81
	H_O	1	0.83	0.77	0.71
87S	A_N	1	2	1	1
	H_E	0	0.34	0	0
	H_O	0	0.42	0	0
50S	A_N	3	6	8	2
	H_E	0.6	0.75	0.87	0.06
	H_O	0.67	0.83	0.79	0.06
28-36S	A_N	3	5	8	3
	H_E	0.73	0.74	0.8	0.11
	H_O	1	0.5	0.83	0.11
32-40S	A_N	2	6	9	6
	H_E	0.33	0.83	0.81	0.72
	H_O	0.33	0.67	0.81	0.71
209-3S	A_N	6	8	11	6
	H_E	1	0.7	0.87	0.73
	H_O	1	0.41	0.31	0.44

the other populations. In general, only four *mt*DNA haplotypes were reported; nucleotide diversity was also moderate to low (Table 4). We suggest that the moderate variability indicated by the set of microsatellite loci designed for *S. ornatus* that we used in our analysis may be at least partially a consequence of applying heterologous markers. Other studies have demonstrated that the use of heterologous markers can reveal artificially high genetic variability in other species (Hellborg *et al.* 2002). We speculate that *S. arizonae* populations have lower levels of genetic diversity than

it appears based on the microsatellite markers given that 5 of the 12 markers we used were designed for another species (*S. ornatus*). The remaining markers used were specifically designed for *S. arizonae* (Ortega *et al*. 2005).

Sky islands can be considered Pleistocene refugia and isolation of the populations may have occurred in the past (Masta 2000). Collaborative efforts between federal governmental agencies are urgently needed to protect sky island ecosystems, including populations of *S. arizonae.* This is particularly relevant now due to the potential impacts of the rapid anthropogenic induced climatic fluctuations on montane species. If shifts in temperature take place faster than the vegetation responses, or occur beyond the boundaries of suitable potential vegetation, then high elevation populations could be forced into areas of marginal habitat where they are likely to experience decreased survival and reproduction (Crick 2004).

Before we can provide management recommendations, additional research should focus on the Animas (New Mexico) population, which displayed relatively low levels of microsatellite variation and no haplotype variation at the mitochondrial level. Although we expended more sample effort in the Animas range than anywhere else, we obtained only three specimens. This suggests that the Animas population may be smaller and more restricted than in other mountain ranges. Due to its ecological and evolutionary significance, more shrews from this mountain range are needed to better characterize population-genetic processes.

In this study, we have provided baseline data on both the nuclear and *mt*DNA genetic variation of the Arizona shrew. Additional well-designed inventory monitoring throughout the range of this species, linked to genetic analyses, particularly in New Mexico and northern Mexico, are needed to understand the status and conservation needs of this unique and elusive species.

Acknowledgements

Resumen

Introducción: Actualmente, la relación filogenética y estructura poblacional de la musaraña de Arizona (*Sorex arizonae*) es poco conocida. No obstante, tener información sobre su variabilidad genética es fundamental debido a que es una especie que se encuentra restringida a pequeños fragmentos de hábitat llamados "sky islands", que se encuentran en las zonas altas de EU y del norte de México.

Material y métodos: Se determinó la estructuración genética utilizando 64 individuos de cuatro regiones montañosas de Arizona y Nuevo México: Ánimas, Huachuca, Santa Rita y Chiricahua, utilizando secuencias de citocromo-*b* mitocondrial y 12 microsatélites.

Resultados: Los resultados del análisis del citochromo-*b* revelaron que existe un haplotipo único para la población de las Ánimas, mientras que las poblaciones restantes comparten un haplotipo. Además cada población tiene un haplotipo único en frecuencias muy bajas. Los estadísticos de F_{ST} indican que aunque la diferenciación genética es baja, es significativa entre todas las poblaciones, siendo la más alta la existente entre las poblaciones de Chiricahua y de Ánimas. Por otro lado, los análisis de STRUCTURE no revelaron diferenciación significativa entre las poblaciones

de Chiricahua y Ánimas, pero si se detectaron diferenciación entre el grupo formado por estas dos poblaciones y entre Huachuca y Santa Rita. Los análisis filogenéticos, utilizando las secuencias de citocromo-*b* disponibles de todas las musarañas norteamericanas, confirman que las musarañas de Arizona se encuentran relacionadas con *S. trowbridgii*, ambas comparten un grupo monofilético que es basal al grupo de las musarañas Neárticas, siendo parte de un grupo monofilético que es hermano del clado del subgénero *Otiosorex*.

Discusión: Nuestros resultados sugieren que las poblaciones de *S.arizonae* tienen un alto nivel de divergencia evolutiva respecto a lo encontrado para otras musarañas norteamericanas. También muestran que existe estructuración genética entre las poblaciones de acuerdo a ambos marcadores, aunque el patrón de estructuración es diferente entre ellos. Dada la inherente vulnerabilidad de estas poblaciones locales aisladas y distribución fragmentada, los datos genéticos apoyan la necesidad incrementar los esfuerzos de conservación enfocados en la protección de las zonas vegetativas riverinas que están asociadas a las zonas altas y cargadas de humedad, en las zonas altas de las montañas en donde nuestros estudios de muestreo indican que hay suficiente humedad para mantener vegetación para proporcionar suficiente base de alimentos para sostener poblaciones de musarañas *S. arizonae*.

Palabras clave: Citocromo-*b* mitocondrial; diversidad haplotípica; islas del cielo; musaraña de Arizona.

References

Avise, J. C., J. Arnold, R. M. Ball, Jr., E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489-522.

Bickham, J. W, C. C. Wood, and J. C. Patton. 1995. Biogeographic implications of cytochrome b sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86:140–144

Brant, S. V., and G. Orti. 2002. Molecular phylogeny of short-tailed shrews, *Blarina* (Insectivora: Soricidae). Molecular Phylogenetics and Evolution 22:163-173.

Brünner, H., N. Lugon-Moulin, F. Balloux, L. Fumagalli, and J. Hausser. 2002. A taxonomical re-evaluation of the Valais chromosome race of the common shrew *Sorex araneus* (Insectivora: Soricidae). Acta Theriologica 47:245–275.

Carraway, L. N. 2007. Shrews (Eulypotyphla: Soricidae) of Mexico. Monographs of the Western North American Naturalist 3:1–91.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1659.

Crick, H. Q. P. 2004. The impact of climate change on birds. Ibis 146:48-56.

Darriba, D., G. L Taboada, R. Doallo, and D. Posada. 2012. JModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

DeChaine, E. G., and A. P. Martin. 2005. Marked genetic divergence among Sky Island populations of *Sedum lanceolatum* (Crassulacea) in the Rocky Mountains. American Journal of Botany 92:477-486.

Demboski, J. R., and J. A. Cook. 2001. Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora: Soricidae): insight into deep and shallow history in northwestern North America. Molecular Ecology 10:1227-1240.

Demboski, J. R., and J. A. Cook. 2003. Phylogenetic diversification within shrews of the *Sorex cinereus* group (Soricidae). Journal of Mammalogy 84:144-158.

Diersing, V. A., and D. F. Hoffmeister. 1977. Revision of the shrew *Sorex merriami* and a description of a new species of the subgenus *Sorex*. Journal of Mammalogy 58:321-333.

Earl, D. A., and B. M. vonHoldt. 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resources 4: 359-361.

Esteva, M., F. A. Cervantes, S. V. Brant, and J. A. Cook. 2010. Molecular phylogeny of long-tailed shrews (genus *Sorex*) from Mexico and Guatemala. Zootaxa 2615:47-65.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Molecular Ecology 14: 2611-2620.

Excoffier, L., P. Smouse, and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fumagalli, L., P. Taberlet, D. T. Steward, L. Gielly, J. Hausser, and P. Vogel. 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 11:222-235.

George, S. B. 1988. Systematics, historical biogeography, and evolution of the genus *Sorex*. Journal of Mammalogy 69:443-461.

George, S. B. 1989. *Sorex trowbridgii*. Mammalian Species 337:1-5.

Hanski, I. 1994. Population biological consequences of body size in *Sorex*. Pp. 15-26 in Advances in the biology of shrews (Merritt, J. F., G. L. Kirkland, Jr., and R. K. Rose, eds.). Special Publication of Carnegie Museum of Natural History, Chicago.

Hellborg, L., C. W. Walker, E. K. Rueness, J. E. Stacy, I. Kojola, H. Valdmann, C. Vila, B. Zimmermann, K. S. Jakobsen, and H. Ellegren. 2002. Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. Conservation Genetics 3:97-111.

Hoffmeister, D. F. 1986. Mammals of Arizona. The University of Arizona Press. Tucson, EE. UU.

Junge, J. A., and R. S. Hoffmann. 1981. An annotated key to the long-tailed shrews (genus *Sorex*) of the United States and Canada, with notes on Middle American *Sorex*. Occasional Papers of the Museum of Natural History, The University of Kansas 94:1-48.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

Knowles, L. L. 2001. Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in the montane grasshoppers. Molecular Ecology 10:691-701.

Madison, D. R., and W. P. Madison. 2000. MacClade 4. Analysis of Phylogeny and Character Evolution. Sinauer Associates, Inc. Publishers. Sunderland, EE. UU.

Maldonado, J. E., C. Vila, and R. K. Wayne. 2001. Tripartite genetic subdivision in the ornate shrew (*Sorex ornatus*). Molecular Ecology 10:127-147.

Maldonado, J. E., F. Hertel, and C. Vila. 2004. Discordant patterns of morphological variation in genetically divergent populations of ornate shrews (*Sorex ornatus*). Journal of Mammalogy 85:886-896.

Maldonado, J. E., J. Leonard, G. A. Miranda, J. Ortega, R. K. Wayne, and R. J. Aguilera. 2006. Ten polymorphic microsatellite loci for the endangered Buena Vista Lake shrew (*Sorex ornatus relictus*). Molecular Ecology Notes 6:349-352.

Marshall, J. T. 1957. Birds of the pine-oak woodland in southern Arizona and adjacent Mexico. Pacific Coast Avifauna, No. 32, Cooper Ornithological Society. Berkeley, EE. UU.

Mascheretti, S., M. B. Rogatcheva, I. Gunduz, K. Fredga, and J. B. Searle. 2003. How did pygmy shrews colonize Ireland? Clues from a phylogenetic analysis of mitochondrial cytochrome b sequences. Proceedings of Biological Sciences 270:1593-1599.

Masta, S. E. 2000. Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): Recent vicariance of sky island populations? Evolution 54:1699-1711.

McAliley, L. R., M. B. O'Neill, and R. J. Baker. 2007. Molecular evidence for genetic subdivisions in the desert shrew, *Notiosorex crawfordi*. The Southwestern Naturalist 52:410–417

Moritz, C. 1994. Defining Evolutionary Significant Units for conservation. Trends in Ecology and Evolution 9:373-375.

Mouchaty, S.K., A. Gullberg, A. Janke, and U. Arnason. 2000. T he phylogenetic position of the Talpidae within eutheria based on analysis of complete mitochondrial sequences. Molecular Biology and Evolution 17:60-67.

Nishida, F. H., W. J. Sundberg, J. A. Menge, J. S. States, R. E. Tulloss, and B. J. Cifuentes. 1992. Studies in the mycoflora of the Chiricahua Mountains. I. Preliminary report on species distribution, ecology, and biogeographical affinities. Pp. 35-39 in Chiricahua Mountains Research Symposium, Proceedings (Barton, A. M., and S. Sloane, eds.). Southwest Parks and Monument Association, Tucson, EE. UU.

Ohdachi, S. D., R. Masuda, H. Abe, J. Adachi, N. E. Dokuchaev, V. Haukisalmi, and M. C. Yoshida. 1997. Phylogeny of Eurasian soricine shrews (Insectivora, Mammalia) inferred from the mitochondrial cytochrome b gene sequences. Zoological Sciences 14:527-532.

Ohdachi, S. D., N. E. Dokuchaev, M. Hasegawa, and R. Masuda. 2001. Intraspecific phylogeny and geographical variation of Northeastern Asiatic *Sorex* shrews based on the mitochondrial cytochrome b sequences. Molecular Ecology 10:2199-2213.

Ohdachi, S. D., M. A. Iwasa, V. A. Nesterenko, H. Abe, R. Masuda, and W. Haberl. 2004. Molecular phylogenetics of Crocidura shrews (Insectivora) in East and Central Asia. Journal of Mammalogy 85:396–403.

Ohdachi, S. D., M. Hasegawa, M. A. Iwasa, P. Vogel, T. Oshida, L. K. Lin, and H. Abe. 2006. Molecular phylogenetics of the soricid shrews (Mammalia) based on mitochondrial cytochrome b gene sequences: with special reference to the Soricinae. Journal of Zoology (London) 270:177-191.

Ortega, J., S. Young, L. H. Simons, and J. E. Maldonado. 2005. Characterization of six microsatellite loci for *Sorex arizonae*. Molecular Ecology Notes 5:851-853.

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

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

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

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Rousset, F. 1996. Equilibrium Values of Measures of Population Subdivision for Stepwise Mutation Processes. Genetics 142:1357-1362.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstruction phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Sambrook, I., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor Laboratory Press. New York, EE. UU.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva. Geneva, Switzerland.

Sikes, R. S., W. L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235-253.

Simons, L. H., and D. F. Hoffmeister. 2003. *Sorex arizonae*. Mammalian Species 732:1-3.

Simons, L. H. and J. E. Maldonado. 2007. Genetics of Arizona Shrew (*Sorex arizonae*). Final Report on Heritage Grants-in-Aid Project Number I99023. Arizona Game and Fish Department. Phoenix, EE. UU.

Simons, L. H., R. C. Szaro, and S. C. Belfit. 1990. Distribution of *Notiosorex crawfordi* and *Sorex arizonae* along an elevational gradient. Journal of Mammalogy 71:634-640.

Simons, L. H., and W. E. Van Pelt. 1999. Occurrence of *Sorex arizonae* and other shrews (Insectivora) in southern Arizona. The Southwestern Naturalist 44:334-342.

Sipe, T. W., and R. A. Browne. 2004. Phylogeography of masked (*Sorex cinereus*) and smoky shrew (*Sorex fumeus*) in the southern Appalachian. Journal of Mammalogy 85:875-885.

Swofford, D. L. 2001. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0 b10. Sinauer Associates Inc. Sunderland, EE. UU.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitution in the control region of

mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

WARSHALL, P. 1986. Biogeography of high peaks of the Pinalenos. Environmental Data Book, U.S. Forest Service, Coronado National Forest, by Maricopa Audubon Society. Phoenix, EE. UU.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

WOODMAN, N., J. MATSON, AND I. CASTRO-ARELLANO. 2008. *Sorex arizonae*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 13 October 2014.