Phylogeographic diversification of antelope squirrels (Ammospermophilus) across North American deserts

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We investigated the biogeographic history of antelope squirrels, genus Ammospermophilus, which are widely distributed across the deserts and other arid lands of western North America. We combined range-wide sampling of all currently recognized species of Ammospermophilus with a multilocus data set to infer phylogenetic relationships. We then estimated divergence times within identified clades of Ammospermophilus using fossil-calibrated and rate-calibrated molecular clocks. Lastly, we explored generalized distributional changes of Ammospermophilus since the last glacial maximum using species distribution models, and assessed responses to Quaternary climate change by generating demographic parameter estimates for the three wide-ranging clades of A. leucurus. From our phylogenetic estimates we inferred strong phylogeographic structure within Ammospermophilus and the presence of three well-supported major clades. Initial patterns of historical divergence were coincident with dynamic alterations in the landscape of western North America, and the formation of regional deserts during the Late Miocene and Pliocene. Species distribution models and demographic parameter estimates revealed patterns of recent population expansion in response to glacial retreat. When combined with evidence from co-distributed taxa, the historical biogeography of Ammospermophilus provides additional insight into the mechanisms that impacted diversification of arid-adapted taxa across the arid lands of western North America. We propose species recognition of populations of the southern Baja California peninsula to best represent our current understanding of evolutionary relationships among genetic units of Ammospermophilus. © 2013 The Linnean Society of London, Biological Journal of the Linnean Society, 2013, 109, 949–967.


INTRODUCTION

Genetic differentiation within and between species often coincides with significant geological or climatic changes that have shaped species ranges and altered the connectivity between populations over time. Within the North American deserts, many endemic taxa experienced high levels of initial divergence associated with the geological transformations of the Neogene, with subsequent diversification and geographical structuring of populations associated with climatic changes during the Quaternary (Riddle, 1995; Hafner & Riddle, 1997, 2005, 2011). Climatic oscillations throughout the Pleistocene led to repeated cycles of glacial expansion and retreat, with expansion reaching its maximum extent during the last glacial maximum (LGM), approximately 21 000 BP. The distributions of many arid-land (a general term we use to describe deserts as well as...
semi-arid shrublands and grasslands) species show genetic signatures of a history of population isolation and reconnection, as well as distribution changes resulting from glacial cycles, with the most recent expansion of xeric habitats following the LGM. Comparative analyses of similarly distributed species have identified complex patterns of genetic relationships within and between areas of endemism across the major warm deserts (Riddle & Hafner, 2006), but with an underlying signature of congruent genetic discontinuities between evolutionarily distinct lineages across taxa at several pronounced biogeographic barriers. These complex patterns are the results of both shared and unique responses to various events isolating and reconnecting populations.

The biogeographic history of *Ammospermophilus* (antelope squirrels) represents an opportunity to add to the growing literature that addresses the development and assembly of the arid-lands biota of North America. *Ammospermophilus* represents a distinct genus of four (following Álvarez-Castañeda, 2007) or five extant species within the rodent family Sciuridae that are distributed across the deserts and arid lands of western North America (Hall, 1981; Wilson & Reeder, 2005). *Ammospermophilus* first appears in the fossil record during the mid-Miocene, 11.5 Mya, in the Cuyama Valley of southern California (James, 1963), prior to the expansion of the semi-desert ecosystems during the Pliocene. Black (1963) provisionally assigned material of a similar age (Clarendonian North American Land Mammal Age, NALMA, 13.6–10.3 Mya) from eastern Oregon to *Ammospermophilus*, while noting the contrary opinion of Shotwell & Russell (1963). Miller (1980) reported the early Pliocene (Blancan NALMA, 4.75–1.8 Mya) *Ammospermophilus jeffriesi* from the Cape Region, southernmost Baja California peninsula, and Gustafson (1978) reported the early Pliocene (Blancan) *Ammospermophilus hanfordi* from south-central Washington. The depth of this fossil history suggests a causal association between the formation of North American regional deserts and the origin and diversification of *Ammospermophilus*, which is found throughout, and is restricted to, most of the desert and semi-desert regions in North America (except the southern portion of the Chihuahuan Desert; Fig. 1).

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Figure 1. (A) Distribution of North American regional deserts (shaded area; following Shreve, 1942; modified according to Hafner & Riddle, 1997, 2011): Great Basin, San Joaquin, Mojave, Peninsular, Sonoran, and Chihuahuan. Abbreviations: AZ, Arizona; BC, Baja California; BCS, Baja California Sur; CA, California; CFB, Cochise filter-barrier (Morafka, 1977); CHI, Chihuahua; ID, Idaho; ISM, Isla San Marcos; MH, Mesa Huatamote (Hafner & Riddle, 2011); NM, New Mexico; OR, Oregon; SFB, Southern Coahuila filter-barrier (Baker, 1956); SON, Sonora; ST, Salton Trough; TX, Texas; UT, Utah; VS, Vizcaino Seaway (Upton & Murphy, 1997). (B) Distribution of *Ammospermophilus* species (light and dark shaded areas; Hall, 1981; modified according to Hall, 1946; Armstrong, 1972; Findley et al., 1975; Schmidly, 1977; Hafner, 1981; Hoffmeister, 1986; Verts & Carraway, 1998; Yensen & Valdés-Alarcón, 1999; and Geluso, 2009). White circles indicate the localities sampled in this study (numbered in Figure 3 and listed in Appendix S1).
The white-tailed antelope squirrel, *Ammospermophilus leucurus*, is the most widespread member of this genus, occurring from the northern Great Basin to the southern tip of the Baja California peninsula, and into central New Mexico. This distribution encompasses an ecologically broad area throughout both warm desert regions in the south and cold shrubsteppe regions in the north.

Recent molecular evidence suggests that populations of *A. leucurus* from the northern Baja California peninsula expanded northwards into the continental deserts, and following an episode of isolation, formed a lineage distinct from a southern peninsular lineage that includes both *A. leucurus* and *Ammospermophilus insularis* (Riddle et al., 2000a; Whorley, Álvarez-Castañeda & Kenagy, 2004; Álvarez-Castañeda, 2007). Based on a preliminary mitochondrial DNA (mtDNA) analysis with small sample sizes (Riddle et al., 2000a), these northern populations probably share a more recent common evolutionary history with *Ammospermophilus harrisii*, a morphologically distinct species that is geographically separated by the Colorado River, indicating that *A. leucurus* may represent a paraphyletic assemblage with regard to *A. harrisii* and *A. insularis*. Separate mtDNA sequence and morphological analyses of *A. insularis*, on Isla Espíritu Santo in the Gulf of California, and a population of *A. leucurus*, on Isla San Marcos in the Gulf of California (Álvarez-Castañeda, 2007; Fig. 1B), indicated that these insular forms may both represent recently isolated populations of *A. leucurus*, and Álvarez-Castañeda (2007) recommended that *A. insularis* be considered a subspecies of *A. leucurus* (*Ammospermophilus leucurus insularis*), whereas the population on Isla San Marcos represented the mainland subspecies *Ammospermophilus leucurus extimus*. Álvarez-Castañeda (2007) identified one locality (Volcán Tres Virgenes) where northern and southern haplotypes were found in sympathy (one individual each), and suggested that the two lineages may be morphologically as well as genetically diagnosable, and may represent distinct species. Collectively, these studies suggest that the biogeographic history of *Ammospermophilus* is more complex than that suggested by current taxonomy.

Although previous studies have examined the phylogeography of some of the currently recognized species of *Ammospermophilus* (Riddle et al., 2000a; Whorley et al., 2004; Álvarez-Castañeda, 2007), to date none have included individuals from all species and from across the broad geographical ranges of the more widespread species. Here, we combine range-wide sampling of all *Ammospermophilus* species with a broad multilocus data set and a tiered methodology to present a comprehensive examination of the biogeographic and evolutionary history of this genus, and revise the species-level taxonomy to best reflect currently understood relationships among identified clades.

**MATERIAL AND METHODS**

**TAXONOMIC SAMPLING**

We collected tissue from 86 *Ammospermophilus* specimens, including representatives of all five nominal species (Appendix S1). We included geographically widespread and representative samples for *A. leucurus* (62 individuals from 28 localities), *A. harrisii* (nine individuals from four localities), and *Ammospermophilus interpres* (ten individuals from five localities). The two species with restricted distributions, *Ammospermophilus nelsoni* and *A. insularis*, were represented by two and three individuals, respectively. The localities sampled are mapped in Figures 1B and 3, and are listed in Appendix S1. Based on previous molecular research into the higher-level systematic relationships within Sciuridae (Harrison et al., 2003; Mercer & Roth, 2003; Herron, Castoe & Parkinson, 2004), we used *Cynomys gunnisoni* and *Xerospermophilus tereticaudus* as outgroups (Appendix S1).

**LABORATORY PROTOCOLS**

We extracted total genomic DNA from liver or kidney tissues following either a lysis buffer protocol (Longmire, Maltbie & Baker, 1997) or a Qiagen DNeasy Tissue Extraction Kit (Qiagen Inc.). To explore range-wide geographical variation in *Ammospermophilus*, we sequenced the mitochondrial genes cytochrome oxidase 3 (CO3) and a section of D-loop within the control region (CR) for all 86 samples of *Ammospermophilus* and the two out-group taxa. We then sequenced six additional genes for 22 individuals of *Ammospermophilus*, representing each major clade inferred from the analyses of the complete CO3 and CR data. These additional six genes included two nuclear markers, exon 1 of the interphotoreceptor retinoid-binding protein (IRBP) and the recombination activating gene 2 (RAG2), and four mitochondrial genes, including cytochrome oxidase 1 (CO1, the putative animal DNA barcoding gene), cytochrome b (Cytb), the small subunit 12S ribosomal RNA (12S), and the large subunit 16S ribosomal RNA (16S). We amplified all genes using the polymerase chain reaction (PCR) with gene-specific primers and temperature profiles (Appendix S2). Double-stranded PCR products were qualitatively examined using a 0.8% agarose gel. The amplified PCR fragments were purified using the GeneClean II Kit (BIO 101 Inc.), Qiaquick PCR Purification Kit (Qiagen Inc.), or ExoSAP IT (USB Corp.), following the manufacturers'
protocols. The purified PCR fragments (including both the light and heavy DNA strands) were sequenced using the ABI PRISM BigDye v3.1 Cycle Sequencing chemistry (Applied Biosystems Inc.), using the sequencing primers identified in Appendix S2. Unincorporated dye terminators were removed using Sephadex spin columns (Centri-Sep Inc.), and sequence data were generated on either an ABI 310 or 3130 Genetic Analyzer (Applied Biosystems Inc.). We unambiguously aligned complementary strands of each gene using SEQUENCHER 4.9 (Gene Codes Corp.), followed by manual proofreading. The protein-coding sequences were translated into amino acids using MACCLADE 4 (Maddison & Maddison, 2005), and were compared with Rattus and Mus to confirm the correct reading frame and to check for the presence of stop codons. All aligned sequences were deposited in the Dryad repository (http://www.datadryad.org/; Appendix S1).

MATERNAL PHYLOGENY
We assessed range-wide geographical variation in Ammospermophilus using maximum likelihood (ML) and Bayesian inference (BI) phylogenetic methods. For these analyses, variation within a portion of the protein-coding CO3 (691 bp) and non-coding CR (503 bp) from the mitochondrial genome was examined. We used JMODELTEST 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) to identify the most appropriate model of nucleotide evolution chosen under the Akaike’s information criterion (AIC) for the concatenated data set. We conducted ML phylogenetic analysis in TREEFINDER 2008 (Jobb, von Haeseler & Strimmer, 2004) with non-parametric bootstrapping (100 replicates; Felsenstein, 1985). The BI analyses were implemented in MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). We ran analyses for 10 × 10⁶ generations with an initial burn-in of 2 × 10⁶ generations (25 000 trees), with four Monte Carlo Markov chains, and a temperature value of 0.05. Convergence of runs was estimated by examining the posterior probabilities of clades for non-overlapping samples of trees using AWTY (Wilgenbusch, Warren & Swofford, 2004).

MULTILOCUS PHYLOGENY
We examined higher-level relationships of all nominal species and the major genetic lineages of Ammospermophilus using ML and BI analyses of our multilocus data set. We used JMODELTEST to select the appropriate models of sequence evolution for each of the seven genes. TREEFINDER was used to perform the ML analyses and calculate bootstrap values after 1000 replicates. BI analyses were run for 4 × 10⁶ generations using the default parameters of four Markov chains per generation, with random starting trees, and subsequent trees sampled every 100 generations. We assessed the stationarity of the analyses by examining the stabilization of cold-chain likelihood scores and parameter estimates using TRACER 1.4.1 (Rambaut & Drummond, 2007). The convergence of runs was assessed by examining the posterior probabilities of clades for non-overlapping samples of trees using AWTY. After excluding the trees generated during the ‘burn-in’ period prior to stable equilibrium (10 000 trees), we generated a 50% majority-rule consensus tree.

DIVERGENCE TIMES
We estimated divergence times within Ammospermophilus from our multilocus data set using an uncorrelated lognormal relaxed clock model implemented in BEAST 1.4.8 (Drummond et al., 2006; Drummond & Rambaut, 2007). The data set was partitioned into three separate alignments: one alignment contained the concatenated mtDNA data set (four protein-coding and two ribosomal genes, all linked within the mitochondrial genome); a second alignment with the IRBP data; and a third alignment with the RAG2 sequences. JMODELTEST was used to select a model of sequence evolution for each separate alignment, and a Yule process speciation model was used to set the prior on the tree.

We used fossil data to calibrate our phylogeny, setting Ammospermophilus fossilis from the Clarendonian NALMA of the mid-Miocene (James, 1963) as the Ammospermophilus stem-node calibration point. By rooting at the stem of Ammospermophilus, the minimum constraint on the out-group node was established, yielding a conservative estimate for minimum divergence time within Ammospermophilus. This stem placement was further supported by a mid-Miocene estimate of the divergence of Ammospermophilus from the out-group taxa (Cynomys and Xerospermophilus) generated from several independent and external fossil calibrations throughout the sciurid phylogeny (Mercer & Roth, 2003). Analyses in BEAST were run for 4 × 10⁷ generations, sampling every 1000 generations. The first 4 × 10⁶ (10%) generations were discarded as burn-in. TRACER was used to verify proper mixing of the chains and to ensure that the analyses reached stationarity. To increase the effective sample size (ESS) values, the analyses were repeated, and the data from the two separate runs were combined.

Two additional calibration methods were tested to compare divergence estimates within the phylogeny. Using the same BEAST methods, the fossil calibration was placed at the basal node (crown group) of
Ammospermophilus divergence. We additionally used a mutation rate-calibrated estimation of divergence times performed on just the Cytb data set. We used the standard mutation rate of 2% per Myr (Arbogast & Slowinski, 1998). JMODELTEST was used to choose the appropriate models of sequence evolution for the Cytb data set. We employed an uncorrelated exponential relaxed clock with a coalescent model of exponential growth, and ran analyses for $4 \times 10^7$ generations, sampling every 1000 generations, and discarded the first 10% as burn-in.

**DISTRIBUTION AND DEMOGRAPHIC CHANGES**

To explore the generalized distributional changes of *Ammospermophilus* since the LGM, we constructed species distribution models (SDMs) for each of the three major lineages inferred from our phylogenetic analyses. We built models for present (0 kya) and past (LGM, c. 21 kya) climatic conditions based on occurrence data. This data set included occurrence records of individuals examined in this study (Appendix S1) as well as a subset of available records accurate to within 5 km listed in ManIS (http://manisnet.org/). In total we compiled 44 records for the interpres clade, 255 records for the leucurus north clade, and 50 records for the leucurus south clade. These occurrence records spanned the geographical distribution of each of the three lineages. The maximum entropy method implemented in MAXENT 3.2.1 was used to generate these models (Phillips *et al*., 2006a; Phillips, Anderson & Schapire, 2006b). MAXENT has been shown to outperform similar habitat estimators (Phillips & Dudik, 2008; Elith & Graham, 2009), and its utility in phylogeographic studies was summarized by Carstens & Knowles (2007) and Waltari & Guralnick (2009). The predictions for this analysis were based on elevation plus a suite of 19 bioclimatic parameters previously compiled from the WorldClim climate layers (Hijmans *et al*., 2005; Waltari *et al*., 2007), with a 2.5-arcminute/pixel resolution.

Model calibrations were performed using 75% of the data as a training group, and the predicted distribution models were then tested with the remaining 25% (Evans *et al*., 2009). Default parameters were used (500 maximum iterations; convergence threshold of 0.00001; regularization multiplier of 1; 10 000 background points) with random seeding, removal of multiple presence records from individual cells resulting from sampling localities within 5 km² (i.e. one pixel), and logistic probabilities for the output (Phillips & Dudik, 2008). A split-sample approach was used to separate the geographically closest sample pairs between the training and test groups reduced the effects of spatial autocorrelation (Fielding & Bell, 1997; Parolo, Rossi & Ferrarini, 2008).

An initial model (including all 20 variables) was run to produce ‘area under the receiver operation characteristic curve’ (AUC) values for each bioclimatic parameter. A minimum AUC of 0.75 for the test group was considered the threshold for good model performance (Elith *et al*., 2006; Suárez-Seaone *et al*., 2008; Elith & Graham, 2009). Models were then run using temporal transfer modelling from the current distribution to the LGM, incorporating information in the Community Climate System Model (CCSM 3; Collins *et al*., 2006) and the Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori, 2004). MAXENT analyses were performed three separate times using both the CCSM and MIROC climate reconstructions, and the habitat model results from both were averaged, accepting only those areas that both methods agreed were suitable (Waltari & Guralnick, 2009). Averaging the three independent MAXENT runs using the Spatial Analyst feature in ArcGIS produced presence/absence binary habitat models using ArcGIS 9.2 (ESRI Corp., Redlands, CA, USA). The models were evaluated across four logistic thresholds: fixed cumulative value of 10.0; equal training sensitivity and specificity; equal test sensitivity and specificity; and equal entropy of thresholded and non-thresholded distributions. These threshold values were used to assess a range of sensitivities and specificities to ensure that model interpretations were robust. Ultimately, the chosen cut-off of suitable habitat had a fixed cumulative probability of 10, a level that rejected the lowest 10% of predicted logistic values. This value, although conservative, maintained a low omission rate (Pearson *et al*., 2007), consistent with the expectation that the occurrence records contain some georeferencing errors.

We additionally assessed responses to Pleistocene climatic oscillations by exploring the demographic history of three widespread geographical clades of *A. leucurus* inferred from our maternal phylogeny (see below). We created mismatch distributions of pairwise distances among haplotypes and estimated several demographic parameters for each clade, including nucleotide diversity ($\pi$), haplotype diversity ($h$), and Tajima’s $D$ (Tajima, 1989), using DNASP 5.0 (Rozas *et al*., 2003). If SDMs generally predicted an expansion of favourable habitat since the LGM for most lineages of *Ammospermophilus*, we hypothesized that demographic parameter estimates for the three wide-ranging clades of *A. leucurus* would similarly suggest population expansion.

**RESULTS**

**MATERNAL PHYLOGENY**

The HKY + I + G model of nucleotide evolution was selected for the combined CO3 and CR data set.
Analyses of the CO3 and CR sequence data for all samples indicated strong phylogeographic structure within *Ammospermophilus* (Fig. 2). We inferred three well-supported major lineages: *A. interpres* (hereafter referred to as the *interpres* clade), *A. insularis* + *A. leucurus* from the southern Baja California peninsula (leucurus south clade), and *A. harrisii* + *A. nelsoni* + *A. leucurus* from the northern Baja California peninsula and the USA (leucurus north clade). Samples of *A. insularis* formed a distinct clade within the leucurus south clade. *Ammospermophilus harrisii* appeared to be polyphyletic, with two lineages falling out in different places within the leucurus north clade. Samples of *A. harrisii* collected from Sonora, Mexico, and one sample from western Arizona, were basal to a clade that contained all other *A. harrisii*, as well as *A. nelsoni* and two geographical clades of *A. leucurus* within the leucurus north clade (one in the northern Baja California peninsula, the other including all US samples of *A. leucurus*). Relationships among samples within each of the three clades of *A. leucurus* (USA, northern peninsula, and southern peninsula; Fig. 3) lacked resolution (based on posterior probabilities and bootstrap values), consistent with a relatively recent diversification within each lineage. Individuals with alternate mtDNA complements representative of either the northern peninsula or southern peninsula clades (representing two of the three major clades within the genus) were found together at two localities in northern Baja California Sur: 32 km east of San Ignacio (one northern and one southern individual) and 32 km south-east of Santa Rosalía (two northern and one southern individual; Fig. 3B). These localities are in the vicinity of Volcán Tres Vírgenes, a sympatric site previously identified by Álvarez-Castañeda (2007).

**MULTILOCUS PHYLOGENY**

The aligned multilocus data set contained 5962 bp and variable numbers of informative sites per gene: CO1, 76 informative sites/691 bp; CO3, 115/690 bp; Cytb, 173/1143 bp; 12S, 54/832 bp; 16S, 36/550 bp; IRBP, 43/1087 bp; Rag2, 17/969 bp. Models of nucleotide evolution selected for each gene were GTR + I (Cytb, CO1, CO3), GTR + I (12S, 16S), HKY + I (Rag2), and HKY + I + G (IRPB). We were unable to obtain complete sequence data for western samples of *A. harrisii* (Sonora and western Arizona), so this lineage was not included in the multigene data set.

Both ML and BI phylogenetic analyses resulted in a phylogenetic tree with three well-supported clades (Fig. 4). One clade was composed of *A. harrisii*, *A. leucurus* samples from the northern Baja California peninsula and USA, and *A. nelsoni*. Samples of *A. leucurus* from the southern Baja California peninsula formed a well-supported clade that included *A. insularis*, consistent with previous studies (Riddle et al., 2000a; Álvarez-Castañeda, 2007). A third, well-supported major clade was composed of samples of *A. interpres*. These three clades corresponded to the same clades inferred in our mtDNA range-wide assessments, referred to as our leucurus north, leucurus south, and interpres clades, respectively.

Although the monophyly of *Ammospermophilus* was strongly supported, the relationship among the three major clades within this genus was unclear. Analysis of the nuclear data set alone (results not shown) confirmed the monophyly of *Ammospermophilus*, but did not show support for any structure within the genus. Importantly, however, the mtDNA and combined nuclear data were not in conflict, although western samples of *A. harrisii* were lacking from the nuclear data set.

**DIVERGENCE TIME ESTIMATES**

Based on our *Ammospermophilus* stem-calibrated phylogeny, the three major clades of *Ammospermophilus* may have diverged at around 4 Mya (event B in Fig. 5, Table 1). Divergences within *A. interpres* (Fig. 5, event D) probably started near the end of the Pliocene, around 3 Mya. Western samples of *A. harrisii* were lacking from the nuclear data set, and were not included in divergence time estimates, but the estimated date of divergence...
Figure 3. (A) Relationships among species of *Ammospermophilus* (exclusive of *A. interpres*) based on Bayesian and maximum likelihood analyses of 1194 base pairs of mitochondrial sequences (CO3 + CR), depicting three geographically widespread clades of *Ammospermophilus leucurus* in the USA, northern Baja California (BC) peninsula, and southern Baja California peninsula. Strongly supported nodes (≥ 0.95 Bayesian posterior probability, ≥ 70% ML bootstrap) are denoted by asterisks. AZ, Arizona; NM, New Mexico. (B) Distribution of *Ammospermophilus* (light and dark shaded area) and location of sample localities (numbered as in Figure 3A). Inset: detail of localities (numbers from this study, unnumbered circles from Álvarez-Castañeda, 2007) in relation to the purported former location of a Vizcaíno Seaway: northern peninsular clade (dark shading and black circles), southern peninsular clade (light shading and white circles), and three localities (half white, half black; boldface in Figure 3A) in which mtDNA haplotypes of both northern and southern Baja California peninsular clades are found.

Table 1. Estimated divergence dates within *Ammospermophilus* for each node depicted in Figure 5 based on three calibration methods: fossil calibration placement at node A; fossil calibration placement at node B, and a rate calibration using cytochrome *b* (*Cytb*) data

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>Cytb</th>
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<tbody>
<tr>
<td>B</td>
<td>4.13 (2.11–6.6)</td>
<td>11.77 (10.1–13.37)*</td>
<td>5.05 (1.19–5.18)</td>
</tr>
<tr>
<td>C</td>
<td>3.58 (1.34–4.66)</td>
<td>5.08 (2.22–8.21)</td>
<td>1.05 (0.48–1.95)</td>
</tr>
<tr>
<td>D</td>
<td>2.91 (0.47–3.75)</td>
<td>2.37 (0.5–5.02)</td>
<td>0.53 (0.03–0.76)</td>
</tr>
<tr>
<td>E</td>
<td>2.52 (0.99–4.25)</td>
<td>4.17 (1.6–7.34)</td>
<td>0.63 (0.13–1.16)</td>
</tr>
<tr>
<td>F</td>
<td>2.01 (0.95–3.69)</td>
<td>3.42 (1.33–5.83)</td>
<td>1.05 (0.48–1.95)</td>
</tr>
<tr>
<td>G</td>
<td>0.64 (0.02–1.13)</td>
<td>0.85 (0.05–2.31)</td>
<td>0.27 (0.0–0.38)</td>
</tr>
<tr>
<td>H</td>
<td>0.15 (0.03–0.92)</td>
<td>1.05 (0.09–2.78)</td>
<td>0.54 (0.0–0.51)</td>
</tr>
<tr>
<td>I</td>
<td>0.17 (0.01–0.98)</td>
<td>0.64 (0.04–1.68)</td>
<td>0.06 (0.0–0.19)</td>
</tr>
</tbody>
</table>

Divergence times in Mya followed by 95% posterior credibility intervals in parentheses. Asterisks denote fossil calibration points.
between *A. nelsoni* and northern peninsular–USA
*A. leucurus + A. harrisii* (event C) was near 3.6 Mya, and the divergence between *A. harrisii*, northern peninsular *A. leucurus*, and USA *leucurus* (event F) was estimated at about 2 Mya. The time to the most recent common ancestor (tmrca) of southern *A. leucurus + A. insularis* (event E) was estimated at 2.5 Mya, whereas within-population diversification in *A. insularis* (event G), *A. nelsoni* (event H), and *A. harrisii* (event I) was more recent, and well within the Pleistocene.

Our alternative calibrations resulted in different estimates of divergence times. Based on an *Ammospermophilus* crown-calibrated phylogeny, the divergence of *Ammospermophilus* from the out-group taxa was estimated to have occurred at 25.2 Mya, and estimates of clade and lineage divergence within *Ammospermophilus* were generally much older than estimates generated with the placement of fossil calibration on the stem node (Table 1). Dates from a rate-calibrated Cytb data set were more congruent with the placement of the fossil calibration at the stem node of the phylogeny, and suggested that *Ammospermophilus* diverged from the out-group taxa at approximately 12.5 Mya. This rate calibration method also indicated a basal divergence among the major *Ammospermophilus* lineages at around 5 Mya,
consistent with the placement of a fossil calibration at the stem node of the phylogeny.

**Distribution and demographic changes**

The results of all SDMs were significantly better than random samples (AUC = 0.5) in receiver operating characteristic analyses (interpres clade, training AUC = 0.977, test AUC = 0.962; leucurus north clade, training AUC = 0.960, test AUC = 0.950; leucurus south clade, training AUC = 0.996, test AUC = 0.995). The AUC values for all variables were greater than 0.75. The predicted high-quality habitat represented in the present-day SDMs for each of the three clades (Fig. 6A, C, and E) largely captured the current distribution of the species in each clade (Fig. 1). For the leucurus north clade, the present-day SDM (Fig. 6A) indicated continuous, high-quality habitat from the central Baja California peninsula north throughout the Mojave and Great Basin Deserts, and into the San Joaquin Valley of California. Disjunct or lower-quality habitat extended into the eastern reaches of the Great Basin (see Fig. 1), into the Sonoran Desert, and into the westernmost portion of the Chihuahuan subregion of the Chihuahuan Desert (considered to be transitional between Sonoran and Chihuahuan elements by Hafner & Riddle, 2011). The present-day model for the leucurus south clade (Fig. 6C) indicated that continuous high-quality habitat was restricted to the southern half of the Baja California peninsula and to the adjacent, coastal continental Sonoran Desert. The present-day SDM for the interpres clade (Fig. 6E) indicated that appropriate habitat was generally concentrated throughout the two northern subregions of the Chihuahuan Desert (Chihuahuan and Trans-Pecos; Hafner & Riddle, 2011).

The reconstructions of LGM models for each of the three clades of *Ammospermophilus* predicted an overall loss of suitable habitat for each clade, although these models do not portray significant exposures of potential habitat that resulted from lowered full-glacial sea levels (perhaps 200 km of dry land at the head of the Gulf of California and extensive continental shelf along the west coast of the Baja California peninsula; Hafner & Riddle, 2011: fig. 4.3). The high-quality habitat for northern leucurus (Fig. 6B) was compressed to the central Baja California peninsula and the western Mojave Desert. The predicted habitat for the southern leucurus clade (Fig. 6D) was similarly compressed, confined within the southern extent of the Baja California peninsula. The LGM model for the interpres clade (Fig. 6F) indicated that habitat for this clade was dramatically compressed to a much smaller pocket of suitable habitat in the Coahuilan and Zacatecan subregions (Hafner & Riddle, 2011) of the southern Chihuahuan Desert.

Demographic parameter estimates calculated for each of the three widespread clades of *A. leucurus* (USA, southern peninsula, and northern peninsula) yielded similar values. Values indicated that each clade had low nucleotide diversity (USA, 0.0019; southern peninsula, 0.00319; northern peninsula, 0.00315) and relatively high haplotype diversity (0.616; 0.889; 0.805). Tajima’s *D*-values for each clade was significantly negative (−2.16215; −1.87675; −2.16208), consistent with probable population expansion. Mismatch distributions of haplotypes from each lineage (Appendix S3) indicated a unimodal distribution of haplotypes for all three clades, congruent with the demographic parameters and with a model of recent demographic expansion.

**Discussion**

Genetic patterns in a suite of arid-land taxa have been used to investigate the biogeographic history of North American deserts. These include mammals (Riddle et al., 2000a; Riddle, Hafner & Alexander, 2000b, c; Álvarez-Castañeda & Patton, 2004; Jezkova et al., 2009; Bell et al., 2010), birds (Zink et al., 2001; Zink, 2002), reptiles (Upton & Murphy, 1997; Lindell, Mendez-de la Cruz & Murphy, 2005; Douglas et al., 2006; Leaché, Crews & Hickerson, 2007; Leaché & Mulcahy, 2007), amphibians (Jaeger, Riddle & Bradford, 2005; Bryson et al., 2012), invertebrates (Ayoub & Reichert, 2004; Crews & Hedin, 2006; Wilson & Pitta, 2010; Graham et al., 2013), and plants (Nason, Hamrick & Fleming, 2002; Garrick et al., 2009), as well as fish species bordering warm desert regions (Bernardi & Lape, 2005; Reginos, 2005). This broad set of exemplar taxa has demonstrated a complex history of vicariance and dispersal in response to both geological forces and climatic cycles. Our results suggest that *Ammospermophilus* has responded in similar fashion to this shared history, and provide additional insight into the mechanisms that impacted diversification of arid-adapted taxa across the arid lands of western North America.

**Pre-Quaternary diversification**

Initial patterns of historical divergences within *Ammospermophilus* appear coincident with dynamic alterations in the landscape of western North America and the formation of the regional deserts during the late Neogene (Riddle et al., 2000a). Following a basal divergence of *Ammospermophilus* into three major lineages at the Miocene–Pliocene boundary (Fig. 5), diversification within lineages occurred in rapid succession. Many of these divergence events
appear attributable to a number of vicariant events that occurred prior to the Quaternary period.

*Ammospermophilus interpres* in the Chihuahuan Desert, the easternmost component of the North American deserts, represents a divergent lineage that may have been isolated during the Late Miocene–early Pliocene. During this time, secondary uplifting of the Sierra Madre Occidental occurred simultaneously with global climate change and the development of regional deserts (Axelrod, 1979; Webb, 1983; Zink et al., 2000; Riddle et al., 2000a). The synergistic effects of these Neogene events probably subdivided many taxa spanning the Sonoran and Chihuahua deserts (Riddle & Hafner, 2006; Bryson, García-Vázquez & Riddle, 2011), with the more strongly desert-adapted taxa likely to be isolated in arid regions subdivided by the Sierra Madre Occidental (Pyron & Burbrink, 2010).

*Ammospermophilus leucurus*, as currently recognized, represents a geographically structured polyphyletic species forming at least three distinct lineages: the USA lineage and northern peninsular lineage (aligned with *A. nelsoni* and *A. harrisii* in the *leucurus* north clade), and the southern peninsular lineage of the *leucurus* south clade (Fig. 3). The northern and southern peninsular lineages meet at the eastern edge of the Vizcaíno Desert of the central Baja California peninsula. This peninsular pattern, inferred previously in *Ammospermophilus* (Riddle et al., 2000a), is similar to patterns of mid-peninsular divergences detected in a suite of other taxa (e.g. Riddle et al., 2000b, c; Lindell et al., 2005; Crews & Hedin, 2006; Lindell, Méndez-de la Cruz & Murphy, 2008; Bryson et al., 2012). Spatial and temporal divergences in these taxa support a mid-peninsular vicariance event potentially caused by the Vizcaíno Seaway (Figs 1A, 3B) during the late Miocene or early Pliocene (Upton & Murphy, 1997; Riddle et al., 2000a; Whorley et al., 2004; Hafner & Riddle, 2011). Contact localities between the two clades of *Ammospermophilus* occur along the eastern edge of the hypothesized seaway, which may have been bridged by regional uplift and associated eruption of the Tres Virgenes volcanic field, initiated 1.2 Ma and continuing today (Hafner & Riddle, 2011). Although direct geological evidence is seemingly absent for the Vizcaíno Seaway (Crews & Hedin, 2006; Lindell, Ngo & Murphy, 2006), there may be a strong set of environmental factors driving divergences across this area (Grismer, 2000, 2002). Regardless of the exact causal mechanism, diversification across the mid-peninsular region during the Late Miocene and Pliocene appears to be a robust pattern.

Although the distinction between the northern and southern peninsular lineages of *A. leucurus* is based primarily on mtDNA sequence differences, studies based on other data also support this division. Hafner (1981) recognized that the southern peninsular form was basal to all other recognized species in the genus (except *A. interpres*) based on limited allozyme data and morphometric analyses, and tentatively suggested the recognition of a new species, *Ammospermophilus canfieldiaceae*. Cladistic analysis of 23 presumptive loci encoding for 13 proteins, with *A. interpres* designated as the out-group, placed the southern peninsular form (represented by a single population from the southernmost Cape Region) outside all remaining samples of (in turn) *A. nelsoni*, *A. harrisii*, and *A. leucurus* (including two populations from the northern peninsula; Hafner, 1981). Similarly, phenetic analysis of bacular morphology (Hafner, 1981) indicated that those of *A. interpres* were the most divergent, followed (in order) by those from the southern peninsula, *A. nelsoni*, *A. harrisii*, and other *A. leucurus*. Multivariate analysis of 26 cranial and dentary measurements from a total sample including 253 specimens from the Baja California peninsula (Hafner, 1981) indicated an abrupt shift in morphology at approximately 29°30′N latitude, the northernmost margin of the peninsular fog desert (Mesa Huatamote filter barrier; Hafner & Riddle, 2011; Fig. 1A). This morphological shift was more abrupt than that across the Colorado River between the recognized species *A. leucurus* and *A. harrisii*, but was substantially north of the Vizcaíno Desert contact between northern and southern peninsula clades identified herein by mtDNA sequence analysis. Hafner (1981) indicated a subtle shift in morphology in the Vizcaíno region (based on condylobasal length), and also demonstrated a geographically separate shift in pelage coloration (banding pattern of tail undersurface, a character used to distinguish species of *Ammospermophilus* in New Mexico; Findley et al., 1975). This shift was even
farther north, coinciding with the versants of the Peninsular Range (Sierras Juárez and San Pedro Mártir): *Ammospermophilus* of the more mesic western slopes and south on the peninsula have the pattern characteristic of *A. harrisii* and *A. interpres*, whereas those of the drier eastern slopes have the typical *A. leucurus* banding pattern.

The evolutionary histories of *A. harrisii*, *A. nelsoni*, and northern populations of *A. leucurus* appear to be closely linked. Together, these taxa probably diverged in the early Pliocene, around 3.6 Mya, into western (*A. nelsoni*) and eastern (*A. harrisii + northern A. leucurus*) groups, coincident with geological events that isolated the Mojave Desert and San Joaquin Valley from the Peninsular and Sonoran Deserts (Bell et al., 2010). These included the intensified uplift of the Sierra Nevada and Transverse ranges (Norris & Webb, 1990), marine inundation of the Salton Trough (Boehm, 1984), and the formation of a through-flowing Colorado River (the freshwater interpretation of the Bouse Embayment of Luchitta, 1979; Spencer & Pearthree, 2001, 2005; Fig. 1A).

Interestingly, a distinct second lineage of *A. harrisii* from Sonora and western Arizona may have also diverged from other lineages within our *leucurus* north clade during the late Miocene–Pliocene. Although we lacked nuclear sequence data for *A. harrisii* from Sonora and western Arizona, and so did not include this lineage in multilocus estimates of divergence times, our phylogenetic analyses of mtDNA data (Figs 2, 3) suggest a basal split of this lineage from other lineages within our *leucurus* north clade. Assuming multilocus data are in accord with this estimate, this divergence may date to sequential development of the through-flowing Colorado River (Spencer & Pearthree, 2001, 2005) along the Bouse Embayment (Buising, 1990). This geological event is thought to have driven diversification in a number of co-distributed taxa (Lamb, Avise & Gibbons, 1989; Jones, 1995; Riddle et al., 2000b, 2000c; Pellmyr & Segraves, 2003; Murphy, Trépanier & Morafka, 2006; Castoe, Spencer & Parkinson, 2007). The region surrounding the head of the Gulf of California, at the meeting point of the Mojave, Peninsular, and Sonoran regional deserts, has had a complex geological history, resulting in complex patterns of species diversity (e.g. Hafner & Riddle, 2011) and within-species groups (e.g. *Neotoma lepida* species group; Patton, Huckaby & Álvarez-Castañeda, 2007). Genetic relationships within *A. harrisii* in this region should be evaluated in future studies employing multilocus data and larger sample sizes of *A. harrisii* from the western part of the Sonoran Desert, and investigating possible introgression between the two lineages of *A. harrisii*: our sample from locality 32 (Fig. 3B) contained haplotypes from eastern and western *A. harrisii* (Fig. 3A).

All of the species of *Ammospermophilus* possess large, conservatively retained blocks of constitutive heterochromatin (accounting for a 75% increase in total DNA versus other ground squirrels), despite extensive non-Robertsonian chromosomal rearrangements among the species (Mascarello & Mazrimas, 1977). Mascarello & Mazrimas (1977: 215) concluded that these unusual blocks of heterochromatin serve an important function, perhaps of ‘a regulator of recombination and, hence, of variation in populations.’ If these blocks of heterochromatin limit nuclear DNA variation, it may partially explain the absence of geographic structuring found in the two nuclear DNA markers compared with the extensive differentiation in mtDNA genes.

### Quaternary Climate Change and Genetic Consequences

Population contractions during the LGM and subsequent expansions northward are characteristic of a number of North American warm desert taxa (e.g. Riddle et al., 2000a; Jaeger et al., 2005; Cicero & Koo, 2012). Based on our species distribution models, there was an overall restriction of suitable habitat for each of the three major clades of *Ammospermophilus* at the LGM. Suitable habitat subsequently expanded along with the desert regions of western North America following glacial retreat.

Genetic patterns evident in the three geographically widespread clades of *A. leucurus* suggest that these clades tracked this expanding habitat. Our population demographic analyses indicate that each clade of *A. leucurus* has undergone recent expansions from smaller ancestral populations, leading to an excess of low-frequency polymorphisms. These results are in general agreement with the data reported by Whorley et al. (2004), who found evidence of population expansion in *A. leucurus*. In particular, the USA *leucurus* lineage is widespread across the Mojave Desert and Great Basin, displaying generally smooth, clinal variation in cranial morphology, with more abrupt change occurring along the Peninsular Ranges of the northern Baja California peninsula (*Ammospermophilus leucurus peninsulae*), and along the upper Colorado River and lower San Juan River of south-eastern Utah (*Ammospermophilus leucurus pennipes*; Hafner, 1981). While noting often marked variation in pelage coloration associated with more mesic climate (darker coloration) or substrate (reddish), Hafner (1981) recommended combining the five recognized subspecies (Hall, 1981) spanning the Mojave Desert and Great Basin into a single subspecies (*Ammospermophilus leucurus leucurus*).

Soon after the marine inundation of the Salton Trough, sediment from the fully formed Colorado
River had filled in much of the Salton Trough and isolated it from the Gulf (Spencer & Pearethree, 2001, 2005); however, portions of the Salton Trough were periodically submerged during the Late Pleistocene–Holocene (Stokes et al., 1997). Collectively, these historical inundations of the Salton Trough appear to have driven movement and maintained the phylogenetically distinct lineages of USA and northern peninsular Ammospermophilus. Similarly, abrupt ecological and climatic shifts in the Vizcaíno Desert region of the central Baja California peninsula (Grismer, 2002), coupled with climatic oscillations of the Late Pleistocene, may have produced alternate pulses of contact and isolation between the northern and southern peninsular forms of Ammospermophilus subsequent to their divergence in isolation.

Rivers appear to represent partial or complete barriers to dispersal in Ammospermophilus. For example: the Colorado River between A. leucurus and A. harrisii downstream from the central Grand Canyon of Arizona; the Río Grande between A. leucurus and A. interpres in New Mexico; the western (Río Grande) and eastern (Pecos River) limits of A. interpres in Texas; and the few records of A. leucurus north of the Snake River in Idaho are believed to have resulted from crossings of man-made bridges (Davis, 1939), as is a single record on the south side of the bridge across the Grand Canyon of the Colorado River in Arizona (Hoffmeister, 1986). It is not clear whether these limits are imposed by the rivers themselves or by the absence of suitable habitat in the vicinity of the rivers. The lower reaches of the Colorado River and the Río Grande have both been historically subject to extensive meanderings, effecting a river ‘crossing’ in static rodent populations. The Río Grande has historically been reduced seasonally to a shallow stream and mudflats, whereas the Colorado River was fully diverted for 2 years in 1905 (forming the Salton Sea; Sykes, 1937), and flow to the Gulf was reduced substantially by completion of the Hoover Dam (in 1935), and ceased entirely for 20 years following construction of the Glen Canyon Dam in 1962 (Brusca et al., 2005). However, two of three specimens (MVZ) assigned to A. harrisii from the vicinity of Yuma, Arizona, and collected prior to these modern perturbations (1892 and 1899), were morphologically identified as A. leucurus with a posterior probability of > 0.99 (Hafner, 1981).

The isolation of A. harrisii and A. interpres appears to have been maintained by the Sierra Madre Occidental and the ranges of eastern Arizona and western New Mexico. The Cochise filter-barrier (Morafka, 1977; Fig. 1A), a lower-elevation gap in these elevated highlands along the southern Arizona–New Mexico border, probably posed a complete barrier to Ammospermophilus during full-glacial periods, when pluvial lakes filled the adjacent closed basins (pluvial lake Cochise in Arizona, Animas in New Mexico, and Palomas in Chihuahua; Smith & Street-Perrott, 1983). Although the Cochise filter-barrier has maintained isolation in a large number of co-distributed taxa (Pyron & Burbrink, 2010), A. harrisii has subsequently spread east (Geluso, 2009), yet remains isolated from A. interpres by the intervening playas and the Río Grande in southern New Mexico (Fig. 1B). In central New Mexico, A. leucurus occurs on bajadas on the west bank of the Río Grande, whereas A. interpres is restricted to piñon–juniper woodland habitat 10–15 km east of the river (Fig. 1B; D. J. Hafner, pers. observ.). Although we did not analyse the demographic history of A. interpres because of the low sample size, this species is likely to have contracted and expanded in response to glacial–interglacial cycles throughout the Pleistocene. Our SDM at the LGM (Fig. 6F) indicates a severe compression of the distribution of A. interpres into the southernmost Chihuahuan Desert, very similar to a recent SDM constructed for the desert pocket mouse, Chaetodipus eremicus (Jezkova et al., 2009). In stark contrast, our present-day models (Fig. 6E) suggest large swathes of suitable habitat extending from the southern Chihuahuan Desert, across all of Trans-Pecos Texas, and most of New Mexico, in broad agreement with the current distribution of A. interpres. Presumably future studies will uncover a marked genetic response in A. interpres as this species tracked habitat expansion northwards.

**CONCLUSION**

The apparent lack of zones of sympatry between neighboring species of Ammospermophilus has frustrated attempts to evaluate the degree of genetic isolation between recognized species (Findley et al., 1975; Hafner, 1981). Clear morphological and ecological differences exist among A. leucurus and the peripheral allopatric species A. nelsoni (larger body size; more yellowish pelage coloration; more open desert habitat) and A. interpres (longer relative tail and hindlimbs; closer association with piñon–juniper woodland and rocky habitat); however, such distinctions are far less pronounced between A. leucurus and A. harrisii, separated only (and perhaps incompletely) by the lower Colorado River (Hafner, 1981). There are no obvious morphological or ecological differences between the northern peninsular and southern peninsular lineages of A. leucurus described herein (Hafner, 1981), although Álvarez-Castañeda (2007) indicated possible morphological differences. Although the two lineages are defined primarily on mtDNA molecular sequence differences, these are nonetheless greater than those among A. nelsoni,
A. leucurus, and A. harrisii. In order to reconcile these patterns pending more intensive genetic analysis of populations along purported or possible areas of contact (Vizcaíno Desert and lower Colorado River), we propose the recognition of the southern peninsular lineage of A. leucurus as a distinct species. We believe that this alternative best represents our current understanding of evolutionary relationships among genetic forms of Ammospermophilus, rather than retaining obvious paraphyly of A. leucurus relative to A. nelsoni and A. harrisii (based on molecular sequence data and supported by preliminary allozyme evidence; Hafner, 1981), or to resolve the paraphyly by combining A. leucurus (Merriam, 1889) and A. nelsoni (Merriam, 1893) under A. harrisii (Audubon & Bachman, 1854).

AMMOSPERMOPHILUS INSULARIS NELSON & GOLDMAN, 1909
PENINSULAR ANTELOPE SQUIRREL
(SYNONYMY UNDER SUBSPECIES)

Geographic range: Inhabiting generally rocky desert habitat in Baja California Sur, Mexico (including Isla Espíritu Santo and Isla San Marcos) from the Cape Region (excluding higher elevation coniferous forests of the Sierra Laguna) north to the eastern edge of the Vizcaíno Desert, in the vicinity of the Tres Virgenes volcanic field. Elevational range from approximately sea level to approximately 600 m a.s.l. (possibly higher in the Tres Virgenes, Sierra Giganta, and other arid ranges of Baja California Sur).

Description: Mid-dorsal summer pelage coloration variable, but generally among the darker (higher eumelanin) and more rufous (higher phaeomelanin) for the genus; specimens from the eastern edge of the Vizcaíno Desert and the Llano de Magdalena notably lighter and less rufous; two clearly defined black bands alternating with three white (including distal) bands on underside of tail (Hafner, 1981). Small to average sized for the genus, with a relatively long tail; specimens from eastern edge of Vizcaino Desert notably smaller than others; short, robust rostrum and short tooth row; small auditory bullae; baculum with long distal recurved wings, short proximal lateral expansions, deep shaft angle, and deep distal cup (Hafner, 1981).

AMMOSPERMOPHILUS INSULARIS INSULARIS NELSON & GOLDMAN, 1909

Ammospermophilus leucurus insularis Nelson & Goldman, 1909: 24. Type locality 'Espíritu Santo Island, Gulf of California, Baja California [Sur], Mexico.' Type specimen adult female, skin and skull, United States National Museum (USNM) 146783 collected 7 February 1906 by E. W. Nelson and E. A. Goldman, original number 19072.


Geographic range: Known only from Isla Espíritu Santo and Isla Partida Sur (the two islands in Bahía de la Paz are connected by a narrow isthmus).

Description: Similar to Ammospermophilus insularis extimus of the peninsular mainland except slightly larger and darker, with vestigial or absent upper premolar (Pm3). Skull larger, particularly zygomatic arches, nasals, and auditory bullae (Álvarez-Castañeda, 2007). The Pm3 is extremely variable in the family Sciuridae, and is occasionally absent, unilaterally or bilaterally, in all members of the genus. Whereas the frequency of an anomalous Pm3 is below 15% in other species of Ammospermophilus, it is over 50% in A. i. insularis. In addition to the absence of one or both permanent Pm3, anomalies include the absence of one or both deciduous Pm3 (dPm3) or retention in adults of the dPm3 (Hafner, 1981; Álvarez-Castañeda, 2007).

AMMOSPERMOPHILUS INSULARIS EXTIMUS NELSON & GOLDMAN, 1929

Ammospermophilus leucurus extimus Nelson & Goldman, 1929: 281. Type locality ‘Saccaton, 15 mi. N of Cape San Lucas, Lower California [Baja California Sur], Mexico.’ Type specimen adult female, skin and skull, USNM 146587, collected 29 December 1905 by E.W. Nelson and E.A. Goldman, original number 18805.

Geographic range: Eastern edge of the Vizcaíno Desert (vicinity of Tres Virgenes volcanic field) in the central Baja California peninsula, northern Baja California Sur, south throughout the peninsula to the Cape Region, excluding the higher elevation, coniferous forests of the Sierra Laguna.

Description: Same as for species.

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REFERENCES
Crews SC, Hedin M. 2006. Studies of morphological and molecular phylogenetic divergence in spiders (Araneae: *Homalonychus*) from the American southwest, including
divergence along the Baja California Peninsula. *Molecular Phylogenetics and Evolution* 38: 470–487.

Davis WB. 1939. *The Recent mammals of Idaho.* Caldwell: Caxton Printers, Ltd.


Jaeger JR, Riddle BR, Bradford DF. 2005. Cryptic Neogene vicariance and Quaternary dispersal of the red-spotted toad (*Bufo punctatus*): insights on the evolution of
North American warm deserts biota. Molecular Ecology 14:
3033–3048.


Riddle BR. 1995. Molecular biogeography in the pocket mouse (Perognathus and Chaetodipus) and grasshopper mice (Ony- chomys): the late Cenozoic development of a North Ameri- can aridlands rodent guild. Journal of Mammalogy 76: 283–301.


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web-site:

Appendix S1. Voucher specimens for tissue used in this study are housed in the New Mexico Museum of Natural History (NMMNH), Museum of Southwestern Biology, University of New Mexico (MSB), Burke Museum of Natural History and Culture, University of Washington (UWBM), Monte L. Bean Life Science Museum, Brigham Young University (BYU), Colección Nacional de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México (CNMA), or The Museum, Texas Tech University (TTU). Tissue samples are stored in NMMNH, UWBM, BYU, CNMA, TTU, or Louisiana State University Museum of Natural Science (M-numbers); corresponding LVT numbers refer to sequences deposited in Dryad (http://www.datadryad.org; doi:10.5061/dryad.gs94r). Boldface numbers in parentheses before locality names refer to mapped localities in Figs. 1 and 3. Localities for *A. interpres* are listed north to south and mapped in Fig. 1.

Appendix S2. Oligonucleotide primers used to amplify and sequence mitochondrial and nuclear DNA in *Ammospermophilus* and the two outgroup taxa *Cynomys* and *Xerospermophilus*. IRBP (nuclear Interphotoreceptor Retinoid-Binding Protein), Rag2 (nuclear Recombining Activating Gene 2), 12S (small subunit mitochondrially-encoded ribosomal RNA), 16S (large subunit mitochondrially-encoded ribosomal RNA), Cytb (mitochondrial cytochrome b), CO1 (mitochondrial cytochrome oxidase 1), CO3 (mitochondrial cytochrome oxidase 3), and CR (mitochondrial Control Region). Primer names and sequences follow the cited sources, except Ammo LS1, which was generated specifically for this study.

Appendix S3. Mismatch distributions (expected = solid line; observed = dashed line) representing the haplotype frequency distribution of three geographically widespread clades of *Ammospermophilus leucurus*: (A) USA; (B) southern Baja California peninsula; and (C) northern Baja California peninsula.