Journal of Mammalogy, 88(5):1160–1169, 2007

SYSTEMATICS OF THE ANTELOPE GROUND SQUIRREL (*AMMOSPERMOPHILUS*) FROM ISLANDS ADJACENT TO THE BAJA CALIFORNIA PENINSULA

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I assessed the systematics of *Ammospermophilus leucurus* from the islands of Espíritu Santo and San Marcos adjacent to the Baja California peninsula, using sequences of the mitochondrial cytochrome-*b* (*Cytb*) gene and morphological analyses from the island and the peninsular samples. For the molecular analysis, the *Cytb* gene (500 base pairs) was sequenced from individuals from 34 different localities. Maximum-parsimony, maximum-likelihood, and neighbor-joining analyses indicated a phylogenetic break in the central part of the Baja California peninsula. Samples from the islands of Espíritu Santo and San Marcos were grouped with the southern clade with an average of 1.2 substitutions that separated adjacent haplotypes in a network. Two haplotypes occurred on San Marcos Island; one was unique and the other also was present in peninsular samples. The only haplotype found on Espíritu Santo Island also occurred on the peninsula. Both univariate and multivariate morphometric analyses and the pattern of presence–absence of the 3rd upper premolar distinguished samples from Espíritu Santo and the Cape region of the peninsula but not between San Marcos Island and Francisco de la Sierra on the peninsula. Thus, the San Marcos Island ground squirrel could not be distinguished from those of the peninsula and should be recognized as the same subspecies that occupies the adjacent peninsular areas (*A. leucurus extimus*). In addition, neither molecular nor morphological data supported the recognition of *A. insularis* (from Espíritu Santo Island) as a distinct species from peninsular samples of *A. leucurus*, although this taxon showed morphological differentiation in dental characters that still support its recognition as a valid subspecies (*A. leucurus insularis*).

Key words: *Ammospermophilus insularis*, analysis of molecular variance, island populations, mitochondrial DNA, rodents

The antelope ground squirrel (*Ammospermophilus*) has 2 species in the Baja California peninsula. *Ammospermophilus leucurus* ranges throughout the Baja California peninsula and is characteristic of the Great Basin, Mojave, Sonora, and Baja California deserts (Howell 1938). There are 9 subspecies; 4 are distributed along the Baja California peninsula (Belk and Smith 1991): *A. l. extimus* in the south, *A. l. canfieldae* in the center, *A. l. peninsulae* in the northern Pacific side, and *A. l. leucurus* in the northern Gulf side of the peninsula (Yensen and Valdéz-Alarcón 1999).

The 2nd species, *A. insularis*, is restricted to Espíritu Santo Island in the Gulf of California (Fig. 1). It was originally described as *A. leucurus insularis* (Nelson and Goldman 1909), but Howell (1938) considered it a full species, based mainly on the absence of the 3rd upper premolar. However, Best et al. (1990) stated that an adequate analysis of variation in this character had yet to be made. *A. insularis* was classified as a separate species because it lacks the 3rd upper premolar in about 50% of the specimens and the premolar is rudimentary in the rest (Howell 1938). In contrast, only 5% of the specimens from the Cape Region in the southeastern part of the peninsula showed absence of the 3rd upper premolar. A cladistic analysis based on cytochrome *b* (*Cytb*) suggested that *A. insularis* was closest to *A. leucurus* and *A. harrisii* (Best et al. 1990), but chromosome-banding similarities indicated that *A. insularis* might be most closely related to *A. harrisii* (Mascarello and Bolles 1980). Other authors have considered *A. insularis* to be most similar to *A. leucurus extimus* (Best et al. 1990; Howell 1938; Yensen and Valdéz-Alarcón 1999).

Ammospermophilus insularis is the only species of *Ammospermophilus* that is endemic to a single island (Hall 1981; Lawlor et al. 2002; Yensen and Valdéz-Alarcón 1999), although antelope squirrels had been collected earlier on San Marcos Island. The taxonomic status of this population is unknown although it is believed that it derived from pets introduced by inhabitants of a mining town on the island (D. Haffner, pers.

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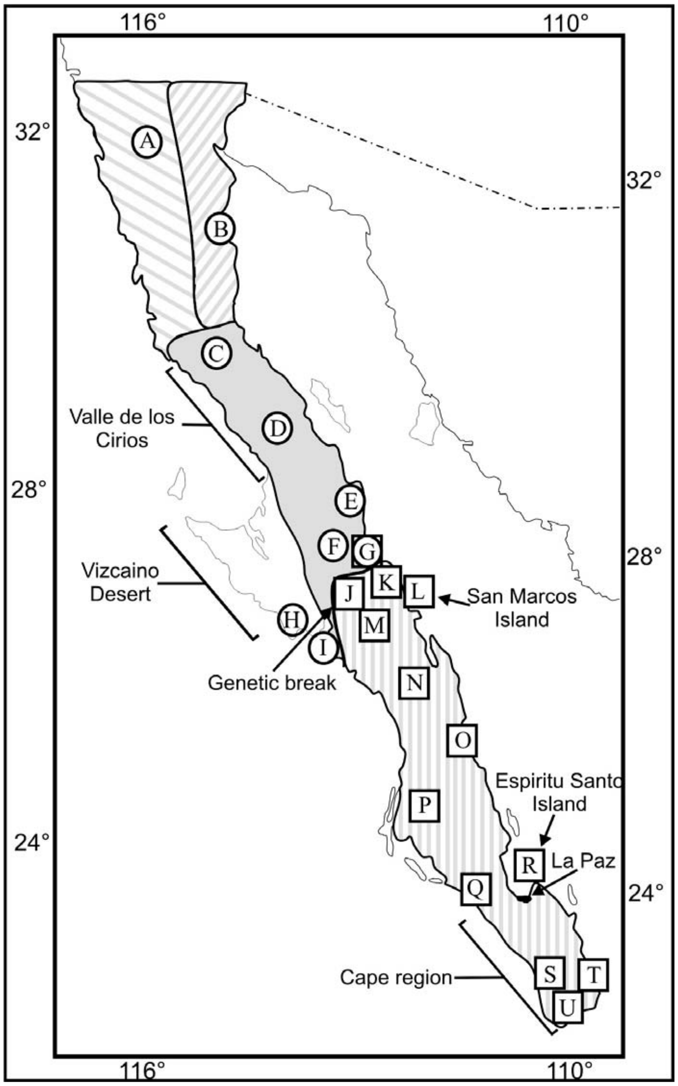

FIG. 1.—Location of specimens examined for sequencing of *Ammospermophilus leucurus*. Open circles are the northern group and squares are the southern group. The list of specimens and geographic groups is given in Table 1. The line in the middle part of the peninsula shows the break between the northern and southern clades. The geographic groups are: A) Valle de la Trinidad, B) San Felipe, C) Misión San Fernando, D) Punta Prieta, E) Barril, F) San Francisco de la Sierra, G) Volcán Tres Vírgenes, H) Abreojos, I) Cadejé, J) San Ignacio, K) Santa Rosalía, L) San Marcos Island, M) San Juan de Las Pilas, N) Comondú, O) Loreto, P) Ciudad Constitución, Q) Santa Rita, R) Espíritu Santo Island, S) Punta Lobos, T) El Pulmo, and U) Santiago. The shaded areas show the distribution of the subspecies in the Baja California peninsula; *A. l. extimus* (vertical lines), *A. l. canfieldae* (solid), *A. l. leucurus* (lines upper right to left), and *A. l. peninsulae* (lines upper left to right).

comm). Mining in the island began in the 1st half of the 20th century and the introduction of the species could have happened during or after this time.

Classical and current taxonomy of nearly all species and subspecies of mammals that inhabit the Baja California peninsula developed from morphological analyses, including various morphometric methods. The most recent applications of molecular methodologies, particularly those of DNA sequencing, have provided a stronger understanding of lineage diversity within species, as well as phylogenetic relationships among them. As a result, the phylogeographic structure of species distributed along the peninsula have been the subject of recent interest (Maldonado et al. 2001; Patton and Álvarez-Castañeda 2005; Riddle et al. 2000) rather than a reevaluation of the morphological characters originally used to define the taxa. This is particularly true of insular populations and taxa (e.g., Álvarez-Castañeda and Patton 1999, 2000; Lawlor et al. 2002).

Analyses of *Cytb* and cytochrome oxidase subunit 3 (*COIII*) genes in the mitochondrial DNA of rodents and other vertebrates from the Baja California peninsula support the hypothesis for the presence of a midpeninsular seaway (Riddle et al. 2000), which divided taxa into 2 or more groups at varying taxonomic levels (Patton and Álvarez-Castañeda 2005; Riddle et al. 2000; Whorley et al. 2004). For some species widely distributed to the north (e.g., *Chaetodipus fallax*, *C. formosus*, *Perognathus longimembris*, and *Reithrodontomys megalotis*—Álvarez-Castañeda and Patton 1999), this area marks the southern boundary of their range. For others, there is no apparent phylogenetic break and they are continuously distributed through the southern part of the peninsula (e.g., *Chaetodipus spinatus*—S. T. Álvarez-Castañeda, in litt.). However, there are few phylogenetic studies for small mammals in the region and fewer studies yet have focused on the levels of genetic differentiation of small mammals from the islands of Espíritu Santo and San Marcos, where populations appear to be highly vulnerable to human activity. Therefore, it would be very useful to understand the phylogenetic relationships of the insular forms with the peninsular forms to determine and implement conservation and management recommendations. For this reason, I collected antelope ground squirrels from throughout the Baja California peninsula including the islands of Espíritu Santo and San Marcos, and compared them with subspecies from throughout their entire distributional range using morphological and genetic data. I hypothesized that these island populations would be closely related to and show low levels of morphologic and genetic differentiation from adjacent peninsular animals.

MATERIALS AND METHODS

Surveys were conducted from 1992 to 2005 throughout the Baja California peninsula and the islands of Espíritu Santo and San Marcos. The specimens were collected using Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida). The trap lines consisted of 40 traps at 10-m intervals, using rolled oats as bait. Specimens were captured and handled following the guidelines for the capture, handling, and care of mammals approved by the American Society of Mammalogists (Animal Care and Use Committee 1998). Voucher specimens and tissues are housed in the following mammal collections: Centro de Investigaciones Biológicas del Noroeste (CIB); Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); Museum of Natural History of New Mexico (MNHNM); University of New Mexico Museum of Southwestern Biology (MSB); and United States National Museum, Smithsonian Institution (USNM).

TABLE 1.—Geographic groups ($n = 21$) and localities ($n = 34$) of *Ammospermophilus leucurus*. Nomenclature follows that of Yensen and Valdéz-Alarcón (1999). The first 3 columns indicate geographic groups that are shown in Fig. 1, including locality (L), haplotype number (H), and state (ST). All latitudes (Lat.) are north and longitudes (Long.) are west. GenBank specimens with accession numbers beginning with AY also were used by Whorley et al. (2004).

L	H	ST[a]	Locality (nearby town)	Taxon	Lat.	Long.	Specimen no.[b]	GenBank no.
North group								
A1	H1	BC	Valle de la Trinidad	*A. l. peninsulae*	31°18′	115°34′	409233 MSB	AY685542
B2	H1	BC	San Felipe	*A. l. leucurus*	31°10′	114°56′	40140 MSB	AY685539
C3	H1	BC	San Fernando	*A. l. canfieldae*	29°59′	115°13′	808 NHNNM	IDEM AY685542
C4	H1	BC	Santa Catarina	*A. l. canfieldae*	29°36′	115°11′	42893 MSB	AY685545
C5	H2	BC	Cataviña	*A. l. canfieldae*	29°49′	114°51′	759 NHNNM	IDEM AY685544
D6	H1	BC	Punta Prieta	*A. l. canfieldae*	28°54′	114°08′	773 NHNNM	IDEM AY685542
D7	H2	BC	Punta Prieta	*A. l. canfieldae*	28°40′	114°10′	2875 CIB	AY685544
D8	H1	BC	Chapala	*A. l. canfieldae*	29°27′	114°26′	796 NHNNM	IDEM AY685542
E9	H2	BC	Barril	*A. l. canfieldae*	28°18′	112°54′	6605 CIB	IDEM AY685544
F10	H1	BCS	San Francisco de la Sierra	*A. l. canfieldae*	27°33′	113°04′	8508 CIB	IDEM AY685542
G11	H4	BCS	Volcán Tres Vírgenes	*A. l. canfieldae*	27°29′	112°31′	8500 CIB	DQ866059
H12	H3	BCS	Abreojos	*A. l. canfieldae*	26°58′	113°28′	8518 CIB	DQ866060
H12	H5	BCS	Abreojos	*A. l. canfieldae*	26°58′	113°28′	6610 CIB	DQ866061
I13	H1	BCS	Cadejé	*A. l. canfieldae*	26°21′	112°31′	7716 CIB	IDEM AY685542
I14	H1	BCS	Ballena	*A. l. canfieldae*	26°25′	112°38′	7715 CIB	IDEM AY685542
South group								
G11	H12	BCS	Volcan Tres Vírgenes	*A. l. extimus*	27°29′	112°31′	8501 CIB	IDEM AY685507
J15	H13	BCS	San Ignacio	*A. l. extimus*	27°13′	112°52′	1658 NHNNM	DQ866053
K16	H11	BCS	Santa Rosalía	*A. l. extimus*	27°18′	112°17′	1369 NHNNM	DQ866056
K17	H15	BCS	Santa Agueda	*A. l. extimus*	27°16′	112°18′	531445 USNM	AY685514
L18	H11	BCS	San Marcos Island	No subspecies	27°11′	111°56′	8527, 8532 CIB	IDEM DQ866057
L18	H17	BCS	San Marcos Island	No subspecies	27°11′	111°56′	8529, 8531 CIB	DQ866057
M19	H16	BCS	San Juan de Las Pilas	*A. l. extimus*	26°52′	112°35′	7712 CIB	DQ866062
N20	H11	BCS	Comondu	*A. l. extimus*	26°17′	111°48′	531430 USNM	IDEM DQ866057
O21	H11	BCS	Loreto	*A. l. extimus*	26°05′	111°19′	1362 NHNNM	IDEM DQ866057
O22	H12	BCS	Puerto Escondido	*A. l. extimus*	25°46′	111°18′	1363 NHNNM	IDEM AY685507
P23	H18	BCS	Ciudad Insurgentes	*A. l. extimus*	25°26′	111°15′	7653 CIB	DQ866058
P24	H8	BCS	Puerto López Mateos	*A. l. extimus*	25°14′	110°58′	6101 CIB	AY685486
P25	H19	BCS	Ciudad Constitución	*A. l. extimus*	24°59′	111°36′	6110 CIB	AY685516
Q26	H9	BCS	Santa Rita	*A. l. extimus*	24°13′	111°30′	6611 CIB	AY685494
R27	H11	BCS	Espíritu Santo Island	*A. insularis*	24°26′	110°23′	112, 115−120 CIB, nk6701 MSB, 58107−58111 MSB	IDEM DQ866057
S28	H12	BCS	Punta Lobos	*A. l. extimus*	23°26′	110°13′	531436 USNM	AY685507
S29	H12	BCS	Pescadero	*A. l. extimus*	23°21′	110°10′	1354 NHNNM	IDEM AY685507
S30	H9	BCS	Migriño	*A. l. extimus*	23°01′	110°04′	6149 CIB	AY685506
T31	H7	BCS	El Pulmo	*A. l. extimus*	23°26′	109°34′	531432 USNM	AY685502
U32	H14	BCS	Burrera	*A. l. extimus*	23°30′	110°03′	531434 USNM	AY685500
U33	H10	BCS	Santiago	*A. l. extimus*	23°29′	109°49′	8086 CIB	DQ866054
U34	H6	BCS	Miraflores	*A. l. extimus*	23°11′	109°46′	1351 NHNNM	DQ866055

[a] BC = Baja California; BCS = Baja California Sur.

[b] MSB = University of New Mexico Museum of Southwestern Biology; NHNNM = Museum of Natural History of New Mexico; CIB = Centro de Investigaciones Biológicas del Noroeste; USNM = United States National Museum, Smithsonian Institution.

Phylogenetic relationships among specimens of the peninsula and the islands were assessed by analyzing a 500-base pair (bp) fragment of the *Cytb* gene. Fifty-one individuals from 34 localities were assembled into 21 geographic groups. Seventeen of these specimens were from the islands of San Marcos and Espíritu Santos (Fig. 1; Table 1). All localities from the same geographic area were considered in the same geographic groups.

Genomic DNA was extracted in the laboratory from liver tissues preserved in 95% ethanol and frozen at −80°C, using the DNeasy kit (QIAGEN, Inc., Valencia, California) or Chelex 5% (Walsh et al. 1991). Primers MVZ05 and MVZ16 (Smith 1998) were used to amplify an approximately 800-bp fragment of the *Cytb* gene. Amplification reactions included: 12.5 μl of template (10 ng), 4.4 μl of double-distilled H_2O, 2.5 μl of each primer (10 nM concentration), 0.474 μl of (0.4 nM) deoxynucleoside triphosphates, 0.5 μl of (3 mM) $MgCl_2$, 0.125 μl of Taq polymerase (Platinum; Invitrogen Corp., Carlsbad, California), and 1x Taq buffer to a final volume of 25 μl. Amplification conditions consisted of 3 min of initial denaturation at 94°C followed by 37 cycles of denaturation at 94°C for 45 s, 1 min annealing at 50°C, and 1 min extension at 72°C. Amplified products were purified using the QIAquick PCR purification kit (QIAGEN), and this template was cycle-sequenced with MVZ05, using the Taq FS kit and run on an ABI 377 (Foster City, California) automated sequencer. A 500-bp gene fragment (including the start codon position 14115 with reference to

Mus musculus sequence) was obtained from 51 individuals representing 21 geographic groups from throughout the peninsula, including samples from both islands (Espíritu Santo and San Marcos) for estimations of gene diversity and haplotype diversity. Representative haplotypes generated for this study have been deposited in GenBank (accession numbers DQ866053–DQ866062; specimens with an acronym beginning with AY also were used by Whorley et al. [2004]). *Ammospermophilus interpres* was included as the outgroup (GenBank accession number AU46174—Herron et al. 2004). Sequences were edited and aligned using Sequencher version 4.1.1 software (Gene Codes Corp., Ann Arbor, Michigan) and translated into amino acid for confirmation of alignment.

Representative and nonredundant haplotypes (Collapse version 1.1 software—Posada 2004) were used for each population for the phylogenetic methods. Phylogenetic analyses under the maximum-parsimony, maximum-likelihood, and distance-optimality criterion were performed using PAUP 4.0b10 (Swofford 2002). In addition, Bayesian inference was performed using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). Maximum-parsimony analyses, with all characters equally weighted and heuristic searches with 1,000 random addition sequences, were conducted implementing the tree-bisection-reconnection algorithm for branch swapping. For the maximum-likelihood analysis, the most appropriate evolution model for the data was selected using Modeltest 3.06 (Posada and Crandall 1998) with Akaike information criterion. The Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) also was selected as the best-fit model of nucleotide substitution. This model was then used for maximum-likelihood searches consisting of 100 random replicates with tree-bisection-reconnection branch swapping. Under the distance criterion, phylogeny was estimated using the neighbor-joining algorithm. To make comparisons with the amount of percent sequence divergence values from previous studies (Bradley and Baker 2001), I used the Kimura 2-parameter model. The support of the nodes was assessed with bootstrap analyses, including a fast heuristic procedure with 1,000 pseudoreplicates. Only bootstrap values $\geq$ 50% were shown in the trees.

A Bayesian phylogenetic analysis was performed using the Hasegawa–Kishino–Yano distance model selected by MrModeltest version 2 (Nylander 2004) and implementing the Metropolis coupled Markov chain Monte Carlo simulation. Four separate runs were performed starting from a random tree with 4 simultaneous chains. Each run was conducted with 5,000,000 generations and sampled at intervals of 1,000 generations. The first 500 samples of each run were discarded as burn-in, and the remaining topologies were used to calculate posterior probabilities from the 50% majority-rule consensus trees. Determination of a stationary condition was evaluated by plotting the log of the likelihood score of sample points against generation.

Arlequin version 2.001 (Schneider et al. 2000) was used to estimate a matrix of pairwise differences that was then used to construct a minimum-spanning network from all the unique haplotypes of the peninsular and island populations. An analysis of molecular variance was used to examine the hierarchical apportionment of haplotypes between northern and southern populations with 1,000 permutations using the Kimura 2-parameter model. A coalescence analysis also was conducted in Arlequin version 2.001 to obtain estimates of τ (time period over which the population expansion has taken place), Θ_0 (population size before the expansion), and Θ_1 (the population size after expansion). Migration rates ($M = 2mN_f$) among the groups were estimated using the Markov chain Monte Carlo coalescent approach. The program MIGRATE 2.0.3 (Beerli 2003) was used with the empirical transition–transversion (Ti/Tv) ratio and base frequencies, using maximum likelihood. Other parameters were set as defaults of MIGRATE, where different populations were grouped for each of the main clades. The program was run with all the populations at the same time.

Morphological analyses were limited to 168 adult specimens, 131 from the southern part of the Baja California peninsula, 27 from Espíritu Santo Island, and 10 from San Marcos Island (Appendix I). The taxonomy follows that of Yensen and Valdéz-Alarcón (1999). Nine measurements of each cranium were taken with calipers (0.01-mm resolution). Measurement definitions are given below; letters in parentheses are used to identify measurements in some figures. All measurements given in the text are expressed in millimeters. Skull measurements were taken following Best (1978): total skull length (GLS), zygomatic width (ZW), basal length (BL), anterior nasal width (ANW), braincase breadth (BB), bulla width (BW), bulla length (BL), mandible toothrow length (MTR), and maxillary toothrow length (XTR).

Principal component analyses of characters on a correlation matrix were calculated; to avoid size variation among populations, log-transformed craniodental variables were made and projections of populations were plotted on the first 3 components. The degree of variability in the presence and prominence of the 3rd upper premolar (P3) was evaluated in samples from both island and peninsular populations from La Paz to the Cape Region as well as from San Francisco de la Sierra. This character has been previously used to diagnose *A. insularis* (Howell 1938).

RESULTS

Phylogenetic analysis.—The maximum-parsimony analysis yielded 30 trees of equal length (length = 41, consistency index = 0.951, retention index = 0.961). The northern and southern groups previously described by Whorley et al. (2004) were monophyletic with a boostrap support of 100%; however, a great number of polytomies were found in each of the main groups. The best-fit model of nucleotide substitution was the Hasegawa–Kishino–Yano model (Ti/Tv = 20.92; A = 0.277, C = 0.259, G = 0.156, and T = 0.307). The maximum-likelihood analysis with the Hasegawa–Kishino–Yano evolution model produced only 1 tree (score = 948.59; Fig. 2). Neighbor-joining analysis showed similar results to those obtained under the maximum-likelihood criteria (not shown). In addition, Bayesian inference (4 replicates) converged on essentially identical tree topologies (not shown). These trees showed 2 monophyletic clades: 1 representing samples from the north (100% support) and 1 from the south (85% support). Nevertheless, a polytomy of specimens from each of the main clades was found. Significantly, in all analyses, haplotypes

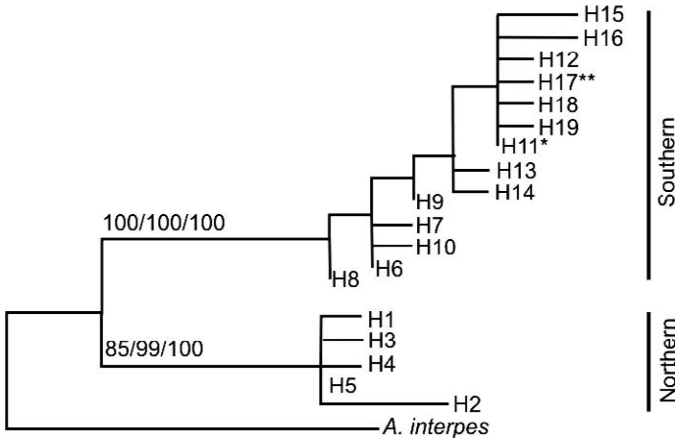

Fig. 2.—The likelihood analysis with the Hasegawa–Kishino–Yano evolution model used in 2 searches of 1,000 replicas each with maxtrees = 100 and swapping with the tree-bisection-reconnection algorithm and with only 1 tree in the 1st island (score = 948.59), illustrating the reciprocal monophyly of samples of *Ammospermophilus leucurus* from the southern and northern halves of the peninsula. Espíritu Santo Island has 1 haplotype (H11), and San Marcos Island has 2 haplotypes (H11 and H17), all in the southern clade. The key to the haplotypes is given in Table 1. Some of the haplotypes are present in more than 1 population. * = haplotype present in mainland, Espíritu Santo Island, and San Marcos Island; ** = haplotype unique to San Marcos Island. Values over the main branch are the bootstrap support for Bayesian inference/distance optimality criterion/maximum parsimony.

from both Espíritu Santo and San Marcos islands fell within the southern clade (Fig. 2).

The percentage of sequence divergence between the 2 Baja Californian clades was 3.7%. All 13 specimens from Espíritu Santo Island shared haplotype H11, which also was found on the peninsula. Specimens from San Marcos Island had 2 haplotypes, 1 unique to the island (H17), and the other (H11) that was also found on the peninsula, and differed by a single base pair (Fig. 3).

Haplotype network.—The haplotype network was divided into 2 geographic groups with different numbers of haplotypes (Fig. 3). Seventy-three percent of the haplotypes were found in the south but only 27% of those occurred in more than 1 locality. On the other hand, 2 of the 5 northern haplotypes were found in more than 1 locality. In the northern part of the Baja California peninsula, there were 5 haplotypes from 15 specimens from 14 localities in 9 geographic groups with an average of 1.2 mutational steps between haplotypes and with 1 haplotype (H1) widely dispersed in 6 geographic groups (42.8%). In the southern part, there were 14 haplotypes from 36 specimens from 21 localities of 13 geographic groups with an average of 1.2 mutational steps between haplotypes, and with 1 haplotype (H11) widely dispersed among 5 localities (23.8%). Haplotypes were not shared between the northern and the southern groups, except at Volcán de las Tres Vírgenes where specimens with northern and southern haplotypes were collected (locality G in Fig. 1; haplotypes H4 and H12 in Fig. 3).

Espíritu Santo Island had 1 haplotype (H11) that also was the most common of the southern groups and also was found in

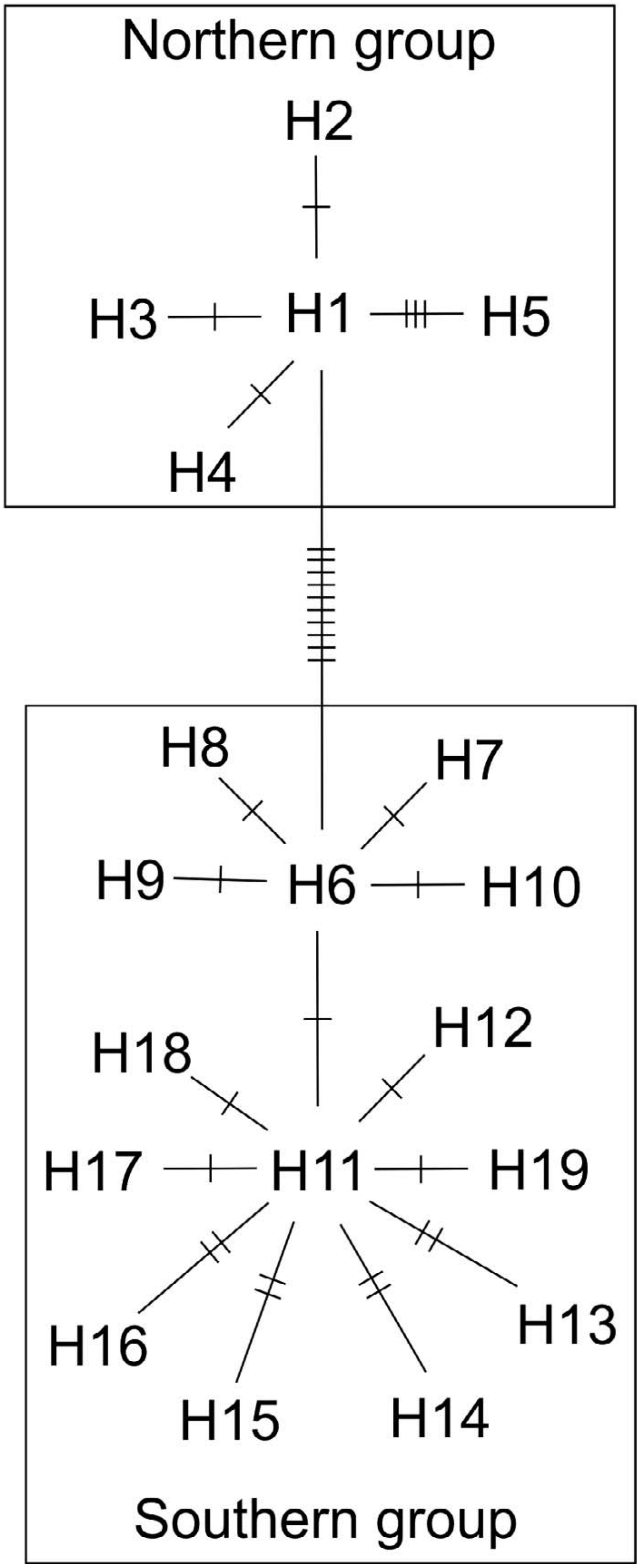

Fig. 3.—Haplotype network for the 500-base pair cytochrome-*b* data set, including populations from the Baja California peninsula. Each perpendicular hash mark across the line between adjacent haplotypes in the network represents a single-base substitution. The key to the haplotypes is given in Table 1. Some of the haplotypes are present in more than 1 population.

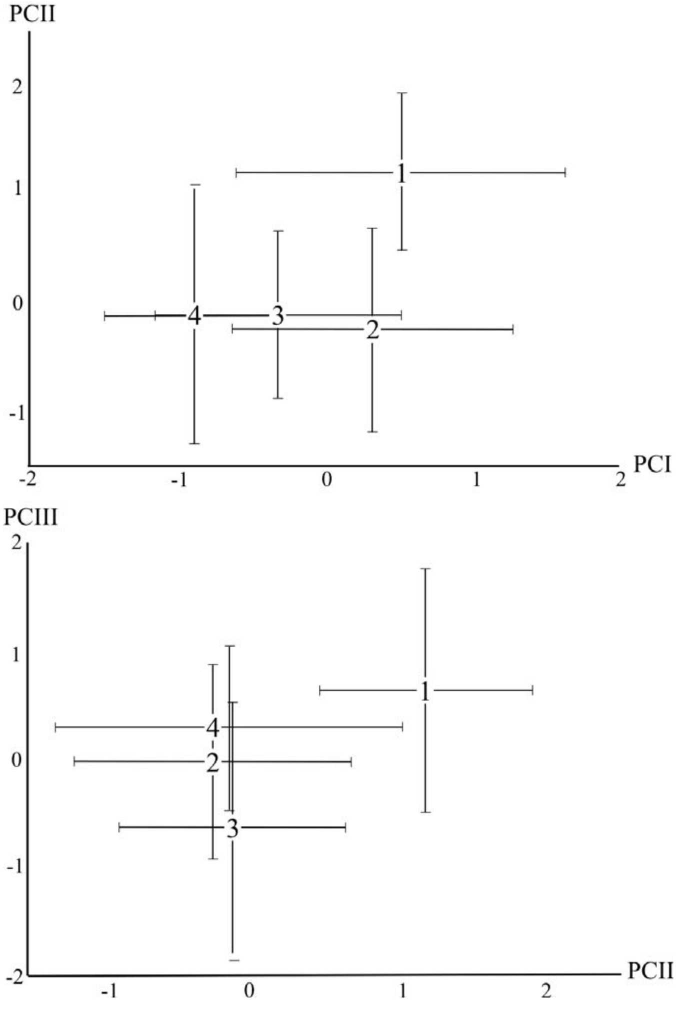

FIG. 4.—Results of the principal component (PC) analysis of 9 skull measurements of *Ammospermophilus* from Baja California. Upper) PCI and PCII; lower) PCII and PCIII. 1) Area near San Francisco de la Sierra, 2) Espíritu Santo Island, 3) San Marcos Island, and 4) area near La Paz.

Puerto Escondido, Miraflores, Santa Rosalia, Loreto, and San Marcos Island. An additional haplotype (H17) that differed at 1 position also was found only on San Marcos Island.

The haplotype network showed a general north-to-south pattern but no clear phylogeographic structure was detected within the 2 haplotype groups. This corresponds to an overall low level of sequence divergence within the network (Fig. 3) with few mutational steps among all haplotypes. The large difference between the southern and northern groups (Whorley et al. 2004) is indicative of a strong increase in effective population size in their coalescence history (Schneider and Excoffier 1999).

The time period over which this population expansion has taken place can be estimated from the formula $\tau = 2ut$. In this equation u is the mutation rate for the entire sequence being analyzed and t is generation time that is based on the stepwise growth model and nonlinear least-squares approach processed in Arlequin (Schneider et al. 2000). Using the value of the mutation rate for the *Cytb* gene of 1.3×10^{-5} (Zheng et al. 2003) and the empirical value of τ for our data, 1.25 for the north and 3.34 for the south, I estimated the coalescence time to be 47,307 generations (4,615–123,230) for the north versus 121,538 generations (15,769–128,461) for the south. The average generation time for the antelope squirrel in the Baja California peninsula is 1 year (Kenagy et al. 2005); the initiation of population expansion throughout the northern half of the Baja California peninsula is believed to be very recent, which is supported by the analysis of Whorley et al. (2004) for a 20° latitude transect.

The statistical analysis between the areas north and south of the phylogenetic break in the peninsular and in the 2 island populations showed a variation of 3.16% among groups, 34.95% among populations within groups, and 62.89% within populations. When the analysis was conducted at the same latitude between the island and the peninsular populations (San Marcos Island with San Francisco de la Sierra and Espititu Santo Island with La Paz), the percentages of variation were 2.84%, 23.26% among groups; 6.63%, 17.62% among populations within groups; and 90.53%, 69.12% within populations, respectively. These results showed that the variation of haplotypes in the same population is greater than that among groups and showed no strong differences between island and peninsular populations.

Morphometric analysis.—Specimens from Espíritu Santo Island had the largest skulls, followed by those on the peninsula on the east side of La Paz Bay. Significant differences were found in basilar and mandibular toothrow length, and in width of the zygomatic arches, nasals, and bullae (Student's *t*-tests; $P < 0.5$). The specimens from San Marcos Island had slightly larger skulls than those of nearby populations (San Francisco de la Sierra); however, no significant differences ($P < 0.5$) were observed between these 2.

The first 3 principal components (PCs) explain 75.6% (PCI 52.6%, PCII 13.5%, and PCIII 9.4%) of the variation. Values in the 1st factor were positive in spite of the log-transformed craniodental variables that represented a size effect between the populations (Fig. 4). The measurements that explained the great variation were bulla length in PCI, anterior nasal width in PCII, and bulla width in PCIII. No substantial differences were found among island and La Paz populations. However, the population of San Francisco de la Sierra had a small overlap with the other 3 populations. Island populations (Espíritu Santo and San Marcos) were generally larger than the peninsular population at the same latitude.

Morphological analysis.—In relation to the presence of the 3rd upper premolar (P3) of 27 specimens from Espíritu Santo Island, including 14 paratypes, 15 (55%) lost at least 1 P3 (12 lacked both, and 3 were missing 1 premolar). Of the 12 specimens with both premolars, 8 (66.6%) were subadults. Of 131 specimens from the southern part of the peninsula, only 11 (8.4%) did not have 1 P3, although 10 specimens from San Marcos Island and 45 from the San Francisco de la Sierra area on the adjacent peninsula all have the 2 P3s.

DISCUSSION

The phylogenetic analysis showed northern and southern monophyletic groups with a break south of the Vizcaino Desert.

This division corresponds to the 2 physiographic areas characterized by Goldman and Moore (1946—Vizcaino Desert–Baja California Sur) and Álvarez and Lachica (1974—Baja California–Vizcaino). Both populations from the islands were clustered within the genetic diversity of the southern clade. Individuals carrying haplotypes from both northern and southern clades were found in sympatry only in the area of Volcán de las Tres Vírgenes (locality 11), north of the town of Santa Rosalía. Additional field and genetic research in this area between Santa Rosalía and San Ignacio will be needed to determine the degree of genetic exchange between the regional groups of each haplotype clade.

The unequal distribution of haplotypes in the haplotype network (Fig. 3) between the south and north suggests that the populations in the southern clade were older because with similar sample sizes from the north and south (with the exception of the great number of replicates from island specimens), the southern clade had a greater number of haplotypes. In both clades the haplotype network suggested a star-shaped phylogeny with few internal haplotypes (1 in the north and 2 in the south) with tightly linked distal haplotypes. These patterns further indicated recent population expansions as described above by the estimated coalescent times. This coalescing time is in concordance with the Pleistocene origin of the island (Carreño and Helenes 2004), so both islands (considered as bridge islands) could have been invaded before a seaway separated them from the peninsula approximately $<$10,000–15,000 years ago (Carreño and Helenes 2004). Island populations were of very recent origin and shared a common haplotype with specimens from the peninsula.

There were no morphological differences (pairwise univariate comparisons, all with $P < 0.05$; complete multivariate overlap along PCI to PCIII; Fig. 4) between island populations and the southern population of the peninsula (La Paz). The 3 groups appeared to be part of the same group and should be considered 1 subspecies for this area. Island populations were morphologically different compared to the specimens of the northern group (San Francisco de la Sierra) that were from another genetic group and are considered a different subspecies.

These results suggest that the northern and southern clades are different species that can be diagnosed both morphologically and genetically, although individuals from the transition area are similar in morphology because they share the same environment or because hybridization may be occurring there. Alternatively, the northern and southern clades may represent distinct phylogenetic units, but are not separate species because intermediate geographical populations fully intermix. However, more concentrated studies with larger sample sizes and nuclear markers need to be conducted along the transition zone between the 2 clades to evaluate these hypotheses.

The results also suggest that the 2 island populations are closely associated with the southern clade. For the San Marcos Island population, D. J. Hafner (in litt.) hypothesizes that all *Ammospermophilus* were recently introduced as pets to the mining settlement on the island from Santa Rosalía. If this is the case, the population is the same as the one on the southern part of the peninsula. The 5 specimens examined from different areas of the island included 2 haplotypes, 1 was found on the peninsula and the other, differing by only 1 base pair substitution, was apparently unique to the island. It is unlikely to find a haplotype that is unique to San Marcos Island if it is indeed a recent introduction.

There are 2 possible explanations for this finding: both haplotypes from San Marcos Island do occur on the peninsula but haplotype H17 was not uncovered in my limited surveys; or this unique haplotype represents a new mutation that increased in frequency due to drift or there was an endemic population with a fixed haplotype, but other squirrels were introduced recently from the peninsula or there has been recent or ongoing gene-flow introducing the peninsular haplotype, or both. If the other haplotype is indeed found on the peninsula, then Hafner's human introduction hypothesis remains a possibility. However, if haplotype H17 is only found in San Marcos, the population could be considered as endemic, and H11 would be an ancestral haplotype. Measurable migration rates were higher from peninsular to island populations, $M = 174.45$, than in the other direction, $M = 0.24$, which supports the peninsular origin of the island populations. Unfortunately, the limited sample size was insufficient to verify any of these hypotheses. More detailed studies using larger sample sizes, D-loop, and microsatellite data may be necessary to provide further evidence for levels of gene flow between island and peninsular populations.

The morphometric data showed a slight difference in size between the San Marcos Island sample and the peninsular forms; however, examination of the genetic data did not support a long time of divergence. The change in size could be an ecophenotypic plastic response over short periods of times and few generations as has been reported for pocket gophers (Patton and Brylski 1987). In contrast, a high level of molecular and morphological divergence has been found in another rodent species (*C. spinatus*) from San Marcos Island and the peninsula (S. T. Álvarez-Castañeda, in litt.), for which there has been no evidence of recent introduction to the island. Finally, the large size of the San Marcos Island ground squirrels is similar to that of most Gulf of California island populations of rodents (Case 1978; Lawlor 1982), a phenomenon commonly known as the ''Island Rule'' (Van Valen 1973a, 1973b) with dwarfism and gigantism of populations on islands (Foster 1964; Sondaar 1977).

Neither the morphological nor the genetic analyses showed diagnostic characters for the San Marcos Island population and did not support considering it as a different population from those present in the Baja California peninsula. Therefore, I recommend that this population be recognized as *A. l. extimus*, which is the same subspecies as the one in the southern part of the peninsula.

No significant genetic differentiation was found between Espíritu Santo Island and southern peninsular forms. Of the 13 specimens examined from Espíritu Santo Island, only 1 mitochondrial DNA haplotype (H11) was found, which was also the most common haplotype in the southern part of the peninsula. However, even if examination of the genetic data suggests that the Espíritu Santo Island ground squirrels should not be recognized as a distinct species, these specimens did show significant differences in cranial morphology and they did show a high rate of loss of the 3rd upper premolar in relation to the peninsular population. Based on the fact that there are clear diagnostic morphological characteristics that can easily be used to distinguish among populations, I recommend that the Espíritu Santo antelope ground squirrel be recognized as a subspecies of the peninsular form, *A. leucurus insularis* following the definition of subspecies given by Lidicker (1962).

That specimens associated with both clades had different geographic origins as shown by the monophyletic pattern of each group and that the 2 groups entered the central part of the peninsula will be a working hypothesis for the next stage of this research. At present, no geographic barrier exists in the central part of the peninsula to delimit the 2 groups. Part of the Vizcaino Desert is a large area of stabilized dunes and may serve as a partial barrier. However, to the east, both San Francisco de la Sierra and the Santa Marta Mountains form a corridor between the southern and northern groups. At this time, the break appears to be associated with habitats within ecotone areas and there is a possibility of a wide hybrid zone in this area.

Following are my suggested revisions to the taxonomy of the *A. leucurus* from the Baja California peninsula and surrounding islands.

Ammospermophilus leucurus extimus

Ammospermophilus leucurus extimus Nelson and Goldman, 1929:281. Type locality ''Saccaton, 15 mi N of Cape San Lucas, Lower California [Baja California Sur], Mexico.''

Distribution.—From the middle part of the Baja California peninsula to the south including the population of San Marcos Island.

Ammospermophilus leucurus insularis

Ammospermophilus leucurus insularis Nelson and Goldman, 1909:24. Type locality ''Espíritu Santo Island, Lower California [Baja California Sur], Mexico.''
Ammospermophilus insularis Hall and Kelson, 1959:334.

Distribution.—Restricted to Espíritu Santo Island.

There are conservation and management implications to these results. The population of *A. l. insularis* from Espíritu Santo Island is considered ''threatened'' by the Mexican government (SEMARNAT 2002), but the conservation protection status of *A. l. extimus* from the San Marcos Island has not yet been determined because there is no previous formal published record of this population. However, further studies need to be conducted to evaluate the conservation management status of this population because the island is small and there is an open pit gypsum mine near the sea and a mining town. Our survey on San Marcos Island shows that the largest number of *Ammospermophilus* was found in the following 3 areas, 2 of which have a high level of human activity: a relatively flat area in the northwest; a canyon in the central part of the island at the north end of the gypsum mine; and ''El Palmillar,'' an area near the town with a small arroyo (dry wash) containing a few date palm trees. This is important because if the population was not recently introduced to the island, it may be on its own evolutionary trajectory and be considered as an evolutionary or management unit that deserves to be considered for listing by the Mexican Federal Government.

RESUMEN

Se revisa la sistemática de las poblaciones insulares de *Ammospermophilus leucurus* en Espíritu Santo y San Marcos con relación a las poblaciones que habitan la península de Baja California, mediante técnicas morfométricas y moleculares. Utilizando secuencias del gen que codifica para el citocromo *b* (500 pb) de individuos de 34 poblaciones diferentes. Se realizaron análisis de máxima parsimonia, máxima verosimilitud y distancia genética, los cuales indican la presencia de una discontinuidad genética en la parte media de la península de Baja California. Las muestras de las islas Espíritu Santo y San Marcos se agrupan dentro del clado del sur con un promedio de sustituciones de 1.2. En la Isla San Marcos se encontraron dos haplotipos, uno exclusivo y el otro compartido con la península. En la isla Espíritu Santo sólo se encontró un haplotipo, el cual también está presente en la península. Los análisis univariados y multivariados, y el patrón de ausencia/presencia del tercer molar superior utilizados para diferenciar a las poblaciones de Espíritu Santo de las del resto de la península, no mostraron diferencias entre ejemplares de la isla con los de la región sur de la península. Los resultados obtenidos no permiten distinguir entre ejemplares de Isla San Marcos de los de la península, además, ninguno de los datos moleculares o morfológicos son contundentes para seguir reconociendo a *A. insularis* (Isla Espíritu Santo) como una especie diferente de la presente en la península, por lo que se propone sea considerada como subespecie (*A. leucurus insularis*).

ACKNOWLEDGMENTS

I thank J. Kenagy, J. Whorley, C. Conroy, P. Cortés-Calva, and especially to J. Bowles for the revision of the final manuscript and their comments; to J. L. Patton for reviewing the manuscript and providing helpful comments; to J. Maldonado and 2 anonymous reviewers who greatly enhanced the final version of this manuscript although neither should be held responsible for its contents; to M. de la Paz, J. Whorley, P. Cortés-Calva, E. Rios, A. Trujano, and A. Gutiérrez for their help in the collection of specimens; to I. Leyva in the laboratory analysis; and to D. Dorantes for her English editing and tutorial. Tissue and voucher samples used in this study were provided by J. L. Patton of the Museum of Vertebrate Zoology, Michael Carleton of the United States Museum of Natural History, D. Hafner of the Museum of New Mexico Natural History, and T. Yates from the University of New Mexico. Funding was received from Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT grant I25251N, 39467Q; SEMARNAT-2002-CO1-019), and the University of California MEXUS-CONACYT project together with a grant for a faculty visit.

LITERATURE CITED

ÁLVAREZ, T., AND F. DE LACHICA. 1974. Zoogeografía de los vertebrados de México. Pp. 217–302 in El escenario geográfico, recursos naturales (J. L. Lorenzo, ed.). Instituto Nacional de Antropología e Historia, México City, México.

Álvarez-Castañeda, S. T., and J. L. Patton. 1999. Mamíferos del noroeste Mexicano. Centro de Investigaciones Biológicas del Noroeste, La Paz, México.

Álvarez-Castañeda, S. T., and J. L. Patton. 2000. Mamíferos del noroeste Mexicano II. Centro de Investigaciones Biológicas del Noroeste, La Paz, México.

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Beerli, P. 2003. MIGRATE—a maximum likelihood program to estimate gene flow using the coalescent. http://evolution.genetics.washington.edu/lamarc/migrate/html. Accessed June 2004.

Belk, M. C., and H. D. Smith. 1991. *Ammospermophilus leucurus*. Mammalian Species 368:1–8.

Best, T. L. 1978. Variation in kangaroo rats (genus *Dipodomys*) of the Heermanni groups in Baja California Mexico. Journal of Mammalogy 59:160–175.

Best, T. L., K. Caesar, A. S. Titus, and C. L. Lewis. 1990. *Ammospermophilus insularis*. Mammalian Species 364:1–4.

Bradley, R. D., and R. J. Baker. 2001. A test of genetic species concept: cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960–973.

Carreño, A. L., and J. Helenes. 2004. Geology and ages of the islands. Pp. 14–40 in A new island biogeography of the Sea of Cortéz (T. J. Case, M. L. Cody, and E. Ezcurra, eds.). 2nd ed. Oxford University Press, Oxford, United Kingdom.

Case, T. J. 1978. A general explanation for insular body size trends in terrestrial vertebrates. Ecology 59:1–18.

Foster, J. B. 1964. Evolution of mammals on islands. Nature 202: 234–235.

Goldman, E. A., and R. T. Moore. 1946. Biotic provinces of Mexico. Journal of Mammalogy 26:347–360.

Hall, E. R. 1981. The mammals of North America. John Wiley & Sons, Inc., New York.

Hall, E. R., and K. R. Kelson. 1959. The mammals of North America. Ronald Press, New York.

Hasegawa, M., K. Kishimo, and T. Yano. 1985. Dating the human–ape splitting by molecular evolution of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

Herron, M. D., T. A. Castoe, and C. L. Parkinson. 2004. Sciurid phylogeny and the paraphyly of Holartic ground squirrels (*Spermophilus*). Molecular Phylogenetics and Evolution 31:1015–1030.

Howell, A. H. 1938. Revision of the North American ground squirrels, with a classification of the North American Sciuridae. North American Fauna 56:1–256.

Kenagy, G. J., J. Whorley, P. Cortés-Calva, and S. T. Álvarez-Castañeda. 2005. Timing of reproduction in antelope ground squirrels, *Ammospermophilus leucurus*, near La Paz, Baja California Sur. Pp. 259–264 in Contribuciones mastozoológicas en homenaje al Dr. Bernardo Villa Ramírez (V. Sánchez-Cordero and R. Medellín, eds.). Universidad Nacional Autónoma de México, México City, México.

Lawlor, T. E. 1982. The evolution of body size in mammals. Evidence from insular populations in Mexico. American Naturalist 119:54–72.

Lawlor, T. E., D. J. Hafner, P. Stapp, B. R. Riddle, and S. T. Álvarez-Castañeda. 2002. The mammals. Pp. 326–361 in A new island biogeography of the Sea of Cortéz (T. J. Case, M. L. Cody, and E. Ezcurra, eds.). 2nd ed. Oxford University Press, Oxford, United Kingdom.

Lidicker, W. Z., Jr. 1962. The nature of subspecies boundaries in a desert rodent and its implications for subspecies taxonomy. Systematic Zoology 4:160–171.

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

Mascarello, J. T., and K. Bolles. 1980. C- and G-banded chromosomes of *Ammospermophilus insularis* (Rodentia: Sciuridae). Journal of Mammalogy 61:714–716.

Nelson, E. W., and E. A. Goldman. 1909. Eleven new mammals from Lower California. Proceedings of the Biological Society of Washington 22:23–28.

Nelson, E. W., and E. A. Goldman. 1929. A new antelope squirrel from Lower California. Journal of the Washington Academy of Sciences 19:281–282.

Nylander, J. A. 2004. MrModeltest v2.2. Distributed by the author, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden.

Patton, J. L., and S. T. Álvarez-Castañeda. 2005. Phylogeography of the desert woodrat, *Neotoma lepida*, with comments on systematics and biogeography. Pp. 375–388 in Contribuciones mastozoológicas en homenaje al Dr. Bernardo Villa Ramírez (V. Sánchez-Cordero and R. Medellín, eds.). Universidad Nacional Autónoma de México, México City, México.

Patton, J. L., and P. V. Brylski. 1987. Pocket gophers in alfalfa fields: causes and consequences of habitat-related body size variation. American Naturalist 130:496–506.

Posada, D. 2004. Collapse ver. 1.1. http://darwin.uvigo.es/software/collapse.html. Accessed April 2004.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Riddle, B. R., D. J. Hafner, L. F. Alexander, and J. R. Jaeger. 2000. Cryptic vicariance in the historical assembly of a Baja California peninsular desert biota. Proceedings of the National Academy of Sciences 97:14438–14443.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from distribution of pairwise differences when mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

Schneider, S., D. Roessle, and L. Excoffier. 2000. Arlequin, version 2.0. A software for population genetic data analysis. http://anthropologie.unige.ch/arlequin. Accessed February 2003.

SEMARNAT. 2002. Norma Oficial Mexicana NOM-059-ECOL-2001, protección ambiental—especies nativas de México de flora y fauna silvestres—categorías de riesgo y especificaciones para su inclusión, exclusión o cambio—lista de especies en riesgo. Diario Oficial de la Nación, 6 March 2002:56–85.

Sondaar, P. Y. 1977. Insularity and its effect on mammal evolution. Pp. 671–707 in Major patterns of vertebrate evolution (P. C. Goody, B. M. Hecht, and M. K. Hechtieds, eds.). Plenum Press, New York.

Smith, M. F. 1998. Phylogenetic relationships and geographical structure in pocket gophers in the genus *Thomomys*. Molecular Phylogenetics and Evolution 9:1–14.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Van Valen, L. 1973a. Body size and number for plants and animals. Evolution 27:27–35.

Van Valen, L. 1973b. Pattern and the balance of nature. Evolution Theory 1:31–49.

WALSH, P. S., D. A. METZGER, AND R. HIGUCHI. 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513.

WHORLEY, J., S. T. ÁLVAREZ-CASTAÑEDA, AND G. J. KENAGY. 2004. Genetic structure of desert ground squirrels over a 20-degree-latitude transect from Oregon through the Baja California peninsula. Molecular Ecology 13:2709–2720.

YENSEN, E., AND M. VALDÉZ-ALARCÓN. 1999. Family Sciuridae. Pp. 239–320 in Mamíferos del noroeste de México (S. T. Álvarez-Castañeda and J. L. Patton, eds.). Centro de Investigaciones Biológicas del Noroeste, La Paz, México.

ZHENG, X., B. S. ARBOGAST, AND G. J. KENAGY. 2003. Historical demography and genetic structure of sister species: deer mice (*Peromyscus*) in the North American temperate rain forest. Molecular Ecology 12:711–724.

Submitted 28 February 2006. Accepted 30 April 2007.

Associate Editor was Jesús E. Maldonado.

APPENDIX I

Localities of the specimens used for the morphometric analysis. Espíritu Santo Island (27): Espíritu Santo Island (112–120, 2805 CIB; 79074, 146774–146787 USNM; 42811–24814 MVZ). San Marcos Island (10): San Marcos Island (108–111, 8527–8532 CIB). Cape Region (19): 6 miles N San José del Cabo (110929 MVZ); San José del Cabo (42816 MVZ); San Lucas (42817–42821 MVZ); Agua Caliente (42822–42825 MVZ); Buena Vista (110923–110925 MVZ); El Chorro (110927 MVZ); El Carrizalito (110926 MVZ); San Juan de la Sierra (110930 MVZ); Los Burros (42815 MVZ); Todos Santos (42810 MVZ). El Triunfo (10): El Triunfo (110931–110940 MVZ). Ensenada de Muertos (16): 1.5 km S Los Planes (5500–5510 CIB); 4.5 km W Ensenada de Muertos (5495–5498 CIB); Ensenada de Muertos (5499 CIB). La Paz (61): 4 miles N La Paz (110928 MVZ), El Comitán, 17.5 km W La Paz (1641, 1642, 3485, 4914, 6139 CIB); 25 km W La Paz (6135 CIB); 26 km W La Paz (6136 CIB); 27 km W La Paz (6137 CIB); 28 km W La Paz (6138 CIB); 35 km W La Paz (6123–6134 CIB); 4 km N, 20 km W La Paz (6125, 6126 CIB); 4 km N, 23 km W La Paz (6127–6131, 8895–8898 CIB); 5 km S, 28 km W La Paz (6142–6145 CIB); 6 km N, 20 km W La Paz (6114–6124 CIB); Brisamar, 25 km W, La Paz (104–107, 1639, 1640, 4911–4915, 5492–5494, 6612, 8879, 8880 CIB). Ciudad Constitución (10): Ciudad Constitución (6102–6110 CIB). San Pedro de La Presa (2): San Pedro de la Presa 460 m (103, 5921 CIB). San Francisco de la Sierra (13): San Francisco de la Sierra (2876–2881, 8516 CIB); Rancho San Gregorio, 30.2 km N, 11 km W San Ignacio (8873–8875 CIB); Rancho San Marcos, 41.2 km N, 20 km E Guerrero Negro (10120–10122 CIB), 43.7 km N, 19.5 km E Guerrero Negro (10119 CIB).